MAPPING EXPERIMENTS WITH r MUTANTS OF BACTERIOPHAGE T4D[1]

R. S. EDGAR, R. P. FEYNMAN, S. KLEIN, I. LIELAUSIS, AND C. M. STEINBERG

Division of Biology, California Institute of Technology, Pasadena, California

Received September 22, 1961

ONE of the most frequent types of plaque morphology mutants with the T-even bacteriophages are the *r* (rapid lysis) mutants. A special subclass, the *r*II mutants, do not grow on certain bacterial hosts upon which the wild-type phage grows normally. This property allows a small fraction of wild-type recombinants from crosses to be easily enumerated, and it has been exploited by BENZER (1955, 1957, 1961) and by others to study the detailed genetic fine structure of the *r*II region. Most of the large number of *r*II markers isolated to date are in the phage strain T4B. Due to the availability of a large number of plaque morphology markers in another phage strain T4D, it was desirable to isolate and map a reasonably large number of *r*II markers in this strain, in order to provide material for various types of genetic studies. The mapping data and the data on the distribution of *r* markers are, however, of interest in themselves and are presented here.

MATERIAL AND METHODS

A detailed description of media, bacterial strains, and procedures for crosses and preparation of phage stocks is given by STEINBERG and EDGAR (1962).

The *r*I mutant, *r*48, is described by DOERMANN and HILL (1953). The *r*III mutant, *r*67, was obtained from DR. A. H. DOERMANN.

The *r*II mutants, *r*43, *r*65, *r*73, *r*61, *r*47, *r*64, *r*59, *r*70, *r*62 and *r*71, are described by EDGAR (1958). The *r*II mutants, *r*76 and *r*77, were recently isolated by and obtained from DR. A. H. DOERMANN. The *r*II mutants, s1, s2 and s3, were isolated by one of us (R.P.F.) in a special study of "partial revertants" to be reported elsewhere.

The remaining mutants were newly isolated in this laboratory. Each mutant was obtained from the contents of a different plaque of wild-type T4D and thus represents an independent mutational event. The mutants from this experiment carry the designation *r*ED followed by a number.

Criteria for acceptable crosses are those of CHASE and DOERMANN (1958). In a few cases, a given cross was repeated three or more times and one recombination

[1] This investigation was aided by a grant from the National Foundation (CVRE-120) and by a grant from the U.S. Public Health Service (RG-6965).

value differed from the mean of the others by a factor two or more; the aberrant value was assumed to be the result of a gross error and was discarded. Otherwise, all acceptable crosses are included in the data reported here.

EXPERIMENTAL RESULTS

Distribution of the mutants: Previously described *r* mutants of phage T4 fall into three clusters, each of which is essentially unlinked to the others. Since there is one cluster in each of the three so-called linkage groups, we will refer to the clusters as *r*I, *r*II, and *r*III, while noting that this classification differs somewhat from that of BENZER (1957). Typically, *r*II mutants fail to grow on lambda-lysogenic strains of *Escherichia coli* K-12. Out of 194 newly isolated *r* mutants, 64 were *r*II mutants by this criterion; detailed data on crosses of these mutants to each other and to previously described *r*II mutants are reported below. Some of the remaining mutants were crossed to one or more "tester" strains, *r*48, *r*EDb50, *r*67, which are respectively *r*I, *r*II, and *r*III. All proved to be closely linked to one of the testers. Out of 74 mutants studied in this way, there were 65 *r*I, seven *r*II, and two *r*III mutants.

The *r*I mutants are of two easily distinguishable phenotypes. Most exhibit a plaque morphology quite similar to *r*48. A few, however, give plaques which have fuzzy borders; these we designate as *rf* mutants. The *rf* mutants also require a longer time to lyse a bacterial culture in liquid medium. In addition, one of the *r*III mutants shows the *rf* phenotype. Several *rf* mutants were backcrossed to wild type, and, in each case, the *rf* character segregated as a single marker.

The existence of a class of *r*II mutants which are capable of plating on K-12 (λ) with near unit efficiency should also be noted.

The *r*II mutants which fail to plate on K-12 (λ) strains may be subdivided into two functional groups or cistrons by complementation tests (BENZER 1955). These tests were performed as spot tests. Out of 64 mutants, 26 proved to be in the B cistron, 37 in the A cistron, and one (*r*EDdf41) was a large multisite mutant covering the entire *r*II region. It is rather surprising that, aside from the single multisite mutant, all mutants were capable of spontaneous reversion. Of the A cistron mutants, 14 are apparently allelic to the previously described mutant *r*62, one gives no recombinants with *r*64, and 20 are at different sites (two were not studied). Of the B cistron mutants, ten are apparently allelic to the previously described mutant *r*43, one (*r*EDb50) gives no recombinants with *r*73, nine are at different sites (six were not studied, principally due to high reversion rates). Note that in each cistron over one third of the mutants are at one site. It is interesting that in phage strain T4B there is also one highly mutable site in each cistron (BENZER 1961). The identity of the A and B cistron "hot spots" in the two T4 strains has been established by crosses.

Fine structure mapping: A large number of two-factor crosses among *r*II mutants was performed. Wild-type recombinants were scored by plating upon a selective indicator strain. Many of the mutants came from the experiment described above; the sources of the others were given under materials and methods.

Most of the data from these crosses are given under the maps shown in Figures 1 and 2.

A few markers in the A cistron are not included in the map. The results of crosses with these markers are given in Table 1. Reasons for excluding these

FIGURE 1.—Map of the *r*II A cistron of T4D. Average recombination values (in %) are shown; the superscript is the number of crosses upon which the average is based. Markers enclosed in brackets are too close together to be ordered with certainty. The markers *r*61, *r*47, and *r*64 do not revert, and *r*47, in particular, is known to be a multisite mutant of some length; all other markers are capable of spontaneous reversion.

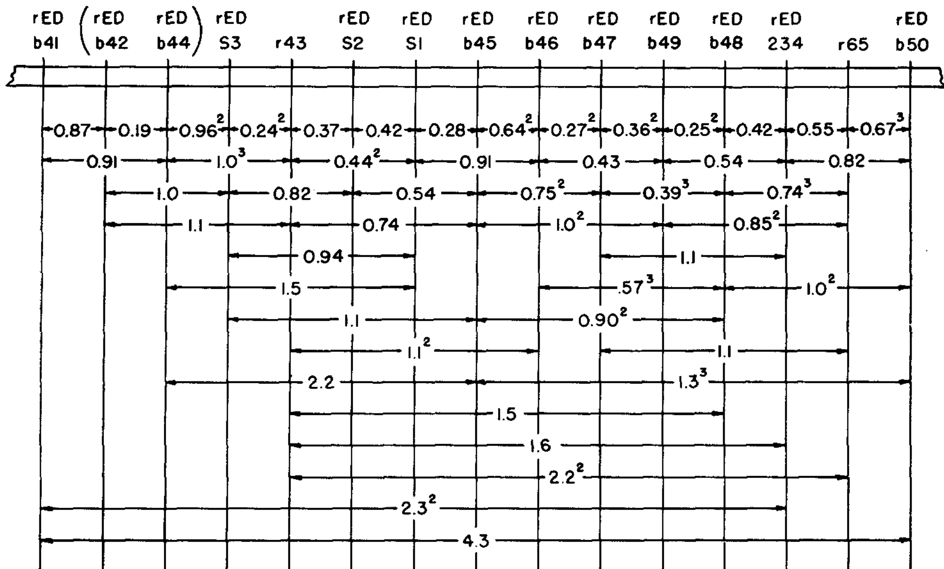

FIGURE 2.—Map of the *r*II B cistron of T4D. Average recombination values (in %) are shown; the superscript is the number of crosses upon which the average is based. Markers enclosed in brackets are too close together to be ordered with certainty. All of the markers are capable of spontaneous reversion.

markers from the map are: (1) Some are exceedingly close to one marker that is on the map and (relatively) distant from any other, making ordering by two-factor crosses impossible; (2) Insufficient crosses were performed so that an order cannot be established; (3) In three cases, the markers could not be placed on the map without serious inconsistency.

In view of the fact that the standard error of a single cross is expected to be about 25 percent of its mean (EDGAR 1958) and that these data were collected by a number of different investigators over a rather long period of time, the few inconsistencies with linear ordering should not be taken too seriously.

DISCUSSION

It had been hoped that among the non-*r*II mutants, new *r* loci would be discovered. This proved not to be the case. Even though most of the *r*II mutants could be screened out by testing for the ability to plate upon K-12 (λ), the frequency of *r*I mutants is so high that the probability of discovering possible additional loci by these means would be small. This is true, even if the postulated loci were represented in frequencies not much less than that of the *r*III locus. The enormous difference in apparent mutation rates between the *r*I and *r*II regions, on the one hand, and the *r*III region on the other is quite striking. Perhaps the *r*III region is very short genetic segment. More plausible, however, is the possibility that the reason for the difference is physiological rather than genetic. From the existence of gross alterations such as the mutant *r*EDdf41, which behaves as

TABLE 1

Recombination frequencies for A cistron crosses not given in Figure 1

Markers	Recombination frequency (percent)*	Markers	Recombination frequency (percent)*
*r*EDa43 × *r*ED348	0.028	*r*59 × *r*ED144	2.5
*r*ED144 × *r*ED159	0.041	*r*59 × *r*ED328	2.0[2]
*r*ED144 × *r*ED113	0.021	*r*59 × *r*ED37	1.8[2]
*r*ED159 × *r*ED113	0.054	*r*59 × *r*ED337	1.0
*r*70 × *r*ED338	0.0078	*r*59 × *r*70	0.98[2]
*r*ED159 × *r*ED19	0.38	*r*59 × *r*76	0.49[2]
		*r*59 × *r*ED230	0.22
*r*ED113 × *r*61	2.3	*r*59 × *r*77	0.39
*r*ED113 × *r*ED19	0.73	*r*59 × *r*62	1.4[2]
		*r*59 × *r*ED220	1.1
*r*ED344 × *r*EDa42	0.57		
*r*ED344 × *r*61	0.58	*r*76 × *r*ED336	0.63[2]
*r*ED344 × *r*EDa43	0.73[2]	*r*76 × *r*70	0.70
*r*ED344 × *r*47	0.81	*r*76 × *r*ED230	0.26
*r*ED344 × *r*EDa44	0.51	*r*76 × *r*77	0.26[2]
*r*ED344 × *r*EDa45	1.3	*r*76 × *r*62	1.1[3]
*r*ED344 × *r*ED19	3.3	*r*76 × *r*ED220	1.1
*r*ED123 × *r*64	0.00		
*r*ED123 × *r*ED35	0.56		
*r*ED123 × *r*71	1.8		
*r*ED123 × *r*ED336	2.4		
*r*ED123 × *r*59	2.9		

* Superscript is the number of crosses upon which the average value given is based.

if it were a deletion of the entire *r*II region, we reason that the physiological function of the *r*II region is completely dispensable for growth in bacterial host strain B. And it is surmised that the *r*I region is similar. Thus all mutations (which produce a difference in plaque morphology) at these loci would be nonlethal and hence isolatable. For a locus whose function is not dispensable, only the class of mutations which differ from wild-type sufficiently to produce a difference in plaque morphology, but which are not complete physiological negatives would be detectable. The *r*II mutants which are able to plate upon K-12 (λ) are examples of this class of mutation. We might postulate that in the *r*III region only such mutants are isolated. Furthermore, it is possible that other loci remain to be discovered. Brenner, Barnett, Crick and Orgel (1961) and Streisinger (personal communication) have evidence that most spontaneous mutations in T-even bacteriophages are of a type that complete physiological negatives result, and that this is not true for base analogue induced mutations. This would suggest that new *r* loci might be uncovered by screening mutants induced by base analogues.

Turning now to the fine structure mapping data, we would like to comment upon the question of the additivity of genetic distances. That is, to what degree does the recombination frequency between two markers approximate the sum of the frequencies of recombination in the elementary intervals between the

markers? In Figures 3 and 4 we have plotted "recombination frequency" *vs.* "map distance" for all of our crosses covering two or more elementary intervals. CHASE and DOERMANN (1958) reported data showing a remarkable degree of additivity despite the high negative interference which was manifested in three-factor crosses. HERSHEY (1958) pointed out that the additivity in CHASE and DOERMANN's data was, in fact, far too good and suggested a hypothesis ("marker stimulated recombination") in order to account for this. Qualitatively, it is evident in Figures 3 and 4 that almost all of the points lie below the line of strict additivity. Quantitatively, the larger recombination values are about a factor of two below the values expected on the basis of strict additivity. The discrepancy between our data and those of CHASE and DOERMANN is probably due to the fact that almost all of our elementary intervals are quite small, and furthermore, complications which occur with crosses between markers in different cistrons are absent.

Rather more basic than the question of additivity is the question of linearity. That is, can the markers be seriated in a linear order at all? BENZER (1961) has developed a new method of partially ordering *r*II markers. This method makes use of overlapping multisite mutants and permits a quantitative ordering of markers without reference to quantitative recombination frequencies. BENZER has unambiguously ordered 16 of our markers by this technique and the agree-

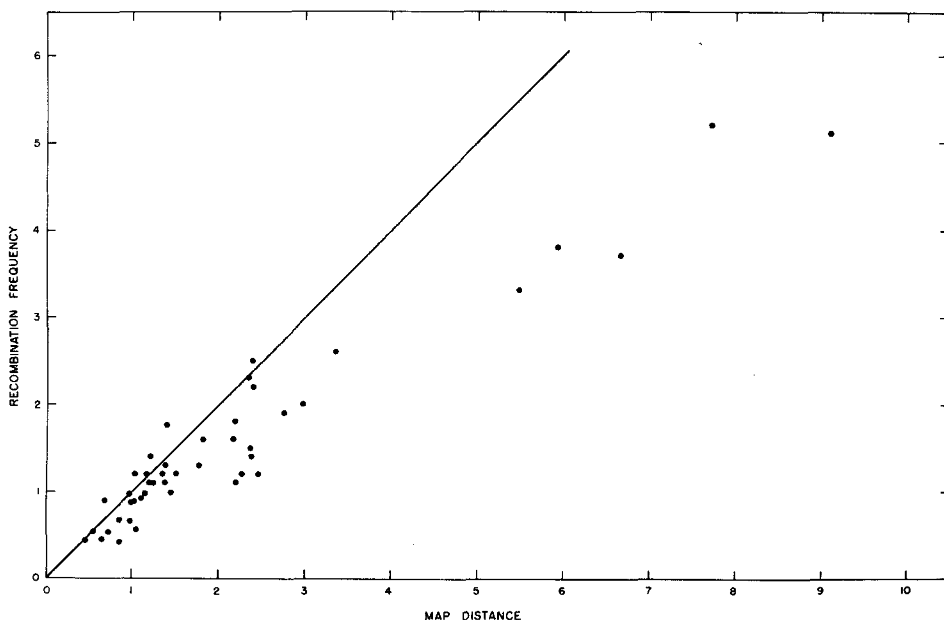

FIGURE 3.—Recombination as a function of map distance for *r*II A cistron markers. For each point, the ordinate ("recombination frequency") is the measured frequency of recombination for a given pair of markers, and the abscissa ("map distance") is the sum of the recombination values for the smallest intervals between the given pair of markers. Data in this figure are taken from Figure 1.

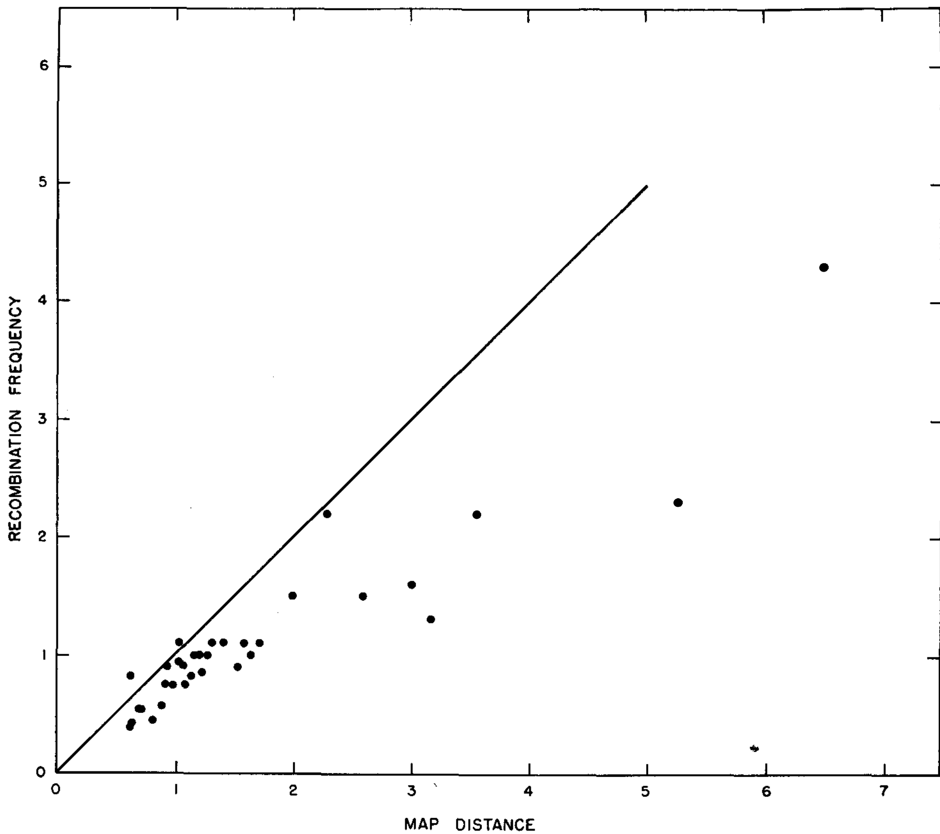

FIGURE 4.—Recombination as a function of map distance for *r*II B cistron markers. For each point, the ordinate ("recombination frequency") is the measured frequency of recombination for a given pair of markers, and the abscissa ("map distance") is the sum of the recombination values for the smallest intervals between the given pair of markers. Data in this figure are taken from Figure 2.

ment with the order established here is perfect. This result is already published and the reference cited and will not be elaborated on here. Suffice it to say that the agreement between the two essentially independent techniques places the linear structure of the gene on a very firm foundation.

SUMMARY

Results of mapping experiments with some 155 spontaneous *r* mutants of bacteriophage T4D are presented. The mutant markers were all located in the *r*I, *r*II, or *r*III region. The *r*II markers were further located within the *r*II region by two factor crosses. An analysis of these latter data shows the existence of high negative interference in two factor crosses. The order of the markers based upon the two factor crosses is in agreement with the order obtained by BENZER using deletion mapping of some of the same markers. The distribution of *r* loci in the T4D genome is discussed.

LITERATURE CITED

BENZER, S., 1955 Fine structure of a genetic region in bacteriophage. Proc. Natl. Acad. Sci. U.S. **41**: 344–354.

1957 The elementary units of heredity. pp. 70–93. *The Chemical Basis of Heredity*, Edited by W. D. MCELROY and B. GLASS. Johns Hopkins Press. Baltimore, Maryland.

1961 On the topography of the genetic fine structure. Proc. Natl. Acad. Sci. U.S. **47**: 403–415.

BRENNER, S., L. BARNETT, F. H. C. CRICK, and A. ORGEL, 1961 The theory of mutagenesis. J. Mol. Biol. **3**: 121–124.

CHASE, M., and A. H. DOERMANN, 1958 High negative interference over short segments of the genetic structure of bacteriophage T4. Genetics **43**: 332–353.

DOERMANN, A. H., and M. B. HILL, 1953 Genetic structure of bacteriophage T4 as described by recombination of factors influencing plaque morphology. Genetics **38**: 79–90.

EDGAR, R. S., 1958 Mapping experiments with *r*II and *h* mutants of bacteriophage T4D. Virology **6**: 215–225.

HERSHEY, A. D., 1958 The production of recombinants in phage crosses. Cold Spring Harbor Symposia Quant. Biol. **23**: 19–46.

STEINBERG, C. M., and R. S. EDGAR, 1962 A critical test of a current theory of genetic recombination in bacteriophage. Genetics **47**: 187–208.