Formation of Concatemeric DNA as an Intermediate in the Replication of Bacteriophage T1 DNA Molecules

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SUMMARY

The structure of intracellular DNA extracted from phage T1 infected cells was analysed by sedimentation through sucrose gradients. DNA labelled with ³H-dThd during a short pulse given at any time during T1 DNA synthesis sedimented in neutral gradients as a broad heterogeneous band with a large fraction of the label sedimenting more rapidly than mature T1 DNA molecules. Rapidly-sedimenting label was also observed when pulse-labelled DNA was denatured and analysed on alkaline sucrose gradients. Electron microscopy of intracellular T1 DNA revealed linear molecules of variable length the longest of which were three to four times the mature genome length. The distribution of lengths derived from electron microscopy are consistent with the molecular length distributions calculated from the sedimentation coefficients. We conclude that the rapidly-sedimenting DNA is in the form of concatemers consisting of linear tandem repeats of the T1 genome. The concatemeric form of replicating T1 DNA is a precursor of progeny T1 genomes since in pulse-chase experiments it was converted efficiently into mature, infectious T1 phage particles. The identification of this concatemeric form of T1 DNA provides supporting evidence for the model proposed by Gill & MacHattie (1976) to account for the formation of the very limited number of cyclic permutations of gene sequence found for mature T1 DNA molecules.

INTRODUCTION

The DNA of bacteriophage T1 is a linear, double-stranded molecule of mol. wt. $31 \times 10^6$ with a terminal repetition of 6.5%, or 2800 base pairs, of the nucleotide sequence (Bresler et al. 1967; Lang et al. 1967; Thomas & MacHattie, 1967; MacHattie et al. 1972; Gill & MacHattie, 1975; MacHattie & Gill, 1977). Direct electron microscopic visualization and band width measurements in CsCl density gradients have detected a mol. wt. heterogeneity of about 1.2% (MacHattie et al. 1972; MacHattie & Gill, 1977). The DNA molecules extracted from phage particles are found with three permutations of nucleotide sequence which differ from each other by circular permutation through either 6% or 12% of the total nucleotide sequence (Gill & MacHattie, 1976).

The presence of three permutations led Gill & MacHattie (1976) to suggest that they arise during maturation and particle assembly by the processive excision of 'headfuls' of DNA from a concatemeric DNA intermediate containing a number of tandem repetitions of the T1 base sequence. Furthermore, these authors proposed that the cutting process begins at a specific initiation site in the nucleotide sequence of the concatemer which locates

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the first end of the 'first-cut' molecule; the other end of the 'first-cut' molecule would be
determined by a cut at a distance of one 'headful' from the first end. This would give a
molecule containing one copy of the nucleotide sequence plus a terminal repetition, i.e.
a molecule of length 1.065 assuming that the T1 sequence has a repeat length of 1.000. The
'second-cut' and 'third-cut' molecules each begin at the end-point of the previous cut. This
mechanism has much in common with that proposed by Tye et al. (1974) to account for the
limited set of permutations found for phage P22 DNA molecules. As the relative proportions
of 'first-', 'second-' and 'third-cut' molecules are 0.4:0.4:0.2, Gill & MacHattie (1976) con-
cluded that the sequential character of the maturation process persisted over only a limited
length of the concatemer or over a limited number of maturation events before reverting
to the specific initiation site. This number would be frequently only two (giving first-cut
and second-cut molecules in equal proportions) and sometimes three (giving half the
number of third-cut molecules) or occasionally four.

In a later study using T1 variants with altered genome repeat length, MacHattie
& Gill (1977) provided evidence to support the 'headful' mechanism for T1 DNA
maturation. They showed that for each variant the total genome length was the same as
wild type T1 and that the length of the terminal repetition varied to compensate for the
altered repeat length. A feature of the 'headful hypothesis' of Streisinger et al. (1967) is
that lengths of genome are excised from a concatemer by a process which does not recognize
specific nucleotide sequences at the ends of molecules (Frankel et al. 1971; Luftig et al. 1971;
Ritchie & White, 1972).

One prediction of these studies is that T1 DNA replicates by way of a concatemeric
intermediate with a minimum length of two to three genome equivalents. For this reason
we have investigated the structure of intracellular replicating T1 DNA. Evidence based on
zone sedimentation through sucrose gradients and supported by electron microscopy,
shows that T1 DNA labelled with short pulses of 3H-dThd is in the form of linear concate-
meric molecules. Pulse-chase studies show this DNA to be a direct precursor of molecules
of identical sedimentation rate to mature T1 genomes and that up to 50 % of pulse-labelled
DNA is transferred to mature phage particles. These observations provide direct experi-
mental support for the type of concatemeric DNA envisaged by Gill & MacHattie (1976)
as the substrate for 'headful' maturation of T1 genomes.

METHODS

Phage and bacteria. Phage T1 wild type (originally described as T1 Ds++ by Dr C. Bresch,
was a gift from Dr L. A. MacHattie), am 221, an amber mutant in gene 2 and having a
DNA negative phenotype in Su- bacteria, was isolated in this laboratory. Escherichia coli
strains B and W3350 (both non-permissive for amber mutants) were used as host bacteria.

Growth and purification of phage. Unlabelled phage stocks were prepared by the confluent
lysis method on nutrient agar plates as previously described (Martin et al. 1976) but without
the dialysis step. 32P-labelled phage were grown in low phosphate medium by the method of
partition and CsCl step gradients was as described by Martin et al. (1976). Marker DNA
for use in sedimentation analysis was extracted from the purified phage by heating at 60 °C
for 45 min in 2 × SSC (0.3 M-NaCl, 0.03 M-sodium citrate; Ritchie, 1970; Ritchie & Mal-
colm, 1970).

Radioactive labelling of intracellular DNA. Bacteria were grown at 30 °C with aeration
in either M9 medium (Anderson, 1946) supplemented with 0.1 % casamino acids or ETCG
Phage T1 DNA replication

medium (MacHattie et al. 1967). Unless otherwise stated the cells were harvested by centrifugation at $1 \times 10^8$ cells/ml, resuspended at $1 \times 10^8$ cells/ml in ice-cold growth medium and chilled in ice for 20 min before the addition of phage at a multiplicity of 5 p.f.u./cell. After an adsorption period of 15 min in ice the phage infection was started by adding 2 vol. of medium at 45 °C to bring the temperature rapidly to 30 °C and incubation with aeration at 30 °C. DNA was labelled by the addition of methyl-$^3$H-thymidine (15000 to 30000 Ci/mmol, Radiochemical Centre, Amersham) for the times indicated below for each experiment. For 'pulse-chase' experiments the incorporation of $^3$H-dThd into DNA was terminated by addition of an excess of unlabelled thymidine (10 mg/ml) and incubation was continued at 30 °C. Similar results were obtained with either growth medium.

**Extraction of intracellular DNA.** At the appropriate times phage replication was stopped by transfer of a sample of the infected culture to an equal vol. of ice-cold lysis medium (0.01 M-KCN, 0.1 M-EDTA, 100 μg/ml lysozyme, 0.1 M-tris, pH 8.0). The bacteria were converted to spheroplasts by incubation for 5 min at 45 °C and lysed either by incubation for 5 min at 45 °C with 0.3% (w/v) SDS (final concentration), a procedure which disrupts T1 phage particles (Bresler et al. 1967; Gill & MacHattie, 1975), or by incubation for 5 min at 30 °C or 45 °C with a final concentration of 0.7% (w/v) sodium deoxycholate (DOC), a procedure which neither disrupted nor reduced the infectivity of mature T1 virions. The SDS lysis method does not completely separate the phage DNA from the capsid of mature virions (Bresler et al. 1967; Gill & MacHattie, 1975) and produces what are known as 'ghost complexes' consisting of released DNA attached to an empty particle. To achieve complete separation, the lysed samples were heated at 75 °C for 10 min and then cooled in ice prior to neutral sucrose gradient sedimentation. This last step was not necessary for centrifugations in either alkaline sucrose or CsCl.

**Sucrose density gradient centrifugation.** Five ml linear sucrose gradients (5 to 20%, w/v) were layered over a shelf of 0.2 ml CsCl (density of 1.5 g/ml) in Spinco nitrocellulose tubes and centrifuged at 10 °C in the SW 50-1 rotor of a Spinco ultracentrifuge. The sucrose was dissolved either in 0.1 M-NaCl, 0.05 M-phosphate, pH 6.8 (neutral sucrose) or 0.9 M-NaCl, 0.1 M-NaOH, pH 12.1 (alkaline sucrose). DNA samples (0.15 to 0.2 ml) were layered gently on top of the gradients with a wide bore pipette to avoid shear breakage. Gradient fractions were collected dropwise from the bottom of the centrifuge tube on 3 cm squares of Whatman 3 MM filter paper, which were washed with trichloracetic acid and water and assayed for radioactivity as described below.

**Radioactivity assays.** Samples of 0.01 to 0.15 ml were applied to 3 cm squares of Whatman 3 MM filter paper each of which was washed twice for 15 min in ice-cold 10% (w/v) trichloracetic acid, thoroughly washed in water and dried. The dried squares were suspended in 10 ml of toluene-based scintillant (Ritchie & Malcolm, 1970) and the radioactivity counted in a liquid scintillation counter.

**Electron microscopy.** Infected cell extracts containing replicating T1 DNA lysed by the SDS method were incubated with pronase (1 mg/ml) at 37 °C for 4 h and the DNA was extracted with phenol and dialysed against 2 x SSC as described by Kelly & Thomas (1969). DNA was mounted on Parlodion coated copper grids and stained with uranyl acetate using the modification of the Kleinschmidt technique described by Davis, Simon & Davidson (1971). Both the aqueous and formamide versions were used without heavy metal shadowing. Samples were examined with a Siemens Mark II electron microscope and micrographs were taken at a magnification of 8000 x. The contour lengths of molecules were measured with a map measuring device from enlarged tracings of the micrographs. ϕX174 open circular (RF II) DNA molecules, prepared from covalently closed circular (RF I) molecules
Fig. 1. Synthesis of T1 DNA in *E. coli* B. Cells growing at 30 °C in ETCG were harvested at 2 x 10⁸ cells/ml and a sample was irradiated with u.v. light. Samples of irradiated and unirradiated cells were each infected with 5 phage/cell of either T1⁺ or T1 am 221 and aerated at 30 °C. At intervals, 0.5 ml samples were transferred to tubes containing 0.1 ml of ³H-dThd at 20 μCi/ml and incubated at 30 °C for 1 min when 50 μl samples were applied to filter paper squares and immediately immersed in ice-cold 10 % TCA. T1⁺ infection: ■—■, unirradiated. ○—○, irradiated cells. T1 am 221 infection: □—□; irradiated; ■—■, unirradiated cells.

by treatment with 0.001 M-dithiothreitol by the method of Bode (1967), were added to each T1 DNA preparation to serve as a length and mol. wt. standard.

*Ultraviolet light irradiation.* Ten ml cultures of *E. coli* strain B growing in ETCG medium at 2 x 10⁸ cells/ml were irradiated with shaking in a 90 mm diam. glass Petri dish. Irradiation was routinely for 1 min at a distance of 100 cm from a 30 W u.v. lamp (total dose of about 300 ergs/mm²). Cells were infected immediately after irradiation.

**RESULTS**

*DNA synthesis in T1-infected bacteria*

The overall pattern of DNA synthesis in T1-infected bacteria is illustrated by the incorporation of ³H-dThd during 1 min pulses given at intervals during the growth cycle (Fig. 1). Results are shown for infections of unirradiated bacteria (filled symbols) and for bacteria which have received a dose of u.v. light sufficient to inhibit host cell DNA synthesis permanently without significantly reducing T1 DNA synthesis (open symbols). Following infection of irradiated bacteria by wild type (T1⁺) phage there was a lapse of about 5 min during which no label was incorporated, thereafter the rate of ³H-dThd incorporation rapidly increased until 15 min after which there was an equally rapid decline presumably related to the onset of lysis. With unirradiated host cells the kinetics of ³H-dThd incorporation was much the same, the label incorporated into DNA immediately following infection reflecting bacterial DNA synthesis occurring prior to the expression of the T1-induced shut-off of host DNA synthesis (Figurski & Christensen, 1974). This shut-off can be seen more clearly after infection of non-permissive (Su−) bacteria with an amber mutant defective for T1 DNA synthesis (Figurski & Christensen, 1974). In these circumstances ³H-dThd was incorporated only during the first few minutes of infection of irradiated bacteria whereas after infection of irradiated cells, in which host DNA synthesis was also eliminated, there was no incor-
Fig. 2. Sedimentation analysis in neutral sucrose gradients of intracellular T1+ DNA. An ETCG culture of E. coli B was infected with T1+ phage and the DNA labelled by the addition of 3H-dThd to the growth medium. At the end of the labelling period samples were lysed by the DOC method, mixed with 32P-labelled T1+ marker DNA and sedimented at 40,000 rev/min for 75 min at 10 °C: (a) 13 to 14 min label with 50 μCi/ml 3H-dThd; (b) 5 to 15 min label with 5 μCi/ml 3H-dThd. Labels: ●—●, 3H; ○—○, 32P. Sedimentation is from right to left.

poration whatsoever (Fig. 1). It will be shown that label incorporated into DNA after 5 min of infection is efficiently transferred to phage particles.

Unirradiated bacteria grown at 30 °C were used for all infections reported in the remainder of this paper. With these conditions, the latent period for T1+ was about 25 min, new progeny phage could be detected by 10 to 12 min after infection and the burst size was 50 to 100 phage per cell.

Zone sedimentation of pulse-labelled intracellular T1 DNA

Intracellular DNA was labelled with 3H-dThd during short (1 min) pulses given after the onset of T1+ DNA synthesis and the cultures were lysed immediately using either the SDS or DOC methods. The newly-synthesized T1 DNA sedimented through neutral pH sucrose gradients as a broad zone (Fig. 2a) which extended from material co-sedimenting with mature T1 DNA marker (32S) to a much faster sedimenting label having a sedimentation coefficient of 60S or greater. The faster sedimenting DNA often showed a peak at about 42 to 45S. With longer pulses the fast-sedimenting material was much less apparent and the bulk of the 3H label sedimented at the same rate as the mature T1 marker DNA. This result for a 10 min continuous label given from the 5th to 15th minute of infection is shown in Fig. 2(b).

Pulse-labelled intracellular T1 DNA was denatured with alkali and sedimented through alkaline sucrose gradients. Following a short pulse, the 3H label showed a broad distribution through the gradient with some label sedimenting with the single strands of the denatured
Fig. 3. Sedimentation analysis in alkaline sucrose gradients of radioactively-labelled intracellular T1+ DNA. An ETCG culture of *E. coli* B infected with phage as described in Methods, was labelled by adding 20 μCi/ml of 3H-dThd at the sixth minute of infection. At 9 and 16 min after infection samples were removed and lysed by the SDS method. Mixtures of 3H-labelled intracellular DNA and 32P-labelled T1+ marker DNA were denatured by incubation in 0.1 M-NaOH for 5 min at 25 °C and sedimented through alkaline sucrose gradients at 40000 rev/min for 75 min at 10 °C: (a) 6 to 9 min pulse label; (b) 6 to 16 min pulse label. Labels: ●, 3H; ○, 32P. Sedimentation is from right to left.

T1 DNA marker and some sedimenting more rapidly (Fig. 3a). With longer periods of incorporation the majority of the radiolabel was found in single-stranded DNA with identical sedimentation properties to the single strands from mature T1 genomes (Fig. 3b).

The sedimentation properties of 3H-dThd incorporated into native or denatured T1 DNA did not noticeably change during the entire infectious cycle, short pulses given at various times being incorporated mostly into the broad zone of fast-sedimenting DNA, suggesting that for the major part of the replication cycle the mode of DNA replication remains unaltered.

We have also examined the structure of host cell DNA following T1 infection and found that from a minute or two after infection the vast bulk of the bacterial DNA sedimented much more slowly than 32S and must be present as very small fragments. This result is to be expected in view of the report that T1 utilizes host cell DNA as a source of nucleotides for the synthesis of phage DNA (Labaw, 1953). Thus it would appear that the fast-sedimenting properties of rapidly-labelled intracellular T1 DNA do not arise from its association, by trapping, for example, with high mol. wt. bacterial DNA.

The fate of pulse-labelled T1 DNA: SDS lysis

SDS treatment of T1 phage particles causes disruption of the particle structure and loss of infectivity. At temperatures below about 50 °C a mixture of free DNA and ‘ghost complex’ DNA is produced; at higher temperatures the DNA is released entirely as free molecules (Bresler et al. 1967; Gill & MacHattie, 1975). The fate of DNA labelled with 3H-dThd during a short pulse and then chased by the addition of a large excess of unlabelled
Fig. 4. Sedimentation analysis of intracellular Ti+ DNA labelled in a ‘pulse-chase’ experiment. *E. coli* W3350 grown to $1 \times 10^8$ cells/ml at $30\,^\circ\text{C}$ in M9C medium was suspended in fresh medium at $5 \times 10^8$ cells/ml, infected by the addition of 5 phage/cell and aerated at $30\,^\circ\text{C}$. $^3\text{H}$-TdR at $10\,\mu\text{Ci/ml}$ was added to the growth medium at 6 min after infection. At 8 min after infection unlabelled thymidine was added (10 mg/ml) and samples were withdrawn at 8, 12, 16 and 20 min after infection and lysed with SDS. For sedimentation through neutral sucrose gradients the samples were heated at $75\,^\circ\text{C}$ for 10 min and quenched in ice before loading. For analysis in alkaline sucrose gradients the DNA was denatured in $0.1\,\text{M-NaOH}$ for 5 min at $25\,^\circ\text{C}$. Gradients were centrifuged at 40000 rev/min for 75 min at $10\,^\circ\text{C}$. (a) Neutral sucrose gradients; (b) alkaline sucrose gradients. The time at which each sample was lysed is given in each panel. Sedimentation is from right to left.
Fig. 5. Separation of T1 phage particles and DNA by sedimentation through sucrose gradients. An ETCG culture of E. coli B was infected with T1 + as described in Methods and labelled with 3H-dThd (25 μCi/ml) from 10 to 12 min after infection. The 3H label was ‘chased’ by incubation with an excess of unlabelled thymidine (10 mg/ml) until 20 min, when the cells were lysed by the DOC method. A sample of the lysate was mixed with 32P-labelled T1 + phage particles and 32P-labelled T1 + marker DNA and sedimented through a neutral sucrose gradient at 22000 rev/min for 45 min at 10 °C. Labels: O---O, 3H; (3--(3, 32P. Sedimentation is from right to left.

thymidine was followed in cell extracts lysed by SDS and then heated before centrifugation through sucrose gradients.

The neutral and alkaline sucrose gradient sedimentation profiles of T1 DNA labelled from 6 to 8 min after infection and sampled at 0, 4, 8 and 12 min after initiation of the chase are shown in Fig. 4(a) and 4(b) respectively. Immediately following the pulse (8 min sample), the bulk of the label was extracted as the rapidly-sedimenting form when analysed on neutral pH gradients (this profile is similar to that shown in Fig. 2(a)). Following a 4 min chase (12 min sample) some of the faster-sedimenting label had been transferred to the 32S form. At later times (16 and 20 min samples) the fast-sedimenting DNA had become totally converted to the 32S form and was identical in sedimentation properties to mature T1 DNA (marker DNA is not shown). Fig. 2(b) shows a similar pattern after a long period of continuous labelling.

When denatured in alkali and sedimented through alkaline sucrose gradients, DNA from the unchased 8 min sample sedimented as a heterogeneous collection of single strands. As the chase progressed, the faster-sedimenting single strands were converted to molecules co-sedimenting with single strands from T1 phage particles, the slower-sedimenting forms remained unchanged. These pulse-chase results resemble those shown in Fig. 3 for short and long pulse-labelled DNA and indicate that newly-synthesized T1 DNA molecules exist as structures with a broad distribution of sizes and that about 50% of the single strands are longer than unit length, i.e. concatenated structures. The fast-sedimenting DNA labelled in a short pulse is therefore a precursor of unit length molecules and is efficiently processed to structures with an identical S value to mature T1 genomes.

The fate of pulse-labelled T1 DNA: DOC lysis

The DOC method for cell lysis affects neither the structure nor infectivity of mature T1 phage particles particularly when the temperature of lysis is 30 °C or below. This method
Phage T₁ DNA replication

Fig. 6. Sedimentation analysis of DOC extracts from a 'pulse-chase' experiment. An ETCG culture of E. coli B was infected with T₁⁺ phage as described in Methods. At 10 min after infection ³H-dThd at a final concentration of 25 μCi/ml was added to the growth medium followed at 12 min by an excess (10 mg/ml) of unlabelled thymidine. At 14, 19 and 25 min after infection samples were lysed and centrifuged through neutral sucrose gradients at 10 °C either at (a) 22,000 rev/min for 45 min or (b) 40,000 rev/min for 75 min. The time at which each sample was lysed is given in each panel. Sedimentation is from right to left.

was therefore chosen to examine the relationship of the fast-sedimenting, concatenated DNA to mature T₁ progeny phage particles.

T₁⁺-infected cells were pulse-labelled with ³H-dThd from the 10th to 12th min of infection and the radioactive label was chased by further incubation in the presence of a large excess of unlabelled dThd. Samples removed at intervals after the pulse were lysed with DOC and centrifuged through neutral sucrose gradients. The gradients were centrifuged either at 40,000 rev/min for 75 min, which resolved the various DNA forms but sedimented phage particles to the bottom of the tube, or at 22,000 rev/min for 45 min which separated free DNA from DNA assembled into virions but did not clearly discriminate between 32S DNA and the faster forms (Fig. 5). At the slow speed it can be seen that with a 2 min chase period the bulk of the label sedimented as free DNA located near the
Table 1. Transfer of radioactivity from DNA to phage particles in a 'pulse-chase' experiment*

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* E. coli strain B infected with T1+ was labelled with 3H-dThd from 10 to 12 min after infection. The radioactive 'pulse' was 'chased' by the addition of excess unlabelled dThd at 12 min. Samples removed at subsequent intervals were lysed with DOC and centrifuged through neutral sucrose gradients to separate phage particles from free intracellular DNA. The radioactivity in the phage and DNA peaks was calculated as a percentage of the total radioactivity loaded on the gradient. Full details are given in the legend to Fig. 7.

† Percentage of total radioactivity in the DNA peak.
‡ Percentage of total radioactivity in the phage particle peak.

The proportion of label transferred from DNA to phage particles observed over a series of similar experiments is given in Table 1. Between 30% and 50% of 3H-dThd incorporated during the 10th and 12th min of infection is transferred to phage particles during the subsequent 13 min chase period. A small but detectable proportion of the label (2 to 4%) was found in phage particles at the end of the 2 min pulse. This rose to 5 to 10% during the first 2 min of the chase period and maximum transfer was effectively complete by 10 min after dilution of the 3H label. Pulse-labelled concatenated DNA is therefore an efficiently used precursor of T1 phage particles.

Electron microscopy of intracellular T1 DNA

The sedimentation properties of fast-sedimenting intracellular T1 DNA, particularly when denatured, suggest structures with a greater contour length than mature, unit length molecules. To obtain more direct information on this point, fast-sedimenting intracellular T1 DNA was purified by phenol extraction and examined by electron microscopy. Extracts prepared from cells infected with T1+ for 17.5 min were centrifuged through a 25 ml neutral sucrose gradient (5 to 20%, w/v) and the fractions sedimenting faster than 32S (between 40 and 60S) were pooled and prepared for electron microscopy as described in Methods. Because the cell extract was prepared by the SDS method at low temperature some of the DNA was derived from 'ghost complexes' and this served as a marker for unit length T1 molecules in addition to the ϕX174 DNA length standard always present.

A control spreading of DNA extracted from T1 phage particles showed a distribution of...
molecules about a mean of 16.7 μm (± 1.2 s.d.) equivalent to a mol. wt. of $31.3 ± 3.5 \times 10^6$ relative to φX 174 (Fig. 7a). A few molecules obviously shorter than unit length were also detected. Of the 45 molecules examined from samples of fast-sedimenting intracellular Tt DNA all were linear and unbranched. Eighteen were distributed about the mean for unit length molecules and were probably released from 40S ‘ghost complex’ structures. The remaining 27 were longer and ranged from 1.5 to 4 times the unit modal length, most being in the dimer to trimer length range (Fig. 7b). No obvious periodicity of length was apparent and apart from their extended length, they showed no unusual structural features.

While the number of fast-sedimenting molecules examined is small, the electron microscopic evidence nevertheless clearly points to the presence of concatenated DNA structures. This confirms the sedimentation patterns, particularly of denatured intracellular DNA, in support of the conclusion that the rapidly-sedimenting, pulse-labelled intracellular Tt DNA molecules have a greater length than virion DNA.

**DISCUSSION**

These experiments describe the first attempt to define the mechanism by which phage Tt DNA molecules are replicated during infection of *E. coli*. The analysis has concentrated on the structure of Tt DNA synthesized during a short pulse of $^3$H-dThd and has identified a rapidly-sedimenting form of Tt DNA which is efficiently processed into mature infectious virions. Analysis of the structure of this intermediate has shown it to be concatenemic.

Host DNA synthesis is switched off within a few minutes of Tt infection (Figurski & Christensen, 1974; Fig. 1 of this paper), it is degraded and utilized for Tt DNA synthesis (Labaw, 1953) and we have observed that by 8 min after infection the vast majority of the host DNA sedimented much more slowly than 32S. To add to this we have shown that
during a 'chase' period the pulse-labelled DNA is converted efficiently into infectious T1 phage particles (Fig. 6). Moreover, fractions from all parts of the neutral sucrose sedimentation zone contain infectious T1 DNA capable of transfecting cells to produce infectious T1 phage particles (N. Ramsay & D. A. Ritchie, unpublished data). Therefore, there seems little doubt that the pulse-labelled DNA observed in T1-infected cells must be phage T1 DNA.

The broad sedimentation zone assumed by the pulse-labelled DNA in neutral sucrose gradients points to extensive heterogeneity of size and/or form. This sedimentation pattern survives phenol extraction indicating the absence of protein. From the observation that denatured, pulse-labelled DNA also sediments heterogeneously in alkaline sucrose gradients, we conclude that this material contains molecules of variable size and that the rapidly-sedimenting component of the pulse-labelled DNA sediments rapidly by virtue of its increased mass rather than decreased volume and/or association with other molecules (Frankel, 1968). That is to say, the pulse-labelled molecules contain concatemers of extended length.

Calculations of mol. wt. based on relative sedimentation rates (Burgi & Hershey, 1963) give values up to four times unit mass for the rapidly-sedimenting native DNA, a conclusion supported by the electron microscopic analysis showing the presence of linear molecules up to tetramer length (Fig. 7). The electron microscopy provides several additional facts. (1) None of the concatemeric molecules were seen to be branched as has been observed at low frequency for T7 (Kelly & Thomas, 1969) and for T4 (Broker & Lehman, 1971) and T5 (R. Everett & M. R. Lunt, personal communication). (2) Linear concatemeric DNA was not seen in association with circular structures as reported for A during the late, rolling-circle phase of replication (Bastia et al. 1975). (3) The length distribution showed no periodicity and was apparently random.

During a subsequent 'chase' period, most of the rapidly-sedimenting, pulse-labelled DNA was converted to structures sedimenting with unit length molecules following SDS lysis (Fig. 4). This was observed for native and denatured DNA alike although some shorter molecules are seen in the latter case. With the more gentle DOC lysis method, which does not disrupt mature particles, up to 50% of the pulse-labelled material entered mature phage particles (Table 1). These results establish clearly that the concatemeric DNA is a major precursor of phage particles. Furthermore, non-permissive infections with amber mutants in several head genes lead to the accumulation of the concatemeric form with no production of 32S DNA (D. A. Ritchie & D. H. Joicey, unpublished results).

The DOC lysis method does not destroy the infectivity of mature T1 phage particles. This raises the question of the origin of the 32S DNA that appears after DOC lysis of 'pulse-chase' label (Fig. 6); does it represent an immediate product of the processing of concatemeric DNA molecules which later becomes packaged, or are the cutting and packaging reactions linked in such a way that free intracellular 32S DNA is never formed and the material found in DOC lysates therefore represents DNA released from premature, DOC-sensitive particles?

At present the concatemeric form of T1 DNA is the only known DNA replication intermediate. Since this DNA form has been detected following 3H-dThd pulses as short as 15 s at 30 °C, we presume it to be an early replicating form. Our recent work indicates it is also an essential intermediate in T1 DNA replication since failure to synthesize concatemeric DNA leads to failure to make infectious particles. This is concluded from the results of non-permissive infections with amber mutants in genes 3·5 and 4 both of which have a DNA-arrest phenotype under these conditions. These infections show that DNA
Phage T1 DNA replication

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synthesis occurs only in the form of molecules sedimenting at about 32S without any concomitant concatemer formation (D. A. Ritchie & D. H. Joicey, unpublished data). We now have evidence that the gene 4 product is required not only for concatemer formation but also for general recombination (D. A. Ritchie & D. Flanagan, unpublished data). This might suggest a recombination event for the formation of T1 concatemeric DNA, perhaps of the kind envisaged for T7 (Watson, 1972). This situation is similar to that observed with gene 6 mutants of phage T7 (Fröhlich, et al., 1975; Kerr & Sadowski, 1975).

The role of the covalently closed circular form of intracellular T1 DNA identified by MacHattie et al. (1972) is unclear. The frequency of this unit length, supercoiled circular form, much less than one per infected cell, led us to consider it an unlikely candidate for an obligatory intermediate in T1 DNA replication. However, as the method of isolation depended on its covalently closed configuration it is possible that intracellular T1 circular molecules are rather more common but are frequently nicked either as a natural part of replication or during isolation and consequently undetected in their study. A similar form of T5 molecule has been recently observed (R. Everett & M. R. Lunt, personal communication).

The major finding reported in this paper is the identification of an intracellular concatemeric T1 DNA precursor of up to three to four times the length of mature T1 DNA genomes. This structure is precisely the form of precursor expected from the prediction of Gill & MacHattie (1976) and MacHattie & Gill (1977) to account for the very limited number of cyclic permutations found for mature T1 DNA molecules.

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REFERENCES


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