FURTHER EVIDENCE FOR POLARITY MUTATIONS IN BACTERIOPHAGE T4

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Received November 4, 1966

AMBER mutants (EPSTEIN et al. 1963) in a few neighboring genes in phage T4 complement each other only poorly (STAHL, MURRAY, NAKATA and CRASEMANN 1966). In some of these cases, the yield of phage obtained from mixed infections of a restrictive host with the amber mutations cis to one another is significantly greater than when those mutations are in the trans configuration. Analysis of these cases of cis-trans position effect suggested that one member (at least) of each pair of mutants showing the effect was a "polarity mutant.">

In this note we report two more characteristics of the cis-trans effect both of which reveal properties of polarity mutants previously described in bacterial systems. (1) There is a gradient in the degree to which amber mutants in a given gene depress the activity of the neighboring gene; ambers close to the neighboring gene give better complementation than do the more distant ones. (2) Multiple ambers in the same gene have no (or only slightly) stronger polarity than does the strongest of the component single ambers.

MATERIALS AND METHODS

Phage: The following amber mutant strains of T4D were employed: A465, B258, B285, B25, A454, B265, and A459 in gene 34, obtained from R. S. EDGAR; N58 in gene 34, B252 in gene 35, and N131 in gene 26, obtained from S. BRENNER. The mutant rII196 from S. BENZER was used in mapping experiments. Multiple mutants as desired were identified among the progeny of standard crosses by spot-test backcrosses. In each case the genotype of the presumptive multiple was then verified by backcrosses conducted in the standard manner.

Bacteria: Host-cell bacteria (E. coli strain BB) for complementation tests, and plating bacteria (E. coli strain CR63) were prepared according to STAHL, MURRAY, NAKATA and CRASEMANN (1966). Strain B and its lysogenic derivative G(λ) obtained from Dr. J. J. WEIGLE were used as indicators in the mapping experiments.

Complementation tests: Conditions were as described previously (STAHL, MURRAY, NAKATA and CRASEMANN 1966) except where noted.

RESULTS

(1) Gradient in polarity. Amber N58 in gene 34 was shown previously (STAHL, MURRAY, NAKATA and CRASEMANN 1966) to depress the activity of gene 35, as deduced from a cis-trans position effect. From R. S. EDGAR we obtained seven other ambers at different sites in gene 34 in order to ascertain the dependence,

1 Supported by National Science Foundation Research Grant GB294.
### TABLE 1

"Polarity" of single ambers

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The ability of each of the listed amber mutants in gene 34 to complement amB252 in gene 35 was tested as described in the text. The entries in the table are yield/productive cell. Ambers A455 and B258 are seen to complement better than any of the other six ambers. A qualitative estimate of the degree of polarity of the gene-34 ambers is provided by a comparison with the yields obtained when amN131 in gene 26 was used in the same fashion to complement amB252. All of the experiments we performed are presented in the table, since the differences claimed are small, and we could find no satisfactory way to summarize the data. It is important not only that the mean values of yield/productive cell for B258 and A455 are (probably significantly) higher than any of the other six ambers, but also that on each day for which a comparison can be made the values for these two mutants are higher than for any others.
if any, of the degree of depression of gene 35 activity upon map position of the amber.

The conduct of trans complementation tests differed in two respects from those described previously (Stahl, Murray, Nakata and Crasemann 1966). We anticipated that the tests would be more sensitive under conditions of unequal input. The mutant (N58) previously studied was not a very strong polar; it gave yields in the trans configuration which varied from 17 to 40% of the yield obtained in the cis configuration. The polar property of a mutant will register as a reduction in burst size only in those cells in which the amount of product of the depressed gene limits the yield. For experiments involving rather weak polar mutants, some of the polar effect may be lost in equal input experiments; the variation in numbers of the two infecting types from cell to cell will result in a class of cells in which the polar mutant is in excess and its gene product no longer limiting. Thus, in the present experiments, the phage bearing the amber mutation in gene 35 were adsorbed to BB at a multiplicity of ten particles per cell while the various ambers in gene 34 were adsorbed at 0.1 particles per cell. Under these conditions of unequal input most of the cells are not mixedly infected. The total yield, therefore, will be sensitive to (proportional to) the number of cells which are successfully infected by the minority parent. We took this number to be the number of cells which yield one or more phage particles. The number of productive cells was determined by plating after serum-inactivation of unadsorbed phage; the total yields were measured following chloroform treatment of a diluted suspension of the serum-treated infection mixture. The data are reported as yield per productive cell.

As shown in Table 1, two of the ambers give yields per productive cell about twice as large as do the other six.

A consequence of using an unequal input in the trans configuration is that an appropriate cis control does not exist. A qualitative indication of the intensity of the polarity effect of the various gene-34 ambers is given by the yield from the complementation of amN131 in gene 26 by amB252 in gene 35 under the same unequal input conditions (Table 1).

The location of each of the two "weak polarity mutations" with respect to the other seven ambers was determined by crosses of the type rIIam\textsubscript{weak} × r+am\textsubscript{x} performed in CR63 at a multiplicity of 5 of each parent. The order of the two ambers with respect to rII was decided by the frequency of rII among am\textsuperscript{+} recombinants. The results (Table 2) show that A455 is the extreme member of the set of eight ambers and that B258 is the next most extreme. Crosses of the same sort were performed to reaffirm the previously published order (see Stahl, Edgar, and Steinberg 1964), rII gene 35 gene 34. The two weak polars are nearer to gene 35 than are any of the six strong polars.

(2) The degree of polarity of multiple ambers in gene 34 was determined by trans tests also under conditions of unequal input. The multiple amber to be tested was adsorbed at a multiplicity of 0.1 along with ten particles per cell of amber B252 in gene 35. Platings before and after lysis permitted the determination of yield per productive cell (Table 3). It may be seen that the trans tests with the multiple ambers gave only slightly lower yields per productive cell than did the
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TABLE 2A

Determination of the map position of the "weak polarity mutant" amA455 in gene 34

<table>
<thead>
<tr>
<th>Cross: r196 amA455 X</th>
<th>Percent am+ recombinants</th>
<th>r196 plaques/total plaques</th>
<th>Marker-order deduced</th>
</tr>
</thead>
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<tr>
<td>amB25</td>
<td>14</td>
<td>273/786</td>
<td>r196 amA455 amB25</td>
</tr>
<tr>
<td>amA454</td>
<td>13</td>
<td>300/877</td>
<td>r196 amA455 amA454</td>
</tr>
<tr>
<td>amB265</td>
<td>17</td>
<td>355/995</td>
<td>r196 amA455 amB265</td>
</tr>
<tr>
<td>amN58</td>
<td>15</td>
<td>358/889</td>
<td>r196 amA455 amN58</td>
</tr>
<tr>
<td>amA459</td>
<td>11</td>
<td>319/867</td>
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<tr>
<td>amB285</td>
<td>11</td>
<td>262/708</td>
<td>r196 amA455 amB285</td>
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<tr>
<td>amB258</td>
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</tr>
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<td>amB252 (gene 35)</td>
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TABLE 2B

Determination of the map position of the "weak polarity mutant" amB258 in gene 34

<table>
<thead>
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<th>Cross: r196 amB258 X</th>
<th>Percent am+ recombinants</th>
<th>r196 plaques/total plaques</th>
<th>Marker-order deduced</th>
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</thead>
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<td>amB25</td>
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<tr>
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<tr>
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<td>435/663</td>
<td>r196 amB252 amB258</td>
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</tbody>
</table>

Standard crosses at a nominal multiplicity of five of each parent were performed on the permissive strain CR63. The lysates obtained were plated with strain B over a previously poured top layer seeded with G(X). Only the am+ recombinants make plaques. Among these am+, r196 and r+ were distinguishable as turbid and clear plaques respectively. The reported frequencies of r196 among am+ are the number of turbid plaques per total plaques on these double-layer plates.

TABLE 3

"Polarity" of multiple ambers

<table>
<thead>
<tr>
<th>Genotype of multiple amber</th>
<th>No. of experiments</th>
<th>Yield per productive cell</th>
<th>Yield/productive cell of strongest polar component mutant</th>
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</thead>
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<tr>
<td>B25–B265</td>
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</table>

The ability of each of the indicated multiple amber mutants in gene 34 to complement amB252 in gene 35 was tested as described in the text. The yields/productive cell of the strongest polar component mutant are taken from Table 1.
tests with the strongest of the component single mutants. The observed reduction in yield per productive cell may mean that multiple ambers have slightly stronger polarity than single ambers. However, the failure of the reduction to depend on the number of ambers in the multiple mutant argues against this. In any case, the reduction is so small that the elimination of other explanations would be a formidable task. The experiments as they stand permit the conclusion that, as in bacterial systems (YANOFSKY and ITO 1966), the polarity of a multiple amber is determined (essentially) by its strongest component single amber.

DISCUSSION

In bacterial operons amber mutants in a given gene have been shown to depress the yield of gene product from genes to one side of it in the same operon. Plausible explanations for this phenomenon invoke the hypothesis that the genes in a given operon are cotranscribed. A recurring feature of the polarity phenomenon is that the nearer the amber is to the gene(s) whose activity is depressed, the less the depression. The demonstration of this same feature in the case of the depression of gene 35 activity by ambers in gene 34 strengthens the previous conclusion (STAHL, MURRAY, NAKATA and CRAESMANN 1966) that this gene pair (as well as gene pair 51–27 described by the same authors) is cotranscribed. Additional cis-trans complementation tests (in progress) indicate that cis-trans position effects are rare, as implied by previous observations (e.g. EDGAR, DENHARDT and EPSTEIN 1964; STAHL and MURRAY 1966). Thus, although cotranscription of some pairs of adjacent genes occurs in T4, it appears likely that this situation will prove to be exceptional.

In bacterial systems (YANOFSKY and ITO 1966) it has been reported that double amber mutants do not show stronger polarity than does the stronger of the individual mutants. Our observations in T4 are not in significant disagreement with this observation and permit the (rather trivial) extension of that statement to cover triple and quadruple amber mutants.

SUMMARY

The degree of complementation between amber mutants in genes 34 and 35 of T4 depends on the position of the mutant in gene 34. Mutants close to gene 35 complement better. This result, and the observation that multiple ambers in gene 34 complement gene 35 about as well as their single components, is reminiscent of the behavior of polarity mutations in bacterial operons. The previously drawn conclusion that some gene pairs in T4 are cotranscribed is thereby strengthened.
LITERATURE CITED


