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Genetic Map of Bacteriophage T3

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About 200 amber mutants of phage T3 were found to lie in 14 different genes. These genes are homologous to known T7 genes. The genetic map of T3 is very similar to that of T7.

Comparative studies with T3 and T7 are of interest because these related phages show several distinctive features which might be helpful for understanding their physiologies of infection. Thus, for instance, simultaneous mixed infection of a host cell by T3 and T7 leads to "mutual exclusion," i.e., each mixedly infected cell generally yields only one type of progeny phage (3). Mutual exclusion is probably at least one of the reasons for the low recombination rates between T3 and T7 (2). These low rates are in contrast to the rather extensive base sequence homologies between these phages (1). As a basis for further comparative genetic analyses of T3 and T7 it seemed indicated to construct a genetic map for phage T3 and to relate it to Studier's (8) detailed genetic map for T7.

For this purpose we have further investigated the amber mutants of T3 whose isolation and preliminary characterization has been described by Hausmann and Gomez (4). First, about 200 independently isolated mutants were assigned to complementation groups (genes) by mixed infection of the nonpermissive host in liquid culture, as described previously (5, 9). The mutants could thus be classified into 14 genes. In four instances representatives of more than one spot-test complementation group (4) showed no complementation among themselves when tested in liquid cultures; therefore, they were here assigned to the same gene. Most or all of the mutants in one gene were then crossed in all possible combinations among themselves; the technique was that of Hausmann and Gomez (4). The two mutants separated by the longest map interval within one gene were then selected as gene representatives. These gene-representing mutants were crossed in all combinations. In some cases, to be described in detail elsewhere (H. Beier, Ph.D. thesis, Univ. Freiburg), three-factor crosses with double mutants were also performed. Thus, it was possible to order the mutational sites on a linear map of about 100 map units. In Fig. 1 this map is compared to the T7 map published by Studier (8). The same nomenclature proposed by this author for T7 has been adopted for T3. The assignment of the T3 genes to their homologous counterparts of T7 was done—except for gene 1, whose product does not complement heterologous phage (4, 5)—through heterologous complementation studies with gene-representing mutants of T3 and T7, as described elsewhere (5). In addition to that, in the cases of genes 1, 3, 5, and 17, assays for the corresponding gene products, respectively: RNA polymerase, DNase, DNA polymerase, and serum blocking protein (5, 7) confirmed the assignments. Further evidence for the postulated homologies came (i) from comparing patterns of T3-directed DNA synthesis by gene-representing amber mutants during abortive infection with those patterns corresponding to T7 mutants (6), (ii) from the gene sequence on the map, and, especially, (iii) from the analysis of phage-coded proteins by the gel electrophoresis-autoradiography method (Issinger and Hausmann, manuscript in preparation).

Five genes for which T7 amber mutants are available, have not yet given origin to known mutants of T3 (Fig. 1). It is noteworthy that these are the genes for which relatively few T7 mutants are known (8). Thus, it seems likely that our lack of mutants in the postulated T3 genes is due to chance; in this case a saturating search for further mutants would be expected to be successful. However, it cannot be excluded that mutants in these genes have not been found because they are not essential for T3 growth and thus escaped detection by our isolation procedure. Some bias in our isolation method is suggested by the disproportionately high number of gene 1 mutants, as compared to mutants in other genes (Table 1). Lack of these genes in T3 is unlikely, since Davis and Hyman

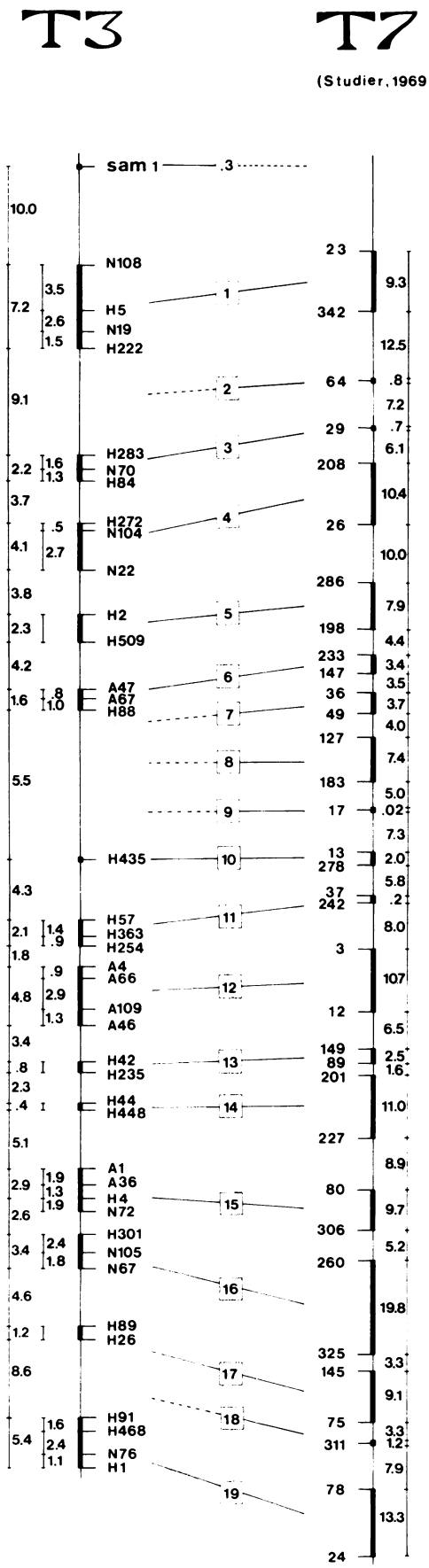

FIG. 1. *The genetic map of phage T3. A sample (0.25 ml) of an exponentially growing culture of permissive host bacteria (Escherichia coli BBw/1) was added to an equal volume of a mixture of two different amber mutants (multiplicity of infection was 5 particles of each parental type). The phage mixture contained potassium cyanide (3 mM final concentration). The phages were allowed to adsorb for 5 min, then specific antiphage serum (final concentration: K = 2) was added. After 5 min, the cells were diluted by a factor of 10^{-4} and allowed to lyse in nutrient broth. Platings were made on E. coli BBw/1 and on E. coli B/1. Further details of the crossing technique are given elsewhere (4). Map distances (to the left of the T3 map, and to the right of the T7 map) are given as twice the percentages of plaques on the nonpermissive host, relative to plaques on the permissive one. The numbers in squares refer to the gene numbers. The symbols and numbers which flank on the gene numbers specify the mutational sites of the mutants used. For comparison, some pertinent data on T7, from Studier (8), have been included in this figure. The "left" ends of the maps are at the top.*

TABLE 1. *Correspondence between the T3 spot-test complementation groups of Hausmann and Gomez (4) and T3 genes, in accordance with Studier's (8) nomenclature*

Gene	Spot-test group	No. of isolated amber mutants
1	1, 2, 3	79
3	15	7
4	4, 5	11
5		4
6	6	8
10		2
11		5
12	7	17
13	12	2
14	13	3
15	9, 11, 14	27
16	8, 17, 18	21
17	10	2
19	16	5

(1) have shown that no major deletions, duplications or inversions occur on the T3 DNA as compared to T7 DNA.

For completeness, the *sam* gene of T3, which codes for the nonessential *S*-adenosylmethionine-cleaving enzyme, and which was mapped by Hausmann and Härle (5), has been included in the T3 map of Fig. 1. This gene, tentatively termed gene 0.3 by Summers, has no apparent functional counterpart in T7.

Since the genetic maps of T3 and T7 are very similar, it will be of interest to look for the specific causative factors of the physiological incompatibility between these phages.

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