Biochemical Changes during Mixed Infections with Bacteriophages T2 and T4

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SUMMARY

Biochemical changes accompanying the partial genetic exclusion of wild-type T2 genes in mixed infections with various mutants of phage T4 have been examined. Total phage yields varied between 14 and 60% of those observed after infections by either wild-type phage alone. DNA synthesis was delayed, but then occurred at rates close to those found with each wild-type phage strain. Among the progeny virus, the frequencies of the excluded wild-type T2 genetic markers ranged between 0.02 and 0.45, and varied with the markers' positions on the genetic map. Measurement of the frequencies of markers among the progeny phage at 15 to 60 min. after infection showed that the weakly excluded T2 genes 1 and 20 increased slightly, while the strongly excluded T2 genes 33 and 42 dropped sharply and then remained constant. The strongly excluded T2 genes 42 and 56 formed low levels of their respective products deoxy-CMP-hydroxymethylase and deoxy-CTP-ase in mixed infections with the corresponding T4 amber mutants in a non-suppressor host. The T2-induced enzymes thymidylylate synthetase and lysozyme reached normal levels during infections with the corresponding defective T4 strains. With mixed infections in an endonuclease-I deficient host, [32P]-labelled T2 phages yielded 14% of their DNA as acid-soluble products, while no breakdown of [32P]-labelled T4 DNA was detected. The possibility is discussed that partial exclusion results from T4-induced nuclease action against T2 chromosomes followed by marker rescue of T2 DNA fragments with intact T4 genomes.

INTRODUCTION

When a bacterium is simultaneously infected with two unrelated kinds of phage only one virus type appears among the progeny. The phenomenon is known as exclusion. With genetically related strains such as T2 and T4, recombination may occur and genetic markers from both parents appear among the progeny, although markers of one parent, in this case T4, predominate. This is known as partial exclusion. It is accompanied by lower yields of phage than are observed with infections by T2 or T4 alone (Delbrück & Luria, 1943; Delbrück, 1945; Streisinger & Weigle, 1956; Adams, 1959; Campbell, 1967).

Genetic aspects of the partial exclusion which occurs between phages T2 and T4 have been extensively studied by Russell (1967) and de Groot (1966; 1967a, b; Pees & de Groot, 1970) who have largely used phage amber mutants which grow on suppressor (su') and not on su- strains of Escherichia coli. Their results, together with those of Stahl & Murray (1966), show that the genetic map of T2 closely resembles that of T4 (Fig. 1; Edgar & Wood, 1966; Mosig, 1970), and that in mixed infections in su- hosts wild-type T2 can supply a
function which cannot be provided by an amber mutant of T4. In addition genetic markers from certain regions of the T2 genome appear much less frequently than others among the phage progeny. In particular, the T2 genes 32, 56, 60 and the region between genes 41 and 55 are strongly excluded in crosses with T4, i.e. they appear with frequencies of about 0.001 to 0.050 among the progeny phage. Less strongly excluded regions appear with frequencies in the range 0.1 to 0.4 (Russell, 1967; de Groot 1967a, b; Pees, 1970).

The mechanism of partial exclusion is not understood. In the experiments described here we have examined some biochemical changes which occur during mixed infections with T2 and T4, particularly with a view to deciding whether T4-induced nucleases are involved in the exclusion process.

**METHODS**

*Bacteriophages.* Phage T2(H) was originally obtained from Dr A. D. Hershey; wild-type T4(D) was obtained from Professor N. Symonds, University of Sussex; rII and amber mutants of T4 were supplied by Dr S. Brenner, MRC Laboratory of Molecular Biology, Cambridge, and Dr R. S. Edgar, California Institute of Technology. Stocks of phages T2, T4 and T4rII mutants were obtained by lysis of *E. coli* BB and T4 amber mutants by lysis of the amber suppressor (su+) strain of *E. coli* CR63. The 'e' mutant T4G59 was supplied by Dr G. Streisinger, University of Oregon, and was grown and assayed according to the procedures described by Emrich (1968); the mutant cannot induce formation of the phage specific endolysin, or lysozyme. The mutant T4td10 cannot induce phage thymidylate synthetase and was described by Simon & Tessman (1963); it was grown and its identity checked by methods described by Wulff & Metzger (1963). Phage labelled with $^{32}$P in their DNA were obtained by lysis of 25 ml. broth cultures of *E. coli* to which 0.1 to 0.25 mc of carrier-free $^{32}$P-phosphate was added 30 min. before infection. Incorporation of $^{32}$P into phage particles varied from about $4 \times 10^4$ to $1.6 \times 10^7$ disintegrations/min./10^{12} particles. Phage preparations were routinely purified by differential centrifuging, and radioactive phage suspensions were also dialysed over 24 hr against 2 changes of 1000 vol. of 0.10 M-NaCl + 0.02 M-MgCl₂. Phage were assayed by the double layer method (Adams, 1959), using appropriate resistant strains of *E. coli* to check purity.
Mixed infections with phages T2 and T4

Bacteria. In addition to the strains already mentioned, the following were used: *E. coli* B3005 (thy-, ade-) as a restrictive host for T4d10; *E. coli* 0-11' (su+); both these strains were obtained from Dr S. Brenner. *E. coli* B, 41 which is deficient in the enzyme endonuclease-I was supplied by Dr H. Hoffmann-Berling, Heidelberg, Germany.

Media. Broth contained 10 g. Oxoid tryptone, 5 g. NaCl/l. Phage absorption medium was described by Hershey & Chase (1952); it was supplemented with DL-tryptophan (2 μg./ml.) immediately before addition of phage. Phage dilution medium contained 0.1 M NaCl + 0.01 M MgCl₂ + 0.01 M tris-HCl, pH 7.2 + 0.07% gelatin. M9 medium was prepared according to Séchaud *et al.* (1965).

Radioactive materials. Carrier-free [³²P]-phosphate and [¹⁴C]-paraformaldehyde were obtained from the Radiochemical Centre, Amersham, Bucks. The paraformaldehyde was depolymerized by maintaining it at 110°C with water in a sealed tube for 2 to 3 days.

Conversion of [³²P]-labelled phage DNA to soluble fragments in mixed infections. One ml. samples of the infected cultures (5 × 10⁸ bacteria) were placed in chilled tubes which contained 0.05 ml. of 5 M-HClO₄ and 0.05 ml. of bovine serum albumin (Armour; 10 mg./ml.) as carrier protein. After 30 min. at 0°C the tubes were centrifuged, the supernatant fluids removed, and each precipitate dissolved in 1 ml. of 0.1 M NaOH; 0.2 ml. samples of these solutions and the original supernatant fluid were neutralized and separately dried down on aluminium planchettes and their radioactivity measured in a low background counter (Low betamat, Isotope Developments, Ltd). For volumes up to 0.3 ml. the counts recorded were directly proportional to volume; more than 1000 counts were recorded for all samples and the standard error was usually not more than 3%. The results were expressed as the percentage of the total [³²P] converted to acid-soluble form.

Growth and infection of bacteria. For all experiments bacteria were grown in broth to a cell density of 5 × 10⁸ to 1 × 10⁹/ml., centrifuged, resuspended in absorption medium at a density of 1 × 10⁸/ml. and starved with gentle aeration for 30 min. Appropriate phage suspensions were then added, and after 5 min. the infected bacteria were diluted into warm, aerated broth.

Measurement of exclusion. Starved bacteria were infected with equal numbers of wild-type T2 and T4, or mutant T4, at total multiplicities of 8 to 12 phages/bacterium. After 5 min. unabsorbed phages were inactivated with T2 and T4 specific anti-sera, and the bacteria diluted into broth. Samples were immediately assayed to measure initial infective centres. After 60 min. the cultures were shaken with chloroform and the progeny phages assayed. Exclusion was measured as the ratio of the number of wild-type plaques to the total number of plaque-formers. This method was used even with amber mutants of T4, despite the fact that the efficiency of plating of wild-type T2(H) on the su+ strain *E. coli* 0-11' is only 0.5 relative to that on *E. coli* B. Since the progeny phage from T2 × T4am crosses must include recombinants for all regions of the genomes not all phage with the am+ genotype will necessarily have the lower plating efficiency on strain 0-11'. Exclusion was therefore measured as the ratio of number of progeny plaques on *E. coli* B to the number formed on *E. coli* 0-11'.

Measurement of DNA synthesis by infected bacteria. The infected bacteria were diluted into warm broth to give a cell density of 5 × 10⁸/ml. At intervals 1 ml. samples were withdrawn, chilled and precipitated with 0.05 ml. of 5 M-HClO₄ and 0.5 mg. bovine serum albumin as carrier prior to hydrolysis and determination of DNA deoxyribose according to Burton (1956).

Protein estimations. The method of Lowry *et al.* (1951) was used.
Enzyme assays

Deoxycytidylate hydroxymethylase. The tetrahydrofolic acid required for this and the following assay was prepared from commercial folic acid (British Drug Houses, Ltd) according to Jones, Guest & Woods (1961). The enzyme assay was based on those of Flaks & Cohen (1959) and Wiberg & Buchanan (1964). A total of 0.35 ml. of enzyme incubation mixture contained: 10 μmole of potassium phosphate buffer, pH 7, 1.25 μmole-[14C]-formaldehyde (0.5 μC/μmole), 0.25 μmole-tetrahydrofolic acid, 5 μmole-MgSO4, 2.5 μmole-deoxycytidine monophosphate (d-CMP; Sigma Chemical Co.), 5 μmole-β-mercaptoethanol and 0.1 to 0.2 mg. protein from crude extracts. The mixtures were incubated at 37°C for 40 min. The reaction was stopped by chilling the tubes and precipitating the proteins with 0.35 ml. of 10% (w/v) trichloroacetic acid. After 30 min. the precipitates were removed and 0.35 ml. portions of the clear supernatant fluids were heated (100°C) for 10 min. with 0.35 ml. of 1% (w/v) FeCl3 solution in 1 N-HCl. Samples (0.1 to 0.2 ml.) of the heated supernatant fluid were placed on glass fibre discs (GF/A, Whatman) with 0.1 to 0.2 ml. of 1 N-HCl and dried down under an infra-red lamp. The dried discs were placed in vials with 5 ml. of scintillation fluid (Butyl-PBD, Ciba, Duxford, Cambs.; 7 g./l. of toluene) and the radioactivity was measured in a Beckman scintillation counter using the isoset for [14C]. The standard error for the counts recorded was usually not more than 2%. Enzyme activity is expressed as μmole of formaldehyde converted to acid-stable, non-volatile form in 1 hr/mg. of protein.

Thymidylate synthetase activity. The assay procedure was identical to that used for d-CMP hydroxymethylase except that deoxyuridylate (d-UMP Sigma Chemical Co.) was used instead of d-CMP. (Flaks & Cohen, 1959).

Assay of deoxycytidine triphosphatase. Five ml. samples of the infected culture (5 × 10^8 bacteria/ml.) were chilled, centrifuged and resuspended in 2 ml. of tris-acetate buffer, 0.02 M, pH 9.1. The suspensions were frozen, thawed and the bacteria broken by sonication. Enzyme activity was measured with these extracts, using the method of Wiberg (1967).

Assay of lysozyme activity. Two ml. samples of infected cultures were chilled and the bacteria broken by sonication. After centrifuging, the extracts were assayed by the method of Greene & Korne (1967) using suspensions of E. coli B as substrate (Jacob & Fuerst, 1958).

RESULTS

Mixed infections: burst sizes and DNA synthesis

The burst sizes were measured, usually in E. coli B; in some instances the amber suppressor strain E. coli CR63 was used to determine if differences in phage yield or exclusion depended on whether all the T4 genes could be expressed. The results are given in Table 1. As expected, the average burst sizes obtained from mixedly infected bacteria were always lower than those from bacteria infected with either T2 or T4 wild-type phage alone. In certain instances, namely when T4 amber mutants in genes 46, 47, or 20 were used in mixed infections, the burst sizes were greater with the amber permissive host E. coli CR63 than when E. coli B was used.

The results summarized in Table 2 indicate that in the mixed infections shown, DNA synthesis was delayed, but then occurred at rates comparable to those in wild-type phage infections.
Mixed infections with phages T2 and T4

Table 1. Average burst sizes in mixed infections between T2 and T4 phages

<table>
<thead>
<tr>
<th>Cross</th>
<th>Average burst size</th>
<th>Burst size range</th>
<th>Bacterial host</th>
<th>No. of experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2 × T2</td>
<td>168</td>
<td>100–240</td>
<td>E. coli</td>
<td>100</td>
</tr>
<tr>
<td>T4 × T4</td>
<td>180</td>
<td>100–250</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>T2 × T4 amC76 (gene 42)</td>
<td>96</td>
<td>65–100</td>
<td>B and CR63</td>
<td>18</td>
</tr>
<tr>
<td>T2 × T4 amB22 (gene 43)</td>
<td>91</td>
<td>62–100</td>
<td>B and CR63</td>
<td>21</td>
</tr>
<tr>
<td>T2 × T4 amN130 (gene 46)</td>
<td>30</td>
<td>25–32</td>
<td>B</td>
<td>2</td>
</tr>
<tr>
<td>T2 × T4 amN130 (gene 46)</td>
<td>75</td>
<td>60–85</td>
<td>CR63</td>
<td>21</td>
</tr>
<tr>
<td>T2 × T4 amA456 (gene 47)</td>
<td>25</td>
<td>14–31</td>
<td>B</td>
<td>25</td>
</tr>
<tr>
<td>T2 × T4 amA456 (gene 47)</td>
<td>49</td>
<td>30–45</td>
<td>CR63</td>
<td>20</td>
</tr>
<tr>
<td>T2 × T4 am50 (gene 20)</td>
<td>50</td>
<td>40–60</td>
<td>B</td>
<td>3</td>
</tr>
<tr>
<td>T2 × T4 am50 (gene 20)</td>
<td>85</td>
<td>82–90</td>
<td>CR63</td>
<td>3</td>
</tr>
<tr>
<td>T2 × T4 amA494 (gene 1)</td>
<td>87</td>
<td>80–94</td>
<td>B and CR63</td>
<td>4</td>
</tr>
<tr>
<td>T2 × T4 am134 (gene 33)</td>
<td>91</td>
<td>85–96</td>
<td>B and CR63</td>
<td>14</td>
</tr>
<tr>
<td>T2 × T4 td10 (deletion)</td>
<td>50</td>
<td>40–60</td>
<td>B and CR63</td>
<td>12</td>
</tr>
<tr>
<td>T2 × T4 G59 (gene e)</td>
<td>65</td>
<td>51–70</td>
<td>B and CR63</td>
<td>21</td>
</tr>
<tr>
<td>T2 × T4 td10 (gene td)</td>
<td>81</td>
<td>73–86</td>
<td>B</td>
<td>8</td>
</tr>
<tr>
<td>T2 × T4 amE56 (gene 56)</td>
<td>56</td>
<td>41–71</td>
<td>B</td>
<td>4</td>
</tr>
<tr>
<td>T2 × T4 amE51 (gene 56)</td>
<td>104</td>
<td>76–139</td>
<td>B</td>
<td>3</td>
</tr>
<tr>
<td>T2 × T4 amB85 (gene 56)</td>
<td>70</td>
<td>47–93</td>
<td>B</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 2. DNA synthesis in cultures of Escherichia coli B infected with wild-type T2 phage and mutants of T4. Each culture contained infected bacteria at a cell density of 5 × 10⁸/ml.

<table>
<thead>
<tr>
<th>Time after infection (min.)</th>
<th>Amount of DNA synthesized (µmoles of DNA-deoxyribose)/l. of culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T2 × T4 am50 gene 20</td>
</tr>
<tr>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>10</td>
<td>47</td>
</tr>
<tr>
<td>15</td>
<td>59</td>
</tr>
<tr>
<td>20</td>
<td>70</td>
</tr>
<tr>
<td>30</td>
<td>88</td>
</tr>
<tr>
<td>60</td>
<td>170</td>
</tr>
</tbody>
</table>

Table 3. Frequencies of different T2 genes in the progeny of mixed infections with wild-type T2 and T4 mutant phages

<table>
<thead>
<tr>
<th>Parental phage types</th>
<th>Gene tested</th>
<th>Fraction of wild-type phage in progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2 × T4 amA494</td>
<td>1</td>
<td>0.22</td>
</tr>
<tr>
<td>T2 × T4 am50</td>
<td>20</td>
<td>0.35–0.40</td>
</tr>
<tr>
<td>T2 × T4 am134</td>
<td>33</td>
<td>0.08–0.10</td>
</tr>
<tr>
<td>T2 × T4 amC76</td>
<td>47</td>
<td>0.05–0.08</td>
</tr>
<tr>
<td>T2 × T4 amB22</td>
<td>43</td>
<td>0.05–0.08</td>
</tr>
<tr>
<td>T2 × T4 amA456</td>
<td>47</td>
<td>0.10–0.15</td>
</tr>
<tr>
<td>T2 × T4 amN130</td>
<td>47</td>
<td>0.12–0.16</td>
</tr>
<tr>
<td>T2 × T4 amE51</td>
<td>56</td>
<td>0.02–0.09</td>
</tr>
<tr>
<td>T2 × T4 amE56</td>
<td>56</td>
<td>0.02–0.09</td>
</tr>
<tr>
<td>T2 × T4 G59</td>
<td>e</td>
<td>0.40</td>
</tr>
<tr>
<td>T2 × T4 h+</td>
<td>h+</td>
<td>0.30–0.40</td>
</tr>
</tbody>
</table>
Table 4. **Mixed infections between wild-type T2 and T4 amN130**
(gene 46): effect of temperature on exclusion

<table>
<thead>
<tr>
<th>Temperature (°)</th>
<th>Host strain</th>
<th>Burst size</th>
<th>Fraction of am+ plaques in progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>B; B41</td>
<td>14–20</td>
<td>0.60–0.70</td>
</tr>
<tr>
<td>37</td>
<td>B; B41</td>
<td>30</td>
<td>0.20</td>
</tr>
<tr>
<td>30; 37</td>
<td>CR63</td>
<td>75</td>
<td>0.06</td>
</tr>
</tbody>
</table>

**Mixed infections: exclusion of different T2 genes**

A series of mixed infection experiments were performed to ensure that the phage strains used here showed the same exclusion behaviour observed by others. The results are summarized in Table 3. Mixed infections with T2 and T4amN130 (gene 46) gave unexpected results indicating that in non-permissive hosts the extent of exclusion was temperature-sensitive (Table 4).

We unsuccessfully attempted to measure exclusion of the T2td gene using the method of Simon & Tessman (1963) to distinguish td and td+ plaques; the method proved unsuitable because wild-type T2 phages gave variable-sized plaques in the assay.

**Mixed infections: frequency of T2 genes at increasing times after infection**

In T-even phage infections the rate of genetic recombination increases with time (Symonds, 1962; Frey & Melechen, 1965). To test whether recombination might be affecting the extent of appearance of T2 genes among progeny, the proportions of am and am+ phages were determined after lysing the bacteria with chloroform at different times after infection. Crosses were performed between wild-type T2 and T4 amber mutants defective in two strongly excluded genes 33 and 42, and between wild-type T2 and T4 mutants defective in the weakly excluded genes 1 and 20. The results are given in Table 5.

**Mixed infections: expression of excluded genes**

The amount of a particular gene product formed is expected to reflect the number of functional copies of the relevant gene. If partial exclusion is caused by the inactivation or destruction of certain regions of the genome, then differences should exist in the amounts of gene products made by weakly and strongly excluded T2 genes in mixed infections with T4. To test this we compared the amounts of an enzyme made on infection with T2 alone with those in a mixed infection with a T4 mutant unable to supply the corresponding enzyme. We chose the products of two genes e and td from regions known or expected to show weak exclusion, and two from strongly excluded regions, namely genes 42 and 56.

**Gene 42 product: synthesis of deoxycytidylate hydroxymethylase**

The results in Fig. 2 show that there is a slight delay in appearance and a marked decrease in the total amount of enzyme formed in a mixed infection compared with the rates and levels found in infections with T2 or T4 alone. Estimation of thymidylate synthetase activities in the same extracts showed that this enzyme was functioning normally even in the mixedly infected bacteria where it reached levels similar to those shown in Fig. 5. Tests with mixed extracts showed that the defective product formed by bacteria infected with T4amC76 did not inhibit the activity of the normal T2-induced hydroxymethylase.
Mixed infections with phages T2 and T4

Table 5. Frequencies of wild-type T2 and T4 am genes at different times after infection. At the times shown the infected cultures were lysed with chloroform and the phage released were measured with Escherichia coli B and O-11 as indicator bacteria

<table>
<thead>
<tr>
<th>Time (min.) after infection</th>
<th>Per ml. of culture</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total am+ as (p.f.u. on B)</td>
<td>(p.f.u. on O-11')</td>
</tr>
<tr>
<td>cross: T2 wild-type x T4am50 (gene 20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>1.8 x 10^6</td>
<td>4.0 x 10^6</td>
</tr>
<tr>
<td>20</td>
<td>3.6 x 10^7</td>
<td>8.0 x 10^7</td>
</tr>
<tr>
<td>25</td>
<td>1.9 x 10^9</td>
<td>3.5 x 10^9</td>
</tr>
<tr>
<td>30</td>
<td>5.7 x 10^9</td>
<td>1.1 x 10^10</td>
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<tr>
<td>35</td>
<td>2.0 x 10^10</td>
<td>3.4 x 10^10</td>
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<td>40</td>
<td>4.3 x 10^10</td>
<td>7.0 x 10^10</td>
</tr>
<tr>
<td>60</td>
<td>5.9 x 10^10</td>
<td>9.0 x 10^10</td>
</tr>
<tr>
<td>cross: T2 wild-type x T4amA94 (gene 1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>2.6 x 10^7</td>
<td>1.5 x 10^8</td>
</tr>
<tr>
<td>20</td>
<td>6.7 x 10^8</td>
<td>3.2 x 10^8</td>
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<td>25</td>
<td>4.0 x 10^9</td>
<td>1.6 x 10^10</td>
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<td>35</td>
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<td>45</td>
<td>2.1 x 10^10</td>
<td>7.0 x 10^10</td>
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<td>2.3 x 10^10</td>
<td>8.2 x 10^10</td>
</tr>
<tr>
<td>60</td>
<td>2.9 x 10^10</td>
<td>9.0 x 10^10</td>
</tr>
<tr>
<td>cross: T2 wild-type x T4amI34 (gene 33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>1.3 x 10^8</td>
<td>2.4 x 10^8</td>
</tr>
<tr>
<td>20</td>
<td>7.0 x 10^8</td>
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</tr>
<tr>
<td>22</td>
<td>1.5 x 10^9</td>
<td>1.1 x 10^10</td>
</tr>
<tr>
<td>25</td>
<td>2.8 x 10^9</td>
<td>2.3 x 10^10</td>
</tr>
<tr>
<td>27</td>
<td>4.0 x 10^9</td>
<td>3.6 x 10^10</td>
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<td>30</td>
<td>6.5 x 10^9</td>
<td>6.0 x 10^10</td>
</tr>
<tr>
<td>60</td>
<td>1.7 x 10^10</td>
<td>1.6 x 10^11</td>
</tr>
<tr>
<td>cross: T2 wild-type x T4amC76 (gene 42)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>1.1 x 10^8</td>
<td>2.3 x 10^8</td>
</tr>
<tr>
<td>20</td>
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<tr>
<td>40</td>
<td>5.6 x 10^9</td>
<td>1.1 x 10^11</td>
</tr>
<tr>
<td>60</td>
<td>5.5 x 10^9</td>
<td>1.2 x 10^11</td>
</tr>
</tbody>
</table>

Gene 56 product: synthesis of deoxycytidine triphosphatase

The results in Fig. 3 show that in a mixed infection between wild-type T2 and T4amE51 less enzyme is formed than in bacteria infected with wild-type T2 alone. Although the actual levels of enzyme varied in different experiments, exactly similar results were obtained when two other amber mutants in gene 56, namely T4amE56 and T4amB85 (kindly supplied by Dr B. de Groot, Leiden) were used. Tests of enzyme activity in mixtures which contained extracts of bacteria infected with T2 and extracts of bacteria infected with T4amE51 showed that the T2 induced activity was unaffected by the product of the mutant T4 gene.

In our experiments the amounts of deoxy-CTP-ase formed in bacteria infected with either T2 or T4 were always lower than those reported by Wiberg (1967). However, in his experiments growing cultures, and in ours starved bacteria were infected with phage, and possibly this may account for the differences observed.
Fig. 2. Formation of deoxycytidylate hydroxymethylase in cultures of Escherichia coli B after infection with phage at m.o.i. of 4 to 6 for each strain. Enzyme activity is expressed as μ moles of [14C]-formaldehyde fixed/hr/mg. protein. O—O, T2; Δ—Δ, T4; ●—●, T2 x T4amC76; △—△, T4amC76.

Fig. 3. Formation of deoxycytidine triphosphatase in cultures of Escherichia coli B infected with phage, at m.o.i. of four for each strain. Enzyme activity is expressed as nmole dCMP formed/hr/mg. of protein. O—O, T2; △—△, T4; △—▽, T4amE51; ●—●, T2 x T4amE51.

Products of genes e and td: synthesis of phage lysozyme and thymidylate synthetase

The results shown in Fig. 4 and 5 indicate that mixedly-infected bacteria formed these enzymes in amounts typical of those produced in cultures infected with T2 alone.

Degradation of T2 DNA in mixed infections with T4

In these experiments the endonuclease-I-deficient strain E. coli B 41 was used as host bacterium to avoid possible effects from superinfection breakdown (Fielding & Lunt, 1970).

[32P]-labelled T2 phage were mixed with an equal number of unlabelled T4 or T2 phage and allowed to adsorb to starved bacteria at a total multiplicity of infection of eight; the infected bacteria were diluted 10-fold out of absorption medium into warm broth and at intervals 1 ml. samples were removed for measurement of acid-soluble [32P] as described in the Methods section. Similar experiments were done with [32P]-labelled T4 and unlabelled T2 phage.

T4 phage which are mutant in genes 46 or 47 are unable to convert bacterial DNA to acid-soluble fragments. To test whether these genes might be involved in the solubilization of T2 DNA, infections were performed with [32P]-T2 phage and each of the T4 mutants amN130 (gene 46) and amA456 (gene 47).

The result of one of these experiments is shown in Fig. 6. The results of experiments with [32P]-labelled T4 are not shown; in these between 1 and 2% of the total [32P] appeared as...
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Fig. 4. Formation of endolysin in cultures of Escherichia coli infected with phage at m.o.i. of 4 to 6 for each strain. Enzyme activity is expressed as drop in E$_{450}$ of +/0/1/min./mg. of protein. O--O, T2; ▲—▲, T4; △—△, T4,G59; ●—●, T2 × T4,G59.

Fig. 5. Formation of thymidylate synthetase in cultures of Escherichia coli infected with phage at m.o.i. of 4 to 6 each strain. Activities are expressed as μmoles of [14C]-formaldehyde fixed/hr/mg. of protein. O—O, T2; ▲—▲, T4; △—△, T4td10; ●—●, T2 × T4td10; □—□, T4 × T4td10.

acid-soluble material at the start and did not increase during the infection. When bacteria were infected with [32P]-T2 and T4amN130 the amount of acid-soluble [32P] formed was slightly less than that with wild-type T4 and [32P]-T2 phages.

DISCUSSION

The pattern of exclusion observed in our experiments agrees with that found by Russell (1967) and de Groot (1966; 1967a, b). Our results differ from theirs in that we obtain consistently higher frequencies for the T2 wild type genes among the progeny phages. The reason for this is not known, but may be related to our use of a different T2 strain, or to differences in the conditions of infection, for example our use of starved bacteria.

The amounts of DNA synthesized in mixedly infected bacteria were less than those formed in cultures infected with either T2 or T4 wild type phage alone, although even in mixed infections the amounts of DNA made were greater than expected from the corresponding phage yields. The low yields may partly result from poor complementation between phage proteins made by the two parental types. This has been shown to occur with the tail-fibre proteins of T2 and T4 (Stahl & Murray, 1966; Russell, 1967) and which, with other unfavourable protein interactions, might contribute to the low yields in all of the crosses described here.

The simplest explanation for the unequal recoveries of the wild-type T2 genetic markers...
Fig. 6. Degradation of [32P]-labelled T2 DNA in cultures of *Escherichia coli* B41. The infected cultures contained bacteria at a cell density of $5 \times 10^8$/ml. and a total of about $1 \times 10^9$ counts/min/ml. of [32P]. The sums of the [32P]-counts recovered in the soluble and pellet fractions for each sample were within 5% of the total counts known to be present. ○—○, [32P]-T2 x T2; ●—●, [32P]-T2 x T4; △—△, [32P]-T2 x T4 amber A456 (gene 47).

is that there is some T4-induced attack on selected regions of the T2 genome which are thereby made less available for genetic transcription or for transmission to progeny phage. Masamune (1968) showed that in mixed infections with wild-type phages, at 4 min. after infection the T2 DNA was present as high mol. wt fragments which sedimented more slowly than whole T2 DNA, while the parental T4 DNA was recovered intact. We observed some conversion of [32P]-labelled T2 DNA but not T4 DNA to acid-soluble fragments in mixedly infected bacteria, re-incorporation of nucleotides into new virus DNA possibly accounting for the relatively small amount of acid-soluble products found (c.f. Wiberg, 1966). The diminished T2 DNA breakdown observed with a mutant of T4 defective in gene 47 suggests that a DNase coded for by this gene may be involved. An interpretation of Masamune's and our results is that initially a few breaks are introduced into the T2 genome and that subsequently non-specific nucleases convert the damaged DNA to acid-soluble fragments.

Selective inactivation of specific regions of the T2 genome is also indicated by our finding that the strongly excluded T2 genes 42 and 56 form only low levels of their respective products, namely deoxy-CMP-hydroxymethylase and deoxy-CTP-ase, in mixed infections with the corresponding T4 amber mutants in an su + host. By contrast the T2 'e' gene is not strongly excluded and T2-induced lysozyme reached normal levels in mixed infections with a T4 'e' mutant. In addition bacteria infected with T2 and T4td10 produced almost as much thymidylate synthetase as bacteria infected with T2 alone. Our attempts to measure exclusion of the T2td gene were not successful. However, the T4td gene lies between genes 32 and 63 (Edgar & Wood, 1966) which are well separated in terms of DNA distances (Mosig, 1970), and Russell (1967) found that although T2 gene 32 was strongly excluded, T2 gene 63 survived about as well as gene 'e' in mixed infection with T4. On genetic grounds it is therefore possible that the T2td gene lies in a region which escapes the exclusion effect.
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operating near gene 32. The formation of normal amounts of T2td gene product in mixed infections appears consistent with this.

The data presented in Table 5 show that the strongly excluded am+ T2 genes 33 and 42 contribute to the yield of progeny phage at about one-tenth the rate of the corresponding T4 am genes. This results in a sharp drop in the frequency of the T2 am+ markers among the progeny, mainly during the early period of maximum DNA synthesis. By contrast, the weakly excluded T2 am+ genes 1 and 20 increase among the progeny phage at rates similar to those of the T4 am markers; the frequencies of these T2 genes therefore remain almost constant throughout the infections, although they are always present in lower amounts than the corresponding T4 genes.

An interpretation of these results is that events required for the rescue of T2 genes occur relatively early in infection and that subsequent changes in the frequencies of T2 markers depend on the balance between their rescue, replication and withdrawal from the intracellular DNA pool as phage maturation occurs. Thus T2 genes which are inefficiently rescued may be effectively diluted out as DNA replication proceeds.

There is some support for this explanation since if T4-induced exclusion causes breaks in T2 chromosomes, the resulting intracellular collections of genomes resemble those studied by Mosig and her collaborators. They have shown that after mixed infections with wild-type T4 partial genomes and whole T4 genomes carrying amber mutations, the yields of am+ markers are smaller than when normal size chromosomes provide the am+ genes (Mosig & Werner, 1969). In single infections, incomplete T4 genomes replicate autonomously only when they possess an initiation site near gene 43, and then they replicate only once and to those ends of their DNA molecules which lie clockwise from gene 43. In mixed infections with normal phage, repeated replication of markers from partial genomes requires their recombination into intact T4 chromosomes (Marsh, Breshkin & Mosig, 1971). Therefore in our experiments, if T2 DNA replication also initiates close to gene 43, the strong exclusion effects near this site must require all T2 markers, before they can replicate, to be integrated with T4 chromosomes. Then T2 genes (e.g. 1 and 20) distant from exclusion sensitive sites would be most readily rescued, and those near excluded sites (e.g. 42 and 33) would be at the ends of T2 DNA fragments and less favoured for rescue by recombination.

The biochemical mechanisms responsible for exclusion remain unknown. More than one T4-induced function appears to be responsible since Pees (1970) has shown that a mutant of T4 is unable to exclude gene 56 of T2 although the other sensitive sites in the T2 genome are excluded normally. This result also shows that there are differences between the exclusion-sensitive sites in the T2 genome. Our results and those of Masamune (1968) indicate that DNases could be involved, but if DNase action is primarily responsible, the unequal extents of exclusion of different T2 genes require the action of site-specific nucleases capable of recognizing relatively short nucleotide sequences. There is no direct evidence that such enzymes are involved in T2-T4 exclusion, although nucleases of this type are known and appear to be responsible for restriction of 'foreign' DNA by E. coli (Linn & Arber, 1968; Arber & Linn, 1969; Meselson & Yuan, 1968) and Haemophilus influenzae (Smith & Wilcox, 1970). Similar phenomena include the restriction of T-even phages which lack glucose on their DNA (Shedlovsky & Brenner, 1963; Symonds, et al. 1963; Hattman, Revel & Luria, 1966), and the phage T5-induced breakdown of the DNA of heterologous super-infecting phages (Fielding & Lunt, 1970). In the case of the closely related phages T2 and T4 the exclusion is only partial, probably because genetic recombination permits rescue and subsequent replication of undamaged regions of the T2 genome.
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