HETERODUPEX HETEROZYGOTES IN BACTERIOPHAGE T4 INVOLVING MUTATIONS OF VARIOUS DIMENSIONS*

By John W. Drake

Department of Microbiology, University of Illinois, Urbana

Communicated by S. Spiegelman, January 6, 1966

Single-factor genetic crosses in bacteriophage T4 produce two distinct types of heterozygous progeny. One kind probably corresponds to a terminal redundancy of the chromosome. The other kind, variously termed a heteroduplex heterozygote, an internal heterozygote, or a recombinational heterozygote, arises as an intermediate in recombination (Fig. 1). Internal heterozygotes are preferentially accumulated in the presence of FUDR, which inhibits DNA replication and therefore inhibits the conversion of internal heterozygotes into homozygous progeny. Furthermore, only certain types of mutational lesions are efficiently recovered from internal heterozygotes. If a cross is performed between two different mutants, one of which (point mutation) contains a base pair substitution and the other an extended deletion, the heterozygous particles which emerge usually contain the point mutant. This result indicates that internal heterozygotes cannot easily accommodate mutations of extended dimensions, presumably because such heterozygotes would contain single-stranded loops. At present it is not clear whether deletion mutations fail to form internal heterozygotes initially, or whether the heterozygotes are formed but are later repaired by a mechanism leading to homozygosity.

The sign mutants of bacteriophage T4 consist of additions and/or deletions of small numbers of base pairs; they are also called "reading-frame mutants" and "acridine-type mutants." An extensive fine-scale map of the rII mutants in the B cistron strongly suggests that sign mutants possess extended dimensions (Fig. 2). An analysis of the ability of internal heterozygotes to encompass sign mutations should therefore yield information about the physical dimensions of the mutations, and also about putative single-stranded regions within the DNA molecule.

Materials and Methods.—Strains of bacteriophage T4B and its rII mutants, and of Escherichia coli, were obtained from the collection of Dr. Sydney Brenner and Mrs. Leslie Barnett in Cambridge. All incubations were at 37°. E. coli strain Bφ plates r phages as large plaques, and r+ phages as small plaques. Strain BB does not distinguish r and r+ phages, and is used to grow stocks and for crosses. Strain OP33 is a K-12(λ) derivative which transmits rII mutants at a very low frequency. Strain QA1 is a K-12(λ) derivative which permits the growth only of certain rII mutants of the amber class, such as rX417. The various rII mutants employed have been extensively mapped, and a preliminary and condensed map appears in Figure 2. All of the mutants except r187 and r196b are capable of reversion.

FUDR crosses were performed according to Shalitin and Stahl. BB cells in M9-casamino acids medium were infected with 5 of each parental phage. At 2 min, FUDR was added to 4 × 10⁻⁴ M, and uracil to 2 × 10⁻⁴ M. The phage-cell complexes were aerated continuously thereafter. At 9 min, chloramphenicol was added to 125 µg/ml. At 90 min the complexes were chilled and centrifuged at low speed. They were resuspended in the same medium without chloramphenicol (but with FUDR and uracil) at about 10⁸/ml and incubated without aeration for an additional 60 min. Lysis was completed with chloroform. Burst sizes ranged from 1 to 10, but were usually about 5.

Enrichment plating for heterozygotes was also performed according to Shalitin and Stahl. OP33 cells were suspended in buffer containing 4 × 10⁻⁴ M KCN. The lysate from an FUDR cross was adsorbed to the cells at a total multiplicity of 0.01 or less. After 4 min of adsorption, during which
over 95% of the phages adsorbed, antiserum was added to inactivate all unadsorbed phages. The complexes were then plated on B cells. From 10 to 80% of the resulting plaques were mottled, indicating heterozygosity.

**Scoring of heterozygotes:** The majority of the crosses were performed between a suppressible rII mutant (rX417, an amber mutant) and an rII mutant (test mutant) not suppressed by QA1. Over 100 mottled plaques were picked from each cross. Each was streaked out on plates which had been previously poured with soft agar containing 2 drops of a mixture of overnight cultures of B and QA1 cells in the ratio 2:1. (The use of this mixed indicator was suggested by Mrs. Leslie Barnett.) Virtually all of the heterozygotes contained wild-type phages, plus one but not both of the parental r phages, in agreement with the results of Hertel.8 Each genotype formed a distinctive plaque type on the mixed indicator, and scoring was unambiguous.

Another series of crosses was performed between a deletion mutant (r187) and various test mutants. When the test mutants were of the amber type, the procedure described above was used for scoring heterozygotes. When the test mutants were not suppressible, mottled plaques were picked and streaked out on B cells, and an isolated r plaque was picked from each heterozygote into about 0.2 ml of broth. To this was added a drop containing the deletion mutant (about 10⁶ phages per drop) and a drop of log phase BB cells. After 4 hr of incubation, the mixtures were spotted onto OP33 cells. Complete lysis in the spot identified the heterozygote as containing the test mutant; no lysis, as containing the deletion mutant.

These tests were shown to be reliable in reconstruction experiments.

**Results.—** Crosses were first performed between an amber mutant, rX417, and a series of test mutants. Only the first 100 heterozygotes to be scored were recorded for each test mutant. The mutants are arranged in Figure 3 in order of their increasing rates of recovery from heterozygotes. The dotted lines delimit the range within which 95 per cent of the values would fall, if rX417 and the test mutant were equally frequently represented. The symbol used for each mutant indicates at least roughly its genetic extent. Thus “small” sign mutants cover only one site (see Fig. 2); “medium” sign mutants cover two sites (fail to recombine with two different mutants which can themselves recombine); and “large” sign mutants cover more than two sites. Ochre mutants are suppressible (although not by strain QA1), and usually contain a base pair substitution.9 The following conclusions may be drawn.

1. The results of Shalitin and Stahl2 are confirmed. The deletion mutant r196b is rarely recovered from heterozygotes, while the six ochre mutants are recovered with an average frequency of 48.8 per cent compared to the expected 50 per cent.

2. The transition from allele exclusion to maximum recovery is not an abrupt change, but rather a gradual increase.

3. The recovery frequencies of the sign mutants are strikingly correlated with

![Figure 1](image-url)
Fig. 2.—Left end of the B cistron. This is a preliminary and condensed version of a much more extensive map prepared by Mrs. Leslie Barnett and Dr. Sydney Brenner. Nonrecombining sites are drawn as overlapping. The named sites are those employed in the present study. Many of the sites arose repeatedly, and thus represent hot spots. Sites falling within the line are sign (0) mutants (ochres and ambers). Sites above the line have sign (+), and below the line sign (−).

their genetic extent. Most of the “large” sign mutants are infrequently recovered from heterozygotes. The “small” mutants are a more heterogeneous set, but are concentrated at the higher recovery frequencies.

(4) Several “small” sign mutants are nevertheless infrequently recovered from heterozygotes, including some (r1074, rFC223) lying in densely marked regions of the map.

Any monotonically arranged set of data will tend to increase gradually, even if actually representing an abruptly changing function, because of accumulated variations in the measurements. It is therefore important to verify the shape of the gradually rising portion of the curve in Figure 3. This was accomplished by performing crosses between a deletion mutant, r187, and a series of test mutants se-
Fig. 3.—Marker recovery frequencies from heterozygotes. Heterozygotes were obtained from FUDR crosses between the test mutants and the amber mutant rX417, and scored for the presence of the test mutant. The expected recovery frequency for a true point mutant is 50% ± 10% (two standard deviations) for sample sizes of 100. Open squares, “large” sign mutants covering more than two sites; open circles, “medium” sign mutants covering just two sites; solid circles, “small” sign mutants occupying a single site; solid squares, ochre mutants.

lected to span the critical region. In this case, the test mutants tend to pre-
dominate over the deletion in heterozygotes, the more so the smaller the mutant
lesion. The results are shown in Figure 4. The rise in heterozygote participation
values in the r187 crosses orders the mutants in very nearly the same sequence as
that shown in Figure 3, except that rFC9 was recovered slightly less frequently
than expected. Four additional amber mutants were also recovered at frequencies
close to 100 per cent.

Two “large” sign mutants (rJ158 and rFC211) from the first set of crosses were
later discovered to map as identical short deletions. They also exhibited the same
frequencies of recovery from heterozygotes (20 and 21%, respectively).

The cross between r187 and r196b revealed that the former was significantly less
frequently represented than the latter among the resulting heterozygotes. The
frequency of heterozygotes on the selection plates was also relatively much smaller
than what was customarily observed in crosses between base pair substitution
mutants.

Discussion.—The method: Of the two types of T4 heterozygotes, internal hetero-
gyotes are preferentially accumulated in FUDR crosses.1 2 Deletion mutations
are rarely recovered from internal heterozygotes. The use of FUDR therefore
greatly magnifies the differences between point mutants and mutants of larger
extent. However, heterozygotes containing deletion mutations are selected to a
limited extent from FUDR crosses by the methods employed here.2 Several per
cent of heterozygotes also appeared on the selection plates from the cross between
r187 and r196b. It is not yet clear whether these heterozygotes represent relatively
rare internal heterozygotes, in addition to terminal redundancy heterozygotes.

The heterozygote enrichment plating method should result in the loss of half of
the internal heterozygotes, since the only phages which will multiply in OP33 cells are those which possess both r+ alleles on the strand of DNA which specifies messenger RNA. However, there is presently no reason to suspect that any allele would be recovered at reduced frequency from either strand of DNA.

In each of the crosses recorded here, the standard mutant and the test mutant were compared within a common lysate, so that physiological differences between crosses may be excluded as sources of the observed mutant-specific heterozygote recovery frequencies. The reproducibility of the results was further confirmed both by comparing two “large” sign mutants which later turned out to map as identical short deletions, and also by comparing crosses against a standard deletion mutant with crosses against a standard ochre mutant.

The extent of mutational lesions: The original investigation of sign mutants characterized them by means of an algebra which assigned to each a sign, usually (+) or (−), but sometimes (0). Double mutants composed of one (+) and one (−) mutation constitute a mutually suppressing pair. The most simple molecular configuration for sign mutants would be additions and deletions of single base pairs. It is clear from the present study, however, that many sign mutants behave as if their mutational lesions were much more extensive. The data suggest a more or less continuous distribution of lesion sizes, from true point mutations to conventionally described deletions. However, nearly all of the sign mutants tested here were of spontaneous origin. Mutants induced by proflavin or by ultraviolet irradiation might exhibit different size distributions.

Heterozygote frequencies have been reported to decline as markers progressively farther from the rII A/B cistron division were tested, although this conclusion was based on small-sized samples. However, this apparent decline might have arisen from the use of fortuitously arranged rII sign mutants with differently sized lesions. When recovery frequencies from the present study were plotted against map order, the result was a wild scatter without any downward trend away from the cistron division.

It should be possible to distinguish between addition and deletion mutants by the tests employed here. Small deletions would fail to recombine with two or more different sites, and would be infrequently recovered from heterozygotes; many mutants of this type were encountered. Small additions would map as points, since they would possess the DNA base sequences required for recombination with any other portion of the cistron, but would nevertheless be infrequently recovered.
from heterozygotes. The more densely mapped the region around such a mutant, the more reliable would be its classification. Mutants r1074 and rFC223 are putative examples of addition-type sign mutants. Streisinger has analyzed the amino acid sequence of a mutant T4 protein resulting from a pair of mutually suppressing sign mutants. The total number of amino acids in the affected region remained constant. One of the sign mutants must therefore have been an addition and the other a deletion of the same number of base pairs.

A third class of sign mutants might also be revealed by these tests: additions and deletions of sign (0). All of the sign (0) mutants in this portion of the B cistron contain chain-terminating amber and ochre codons. These might arise either by base pair substitutions, or by additions or deletions of multiples of three base pairs. Mutants of the latter type might be infrequently recovered from heterozygotes, and might also cover two or more sites on the map. Both the ochre mutation rX511 and the amber mutation r2074 appear to cover two sites on the preliminary map shown in Figure 2. However, none of the six ochre or four amber mutants behaved like extended sites in heterozygote participation tests.

The methods developed here should be useful for quantitatively estimating the extent of sign mutant lesions, especially where additional markers are not available for fine-scale mapping or for measurements of outside marker contraction or expansion. The method may also allow sizing of larger deletions: whatever the nature of the heterozygotes containing r187 and r196b, the relative sizes of the two probably affect their relative recovery frequencies from heterozygotes.

The DNA of internal heterozygotes encompassing sign mutations presumably contains short, looped-out, single-stranded regions. Taking the base pair content of T4 as about $2 \times 10^6$, the total map length as close to 2400 units, the length of the B cistron as 6.5 units, the region of the B cistron shown in Figure 2 as about 40 per cent of the whole cistron, estimated from the total number of sites, and the longer deletion-type sign mutants of Figure 2 as up to 10 per cent of this region, then the looped-out region would contain up to 22 bases. Single-stranded regions of this magnitude are probably too small to detect unambiguously at present.

Summary.—The DNA of an internal heterozygote contains different genetic information on opposite strands in a limited region of the molecule. True point mutations resulting from a base pair substitution will readily form internal heterozygotes during genetic recombination, but extended deletions will not, presumably because of distorted pairing between complementary strands of the DNA. The rII sign mutations of bacteriophage T4 arise from additions or deletions of small numbers of base pairs, and sometimes map as very small deletions. Sign mutants participate in internal heterozygotes at various characteristic frequencies which are measures of the extent of the mutational lesions. Criteria were developed for deciding whether mutants of any algebraic sign contain additions or deletions of base pairs. The DNA of internal heterozygotes encompassing sign mutations must contain very short single-stranded loop-outs.

The author is very grateful to Drs. Sydney Brenner and Francis Crick for providing him with a constantly stimulating environment during his visit to their laboratory in 1964–65. The present work evolved from discussions in Cambridge with Dr. Frank Stahl. The author is also very grateful to Mrs. Leslie Barnett for supplying him with most of the phage and cell stocks.

* This investigation was initiated at the Medical Research Council Laboratory of Molecular
Biology, Hills Road, Cambridge, while the author was a Guggenheim fellow. It was supported in part by U.S. Public Health Service research grant AI-04886 from the National Institute of Allergy and Infectious Diseases.

2 Shalitin, C., and F. W. Stahl, these PROCEEDINGS, 54, 1340 (1965).
3 A repair mechanism which removed single-stranded loops would convert deletion mutation heterozygotes into homozygous mutants, and addition mutation heterozygotes into homozygous wild-type phages. The result would be a curious type of weak, allele-specific selection during mixed growth of sign mutants and wild-type phages.

6 The author is indebted to Mrs. Leslie Barnett and to Dr. Sydney Brenner for making available the results of their ultrasure-scale mapping experiments, and for permission to present the abbreviated map of Fig. 2.
7 Benzer, S., these PROCEEDINGS, 47, 403 (1961).
12 Berger, H., Genetics, 52, 729 (1965).
15 Stahl, F. W., R. S. Edgar, and J. Steinberg, Genetics, 50, 539 (1964).
16 Edgar, R. S., R. P. Feynman, S. Klein, I. Lielausis, and C. M. Steinberg, Genetics, 47, 179 (1962).

DNA REPLICATION

By H. E. Kubitschek* and T. R. Henderson†

Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois, and Biochemistry Department, University of Arkansas School of Medicine, Little Rock

Communicated by George W. Beadle, January 17, 1966

As reported earlier, segregational division could not be detected in continuous, glucose-limited cultures of strains derived from Escherichia coli B when mutation to resistance to bacteriophage T5 was induced with acridine orange and visible light, methylene blue and light, or with 2-aminopurine.1, 2 Since chromosome replication appears to be initiated more or less randomly from different starting points in the B and other F− strains, the absence of segregation cannot be explained as mutation of the T5 locus with or just prior to replication and cell division. In addition, mutant frequencies were so low in these experiments that it is extremely unlikely that both strands were mutated during exposure to the mutagen. Furthermore, it is improbable that the absence of segregation could be attributed to a later alteration of the second strand, leading to mutation of both strands before cell division. Such an alteration might be expected to occur in the presence of DNA "repair" processes that permit the mutant code to be copied from the altered strand to its complement.