The Relation between
Breakdown of Superinfecting Virus Deoxyribonucleic Acid and
Temporal Exclusion Induced by T4 and T5 Bacteriophages

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SUMMARY

Temporal exclusion and breakdown of superinfecting virus deoxyribonucleic acid were measured after infection with T4 and T5 bacteriophages of normal strains of Escherichia coli and strains deficient in endonuclease-I. Bacteria deficient in endonuclease-I when infected with T4 phage excluded superinfecting T4 with little solubilization of the secondary DNA. With wild-type bacteria exclusion was accompanied by extensive superinfection breakdown, probably caused by the bacterial endonuclease-I. In bacteria infected by T5 phage, superinfecting T2 phages could be excluded even when deoxyribonucleic acid degradation was inhibited by maintaining a low [Mg²⁺] in the growth medium. In the presence of 0.01 M-magnesium ions, both wild-type bacteria and bacteria deficient in endonuclease-I infected with T5 phage produced extensive solubilization of the DNA of superinfecting T2 or T4 phages. A nuclease induced by T5 was probably partly responsible for the DNA breakdown which occurred in these conditions.

INTRODUCTION

When bacteria infected by T2 phage are superinfected with further T2 phage after the primary infection has been allowed to proceed, genetic markers of the secondary phage appear infrequently among the progeny virus. This phenomenon, known as temporal exclusion, was originally observed by Dulbecco (1952). Information relating to exclusion with T-even phages was fully reviewed by Adams (1959); similar phenomena have been observed in other phage systems and are also discussed by Adams (1959) and by Campbell (1967).

When T2 phage labelled with ³²P are used for the secondary infection, the superinfecting DNA is largely degraded to acid-soluble fragments which are released into the medium, and very little of the ³²P appears in the progeny virus particles (Lesley et al. 1951; Graham, 1953). Superinfection breakdown also occurs when bacteria infected by phage T5 are secondarily infected with T2 or T4 phages (Graham, 1953). Although DNA degradation could clearly explain genetic exclusion, experiments by French et al. (1952), Graham (1953) and Hershey et al. (1954) suggested that temporal exclusion of superinfecting T2 phage could take place independently of the solubilization of the secondary phages' DNA.

In an attempt to clarify the role of virus and host-cell nucleases in temporal exclusion and superinfection breakdown, we have examined these phenomena using T4 and T5 phage-infected cultures of host strains with normal and subnormal amounts of the bacterial enzyme endonuclease-I (endo-I−-strains; Dürwald & Hoffmann-Berling, 1968).
METHODS

**Bacteriophages.** Phage T2 (H) was originally obtained from Dr A. Hershey; wild type T4 (D) was obtained from Professor N. Symonds, University of Sussex; rII and amber mutants of phage T4 were supplied by Dr S. Brenner, MRC Laboratory of Molecular Biology, Cambridge. Phage T5 was supplied by Professor K. Burton, University of Newcastle upon Tyne. Amber mutants of phage T5 were isolated from phage stocks grown in glucose-salts medium containing 5-bromodeoxyuridine (10 μg./ml.) and selected for their ability to give plaques on *Escherichia coli* strain CR63, but not on strain B/2 or C3000. Stocks of phages T2, T4 and T4 rII mutants were obtained by lysis of broth cultures of *E. coli* BB, and of phage T5 by lysis of *E. coli* B/2. For infections with phage T5, media were made 1 mM with respect to CaCl₂. Stocks of amber mutants of phages T4 and T5 were obtained by lysis of cultures of *E. coli* CR63. Phage λ was obtained by ultraviolet light induction of *E. coli* K12 y10 (λ) which was supplied by Dr B. Fry, University of Sheffield. The procedure was as follows: bacteria were grown for four generations in 50 ml. of broth to a cell density of 2 x 10⁸/ml., centrifuged and resuspended in 10 ml. of saline. The suspension was placed in a sterile Petri dish (9 cm. diameter) and exposed for 20 sec. with gentle rocking to unfiltered u.v. light from a Hanovia Chromolight lamp placed 38 cm. above the Petri dish. The irradiated bacteria were diluted with 40 ml. of broth and aerated at 37° until lysis occurred, which was usually within 2½ hr. All phage preparations were routinely purified by differential centrifugation. They were assayed by the double layer technique (Adams, 1959), using *E. coli* B for T2, T4 and T4 rII, strain B/2 for phage T5, strain CR63 for amber mutants and strain K12.1100 for phage λ. The purity of the stocks was checked by plating on the appropriate resistant strains B/2, B/4, B/5 and K12 y10 (λ).

**Phage preparations labelled with ³²P.** Radioactive T2, T4 and T5 phages were obtained by adding 0.25 mc of [³²P]phosphate to 30 ml. broth cultures of bacteria 30 min. before infection with phage. Radioactive phage λ was made by addition of 0.4 mc of [³²P]phosphate to the 50 ml. broth suspension of bacteria immediately after u.v. irradiation. All preparations of radioactive phage were purified by differential centrifugation and by dialysis against three changes of 0.10 M-NaCl + 0.02 M-MgCl₂. The specific activity of the phosphorus in phage obtained by these methods varied according to the broth's content of inorganic phosphate, but was about 0.33 mc/mg. phosphorus.

**Bacteria.** In addition to the strains already mentioned, the following were kindly supplied by Professor Hoffmann-Berling, Heidelberg, Germany: *Escherichia coli* K12.1100 and *E. coli* B.41 which are deficient in endonuclease-I activity, and their respective parental wild type (endo-I⁺) strains K12.1000 and B.31. During the course of this work we discovered that strains K12.1000 and K12.1100 supported the growth of T4 and T5 phage amber mutants, and must therefore have contained genes for amber suppressors. The isolation and properties of these strains were described by Dürwald & Hoffmann-Berling (1968).

**Chemical analyses.** DNA in bacterial cultures was measured according to Burton (1956), and protein according to Lowry et al. (1951), using crystalline bovine serum albumin (Armour Laboratories) as the standard.

**DNase activity.** The procedures of Stone & Burton (1962) were used. Cell extracts were assayed after RNase treatment, and the substrate was heat-denatured herring DNA. Nuclease activities are expressed as μ-moles of acid-soluble DNA-deoxyribose released/min./mg. protein.

**Breakdown of the DNA of ³²P-labelled superinfecting bacteriophages.** One ml. samples of the infected cultures were withdrawn into chilled tubes which contained 0.1 ml. of bovine
serum albumin (Armour; 10 mg./ml.); 0·1 ml. of 50% trichloracetic acid was added, and after 30 min. on ice the tubes were centrifuged. The supernatant fluids were removed and the pellets dissolved in 1 ml. of 1 N-NaOH and incubated at 70° for 20 min. Samples (0·2 ml.) of these solutions and of the original supernatant fluids were neutralized, dried down on planchettes and their radioactivity was measured. The ratio of acid-soluble to total 32P counts was taken as a measure of superinfection breakdown and expressed as percentage breakdown. In any one experiment the standard error for the total counts recovered was not usually more than 3%.

Radioactive materials and their measurement. Carrier-free [32P]phosphate was obtained from The Radiochemical Centre, Amersham, Bucks. Samples of solutions containing P32 were dried down on aluminium planchettes and counted in a low background counter (Low betamat, Isotope Developments Ltd). The counts were not corrected for self-absorption. For volumes up to 0·3 ml. the counts recorded were directly proportional to the sample volume. At least 1000 counts were recorded for all samples.

Media. Broth contained 8 g. Oxoid tryptone, 5 g. NaCl/l. Phage-adsorption medium was described by Hershey & Chase (1952). Salts medium was the same as that used by Fessler, Keleman & Burton (1960), except that mannitol was replaced by glucose (10 g./l.) which was added separately after autoclaving. Tris-phosphate–glucose (TPG) medium was described by Sedat & Sinsheimer (1964), and tris-calcium–magnesium (TCM) by Kaiser (1968). Phage-dilution fluid contained 0·11 M-NaCl + 0·01 M-MgCl2 + 0·07% gelatin. Saline was 0·85% NaCl solution.

Antiserum. Rabbit antiserum to phages T2 and T4 were kindly provided by Dr R. G. Tucker. Antiserum to phage T5 was obtained by injection of rabbits with purified T5 phage preparations.

RESULTS

Genetic exclusion of superinfecting T-even phages

Bacteria were grown in broth for four to five generations to a cell density of 5 × 107/ml. (for K12 strains) or 2 × 106/ml. (for B strains), centrifuged, washed in saline and resuspended at a cell density of 1 × 109/ml. in phage-adsorption medium. The suspension was aerated at 37° for 15 min. and then infected (m.o.i. = 4) with T4rII phage in the presence of D,L-tryptophan (2 μg./ml.) which was added immediately before the phage. After allowing adsorption for 10 min., the infected bacteria were diluted tenfold into warm broth, and wild-type T4 r+ phage (m.o.i. = 4) added immediately, or at some interval after allowing the primary infection to proceed. Samples (1·0 ml.) of the culture were withdrawn 2 min. after adding the secondary phage, unadsorbed virus particles were inactivated with anti-T4 serum and the infected bacteria suitably diluted and plated on Escherichia coli B to measure total infected bacteria, and on K12 (λ) to measure the number of bacteria successfully superinfected with r+ phage. Exactly similar procedures were used for measuring exclusion when T4 amN134 amber (mutation in gene 33, Edgar & Wood, 1966) was used for the primary, and wild-type T4 phage for the secondary infection. Total infected bacteria were measured after antiserum treatment by plating on E. coli K12.1100 and those yielding wild-type phage by plating on strain B. Genetic exclusion of superinfecting wild-type phage occurred when an rII or amber mutant was used for the primary infection, and regardless of whether the host bacteria contained normal or reduced amounts of endonuclease-I (Tables 1, 2). Genetic exclusion was generally best observed when early log. phase bacteria were used as hosts, and this was particularly important with K12 strains. A high level of primary infection was ensured by using a 10 min. adsorption period.
**T4 phage infected bacteria: effect of bacterial endonuclease-I on superinfection breakdown**

Wild type or endo-I strains of bacteria were infected with T4 rII phage as described above. After dilution of the infected bacteria into warm broth, 10 ml. samples of the culture were withdrawn at different times and superinfected with 32P-labelled T4 r+ phage. Genetic exclusion and the extent of conversion of the 32P-DNA to acid-soluble fragments were measured (Fig. 1). In similar experiments with T2 phage, and with mixed infections involving T2 and T4, 32P-labelled superinfecting DNA was extensively degraded when endo-I+, but not when endo-I-, bacteria were used, although genetic exclusion occurred with both kinds of host strain.

**Table 1. Genetic exclusion in wild-type and endonuclease-I deficient bacteria**

<table>
<thead>
<tr>
<th>Time (min.) of addition of secondary phage</th>
<th>Percentage of infected bacteria which yielded secondary phage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli B (endo-I+)</td>
</tr>
<tr>
<td>1</td>
<td>66.7</td>
</tr>
<tr>
<td>5</td>
<td>8.4</td>
</tr>
<tr>
<td>10</td>
<td>4.9</td>
</tr>
</tbody>
</table>

**Table 2. Genetic exclusion, deoxyribonuclease activity and superinfection breakdown in bacteria infected with T4 phage**

The nuclease activities given are the values obtained at 15 min. after the primary infection, or from part of the same culture before infection.

<table>
<thead>
<tr>
<th>Bacterial host strain of E. coli</th>
<th>Primary phage</th>
<th>Secondary phage</th>
<th>Time (min.) of addition of secondary phage</th>
<th>Deoxyribonuclease activity (μm-moles/min./mg. protein)</th>
<th>Percentage of secondary phage DNA made acid-soluble at 15 min. after secondary infection</th>
<th>Percentage of infected bacteria which yielded secondary phage</th>
</tr>
</thead>
<tbody>
<tr>
<td>K12.1100 (endo-I-)</td>
<td>T4 rII</td>
<td>T4 r+</td>
<td>0</td>
<td>0.48</td>
<td>3.15</td>
<td>5.0</td>
</tr>
<tr>
<td>K12.1000 (endo-I-)</td>
<td>T4 rII</td>
<td>T4 r+</td>
<td>8</td>
<td>0.50</td>
<td>5.0</td>
<td>9.5</td>
</tr>
<tr>
<td>B.41 (endo-I-)</td>
<td>T4 rII</td>
<td>T4 r+</td>
<td>0</td>
<td>3.80</td>
<td>17.00</td>
<td>5.0</td>
</tr>
<tr>
<td>B.31 (endo-I-)</td>
<td>T4 rII</td>
<td>T4 r+</td>
<td>8</td>
<td>3.80</td>
<td>17.00</td>
<td>9.0</td>
</tr>
<tr>
<td>K12.1100 (endo-I-)</td>
<td>T4 amN134</td>
<td>T4 am+</td>
<td>5</td>
<td>0</td>
<td>56.0</td>
<td>6.0</td>
</tr>
<tr>
<td>K12.1000 (endo-I-)</td>
<td>T4 amN134</td>
<td>T4 am+</td>
<td>5</td>
<td>2.2</td>
<td>2.2</td>
<td>8.0</td>
</tr>
</tbody>
</table>

The possibility of a correlation between the appearance of the virus-induced DNase activity (Stone & Burton, 1962) and super-infection breakdown was examined by assaying total DNase activity in cultures infected by T4, together with their ability to exclude and degrade superinfecting virus DNA. Even though appreciable amounts of virus DNase were formed, very little superinfection breakdown was observed with endo-I− host strains, although genetic exclusion occurred; with endo-I+ hosts extensive superinfection breakdown took place (Table 2). Compared with other extracts, those from infected endo-I+ bacteria showed unexpectedly high DNase activities; this suggested that in these cases there might be synergistic effects involving bacterial and virus enzymes. This explanation was supported
DNA breakdown and temporal exclusion

by our finding that the apparent nuclease activity of mixtures which contained extracts of both uninfected endo-I+ bacteria and endo-I− bacteria infected by T4 was greater than that shown by either extract alone (Table 3).

Table 3. Demonstration of increased nuclease activity obtained by mixing extracts of endo-I+ strains of bacteria with extracts of endo-I− strains infected by T4 phage

<table>
<thead>
<tr>
<th>Extract no.</th>
<th>Source of extract</th>
<th>Ratio of volumes extract 1:extract 3</th>
<th>Deoxyribonuclease activity (μmoles/min./mg. protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Uninfected E. coli K12,1000 (endo-I+)</td>
<td>—</td>
<td>Expt 1: 2.5, Expt 2: 2.2</td>
</tr>
<tr>
<td>2</td>
<td>E. coli K12,1100 (endo-I−) immediately after infection</td>
<td>—</td>
<td>Expt 1: 0.7, Expt 2: 0.8</td>
</tr>
<tr>
<td>3</td>
<td>E. coli K12,1100 (endo-I−) at 15 min. after infection</td>
<td>—</td>
<td>Expt 1: 4.0, Expt 2: 2.3</td>
</tr>
<tr>
<td>1 + 3</td>
<td>See above</td>
<td>1:3</td>
<td>Expt 1: 9.1, Expt 2: 4.5</td>
</tr>
<tr>
<td>1 + 3</td>
<td>See above</td>
<td>1:1</td>
<td>Expt 1: 10.6, Expt 2: 5.8</td>
</tr>
<tr>
<td>1 + 3</td>
<td>See above</td>
<td>3:1</td>
<td>Expt 1: 13.0, Expt 2: 7.0</td>
</tr>
</tbody>
</table>

Fig. 1. Superinfection breakdown and genetic exclusion in endonuclease-I deficient and normal bacteria. In the K12 cultures 12%, and in the cultures of B strains 5%, of the infected bacteria yielded secondary phage. Host bacteria: (a) •, E. coli K12,1000 (endo-I+); ○—○, K12,1100 (endo-I−); (b) ●—●, E. coli B.31 (endo-I+); ○—○, B.41 (endo-I−).

Bacteria infected by T5 phage: exclusion and breakdown of superinfecting T5 DNA

The procedures were the same as those already described for the T-even phage infections, except that m.o.i. = 10, and phage dilution fluid adjusted to 0.001 M-CaCl2 was the adsorption medium; phage adsorption was permitted for 20 min. at 37° with very gentle aeration. Wild-type T5 or the amber mutant T5,b1 were used for the primary, and wild-type T5 for the secondary infection. Total infected bacteria were determined by plating on Escherichia
coli CR63 and successful superinfections by plating on *E. coli* c3000 (Table 4). Exclusion was always less than that observed after infection by T4 phage; very little DNA breakdown occurred.

Table 4. Measurement of exclusion and superinfection breakdown after primary infection with phage T5

<table>
<thead>
<tr>
<th>Host strain of <em>E. coli</em></th>
<th>Primary phage</th>
<th>Secondary phage</th>
<th>Percentage of secondary phage DNA made acid-soluble</th>
<th>Percentage of infected bacteria which yielded secondary phage</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.41 (endo-I-)</td>
<td>T5</td>
<td>T5</td>
<td>0.8</td>
<td>—</td>
</tr>
<tr>
<td>B.31 (endo-I+)</td>
<td>T5</td>
<td>T5</td>
<td>0.9</td>
<td>—</td>
</tr>
<tr>
<td>K12.1100 (endo-I-)</td>
<td>T5.B1</td>
<td>T5</td>
<td>2.1</td>
<td>22.0</td>
</tr>
<tr>
<td>K12.1000 (endo-I+)</td>
<td>T5.B1</td>
<td>T5</td>
<td>2.2</td>
<td>32.0</td>
</tr>
<tr>
<td>B.41 (endo-I-)</td>
<td>T5</td>
<td>T4</td>
<td>47.0</td>
<td>22.5</td>
</tr>
<tr>
<td>B.31 (endo-I+)</td>
<td>T5</td>
<td>T4</td>
<td>48.0</td>
<td>14.0</td>
</tr>
<tr>
<td>B.41 (endo-I-)</td>
<td>T5</td>
<td>T2</td>
<td>42.0</td>
<td>10.0</td>
</tr>
<tr>
<td>K12.1100 (endo-I-)</td>
<td>T5.B1</td>
<td>T4</td>
<td>55.0</td>
<td>29.0</td>
</tr>
</tbody>
</table>

Fig. 2. Breakdown of the DNA of superinfecting 32P-labelled T4 phage by a T5-infected culture of *Escherichia coli* B.41. ●—●, DNase activity; ○—○, total DNA; ▲—▲, acid-soluble 32P as per cent of total 32P counts.

**Bacteria infected by phage T5: exclusion and breakdown of superinfecting T2 or T4 DNA**

Bacteria infected with T5 phage were diluted into broth (to give a cell density of $5 \times 10^8$/ml.), aerated at 37° and the changes in the amounts of DNA and DNase in the infected culture were followed. After 5 min., a sample of the infected culture was superinfected with 32P-labelled T4 phage, and the subsequent formation of acid-soluble 32P-labelled material was measured (Fig. 2, Table 4). Bacteria infected with T5 were able to exclude and degrade superinfecting T4 phage DNA to acid-soluble fragments whether the cells contained an active bacterial endonuclease-I or not. The capacity to degrade the superinfecting T4 phage DNA appeared within 5 min. of the initial T5 infection and well before the appearance at 10 to 12 min. after infection of the T5-induced nuclease activity described by Stone &
Burton (1962) and by Paul & Lehman (1966). The synthesis of DNA in the infected culture followed the pattern expected for T5-infected bacteria (Pfefferkorn & Amos, 1958; Crawford, 1959). Exactly similar results were obtained when T2 was the superinfecting phage.

Addition of chloramphenicol (100 μg./ml.) at the time of T5 infection prevented breakdown of superinfecting T4 DNA. However, when chloramphenicol was added at 5 min., and the 32P-labelled T4 at 7 min. after the initial T5 infection, breakdown of the T4 DNA was observed (Fig. 3). The ability of the bacteria to degrade superinfecting T4 phage DNA therefore appeared to depend on prior protein synthesis directed by the T5 genome, and seemed not to require protein synthesis induced by the T4 phage.

![Image](https://via.placeholder.com/150)

**Fig. 3**

Fig. 3. The effect of chloramphenicol on the breakdown of superinfecting T4 phage DNA by T5-infected E. coli K12.1100. ●—●, Chloramphenicol added at time of T5 infection, 32P-labelled T4 phage 5 min. later; ○—○, chloramphenicol added at 5 min., and 32P-labelled T4 7 min. after T5 infection; ▲—▲, no chloramphenicol, 32P-labelled T4 added 5 min. after T5 infection.

![Image](https://via.placeholder.com/150)

**Fig. 4**

Fig. 4. Effect of Mg2+ on breakdown of superinfecting T2 DNA by T5-infected bacteria. In this experiment 8% of the infected bacteria in the medium with Mg2+ ions yielded T2, and in the other culture 12% of the infected bacteria yielded T2 phage. ●—●, medium with Mg2+; ○—○, medium lacking Mg2+.

To investigate whether the exclusion of T-even phages by T5-infected bacteria could occur independently of superinfection breakdown, we tested for exclusion under conditions where DNA degradation was greatly diminished by using a medium lacking Mg2+ (cf. Hershey et al. 1954). For this experiment (Fig. 4) Escherichia coli K12.1100 was grown in TPG medium and then half the bacteria resuspended in TPG medium without glucose, and the remainder in TPG medium lacking glucose and Mg2+. After infection with phage T5.b1, glucose was added, and 5 min. later the cultures were superinfected with 32P-labelled T2 phage. The T2 phages were excluded from the progeny although there was little solubilization of the T2 DNA. The sensitivity of the superinfection breakdown to the external [Mg2+] suggested that the degradative processes might occur at or near the bacterial surface. This suggestion is consistent with our finding that the soluble products formed appeared directly in the medium, and did not require extraction of the bacteria with cold acid, although we normally used this procedure.
Bacteria infected by phage T5: exclusion of phage λ

The above experiments suggested that a T5-induced nuclease might have been responsible for the degradation of superinfecting T-even phage DNA. With a view to eventually testing for a T5-induced enzyme with the phage λ DNA infectivity assay (Kaiser, 1968), we decided to determine whether T5-induced bacteria could exclude and degrade phage λ DNA.

Attempts to get efficient superinfection with phage λ were unsuccessful, so the following procedure was adopted. A 10 ml. broth culture of *Escherichia coli* K12.1100 was grown to a cell density of $5 \times 10^8$/ml.; the culture was centrifuged and the bacteria resuspended in 5 ml. of dilution fluid. $^{32}$P-phage λ was added (m.o.i. = 10) and the culture incubated at 37° for 20 min. without aeration. Broth (5 ml.) was added and the culture gently aerated for 1 min.; the suspension was made 0.001 M with respect to CaCl₂ and phage T5 added (m.o.i. = 10). Samples (1.0 ml.) were taken over a 30 min. period to measure breakdown of λ DNA. The maximum breakdown of λ DNA found in these experiments was observed after 25 min. and amounted to 10% of the total radioactivity added. Examination of the final burst indicated that only T5 appeared in the phage progeny. In the absence of added T5, no λ DNA breakdown was observed.

DISCUSSION

These results indicate that in bacteria infected with T-even phages temporal exclusion of genetic markers and super-infection breakdown are independent phenomena. Since the conversion of secondarily infecting DNA to acid-soluble fragments occurs to only a very small extent in host strains deficient in endonuclease-I, this bacterial enzyme seems to be the major cause of the extensive fragmentation of secondary DNA which is observed with wild-type strains. The presence or absence of endonuclease-I appears to have no effect on the exclusion barrier set up by the primary virus. This agrees with earlier observations which showed that genetic exclusion occurred even when DNA breakdown was prevented by maintaining a low magnesium ion concentration in the growth medium (Hershey et al. 1954). None of the results mentioned eliminates the possibility that some breakage of super-infecting DNA molecules occurs but is insufficient to yield acid-soluble products.

In some of our experiments the amounts of DNase activity in the infected and uninfected cultures were measured. The high nuclease activities in extracts of infected wild-type bacteria can be explained by synergistic effects between bacterial and phage enzymes. Therefore, if the amounts of phage-induced nuclease are the same within the two B and the two K12 strains respectively, the difference in superinfection breakdown could be directly related to the variations in levels of the bacterial endonuclease-I.

The experiments of Hershey et al. (1954) showed that whether superinfection breakdown occurred or not, 40% of the secondary phage DNA could be removed by shearing the infected complexes in a blender. In addition, when low Mg²⁺ concentration prevented breakdown, the ‘non-strippable’ fraction of the secondary DNA remained attached to the bacteria and did not contribute $^{32}$P or genetic markers to the progeny phage. French et al. (1952) also showed that $^{32}$P of secondary phage DNA did not contribute to the progeny, even when breakdown was prevented. It therefore seems likely that temporal exclusion is primarily caused by virus-induced changes at the cell surface which prevent complete injection of superinfecting phage DNA, the degradation of which is a secondary phenomenon caused largely by the host endonuclease-I.

From our experiments with bacteria infected with T5, we conclude that this phage can set up an exclusion barrier to secondary T5, and that this occurs without the solubilization
DNA breakdown and temporal exclusion

of the secondary DNA. The reason for the absence of breakdown is difficult to elucidate since we do not know to what extent the superinfecting T5 DNA molecules are released from phage particles, thereby making them accessible to DNase action. Similarly, in the mixed infections with phages T5 and λ we cannot tell whether impaired release of λ DNA may have contributed to its exclusion and limited breakdown. With T-even phages impaired DNA release presumably has less effect in preventing breakdown since most of the DNA is ejected immediately the particles make contact with bacterial cell walls (Hershey & Chase, 1952).

The breakdown of superinfecting T4 DNA by endo-I− strains of bacteria infected with T5 is particularly interesting, and the results suggest that the effect is caused by an enzyme which degrades heterologous DNA although intracellular T5 phage DNA remains intact. Such nuclease action could account for the results of Weigle & Delbrück (1951), who demonstrated that superinfecting T5 excluded λ in lysogenic cultures which had been induced by u.v. light.

The ability to break down superinfecting T-even phage DNA appears in endo-I− host bacteria within 5 min. of T5 infection and therefore within the period during which protein synthesis induced by the First Step Transfer (FST) portion of the T5 DNA takes place (Lanni, 1968; McCorquodale & Buchanan, 1968). A process induced by the FST portion is the degradation of host cell DNA (Lanni & McCorquodale, 1963), and mutants lacking this function and mapping in the FST region of the viral genome have been isolated by Lanni (1968). It would be useful to know whether bacteria infected with these mutants are able to degrade superinfecting T4 DNA, since it is tempting to suppose that the destruction of host DNA, and that of phages such as T4 and λ, may be initiated by the same process, for example through a nuclease which is sufficiently specific to distinguish between T5 and heterologous DNA. Specificity of this kind would be possible with an enzyme which recognizes particular nucleotide sequences, as appears to occur with the DNA restriction enzyme, endonuclease-III, which is responsible for the initial attack on DNA bearing the ‘wrong’ host modification (Meselson & Yuan, 1968; Linn & Arber, 1968). If T5 phage directs the synthesis of a DNase with a specificity of this kind, the enzyme would be expected to make relatively few breaks in a DNA molecule, and subsequent degradation to low molecular weight fragments would then result from the action of other, less specific nucleases. Even if this does occur, at present we cannot say whether T5-induced exclusion of heterologous phage is primarily caused by nuclease action, or whether DNA degradation is only secondary to some other event more immediately concerned in the exclusion process.

REFERENCES


Evidence for the existence of a T5 phage-induced enzyme which converts viral DNA molecules to high molecular weight fragments without the formation of acid-soluble products is described by Fielding & Lunt (1969).

Note added in proof

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REFERENCE