DNA Replication of Bacteriophage T5. 3. Studies on the Structure of Concatemeric T5 DNA

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SUMMARY

The replication of bacteriophage T5 DNA has been shown to proceed via branched concatemeric intermediates. The structure of this concatemeric DNA was studied with respect to single-stranded regions and single-strand interruptions by digestion with S1 nuclease and agarose gel electrophoresis after alkali denaturation. The results were compared with the pattern of 'nicks' in the mature virion DNA, and the possible origins of these nicks are discussed. The structure of T5 concatemeric DNA was also studied by electron microscopy. Replication forks, loops and rare circular structures were observed, all of which were similar to those seen in replicating DNA of other large phages. Phage capsid structures were detected in association with both concatemeric and mature phage length DNA. These observations are discussed in relation to the replication, maturation and packaging of T5 DNA.

INTRODUCTION

Bacteriophage T5 virion particles contain a linear duplex DNA molecule of mol. wt. approx. 74 x 10^6. This DNA is of unique sequence (Thomas & Rubenstein, 1964) and contains extensive terminal redundancy amounting to about 9% of the total length (Rhoades & Rhoades, 1972; Scheible et al., 1977). An unusual feature of T5 virion DNA is that one strand contains site-specific interruptions or 'nicks' (Abelson & Thomas, 1966; Chen & Bremer, 1976) which may be sealed by treatment with DNA ligase (Jaquemin-Sablon & Richardson, 1970). Recent work has shown that although some of the nicks are at specific sites in a majority of the molecules in a population, there are many other sites in the molecule which may be occasionally nicked (Rhoades, 1977; Scheible et al., 1977). The extensive terminal redundancy and the presence of nicks present some interesting problems for the replication of T5 DNA. The nicks must be sealed before DNA replication can begin, and re-introduced before packaging of replicated DNA. The large terminal redundancy creates potential difficulties for the mechanisms by which mature molecules are cut from the replicated DNA and packaged into phage heads.

Early work on the replication of T5 DNA demonstrated that fast-sedimenting structures were involved (Smith & Burton, 1966; Smith & Skalka, 1966). Later, it was shown that this intracellular pool of DNA was shear-sensitive, would bind to nitrocellulose (and therefore presumably contained single-stranded regions) and produced a distribution of single-stranded fragments upon alkali denaturation which bore some resemblance to that of mature T5 DNA (Carrington & Lunt, 1973). More recently, Bourguignon et al. (1976) examined the early
intermediates of T5 DNA replication and showed that a number of different origins of replication were used prior to the formation of larger, branched molecules.

The experiments reported here describe some structural features of concatemeric T5 DNA, including studies on the number and location of nicks and single-stranded regions, in greater depth and detail than previously reported. The use of S1 nuclease allowed more rigorous studies to be made on the single-stranded regions of T5 concatemeric DNA while the overall pattern of single-strand interruptions was more accurately analysed by agarose gel electrophoresis. In addition, T5 concatemeric DNA was studied by electron microscopy. The samples used for this study were taken after the initial stages of replication had been completed, that is during the period of 'late' protein synthesis and maximum DNA synthesis. The molecules studied showed many of the features of DNA replication previously observed in other phage systems, confirmed some of the results of the study of Bourguignon et al. (1976) on earlier replicative forms and gave examples of replicative molecules of T5 not reported previously. The results are discussed in terms of possible models for the introduction of nicks found in mature T5 DNA and the methods of DNA replication, maturation and packaging used by T5.

METHODS

All the methods used have been previously described (Everett & Lunt, 1980a, b). These include ‘Bacteria and bacteriophage’, ‘Media and buffers’, ‘Preparation of 32P-labelled DNA from T5 virions’, ‘Preparation of intracellular T5 DNA’ and ‘S1 nuclease digestion of DNA’. Sucrose gradient centrifugation was as described by Everett & Lunt (1980a) except that the buffer used throughout was 0.05 M-tris-HCl pH 7.8, 0.001 M-EDTA, 1 M-NaCl. Agarose gel electrophoresis was also as described by Everett & Lunt (1980a), except the agarose concentration was 0.6% (w/v) throughout, and electrophoresis was at 30 V for 15 h at 4 °C. Electron microscopy methods were those of Everett & Lunt (1980b) except that sucrose gradient fractions were dialysed against 0.02 M-NaCl, 0.005 M-EDTA pH 7 before spreading. The measurement of radioactivity and the sources of enzymes, reagents and radioactive materials were all described by Everett & Lunt (1980a, b).

RESULTS

Presence of single-stranded regions in concatemeric T5 DNA

Intracellular DNA labelled with 3H-thymidine (final concentration 5 μCi/ml) was isolated from a culture of Escherichia coli C3000 35 min after infection with T5. A sample (0.2 ml) of the Brij–deoxycholate lysate was centrifuged through a 10 to 25% sucrose gradient and fractions (four drops) collected by upward displacement. The concatemeric DNA peak was identified by taking samples (10 μl) of each fraction for radioactivity estimation (see Fig. 2, Everett & Lunt, 1980a). Those fractions which contained concatemeric DNA were pooled, treated with self-digested Pronase (final concentration 250 μg/ml) for 30 min at 37 °C and then dialysed against S1 buffer. The sample was then treated with S1 nuclease for 30 min and the products analysed by centrifugation through 5 to 20% sucrose gradients. Shishido & Ando (1975) have shown that under appropriate conditions S1 nuclease (which is specific for single-stranded DNA) is also capable of cutting DNA opposite nicks, although increasing the salt concentration abolished almost all this activity. In these experiments the S1 buffer contained 0.2 M-NaCl, which decreased the fragmentation of mature T5 DNA by S1 nuclease to a negligible level (Everett & Lunt, 1980b). Therefore, any cutting of concatemeric T5 DNA by S1 nuclease would occur mainly or exclusively at the sites of single-stranded regions, not nicks.

The concatemeric DNA was extensively cut into fragments of various sizes (Fig. 1a). A
control incubation without enzyme showed that most of the original sample maintained its fast-sedimenting character throughout the manipulations (Fig. 1b); the variable extent of breakdown of the control was probably due to the extreme shear-sensitivity of T5 concatemeric DNA (Carrington & Lunt, 1973). Therefore, it appears that single-stranded regions are abundant in T5 concatemeric DNA.

The gradient profiles showed a reproducible distribution of peaks over several independent experiments. The mol. wt. of the duplex DNA fragments in these peaks can be calculated using the equation of Burgi & Hershey (1963) and are shown in Fig. 1(a). Prominent amongst the products are fragments of mol. wt. approx. $76 \times 10^6$, $56 \times 10^6$, $43 \times 10^6$ and particularly $28 \times 10^6$. Therefore, it appears that single-stranded regions can occur, at least to some extent, at intervals approximating to one mature genome length apart in concatemeric T5 DNA, but other discrete products of smaller size were also evident.

**Pattern of single-stranded interruptions in T5 concatemeric DNA**

The overall distribution of single-stranded interruptions in T5 concatemeric DNA was initially studied using preparations of uniformly labelled DNA. A culture of *E. coli* C3000 at $3 \times 10^8$ cells/ml in low phosphate medium was given $^{32}$P-phosphate to a final concentration of $10 \mu$Ci/ml. When the culture contained $5 \times 10^8$ cells/ml, it was infected with T5, and 30 min later a sample was taken for isolation of intracellular DNA. The sample was fractionated on a sucrose gradient as described above, and fractions (0-1 ml) from the leading edge of the fast-sedimenting peak were diluted with an equal volume of electrophoresis buffer before denaturation with NaOH (final concentration 0-1 M). The single-stranded DNA was then analysed by agarose gel electrophoresis; mol. wt. standards were provided by a sample of denatured mature T5 DNA run on a parallel gel.
Fig. 2. Agarose gel electrophoresis of uniformly $^{32}$P-labelled concatemeric T5 DNA after alkali denaturation. The method of sample preparation is described in the text. Electrophoresis was at 30 V for 15 h. The arrows show the positions and mol. wt. of the major peaks of similarly treated mature T5 virion DNA, run on a parallel gel. ●——○, $^{32}$P concatemeric T5 DNA.

Fig. 3. Structure of mature T5 virion DNA, showing the location of the nicks (data from Chen & Bremer, 1976). The length of the terminal redundancy is indicated by dotted lines, and the mol. wt. of the single-strand fragments of the interrupted strand are given ($\times 10^{-6}$). Mol. wt. are approximate estimates from gel electrophoretic mobility.

The single-strand components of the concatemeric DNA showed some similarity to those of mature T5 DNA (Fig. 2 and 3). However, there was a relatively greater amount of strands of approximately mature length in the concatemeric DNA while that of the smaller fragments was correspondingly reduced. There was little material of greater than mature length in the preparation, although similar experiments with native DNA showed that more than 90% of the fast-sedimenting preparations was retained at the top of the gel (results not shown).

Carrington & Lunt (1973) showed that the distribution of radioactive label among the single-strand fragments of pulse-labelled T5 concatemeric DNA was more heterogeneous than that of uniformly labelled DNA. They suggested that this result could be indicative of repair of single-stranded regions or nicks in vivo. Since it is now clear that this form of intracellular DNA contains single-stranded regions (Fig. 1) their hypothesis was investigated in more detail.

$^3$H-thymidine was added (final concentration 10 $\mu$Ci/ml) to a culture of E. coli C3000 30 min after infection with T5 and samples were taken at intervals afterwards. They were lysed and fractionated on sucrose gradients as described. Fractions from the leading edge of the fast-sedimenting peak were then denatured and analysed on agarose gels as before. Mol. wt.
markers were provided by the addition of $^{32}$P-labelled mature T5 DNA to each fraction before denaturation.

The results (Fig. 4) confirmed and extended the observations of Carrington & Lunt (1973). After a 30 s pulse the labelling pattern was heterogeneous but single-strand fragments of similar size to those of mature T5 DNA were again evident (Fig. 4a). In addition, a fragment of mol. wt. $20 \times 10^6$ to $24 \times 10^6$ was clearly and reproducibly present. After longer pulse periods the profile became smoother with fewer peaks and a greater proportion of radioactivity was in a band of approximately unit mature length (Fig. 4b, c). After a 5 min labelling period, when incorporation of $^3$H-thymidine into acid-insoluble material was more than 90% complete (results not shown), the result was similar to that of the uniform labelling pattern (Fig. 4d, Fig. 2). This profile did not change significantly over the following 5 min (Fig. 4e).

There are several possible reasons why different results were obtained with increasing labelling periods. It seems possible that the size and frequency of single-strand fragments close to the replication fork might be greater than those some distance away, perhaps due to discontinuous replication (Okazaki et al., 1968). In addition, repair of single-stranded regions could contribute to a non-uniform profile which differed from that using extended labelling.

The following conclusions can be drawn from these results. Concatemeric T5 DNA contains a large number of single strand interruptions, especially close to the replication fork. These interruptions can be seen to occur to some degree at sites equivalent to the sites of the interruptions in mature T5 DNA since single-strand fragments of similar size can be released from both forms of DNA. Furthermore, since these fragments are present after a short pulse and are still evident in uniformly labelled DNA, while other fragments not commonly present in mature DNA slowly disappear with increased labelling times, it could be argued that the interruptions which produce these fragments are in some way maintained during DNA replication.

A survey of T5 concatemeric DNA by electron microscopy

Concatemeric T5 DNA, which had been isolated and treated in the same way as that for S1 nuclease digestion, was dialysed against NaCl–EDTA buffer and prepared for electron microscopy. A total of 345 molecules from all regions of the fast-sedimenting peak were examined. Many of the molecules were extremely complex, included many branch points and much single-stranded material (Fig. 5). These molecules, which may have contained several phage equivalents of DNA, were in all cases too long and tangled for study and measurement. This result confirms previous observations (Carrington & Lunt, 1973; Bourguignon et al., 1976) that the fast-sedimenting intracellular T5 DNA is indeed of high mol. wt. All molecules examined (ignoring fragmented DNA) were scored according to the categories in Table 1. Replication forks, seen in about 30% of the total, were of three categories: (i) with no detectable single-stranded DNA (13%), (ii) with one daughter strand single-stranded at the fork (68%) and (iii) with both daughter strands single-stranded (19%). A small percentage of molecules contained replication loops (5% of the total). In one case a second loop was visible inside one arm of the larger loop (Fig. 6), a feature which has been observed previously (Bourguignon et al., 1976). Short, linear single-stranded DNA segments ('whiskers'), probably produced by branch migration during sample preparation (Delius et al., 1971) were sometimes observed at forks. When present in loops, the whiskers were always in association with opposite parental strands (in trans). Where both forks of a loop had single-stranded regions, these were also in trans in 80% of cases. Similar observations in other phage systems have been interpreted as being indicative of chain growth in one direction only (5’ to 3’) (Inman & Schnoss, 1971; Wolfson & Dressler, 1972). On one occasion, a daughter strand
Fig. 5. Electron micrograph of a typically long and branched DNA molecule from a preparation of concatemeric T5 DNA.

Table 1. Classification of concatemeric T5 DNA molecules

<table>
<thead>
<tr>
<th>Description</th>
<th>No. observed</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear*</td>
<td>152</td>
<td>44.1</td>
</tr>
<tr>
<td>Branched†</td>
<td>102</td>
<td>29.6</td>
</tr>
<tr>
<td>Circular‡</td>
<td>8</td>
<td>2.3</td>
</tr>
<tr>
<td>Containing loop</td>
<td>18</td>
<td>5.2</td>
</tr>
<tr>
<td>Linear + capsid§</td>
<td>38</td>
<td>11.0</td>
</tr>
<tr>
<td>Linear + capsid + SS‖</td>
<td>12</td>
<td>3.5</td>
</tr>
<tr>
<td>Branched + capsid</td>
<td>3</td>
<td>0.9</td>
</tr>
<tr>
<td>Linear + capsid + tail</td>
<td>12</td>
<td>3.5</td>
</tr>
</tbody>
</table>

* Molecules which appeared (on inspection alone) to be of equal or greater contour length than mature T5 virion DNA. Shorter fragments were ignored.
† Containing one or more branches, but excluding those with loops or circles.
‡ Circular molecules which appeared to be of similar contour length to mature T5 virion DNA.
§ Excluding those in other categories.
‖ SS, Containing internal single-stranded regions.

with two adjacent single-stranded regions was observed. Such structures have been interpreted as evidence in support of discontinuous replication (Wolfson & Dressler, 1972).

A rare but interesting class of molecule was circular (Fig. 7). It is possible that the
Fig. 6. Electron micrograph of a replicating loop of DNA, which contains a second smaller replicating loop in one arm (arrowed).

proportion of these was underestimated since many tangled specimens were ambiguous; only those which could be clearly followed were regarded as circular. All circular structures contained branch points, and one contained two associated linear DNA strands (daughter tails) (Fig. 7). These tails were of variable length. One measured 38.6 μm which was equivalent to approximately one complete round of circular replication. None of the circles were either unbranched or of the 'θ' type. Measurement of five molecules gave the length of the circle as 38.4 ± 1.7 μm, approx. 10% less than that of mature T5 DNA prepared and measured by the same methods (Table 2). This result is in good agreement with that of Bourguignon et al. (1976) and suggests that the circles could have been formed by recombination between terminal redundant regions of a unit length molecule. However, the low frequency of circular molecules observed implies that replication of T5 DNA by a ‘rolling circle’ method (Gilbert & Dressler, 1968) is uncommon; most replication appears to be mediated through multi-length linear molecules.

Some molecules in the population were associated with capsid-like structures. Of these, most were simply linear, some were linear with single-stranded regions and a few were branched (Table 1, Fig. 8). Some of the capsid-associated structures also included phage tails. The origin of these complexes was unclear. They could have arisen by collapse of pre-formed
Fig. 7. Electron micrograph of a circular T5 DNA molecule, which includes two daughter tails (arrowed).

Table 2. Contour lengths of mature T5 DNA and circular intracellular forms*

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Mean length ± s.d.</th>
<th>No. of molecules measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature T5 DNA</td>
<td>42.13 μm ± 0.18 μm</td>
<td>25</td>
</tr>
<tr>
<td>Circular DNA</td>
<td>38.4 μm ± 1.7 μm</td>
<td>5</td>
</tr>
</tbody>
</table>

* DNA was prepared for electron microscopy by the formamide technique. The method gave longer contour lengths than the aqueous method; mature T5 DNA prepared by the latter method gave a contour length of 36.4 ± 0.89 μm, essentially the same as published values (Lang et al., 1976).

phage particles, phage heads or unstable packaging intermediates or they could be a normal productive intermediate in packaging. It has been shown that formamide (Johnson et al., 1977) and EDTA (Saigo, 1975) can disrupt T5 phage particles. However, intact T5 phage sediment to the bottom of the tube during the conditions of sucrose gradient centrifugation used to prepare concatemeric DNA (Carrington, 1970) and therefore should not have been present in the samples used here. Lunt & Kay (1968) stated that the Brij–deoxycholate lysis procedure had no effect on the viability of T5 phage. However, a small degree of disruption would not have been detected by their method and perhaps would be sufficient to explain the capsid-associated DNA molecules observed here. Therefore, T5 phage labelled with 3H-thymidine were prepared, mixed with a sample of infected bacteria, subjected to Brij–deoxycholate ‘lysis’ and centrifuged through 10 to 25% sucrose gradients with 32P-labelled mature T5 DNA. Approx. 9% of these input 3H counts sedimented in the fast-sedimenting peak region (results not shown). Therefore, some of the capsid-associated molecules observed – particularly those which also included phage tails – may have arisen by
disruption of pre-formed phage particles. However, since the proportion of total DNA deoxyribose present in the form of intact phage particles in a culture 30 min after T5 infection was only about 12% (data from Carrington, 1970), it seems unlikely that all the observed examples could originate in this way. Disruption of pre-formed phage heads or packaging intermediates could explain some of the remainder, but the examples of branched DNA with capsid structures could not have arisen by disruption of filled heads. It is also unlikely that the capsid-associated molecules with single-stranded regions could have been formed by disruption since it is unclear how the single-stranded regions could be repaired once packaged.

A number of capsid-associated, linear and entirely duplex molecules were measured. Their average length was 42 µm, similar to that of mature T5 DNA (Table 2). On finding similar structures in lysates of T7-infected cells, Serwer (1974) concluded that they arose by breakdown of fragile head structures. However, in this case the origin and possible function of these complexes remains unclear. The rare examples of capsid-associated branched DNA indicate that capsids can associate with replicating T5 DNA, and that this association must be strong since the isolation and spreading conditions (1 M-salt in the sucrose gradients, 50% formamide during spreading) would disrupt any weak binding.
DISCUSSION

The results presented in this paper allow a description of certain basic properties of T5 concatemeric DNA. The pattern of single-strand interruptions in the concatamers, revealed by agarose gel electrophoresis of alkali-denatured DNA (Fig. 2 and 4), shows many similarities to that of mature T5 DNA. In agreement with previous data (Carrington & Lunt, 1973) the length of single-stranded segments was rarely greater than mature length, although the double-stranded material sedimented faster than mature DNA and was shown by electron microscopy (Fig. 5) to be mainly of greater contour length than mature DNA. It follows that in these samples of late-replicating DNA, interruptions are common at approximately mature length intervals, as well as at other locations.

Digestion with S1 nuclease showed that many of these interruptions were not simple nicks; under conditions when S1 nuclease cutting at nicks was inhibited, concatemeric DNA was cut by S1 nuclease into duplex fragments mostly smaller than mature length. The sizes of these fragments generated by S1 nuclease treatment are of interest. The major product has a mol. wt. of approx. 28 x 10^6 (Fig. 1), which is about the size expected if single-stranded regions occurred, at least to some extent, at sites corresponding to the sites of the nicks which define the 13.9 x 10^6 and 14.2 x 10^6 mol. wt. segments in mature T5 DNA. The sizes of other major products produced by S1 nuclease digestion could be explained by an extension of this hypothesis to suggest that each unit repeat in the concatamer contains many of the interruptions equivalent to those in mature DNA, and that at least one of these interruptions is a single-stranded region and not a simple nick. In addition, it is possible that single-stranded regions are prevalent at approximately unit length intervals in the late replicating DNA. This observation may have some relevance to the model of concatemer processing suggested by Kelly & Thomas (1969), Schlegel & Thomas (1972), and Watson (1972) for phage T7 and phage T5 DNA.

These data also provide some insight into the origin of the nicks in mature T5 DNA. It has been suggested (Rogers & Rhoades, 1976) that these interruptions are introduced by site-specific endonucleases. Moyer & Rothe (1977) have suggested that the T5 exonuclease, the product of gene D15, may have an as yet undetected in vivo endonuclease activity to introduce these nicks. Recently, Rogers et al. (1979 a, b) has described certain nick-deficient mutants of T5. These were of two classes: either recessive, falling into two complementation groups; or cis-dominant, which could be explained by base changes at individual nick sites. Together with the sequence data of Nichols & Donelson (1977), who showed that at least four bases of the 5' side were common to all major nicks, their data strongly implicate one, or a number of sequence-specific endonucleases in the introduction of the nicks. However, the results presented here suggest that the situation may not be so simple. First, analysis of replicating DNA after a short pulse with ^3H-thymidine (Fig. 4) suggests that many interruptions may be present immediately after the passage of the replication fork, but that those at major nick sites are preferentially retained. Second, at least some of the interruptions are larger than a simple nick. It is possible that the data of Moyer & Rothe (1977) can be re-interpreted to implicate the exonuclease activity of the product of gene D15 in the maintenance of single-stranded regions, not the introduction of the nicks; in the absence of the exonuclease all nicks near the replication fork may be quickly re-ligated. A further consideration is that whereas many of the nicks could be introduced into unpackaged DNA, if the model of concatemer processing discussed below applies to T5 replication, it follows that nicks in the terminally redundant regions must be introduced after processing. Thus the details of nick introduction may differ between individual cases.

The examples of T5 replicating DNA given here (Fig. 5 to 8) indicate that features of the replication fork in T5 concatemeric DNA are common to those of other large DNA phages. Replication loops, sometimes with single-stranded regions in trans and also replication forks
Fig. 9. Possible models for concatemer production. (a) Parental T5 DNA molecule. Vertical lines depict boundaries of terminal redundant region. Unpaired bases are shown at the termini (Watson, 1972). (b) Predicted products of the first round of replication. (c) Possible recombination between redundant regions of two parental molecules. (d) Recombination between the terminal redundant regions of one parental molecule. (e) Product of molecules in (b) and (c). (f) Product of rolling circle replication. In both cases the concatemers produced have lost one terminal redundant region for each additional genome length in the concatemer.

with 'whiskers' were observed. Less typical, however, was the occurrence of extensive and frequent single-stranded regions (Fig. 5); the significance of these remains unknown. It was not possible to correlate positions of these single-stranded regions to the S1 nuclease digestion results because of the lack of defined endpoints and the tangled nature of the exceptionally long molecules observed (see Fig. 5). The most important observation of the electron microscope study was the detection of circular replicating molecules, confirming the results of Bourguignon et al. (1976). The contour length of these circles, approx. 10% less than that of mature DNA, indicates that they may have arisen by recombination between terminal redundant regions (see Fig. 9).

If concatemeric DNA were produced from these molecules by a rolling circle mechanism, the structure of the concatemer produced would include alternating units of terminal redundant and non-redundant regions: the 'repeat' distance would be approx. 10% less than mature DNA, and the net effect would be loss of one unit of terminal redundancy for each unit repeat in the concatemer. Although no unequivocal 'rolling circle' molecules were observed in the preparations, some circles had 'tails' of approximately unit length. However, generation of linear concatemers by either recombination between redundant regions or an extension of the model of Watson (1972), which invokes pairing of predicted exposed single-stranded DNA between daughter molecules of the first round of replication, leads to the same concatemer structure (Fig. 9).

The processing of this concatemer structure during DNA packaging is of interest. In many phages the evidence suggests that packaging of DNA and its excision from the concatemer are intimately linked (Murialdo & Becker, 1978). In the case of T5, the original evidence suggested that unit length molecules were excised from the concatemer prior to packaging (Carrington & Lunt, 1973). However, it is now clear that the intracellular pool of molecules observed, which sedimented at a similar rate to that of mature T5 DNA, were shorter than mature length and therefore could have no productive role in phage maturation (Everett & Lunt, 1980a, b). The most likely hypothesis is that processing of T5 concatemers involves nicking at opposite ends and on opposite strands of the terminal redundant region in the concatemer, allowing polymerase action to separate the mature molecule from the concatemer (Watson, 1972; Kelly & Thomas, 1969; Fig. 10). This model, although
Fig. 10. Model for excision of mature length DNA from T5 concatemers. The terminal redundant regions are depicted by vertical lines. $\uparrow \downarrow$ = sites of action of putative single-strand, site-specific ‘nickase’. $\rightarrow \leftarrow$ = location and direction of polymerase activity (Kelly & Thomas, 1969; Watson, 1972).

attractive, presents a unique problem in the case of T5 through the shear size of the terminal redundancy; some 4500 base pairs must be displaced by DNA polymerase for each mature length excised. The model predicts that the nicks in the redundant regions must be introduced after processing (see above). Since an intracellular pool of mature length molecules has not been observed in T5-infected cells (Everett & Lunt, 1980a, b) it is likely that this processing event occurs, like other related phages, during encapsulation of the DNA. The capsid-association DNA observed in intracellular T5 DNA preparations (Fig. 8) may have some significance in this respect.

However, the precise nature of T5 concatemer processing remains unresolved. The exceptionally large terminal redundant region in T5 DNA provides unusual structural problems which ensure an interesting solution.

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REFERENCES


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