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Revised Location of the *r*III Gene on the Genetic Map of Bacteriophage T4

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Two- and three-factor crosses showed that the T4 *r*III gene is located between genes 31 and 30 rather than between genes 31 and 63.

The current genetic map of bacteriophage T4 shows the arrangement of genes in the gene 31 region as: 63-[*pseT-unf-cd*]-*r*III-31-30 (14). A more recent paper presents evidence that the *unf* and *alc* markers are in the same gene and that *alc* also maps in the 63-31 interval (10). However, discrepancies in the ordering of amber mutations within gene 31 with respect to genes *r*III and 30 as outside markers suggest that this order may be incorrect. Georgopoulos et al. (7) reported the order *r*III-*amN*54 (gene 31)-*amNG*71 (gene 31), whereas Simon et al. (9) have found that apparent arrangement of these gene 31 mutants to be *amNG*71 (gene 31)-*amN*54 (gene 31)-gene 30. We have confirmed these observations by independent crosses shown in Table 1 (crosses 1-4). When *amN*54 carrying *r*67 (gene *r*III) as an unselected outside marker was crossed with *amNG*71, the majority of the am^+ recombinant progeny were r^+. Conversely, when *amNG*71 carrying *r*67 was crossed with *amN*54, the majority of the am^+ recombinants had the *r*67 allele. When *amN*54 carrying *tsB*20 (gene 30) as an outside marker was crossed with *amNG*71, the majority of the am^+ recombinants were ts^+, whereas the reverse was true when *amNG*71 carrying *tsB*20 was crossed with *amN*54.

These results are paradoxical in view of the previously published gene order *r*III-31-30 (6, 8), but can be resolved by assuming that the *r*III gene is located to the right of gene 31. Data from crosses 1 through 4 (Table 1) suggest that genes *r*III and 30 are on the same side of gene 31. The results of cross 5 support this conclusion. The finding that the majority of am^+ r^+ recombinants were *ts* when *amNG*71 carrying *tsB*20 was crossed with *r*67 indicates that the *r*III gene is located between genes 31 and 30. Table 2 gives the results of two three-factor crosses between markers in genes 31, *r*III, and 30, where all progeny genotypes were identified. The most frequent classes represent the parents of the crosses. Assuming that the least frequent class identifies the progeny resulting from a double-crossover event, these results indicate the gene order to be 31-*r*III-30. High negative interference (2) was evident from the frequency of the double-recombinant class, which was higher than

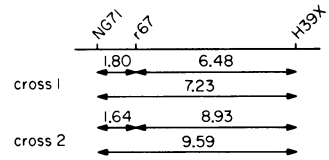
TABLE 1. *Gene 31 region of the T4 genetic map analyzed by three-factor crosses*[a]

	Cross	% Recombination	Outside marker mutant/total
1.	NG71 + + + / N54 r67	1.05	.14
2.	NG71 + r67 + / N54 +	.85	.88
3.	NG71 + + + / N54 tsB20	1.14	.31
4.	NG71 + tsB20 + / N54 +	1.16	.66
5.	NG71 + tsB20 + / r67 +	2.15	.68
6.	+ NG71 + pseT-1 + / N54	1.05	.66
7.	+ N54 r67 pseT-1 + / +	2.0	.63
8.	+ N54 + pseT-1 + / tsB20	12.63	.83

[a] Crosses were in *Escherichia coli* strain B40su_I^+ at 30°C under standard conditions (12, 13). Total progeny were determined by plating on B40su_I^+ at 30°C. am^+ recombinants were determined on S/6/5 su^- at 30°C (crosses 1-6), am^+ *r* recombinants were determined by plaque morphology on S/6/5 su^- at 30°C (cross 7), and am^+ ts^+ recombinants were determined on S/6/5 su^- at 42°C (cross 8). Heavy lines in the figure indicate the designated recombinant with an arrow directed towards the unselected outside marker. Percent recombination = (designated recombinant × 200)/total progeny. The status of the outside marker in the recombinant class selected was determined by differentiating *r* from r^+ plaques (crosses 1 and 2), by plating on S/6/5 at 42°C (crosses 3-5), or by stabbing the selected recombinants to lawns of CTr5x su^- (nonpermissive for *pseT*1 growth) at 30°C (crosses 6-8). Mutants used: *pseT*1 (gene *pseT*), *amNG*71 (gene 31), *amN*54 (gene 31), *r*67 (gene *r*III), and *tsB*20 (gene 30). For simplicity, the *am* prefixes have been omitted.

TABLE 2. *Location of rIII on the T4 genetic map*[a]

Progeny genotypes			Cross 1		Cross 2	
				frequency		frequency
gene 31	rIII	30	Number	%	Number	%
+	r67	+	568	92.2	56	8.4
NG71	+	H39X	656		47	
NG71	+	+	51	5.95	660	89.9
+	r67	H39X	28		437	
NG71	r67	+	6	1.28	2	.49
+	+	H39X	11		4	
+	+	+	5	.53	8	1.15
NG71	r67	H39X	2		6	
Total plaques identified			1327		1220	



[a] Crosses were performed in *Escherichia coli* strain B40su$_I^+$ at 30°C under standard conditions (12, 13). Total progeny was assayed on B40su$_I^+$. Progeny genotypes were analyzed by picking plaques from B40su$_I^+$ into 0.1 ml of H broth in wells in a plastic tray and spotting with a brass replicator into the following lawns of bacteria: (i) CR63su$_I^+$ Str^s; (ii) S/6/5 *su*$^-$ Str^r; (iii) S/6/5 su$^-$ Str^r + 2 × 10^7 *amH*39X (gene 30); (iv) S/6/5 su$^-$ Str^r + 2 × 10^7 *amNG*71 (gene 31). Plates 2 to 4 contained 500 μg of streptomycin per ml of top agar to eliminate phage growth due to transfer of B40su$_I^+$ Str^s cells to these test plates. The test plates were incubated at 30°C overnight and scored for phage growth. The parents of each cross are represented by the most frequent progeny genotype. The recombinant class with the fewest progeny presumably represents progeny arising from a double-crossover event, and it is designated by underlining. Mutants used: *amNG*71 (gene 31) *r*67 (*r*III), *amH*39X (gene 30). For simplicity, the *am* prefixes have been omitted.

expected from a consideration of the frequency of single-crossover events. Although this effect distorts the data in the three-factor crosses, as also observed by Simon et al. (9), the consistency of all two-factor crosses (summarized in Fig. 1) and three-factor crosses presented strongly supports the relocation of *r*III to a position between genes 31 and 30. Furthermore, when an allele of *pseT*, a gene located to the left of the controversial gene 31 region, was the outside marker in similar three-factor crosses (Table 1, crosses 6–8), its segregation indicated that the order of genes in the 31 region is *pseT*-31 (*amNG*71-*amN*54)-*r*III-30.

Independent evidence that the gene 31 alleles are oriented in the order *amNG*71-*amN*54-gene 30 comes from recent investigations of Castillo et al. (1) on the characterization of the gene 31 product (*gp*31) by sodium dodecyl sulfate-urea-acrylamide gradient gel electrophoresis. The protein is made early and thus presumably is transcribed counterclockwise from the light strand. The fact that a relatively large *gp*31 fragment is found in *amN*54 infections but not in *amNG*71 infections indicates that the *amNG*71 locus is near the promoter of gene 31, furthest from gene 30. This arrangement is consistent with the order *amNG*71-*amN*54-*r*67 only if *r*III is also on the promoter-distal side of gene 31.

How can the relocation of the *r*III gene, suggested by the present data, be reconciled with previous assignments of the *r*III gene to a position just clockwise from gene 31 (6, 8)? A survey of the literature suggests that the basis for the previous placement was somewhat arbitrary. In the original map of amber mutants, the *r*III gene

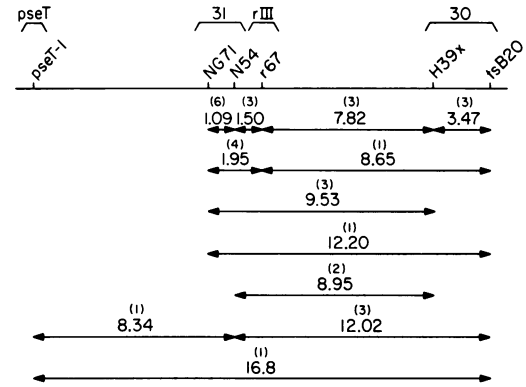


FIG. 1. *Gene 31 region of the T4 genetic map analyzed by two-factor crosses. Crosses were performed in Escherichia coli strain B40su$_I^+$ at 30°C under standard conditions (12, 13). Total progeny was determined on B40su$_I^+$ at 30°C. In crosses between am mutants, the am$^+$ recombinants were determined by plating on S/6/5 su$^-$ at 30°C. In crosses between an am mutant and a ts mutant, am$^+$ ts$^+$ recombinants were selected on S/6/5 su$^-$ at 42°C. In crosses between an am mutant and r67, am$^+$ r$^+$ recombinants were selected on S/6/5 su$^-$ at 30°C. In crosses between a ts mutant and r67, ts$^+$ r$^+$ recombinants were selected on S/6/5 su$^-$ at 42°C. In crosses between pseT1 and an am mutant, am$^+$ pseT$^+$ recombinants were selected on CTr5x su$^-$, which is nonpermissive for pseT1, at 30°C (3). In crosses between pseT1 and a ts mutant, ts$^+$ pseT$^+$ recombinants were determined both by plating on CTr5x at 42°C and by selecting ts$^+$ progeny on S/6/5 su$^-$ at 42°C and analyzing their ability to grow on CTr5x at 30°C. The orientation of amH39x and tsB20 in gene 30 was established by a three-factor cross relative to r67 as an outside marker (data not shown). Figures in parentheses represent the number of times the cross was done. Mutants used: pseT1 (gene pseT), amNG71 (gene 31), amN54 (gene 31), r67 (gene rIII), amH39x (gene 30), and tsB20 (gene 30). For simplicity, the am prefixes have been omitted.*

was placed in this position on the basis of "data to be published" (6). The recombination values, when published, showed that gene 31 is closer to *r*III than is gene 30, but there was no evidence indicating the order of these genes (4, 5). In fact, these authors as well as Stahl et al. (11), who published a composite map including more unpublished data, were noncommittal as to the location of *r*III in relation to genes 31 and 30. They presented two concentric maps, one of *r*III and various standard morphology and host range mutants and the other of amber mutants in essential genes, including genes 30 and 31. In Mosig's elegant experiments to physically map the T4 genome by marker rescue from light T4 particles, the various rapid lysis genes *r*I, *r*II, and *r*III were used as reference points to map distances to *am* mutations and thus provided no information on the absolute position of *r*67 (8). We conclude that there is no essential conflict with previous data, only with their representation, and that the present work establishes the position of the *r*III gene relative to adjacent genes.

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LITERATURE CITED

1. **Castillo, C. J., C. Hsiao, P. Coon, and L. W. Black.** 1977. Identification and properties of bacteriophage T4 capsid forming gene products. J. Mol. Biol. **110**:585–601.
2. **Chase, M., and A. H. Doermann.** 1958. High negative interference over short intervals of the genetic structure of bacteriophage T4. Genetics **43**:332–353.
3. **Depew, R. E., and N. R. Cozzarelli.** 1974. Genetics and physiology of bacteriophage T4 3'-phosphatase: evidence for involvement of the enzyme in T4 DNA metabolism. J. Virol. **13**:888–897.
4. **Edgar, R. S., G. H. Denhardt, and R. H. Epstein.** 1964. A comparative genetic study of conditional lethal mutations of bacteriophage T4D. Genetics **49**:635–648.
5. **Edgar, R. S., and I. Lielausis.** 1964. Temperature-sensitive mutants of bacteriophage T4D: their isolation and genetic characterization. Genetics **49**:649–662.
6. **Epstein, R. H., A. Bolle, C. M. Steinberg, E. Kellenberger, E. Boy de la Tour, R. Chevalley, R. S. Edgar, M. Susman, G. H. Denhardt, and A. Lielausis.** 1963. Physiological studies of conditional lethal mutations in bacteriophage T4D. Cold Spring Harbor Symp. Quant. Biol. **28**:375–394.
7. **Georgopoulos, C. P., R. W. Hendrix, A. D. Kaiser, and W. B. Wood.** 1972. Role of the host cell in bacteriophage morphogenesis: effects of a bacterial mutation on T4 head assembly. Nature (London) New Biol. **239**:38–41.
8. **Mosig, G.** 1968. A map of distances along the DNA molecules of phage T4. Genetics **59**:137–151.
9. **Simon, L. D., D. Snover, and A. H. Doermann.** 1974. Bacterial mutation affecting T4 phage DNA synthesis and tail production. Nature (London) **252**:451–455.
10. **Sirotkin, K., J. Wei, and L. Snyder.** 1977. T4 bacteriophage-coded RNA polymerase subunit blocks host transcription and unfolds the host chromosome. Nature (London) **265**:28–32.
11. **Stahl, F. W., R. S. Edgar, and J. Steinberg.** 1964. The linkage map of bacteriophage T4. Genetics **50**:539–552.
12. **Steinberg, C. M., and R. S. Edgar.** 1962. A critical test of a current theory of genetic recombination in bacteriophage. Genetics **47**:187–208.
13. **Wilson, J. H., and S. Kells.** 1972. Bacteriophage T4 transfer RNA. I. Isolation and characterization of two phage-coded nonsense suppressors. J. Mol. Biol. **69**:39–56.
14. **Wood, W. B., and H. R. Revel.** 1976. The genome of bacteriophage T4. Bacteriol. Rev. **40**:847–868.