MIXED BACTERIOPHAGE INFECTION OF ESCHERICHIA COLI:
EXCLUSION OF SUPERINFECTING T4 BY T7

by

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ABSTRACT

E. coli was infected with T7 as primary infecter and T4 as secondary infecter. When the time between primary and secondary infection was 5 minutes or more, most of the resulting plaques were T7. Varying multiplicity of T4 and T7 from 3 to 9 did not affect exclusion efficiency. Genomes of excluded T4 phages were injected but did not replicate or break down extensively. No T4 proteins were detected in superinfection exclusion. RNA synthesis upon injected T4 genomes was partially inhibited. T7 0.7amJS62a as primary infecter gave a reduced efficiency of exclusion. T7 1am193 as primary infecter abolished exclusion.

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INTRODUCTION

Exclusion among bacterial viruses is a phenomenon in which two different bacteriophages infect the same bacterium, with only one virus infecting successfully and producing progeny. Exclusion was first formally described in 1945 (Delbruck and Luria 1945). Since then a number of bacteriophage pairs have been examined with respect to exclusion.

In the initial mixed infection experiments Delbruck and Luria (1945) studied the phages T1 and T2. When T1 and T2 were mixed with bacteria T2 produced infective centres; no T1 infective centres were detected. In order for T1 to dominate T2 in a mixed infection it required at least a 7 minute temporal advantage, otherwise T2 would dominate. A control experiment showed T1 and T2 did not interfere with the adsorption rate of the other phage. Mixed infection would not result in any single bacterium producing both T1 and T2 particles (Delbruck and Luria 1942). Ultraviolet light-inactivated T2 would still exclude T1 (Luria and Delbruck 1942). In mixed infections of T1 and T4, T4 behaved in qualitatively the same way as T2 in T1 and T2 mixed infections (Delbruck 1945).

Delbruck (1945) defined the "depressor effect" of mutual exclusion: in a mutual exclusion experiment, the burst size of the excluding phage is reduced from the burst size of the same phage in a single infection experiment.

Hershey (1946) observed that mixed infections with $T4r^+$ and $T4r^-$ gave rise to mottled plaques, indicating that infective centres yielded 2 types of phage. $T2r^+$ - $T2r^-$ mixed infections (Delbruck

and Bailey 1946) resulted in a higher than expected number of non-mottled plaques; a result which indicated genetic exchange was taking place. Exchanges of genetic information were also noted between pairs of T6 and T4 r^+ and r^- phages (Delbruck and Bailey 1946).

Exclusion among different T even phages is a partial exclusion. Partial exclusion of T2 by T4 was examined by Streisinger and Weigle (1956). T2 progeny of a T2-T4 mixed infection were not excluded when used for a subsequent T2-T4 mixed infection (Streisinger and Weigle 1956). At least 2 points on the early region of the T2 genome determine the sensitivity of T2 to exclusion by T4 (Russell and Huskey 1974). Russell and Huskey (1974) postulate that exclusion phenomena provide a mechanism for speciation of bacteriophages. They suggest regions of exclusion sensitivity on the T2 map may correspond to loops of nonhomology in the T2-T4 heteroduplex map.

Analysis of pulse labelled proteins from superinfection exclusion of T3 by T7 on SDS polyacrylamide gels provides no evidence for expression of T3 genes in the exclusion (Hirsch-Kauffmann et al 1976). Analysis of superinfecting pairs of T7 which differed by a single amber mutation infecting a suppressor positive host has been performed (Hirsch-Kauffmann 1976). Functions of the superinfecting virus were not expressed. In a genetic analysis of superinfection exclusion of T7 by T7 exclusion occurred if the T7 RNA polymerase gene (and therefore the late genes), protein kinase gene, 1.1 gene, or DNA ligase gene were inactivated in the excluding virus (Hirsch-Kauffmann et al 1976). By the process of elimination this left gene

0.3 or an as yet unidentified, small early protein. A T7 mutant which was 1 am would exclude T7 whereas a mutant which was 1am, 0.3am would not (Hirsch-Kauffmann et al 1976). This result seems to point to the 0.3 gene product as being responsible for exclusion but a T7 phage which was 1 am 0.3am would exclude, suggesting a late function is also involved in superinfection exclusion (Hirsch-Kauffmann et al 1976). Despite the fact that exclusion occurred even if the infection was carried out in the presence of rifampicin or chloramphenicol, the 0.3 gene product could not be detected in SDS polyacrylamide gel electrophoresis of the T7 virion (Hirsch-Kauffmann et al 1976). Gene 0.3 functions in overcoming host restriction and modification (Studier 1975). As a result of these experiments the view has been expressed that superinfection exclusion of T7 by T7 is due to a membrane event which prevents injection of DNA of superinfecting T7 (Schweiger et al 1975). This view is in concord with the results of Benbasat, Burck and Miller (in press) showing primary infecting T7 facilitates inhibition of injection of DNA of T7 phages which attach at a later time. Two other results are related to the membrane alteration hypothesis. First, inactivation of receptors for T5 (which is related to T7) during infection of E. coli takes 30 minutes at 30⁰ (Dunn and Duckworth 1977). Second, the gene 0.3 product has been linked to T7 induced efflux of potassium ions from T7 infected cells (Ponta et al 1976), indicating T7 infection has an effect on bacterial membranes.

Shut-off of E. coli by T7 is relevant to superinfection exclusion since expression of phage early genes is largely dependent on host functions. Inactivation of host RNA polymerase during T7 infection is primarily due to binding of T7 gene 2 product to the polymerase

(Hesselbach and Nakada 1977a) and is enhanced by the T7 gene 0.7 product. The T7 gene 2 product has been purified (Hesselbach and Nakada 1977b). T7 gene 0.7 gene product phosphorylates the beta subunit of E. coli RNA polymerase (Zillig 1975).

Phage lambda excludes T1. Two lambda gene products required for exclusion of T1 have been identified (Christensen, Gawron and Halpern 1978): N and Q gene products. The suggestion has been made that lambda N gene-mediated exclusion of T1 is due to N gene product interfering with T1 replication.

From this overview of exclusion studies of bacteriophages of differing relationships 2 points emerge: 1) Exclusion sensitivity may be due to a defined genetic locus of the excluded phage in addition to functions of the excluding phage. 2) Exclusion may have 2 temporal modes, late and early.

The study reported here examined the exclusion of T4 by T7. In particular, attention was focussed on macromolecular synthesis during exclusion. The molecular events studied in the course of superinfection were: phage attachment and DNA injection, DNA synthesis, RNA synthesis and protein synthesis.

MATERIALS AND METHODS

Bacteria

Escherichia coli B^e and B23 were used as the nonpermissive hosts for amber mutants in all experiments. Both are su⁻. 011' is a su⁺ E. coli B derivative which was used to prepare amber phage stocks. Cultures were grown to between 2 and 3 x 10⁸ cells/ml in H broth before resuspension and growth in defined media.

Bacteriophages

The following T7 strains were from the collection of F. W. Studier: T7⁺, T7 1am193, T7 0.7amJS62a. T4D was from the collection of A. H. Doermann.

Chemicals

5-Bromo-2'-deoxyuridine (5-BrUrd) and 5-fluoro-2'-deoxyuridine (5-FdUrd) were purchased from Sigma Chemical Company. Tetra-methylethylenediamine (TEMED), N,N'-bis-methylene acrylamide (bis) and ammonium persulphate (AP) were from Biorad.

Radiochemicals

5-(³H)-uracil was purchased from Amersham. (³²P) as H₃³²PO₄ and uniformly labelled (¹⁴C)-amino acids were from New England Nuclear.

Media and Buffers

TCG was composed of 0.1 M Tris-Cl, 0.16 mM Na_2SO_4 , 1.0 mM MgSO_4 , 0.05% NaCl, 0.1% glucose, 0.1 mM CaCl_3 , 3 μM FeCl_3 , 0.05% vitamin free casamino acids, final pH 7.4 (Kozinski and Szybalski 1959).

TCG was supplemented with phosphate by the addition of 0.1 M KH_2PO_4 as 0.65 ml per 100 ml TCG for normal PO_4 TCG, 0.128 ml per 100 ml TCG for 1/5 PO_4 TCG and 6.4 ml per 100 ml TCG for 10x PO_4 TCG. 10x PO_4 TCG contained 250 μg of PO_4 per ml.

5-BrUrd TCG was 10x PO_4 TCG supplemented with 100 μg 5-BrUrd per ml, 25 μg per ml uracil and 10 μg per ml Fudr.

H broth contained per 1000 ml of distilled water: 5 g peptone (Difco), 1 g glucose, 5 g NaCl, 8 g nutrient broth (Difco), pH adjusted to 7.0.

TN was 0.01 M Tris-Cl, 0.15 M NaCl, final pH 7.4.

TNE was TN supplemented with 0.015 M EDTA.

T7 Tris Salt was 0.01 M Tris-Cl, 1.0 M NaCl, pH 7.4.

T4 Tris Salt was 0.01 M Tris-Cl, 0.15 M NaCl, pH 7.35.

M9S medium was a modification of that of Bolle et al (1968) and was:

0.04 M Na_2HPO_4 , 0.022 M KH_2PO_4 , 0.05% NaCl, 0.02 M NH_4Cl , 0.01 M FeCl_3 , 1% vitamin-free casamino acids (Difco) and 0.4% glucose, pH 7.2.

M9S.1 was the same as M9S except the concentration of vitamin-free casamino acids was 0.1%.

Top layer agar for phage plating contained, per 1000 ml: 7 g Bacto agar, 10 g Bacto peptone, 8 g NaCl, 1 g glucose, pH 7.0.

Bottom layer agar contained, per 1000 ml: 11 g Bacto agar, 10 g Bacto tryptone, 8 g NaCl, 1 g glucose, pH 7.

RNA extraction buffer was 0.05 M Tris-Cl, 2 mM EDTA, 1% SDS, pH 6.8.

Shearing medium contained 1 mM MgSO_4 , 0.1 mM CaCl_3 , 0.1% gelatin.

TAG was the same as TCG except that the vitamin-free casamino acids were substituted with the twenty amino acids, each at 10 $\mu\text{g}/\text{ml}$. Sample solubilization mixture for SDS polyacrylamide gel electrophoresis was 0.125 M Tris-Cl, 4% SDS, 20% glycerol, 0.01% bromophenol blue, 105 mercaptoethanol, pH 6.8.

Preparation of Labelled Bacteriophages

^3H - ^{32}P -T4 - E. coli B23 was grown at least 2 generation to 3×10^8 cells/ml in H broth. The bacteria were sedimented, washed and resuspended in $1/5 \text{ PO}_4$ TAG without leucine and containing ^{32}P (as $\text{H}_3^{32}\text{PO}_4$) to a specific activity of 1 $\mu\text{Ci}/\mu\text{g}$. The cells were grown 2 generations to 3×10^8 cells/ml at 37° and infected with T4 at a multiplicity of infection (moi) of 10. Ten minutes after infection (^3H)-leucine was added to 5 $\mu\text{Ci}/\text{ml}$ and the culture was incubated at 37° until lysis. The lysates were treated with chloroform and DNase, and the phage were purified by differential centrifugation. An aliquot of the phage preparation was transferred to a glass fiber filter, dried, overlaid with toluene-based 1,4-bis-(2)-(5-phenyloxolyl)benzene and 2,5-biphenyloxazolyl scintillant and assayed for radioactivity with a Nuclear Chicago Isocap 300 scintillation spectrophotometer.

^{32}P -T4 - ^{32}P -T4 was prepared as described for ^3H - ^{32}P -T4 except that (^3H)-leucine was not added and the medium was $1/5 \text{ PO}_4$ TCG.

DNA extraction and purification

Infective centres or bacteria in TNE were treated with 1% sodium dodecyl sulphate (SDS) for 20 minutes at 37⁰ and incubated with self digested pronase (30', 37⁰) at 1 mg/ml for 4.5 hours or overnight at 30⁰. An equal volume of water-saturated phenol was added to the samples which were then placed in a tube roller for 1 hour at room temperature. The phenol layer was removed and the samples washed 3 times with water-saturated ether. Phage DNA was extracted directly with phenol followed by 3 ether washes.

Cesium Chloride Density Gradient Centrifugation

Native DNA was treated as follows: 0.3 ml of sample was mixed with 0.5 ml of TNE and transferred to a polyallomer tube containing 3.2 ml saturated CsCl solution (in distilled water at room temperature). The tube contents were mixed, overlayed with paraffin oil, placed in a SW 50.1 rotor and spun at 10⁰ for 64-72 hours at 30,000 revolution per minute in a Spinco model L ultracentrifuge. The gradient was dripped in 8 drop fractions onto squares of Whatman 3MM filter paper 2 cm by 2 cm. The squares were dried, overlayed with toluene-based scintillant and counted in the scintillation spectrophotometer.

SDS Polyacrylamide Gel Electrophoresis

Samples of infected and uninfected cells which had incorporated (^{14}C)-amino acids were sedimented and resuspended in 0.1 ml of sample solubilization mixture and the sample tubes were placed in a boiling water bath for 2 minutes. The samples were applied to 0.4 x 1 cm wells in a polyacrylamide gel 14 x 11 x 0.16 cm. The stacking gel (0.125 M Tris-Cl, 0.1% SDS, 3% acrylamide, 0.08% Bis, 0.1% TEMED, 0.07% ammonium persulphate, pH 6.8) occupied the top 2 cm of the gel. The running gel (0.375 M Tris-Cl, 0.1% SDS, 10% acrylamide, 0.26% Bis, 0.1% TEMED, 0.07% ammonium persulphate, pH 6.8) occupied the lower 12 cm of the gel. The running buffer was composed of 0.025 M Tris-Cl, 0.192 M glycine, 0.1% SDS, pH 8.8. The gel was run with cooling at about 7 watts power until the tracking dye reached the bottom of the gel. The gels were stained (2% TCA, 1% Coomassie Brilliant Blue, 15% glacial acetic acid, 47.5% ethanol) overnight at room temperature. The gels were destained (2% TCA, 1% Coomassie Brilliant Blue, 15% glacial acetic acid) for 1 hour and washed (7.5% glacial acetic acid) for about 0.5 hours. The gels were dried with heat and vacuum onto a sheet of Whatman 3MM filter paper and exposed to Kodak XR-2 X-ray film.

Preparation of DNA Filters for Hybridization

90 ml of 6x SSC was combined with 720 μg DNA. NaOH was added to 0.33 N and the sample was stirred for 1 hour at room temperature. The DNA solution was brought to neutrality by adding 6 N HCl and applied to a Millipore HAWP 142 50 filter. The filter was washed with 100 6x SSC. The filter was dried in a partial vacuum over dessicant for 2-3 hours at 80 $^{\circ}$. The filter was cut into 9 cm circles containing 6 μg of DNA per filter.

Incorporation of 5-(³H)-uracil

20 μ l of a sample which had been extracted for RNA was placed into a tube containing 1 ml of 1 M KOH and left overnight. 1 ml of 1 N HCl was then added, followed by 50% trichloroacetic acid (TCA) and 50 mM sodium pyrophosphate (PPi) to give a final concentration of 10% TCA, 10 mM PPi. After 1 hour 0.1 ml of *E. coli* B^e at 3×10^8 cells/ml was added as a carrier. The sample was filtered through a Whatman GF/A glass fiber filter. The filter was washed 3 times with 5 ml of 5% TCA, 10 mM PPi and 2 times with ethanol. The filter was dried, placed into a vial containing scintillant and was counted for ³H in the scintillation spectrophotometer. Samples for which alkaline degradation was not performed were treated as above except the 1 M KOH and 1 N HCl were not added.

RNA Extraction Procedure

Samples of infected and uninfected cells which had incorporated 5-(³H)-uracil were sedimented and resuspended in RNA extraction buffer and the sample tube was placed in a boiling water bath for 2 minutes. Then 0.2 ml of 2 M sodium acetate, pH 5.2 was added to each sample, after which 2.2 ml of water-saturated phenol was added. The samples were agitated for 5 minutes and the aqueous phase was removed to a tube containing a further 2.2 ml of phenol. After agitation the aqueous phase was extracted twice with an equal volume of chloroform. Ethanol was added to the aqueous phase to a final concentration of 80% and the sample was placed in the cold for at least 4 hours to precipitate the RNA. The RNA was sedimented in the cold, washed with cold 80% ethanol and resuspended in 0.8 ml of 0.01 M Tris-Cl, 5 mM MgCl₂, pH 7.4. RNase free DNase was added to final concentration of 0.025 mg/ml and

the samples were incubated 10 minutes at 37⁰. EDTA was added to a final concentration of 5 mM and 2 ml of chloroform was added. The aqueous phase was removed and the OD₂₆₀ of each sample was determined with a Unicam SP800 double beam spectrophotometer.

RNA-DNA Hybridization

RNA samples were hybridized in 3x SSC, 0.1% SDS. Hybridization vials contained 8 filters: 2 filters each with E. coli DNA, T7 DNA, T4 DNA and 2 filters without DNA. The total amount of each type of DNA per vial was 12 µg. Not more than 2.7 µg of RNA was added to each hybridization vial. After overnight hybridization at 65⁰ the filters were washed 2 times with 3 ml of 3x SSC. Filters were incubated in 3x SSC with 1 µg/ml RNase for 10 minutes at 37⁰. The filters were washed 3 times with 3 ml of 3x SSC, placed into separate scintillation vials, dried, overlayed with scintillation fluid and assayed for radioactivity.

Plating of Bacteria and Infective Centres

Platings were performed according to methods outlined by Adams (1959). 2 minutes after phage infection or superinfection an aliquot of infected culture was transferred to combined T4 and T7 antiserum and incubated at 30⁰ for 5 minutes. The sample was diluted out of antiserum and dilutions were plated on overlays for infective centres and surviving bacteria.

RESULTS

Kinetics of Superinfection Exclusion

Effectiveness of exclusion was examined as the time of superinfection was varied. The results are shown in Figure 1. The results indicate that T4 is only excluded when T4 superinfection occurs 5 minutes or more after T7 infection. The kinetics of killing of E. coli by T7 are presented in Figure 2. T7 requires 5 minutes to kill E. coli and form maximal numbers of infective centres. The conclusion is that when T7 does not form infective centres T4 is not excluded by T7.

Figure 1

Figure 1 Legend

Kinetics of exclusion of T4 by T7. E. coli was grown to 2×10^8 cells per ml in H broth and T7 was added at an moi of 10. At 1, 2.5, 5 and 9 minutes after T7 infection aliquots of infected culture were transferred to premeasured volumes of superinfecting T4 at an moi of 10. Two minutes after superinfection samples of infected cultures were transferred to T4 and T7 antiserum, incubated for 5 minutes at 30° and diluted out of antiserum. T4 and T7 infective centres and surviving bacteria then were scored. Survivors were less than 2%. Total phage yields for each sample were on the order of 2×10^8 phage per ml. () - T7 infective, () - T4 infective centres.

Figure 2

Figure 2 Legend

Killing of E. coli by T7. Bacteria at 2×10^8 cells per ml in H broth were infected with T7 at an moi of 10 at zero time. Surviving bacteria were plated at 1, 2.5 and 4 minutes after infection.

Effect of Multiplicity of Infection on Superinfection Exclusion

An experiment was performed to examine the effect of varying the multiplicity of infection (moi) on relative yields of T4 and T7 infective centres from superinfection. When T4 infects 5 minutes after T7 infective centres of both phages can be detected. This was chosen as the superinfection time because the relative number of T4 and T7 infective centres is of prime interest. The results are displayed in Table 1. Over the range of mois the relative yield of T4 and T7 infective centres is constant.

<u>moi T4, T7</u>	<u>Yield of infective centres</u>	
	<u>T4</u>	<u>T7</u>
3	2.42×10^8	8.30×10^7
6	2.95×10^8	7.80×10^7
9	2.68×10^8	7.85×10^7

Table 1

Effect of moi on yield of T4 and T7 infective centres after superinfection. T7 was added at given moi at time zero. T4 was added at an identical moi 5 minutes later. Infective centres and survivors were plated as described in Methods and Materials. Survivors were less than 1%.

Depression of T7 Infective Centres During Superinfection Exclusion

A consistent observation made throughout the course of this study was that although T4 was excluded it caused a depression in the yield of T7 infective centres. A typical result is shown in Table 2. When T4 and T7 infected separate cultures the number of infective centres equalled the number of infected bacteria. In the superinfection T7 clearly dominated - less than 3 per cent of the infective centres were T4 - but the number of infective centres did not equal the number of infected bacteria. This depressor effect was also noted by Delbruck (1945) in superinfection exclusion of T2 by T7.

	<u>Yield of infective centres</u>	
	<u>T7</u>	<u>T4</u>
T7	1.5×10^8	-
T4	-	1.8×10^8
superinfection	5.4×10^7	1.5×10^6

Table 2

Depression of infective centres in superinfection exclusion. Infected culture contained 1.4×10^8 cells per ml. Bacteria were infected with phage at an mo_8 of 10. Superinfection with T4 was performed 5 minutes after T7 infection. In all three samples infective centres and surviving bacteria were monitored at 7 minutes after initial infection. Surviving bacteria were less than 0.1%.

Lack of Inhibition of T4 Injection by Prior Infection With T7

T7 might exclude T4 by a cell surface phenomenon. To test this hypothesis an experiment similar to that of Hershey and Chase (1952) was performed to examine phage attachment and DNA injection during superinfection. T4 was labelled with (^{32}P) orthophosphate which labelled phage DNA and (^3H)- leucine which labelled the phage protein coat. After infection cultures were sedimented and resuspended to remove unadsorbed phage and then were sheared in a Waring blender. Aliquots of each culture were set aside as nonsheared controls to monitor spontaneous desorption of phage. After shearing infected cells were sedimented and resuspended. The supernatants and resuspended pellets were analyzed for ^{32}P and ^3H .

Absence of either ^{32}P or ^3H in the pellet would mean that either phage attachment did not take place or that the phage attached but did not inject its DNA and was sheared off. Association of ^{32}P with the pellet and ^3H with the supernatant would mean the phage attached, the DNA was injected and the empty protein coat was sheared off. The control for injection of DNA by T4 alone is shown in Table 2. In the nonblended T4 alone sample ^3H and ^{32}P were associated with the pellet which is the result expected if attachment took place and no shearing was performed. In the T4 alone blended sample ^{32}P was associated with the pellet while the ^3H was associated with the supernatant. This result indicates attachment of the phage, injection of DNA and removal of the empty phage head by shearing. The data for infection with T7 and superinfection with ^3H - ^{32}P -T4 is shown in Table 2. The nonblended control shows that attachment occurred, as both ^3H and ^{32}P were associated with the pellet. The blended sample

shows ^3H associated with the supernatant and ^{32}P associated with the pellet, indicating injection of T4 DNA . The conclusion to be made from the 2 experimental series is the same: When T4 alone infects bacteria, it injects its DNA and when T4 infects bacteria 7 minutes after T7 (a superinfection time at which T4 is excluded) T4 injects its DNA.

Table 3

<u>Distribution of recovered radioactivity (%)</u>		
	<u>^3H</u>	<u>^{32}P</u>
<u>T4 alone</u>		
Unblended		
Supernatant	14.5	11.5
Pellet	85.5	88.5
Blended		
Supernatant	72.7	12.3
Pellet	27.3	87.7
<u>Superinfection</u>		
Unblended		
Supernatant	12.3	7.9
Pellet	87.7	92.1
Blended		
Supernatant	81.3	35.8
Pellet	18.7	64.2

Table 3 Legend

Superinfection samples: Bacteria were grown to 2×10^8 cells per ml in 10xPi TCG. At zero time cells were infected with T7 at an moi of 10. At 7 minutes after infection the culture was infected with ^3H - ^{32}P -T4 at an moi of 10.

T4 alone: Bacteria at a density of 2×10^8 cells per ml in 10xPi TCG were infected with ^3H - ^{32}P -T4. Moi of T4 was 0.5 in order to prevent T4 superinfection exclusion of itself.

T4 alone and superinfection: Thirteen minutes after initial infection unattached phage were removed by sedimenting the infected cells and the pellet was resuspended in shearing medium with chloramphenicol added to 0.1 mg/ml. The samples were sheared for 5 minutes in a Waring blender at 1×10^4 revolutions per minute. An aliquot of culture was set aside as nonsheared control. The samples were sedimented and the supernatant and resuspended pellet were analyzed for radioactivity. In the superinfection sample the yield of infective centres was 1.86×10^8 IC/ml for T7 and 5×10^7 IC/ml for T4.

Exclusion did occur. Infected bacteria were at 2×10^8 cells/ml. The total yield of infective centres relative to infected bacteria indicates that blending did not result in extensive bacterial lysis. For all samples between 54% and 71% of input radioactivity was recovered.

Fate of T4 DNA Injected During Superinfection Exclusion

Given that T4 injects its DNA during superinfection exclusion, an experiment was performed to determine if the injected T4 DNA replicates or is degraded. The experiment was a density transfer experiment. The transfer of density was followed for T4 infecting alone and a superinfection sample as shown in Figure 3. Unreplicated ^{32}P -T4 DNA served as a light density reference. ^{32}P which banded at a density greater than the reference provided evidence of T4 replication. The T4 replication control exhibited 2 bands: a sharp one near the top of the gradient and a broader dense band further down the gradient. The sharp peak is light, unreplicated ^{32}P -T4 marker DNA. The broad, heavy peak is ^{32}P from input T4 which has shifted to heavier density due to replication. The superinfection sample shows no shift in the density of T4 DNA indicating failure to replicate during superinfection exclusion. The shape and position of the ^{32}P Band in the superinfection sample gives clues as to whether the injected DNA is degraded. Mild degradation would make the band wider. Extensive degradation would result in the ^{32}P being distributed throughout the gradient. Inactivation by nicking cannot be detected on neutral CsCl gradients. When T4 DNA is injected during superinfection exclusion it is neither replicated nor extensively degraded.

Figure 3

Figure 3 Legend

Density transfer experiment. E. coli was grown in H broth to 3×10^8 cells/ml, ice chilled, sedimented, washed with TCG, re-suspended in 5-BrUrd TCG medium and grown for 90 minutes at 37° in the dark. Ten minutes before infection the cells were shifted to 30° . At zero time the superinfection sample was infected with T7 at an moi of 10. At 5 minutes the superinfected sample and the T4 alone sample were infected with ^{32}P -T4 at an moi of 5. At 10 and 15 minutes 2 ml aliquots of infected culture were transferred to a tube containing 5 ml of ice cold TNE. At 16 minutes chloramphenicol was added to both samples at a concentration of 100 $\mu\text{g/ml}$. At 20 and 25 minutes 2 ml aliquots were transferred to a tube containing 5 ml of ice cold TNE. The cells in all samples were sedimented and resuspended in TNE. DNA extraction, cesium chloride density gradient centrifugation and gradient fractionation were performed as described in Methods and Materials. Illustrated in Figure 3 are the superinfection and T4 samples from the 20 minute time point. The figure is representative of all time points in the experiment.

Protein Synthesis During Superinfection Exclusion

Examination of proteins synthesized during superinfection exclusion could provide clues as to the nature of superinfection exclusion. Pulse labelled proteins from cells infected with T7, T4 and T4 infecting after T7 were separated by SDS polyacrylamide gel electrophoresis. The resulting gels were autoradiographed. The autoradiograms are presented in Figure 4. No T4 bands can be detected in the superinfection lanes.

Figure 4

Figure 4 Legend

E. coli B^e was grown to 2×10^8 cells/ml in M9S medium at 30°. The cells were sedimented and resuspended in M9S.1 medium. The resuspended cells were split into 4 equal parts. The 4 cultures were incubated for 5 minutes. At zero time cultures A and C were infected with T7 at an moi of 10 and culture B was infected with T4 at an moi of 10. At 5 minutes after zero time culture C was infected with T4 at an moi of 10. At 6, 12, and 18 minutes after zero time 1 ml of each of cultures A, B and C was transferred to a tube containing 1.5 uCi of uniformly labelled (¹⁴C)-amino acids. Four minutes after each transfer 0.1 ml of 10% casamino acids was added to each labelling tube. Eight minutes after each transfer the labelling tubes were chilled. At 6 minutes after zero time 1 ml of culture D was labelled, chased and chilled as described above. All samples were sedimented and prepared for SDS polyacrylamide gel electrophoresis as described in the methods.

A - T7, B - T4, C - T7 followed by T4, D - uninfected.

Assignment of protein bands was made according to Studier (1972, 1973) for T7 and O'Farrell and Gold (1973) and O'Farrell, Gold and Huang (1973) for T4.

Superinfection Experiments Using T7⁺, T7am0.7, T7am1.0

In an attempt to identify the T7 function responsible for exclusion of T4 2 amber mutants of T7 were used in superinfection experiments. T7 gene 0.7 specifies a protein kinase. The kinase phosphorylates about 40 E. coli proteins including the beta subunit of E. coli RNA polymerase. As shown in Table 4 T4 superinfected 2 different cultures for each type of T7 - one at 6 minutes and one at 9 minutes. The relative numbers of infective centres gave a measure of efficiency of exclusion. T7⁺ is seen to exclude T4 when T4 superinfects at 6 and 9 minutes. The exclusion shows characteristic depression of T7 infective centres. T7am0.7 does not exclude completely when T4 superinfects at 6 minutes or 9 minutes but T7 dominates at the later time. Although an amber mutation in T7 gene 0.7 reduces the efficiency of exclusion it does not eliminate it. Unlike T7 gene 0.7, T7 gene 1.0 is an essential gene for expression of T7 infective centres. Despite the fact that when using T7am1.0 superinfected by T4 no T7 infective centres will be observed, if gene 1.0 is not involved in superinfection exclusion of T4 a significant exclusion should occur. This was not the case. The data shows that T4 infective centres are not much below the value of 2.1×10^8 per ml expected if T4 was responsible for the killing of 100% of the cells in the infected culture. Two possibilities exist - 1) T7 RNA polymerase has a direct function in excluding T4 2) a late gene product is responsible for exclusion.

<u>Initial Infector</u>	<u>Yield of infective centres (IC/ml)</u>			
	<u>Superinfection at 6'</u>		<u>Superinfection at 9'</u>	
	<u>T7</u>	<u>T4</u>	<u>T7</u>	<u>T4</u>
T7 ⁺	1.1×10^8	<0.1	2.9×10^7	1.5×10^6
T7am0.7	4.1×10^7	9.1×10^7	7.8×10^7	2.7×10^7
T7am1.0	<0.1	1.8×10^8	<0.1	1.6×10^8

Table 4

Superinfection of T4 upon T7⁺, T7am0.7, T7am1.0. Yield of infective centres per ml. Bacteria at a density of 2.1×10^8 in H broth in three different flasks were infected with T7⁺, T7am0.7 and T7am1.0 at an moi of 7. At 6 and 9 minutes after infection aliquots of each of the infected cultures were transferred to tubes containing T4 at an moi of 7. Infective centres were monitored at 2 minutes after superinfection.

RNA Synthesis during superinfection exclusion

In an attempt to identify the mechanism by which T7 blocks superinfecting T4, RNA synthesis during superinfection exclusion was examined. The technique used was to perform infections in the presence of 5-(³H)-uracil and hybridize the resulting ³H-labelled RNA to T7 DNA and T4 DNA. The results are shown in Table 5. Infection with T7 results in (³H)-labelled RNA hybridizing only to T7 DNA and infection with T4 results in (³H)-labelled RNA hybridizing only to T4 DNA. When T7 infection is followed at 7 minutes by T4 infection hybridization to both T4 DNA and T7 DNA is noted. Does the (³H)-labelled RNA hybridizing to T4 DNA represent the RNA required to produce the 8% nonexcluded T4 phages in this sample or does the injected T4 genome of excluded T4 encounter a post-transcriptional block? To answer this question the ratio of RNA hybridizing per infective centre was calculated for: a) T4 at 6 minutes and b) T4 in the superinfection at 12 minutes. The amount of (³H)-labelled RNA hybridizing to T4 DNA per T4 infective centre in the superinfection is over twice that in the T4 infection. This suggests that injected T4 genomes do encounter a transcriptional block but that transcription may also take place to a small extent on excluded genomes.

<u>Infector</u>	<u>Time (min)</u>	<u>Input ^3H (cpm)</u>	<u>% input ^3H hybridized to</u>	
			<u>T7 DNA</u>	<u>T4 DNA</u>
T7	6	13,262	2.4	<0.1
		26,254	3.1	<0.1
	12	3,259	44.5	<0.1
		6,518	45.0	<0.1
T4	6	5,204	<0.1	32.3
		10,408	<0.1	24.2
	12	14,024	<0.1	24.1
		28,048	<0.1	24.0
Super- infection	6	16,018	5.6	<0.1
		32,036	6.9	<0.1
	12	6,591	36.8	2.0
		13,182	36.4	1.8

Table 5

Table 5 Legend

RNA-DNA hybridization. E. coli B^e was grown to 2×10^8 cells/ml in H broth and split into 3 equal volumes. At zero time flasks A and C were infected with T7 at an moi of 10 and flask B was infected with T4 at an moi of 10. At 7 minutes flask B was infected with T4 at an moi of 10. At 3 minutes all flasks received 5-(³H)-uracil to a final concentration of 10 μ Ci/ml. At 6 and 12 minutes 2 ml aliquots of each flask were transferred to a tube containing 2 ml of ice chilled H broth. All 6 samples were sedimented, re-suspended in RNA extraction buffer and extracted for RNA as described in Materials and Methods. Evaluation of incorporation of 5-(³H)-uracil into acid insoluble and alkali insoluble material was performed as described in Materials and Methods. At least 91% of acid insoluble ³H was alkali labile. In the superinfection sample 8% of infective centres were T4 and 92% were T7. Preparation of filters binding E. coli DNA, T7 DNA and T4 DNA and hybridization of RNA samples to the filters was performed as described in Materials and Methods. Background hybridization to E. coli DNA filters was 0.22% of input ³H or less. Hybridization to blank filters was 0.2% of input ³H or less.

CONCLUSIONS

T7 excluded T4 when T4 superinfected E. coli 5 minutes or more after T7 infection.

In the range of moi from 3 to 9 no change in efficiency of exclusion could be seen.

The number of T7 infective centres in an exclusion experiment was lower than the number of T7 infective centres in a single T7 infection experiment

Excluded T4 injects its DNA

Injected T4 DNA is neither replicated nor extensively broken down.

There is little or no T4 protein synthesis during exclusion.

A low level of transcription occurs on excluded T4 genomes.

Transcription is depressed on primary infecting T7 genomes.

T7 protein kinase is involved in exclusion of T4 as is T7 RNA polymerase or a T7 late gene product.

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