Recombination-dependent Replication of Plasmids during Bacteriophage T4 Infection*

Kenneth N. Kreuzer, Wendy Y. Yap, Anne E. Menkens‡, and Helen W. Engman

From the Department of Microbiology and Immunology, Duke University Medical Center and the Duke University Program in Genetics, Durham, North Carolina 27710

The replication of plasmids containing fragments of the T4 genome, but no phage replication origins, was analyzed as a possible model for phage secondary (recombination-dependent) replication initiation. The replication of such plasmids after T4 infection was reduced or eliminated by mutations in several phage genes (uusY, uusX, 46, 59, 39, and 52) that have previously been shown to be involved in secondary initiation. A series of plasmids that collectively contain about 60 kilobase pairs of the T4 genome were tested for replication after T4 infection. With the exception of those known to contain tertiary origins, every plasmid replicated in a uusY-dependent fashion. Thus, there is no apparent requirement for an extensive nucleotide sequence in the uusY-dependent plasmid replication. However, homology with the phage genome is required since the plasmid vector alone did not replicate after phage infection. The products of plasmid replication included long concatemeric molecules with as many as 35 tandem copies of plasmid sequence. The production of concatamers indicates that plasmid replication is an active process and not simply the result of passive replication after the integration of plasmids into the phage genome. We conclude that plasmids with homology to the T4 genome utilize the secondary initiation mechanism of the phage. This simple model system should be useful in elucidating the molecular mechanism of recombination-dependent DNA synthesis in phage T4.

The processes of DNA replication, recombination, and repair are often interconnected. Important roles for DNA synthesis reactions in homologous recombination and in excision repair have been amply demonstrated, and a second pathway of DNA repair (post-replication recombinational repair) depends on proteins involved in genetic recombination (for reviews, see Kornberg, 1982; Whitehouse, 1982). In the process of replicative transposition, site-specific recombination and DNA replication are intimately related (Kleckner, 1981; Grindley and Reed, 1985). Furthermore, recombination proteins can play a direct role in genomic DNA replication.

The replication of such plasmids after T4 infection was reduced or eliminated by mutations in several phage genes (uusY, uusX, 46, 59, 39, and 52) that have previously been shown to be involved in secondary initiation. A series of plasmids that collectively contain about 60 kilobase pairs of the T4 genome were tested for replication after T4 infection. With the exception of those known to contain tertiary origins, every plasmid replicated in a uusY-dependent fashion. Thus, there is no apparent requirement for an extensive nucleotide sequence in the uusY-dependent plasmid replication. However, homology with the phage genome is required since the plasmid vector alone did not replicate after phage infection. The products of plasmid replication included long concatemeric molecules with as many as 35 tandem copies of plasmid sequence. The production of concatamers indicates that plasmid replication is an active process and not simply the result of passive replication after the integration of plasmids into the phage genome. We conclude that plasmids with homology to the T4 genome utilize the secondary initiation mechanism of the phage. This simple model system should be useful in elucidating the molecular mechanism of recombination-dependent DNA synthesis in phage T4.

The mechanism of secondary initiation involves the use of DNA synthesis in phage T4 apparently requires an exonuclease (gp46/47), the phage-induced Type II topoisomerase (gp39/52/60), and at least two proteins of unknown function (gppusY and gp59) (Mosig, 1983). Mutations in several of these genes also reduce recombination and DNA repair. Instead of the secondary initiation mechanism of the phage, the replication of recombinant plasmids containing cloned tertiary origins is currently being investigated by analyzing the replication of recombinant plasmids containing cloned tertiary origins. The principal advantage of this approach is that one particular mechanism of initiation can be isolated from other aspects of phage DNA metabolism, permitting rapid progress toward an understanding of that mechanism (Kreuzer and Alberts, 1985; Kreuzer and Menkens, 1987; Kreuzer et al., 1988; Menkens and Kreuzer, 1988).

In this study, we analyze the replication of plasmids that possess various fragments of the T4 genome, but which contain no tertiary origin. Such plasmids will be referred to as non-origin plasmids.

The first direct analysis of the replication of plasmids after T4 infection was undertaken by Mattson et al. (1983a). Even when phage-induced breakdown of host DNA was blocked by appropriate phage mutations, the replication of pBR322 was rapidly inhibited after phage infection. However, chimeric plasmids with fragments of the T4 genome were shown to...

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‡Supported in part by Grant GM27219 from the National Institutes of Health. Doctoral candidate in the Committee of Genetics, University of Arizona Health Science Center, Tucson, AZ.

1The abbreviations used are: gp, gene product; kb, kilobase pair; T4 4C, a special multiple-mutant phage strain in which the DNA lacks the hydroxymethylcytosine and glucosyl modifications of wild-type T4 DNA; I/S, insertion/substitution.
Recombination-dependent Plasmid Replication

EXPERIMENTAL PROCEDURES

Materials—Restriction enzymes, DNaI, protease K, and [α-32P]dATP were purchased from commercial sources. T4 DNA polymerase was the generous gift of H. E. Selick and B. M. Alberts (University of California, San Francisco). All recombinant plasmids are derivatives of pBR322 containing fragments of T4 DNA. The following plasmids are described in the indicated studies: pKK025, pKK026, pKK032, pKK045, pKK020, pKK211, pKK221, pKK234, pKK401, pKK404, pKK405, pKK408, pKK415, pKK419, pKK427, and pKK435 (Kreuzer and Alberts, 1986); and pGJF1 (Kreuzer and Menkens, 1987). Plasmid pKK405 was assayed for replication after infection by uusY+ phage (also see Kreuzer and Menkens, 1987; Menkens and Kreuzer, 1988). However, only plasmid pGJF1 was capable of replication after infection by the T4 uusY deletion mutant (compare lane 8 with all other even-numbered lanes). The uusY- mutation in the infecting phage has two apparent effects (Kreuzer et al., 1988). First, it greatly increases the yield of replicated tertiary origin plasmid; and second, it prevents the replication of non-origin plasmids.

RESULTS

Replication of Non-Origin Plasmids after T4 Infection—Mattson et al. (1983a) showed that a plasmid pBR322 derivative, containing a fragment of the T4 rII genes could replicate after infection by T4 mutants deficient in host DNA breakage. Plasmid replication apparently required recombination with the infecting phage genome since it was abolished when the infecting phage carried a deletion of the rII DNA contained in the plasmid (Mattson et al., 1983a).

In our studies of tertiary origin-containing plasmids, a similar replication of non-origin plasmids often presented a very significant background against which replication from the cloned origin was compared (Kreuzer and Alberts, 1985, 1986). It was therefore of considerable interest to find that replication of tertiary origin-containing plasmids could be measured with essentially no background when the infecting phage carried either of two uusY mutations. Plasmid pKK405 contains a 1.36-kb insert that includes a tertiary origin, whereas plasmid pKK405Δ has the same insert except for a 0.12-kb deletion of the origin. Both plasmids replicated after T4 uusY+ infection; but only plasmid pKK405, which contained the tertiary origin, replicated after T4 uusY- infection (Kreuzer et al., 1988).

To begin to explore the mechanism of this non-origin plasmid replication, a series of deletion derivatives of plasmid pKK405 was assayed for replication after infection by uusY+ phage (T4 uusY+ phage and a uusY deletion mutant phage (T4 uusY-)) (also see Kreuzer and Menkens, 1987; Menkens and Kreuzer, 1988). This result implied that non-origin T4 DNA present in both plasmids allowed plasmid replication by a mechanism dependent upon the phage uusY protein.

As shown in Fig. 1B, all four plasmid deletion derivatives replicated to a significant extent after infection with the uusY+ phage (lanes 3, 5, 7, and 9). The only derivative with a functional tertiary origin was plasmid pGJB1, which did not replicate as well as the non-origin plasmid pKK464 in the uusY+ infections (also see Kreuzer and Menkens, 1987; Menkens and Kreuzer, 1988). However, only plasmid pGJB1 was capable of replication after infection by the T4 uusY deletion mutant (compare lane 8 with all other even-numbered lanes). The uusY- mutation in the infecting phage has two apparent effects (Kreuzer et al., 1988). First, it greatly increases the yield of replicated tertiary origin plasmid; and second, it prevents the replication of non-origin plasmids.

Since all three non-origin plasmid derivatives (pKK467, pKK464, and pKK451) exhibited gpwusY-dependent replication, there is no unique sequence element in pKK405Δ that allows plasmid replication. Several other non-overlapping deletion derivatives of the T4 insert similarly showed gpwusY-dependent replication (data not shown), arguing against a requirement for any particular nucleotide sequences in the plasmid.

The conclusion that homology alone is required for non-origin plasmid replication is strengthened by two related approaches. First, as discussed above, Mattson et al. (1983a)
showed that an rI1 gene-containing plasmid could replicate only if the infecting T4 was not deleted for the same segment of DNA. Second, a large number of other recombinant plasmids containing segments of the T4 genome also exhibited gpuvSY-dependent replication. The results of several such plasmid replication tests are shown in Fig. 2, and others are summarized in Table I. The recombinant plasmids collectively included approximately 60 kb of the T4 genome (total size of genome is 167 kb). This collection of recombinant plasmids included many that were selected on the basis of enhanced secondary initiation. Secondary initiation has been shown to require the products of several genes involved in recombination (e.g. uvsY, uvsX, 46, and 59) and has been postulated to involve the conversion of recombinational intermediates into replication forks (Mosig, 1983; Kozinski, 1983; also see “Discussion”).

The similarity of non-origin plasmid replication and phage secondary initiation was tested by examining the requirements for the above-mentioned gene products. A series of phage T4 mutants otherwise isogenic to T4 I/S were used to infect E. coli strains MCS1 (supD) and AB1 (sup), each containing plasmid pKK467 (see above). Consistent with the results shown above, this non-origin plasmid replicated in both uvsY+ infections (Fig. 3, lanes 1A and 1B) and failed to replicate in the uvsY- infections (lanes 2A and 2B). The nearly identical results in hosts MCS1 and AB1 were expected since neither the uvsY+ nor the uvsY- phage strain carried

**FIG. 1.** Replication of recombinant plasmids containing fragments of the uvsY region from the T4 genome. A schematic map of the uvsY region of the phage genome is shown in A. The approximately 1.4-kb HindIII restriction fragment from pKK405 is shown (top), with ori(uvsY) (ori), the promoter for gene uvsY (P), and the uvsY transcript (rightward-pointing arrow) indicated. Various segments of this region have been sequenced by Takahashi et al. (1985), Gründl and Mosig (1986), and Kreuzer and Menkens (1987). The inserts contained in the pBR322 derivatives pKK451, pGJB1, pKK464, and pKK467 are indicated below the map. The replication of each of these plasmids as well as the vector pBR322 is shown in B. E. coli AB1 bearing the indicated plasmid was infected with either T4 I/S (uvsY+; odd-numbered lanes) or T4 KK608 (uvsY-; even-numbered lanes) and incubated for 1 h at 37 °C. Total intracellular DNA was then prepared from each culture and digested with restriction enzymes SspI and HaeIII, and the resulting DNA fragments were separated by agarose gel electrophoresis. A photograph of the ethidium bromide-stained gel is shown, with the locations of plasmid and phage DNA restriction fragments indicated. The molecular size scale on the left was generated from the migration of XbaI fragments of T4 dC DNA (Kutter and Ruger, 1983).

**FIG. 2.** Replication of non-origin plasmids requires gpuvSY. E. coli MCS1 bearing the indicated plasmid was infected with either T4 I/S (uvsY+; odd-numbered lanes) or T4 KK608 (uvsY-; even-numbered lanes) and incubated for 2 h at 37 °C. Packaged DNA was then prepared from each culture and digested with SspI, and the resulting fragments were separated by agarose gel electrophoresis. A photograph of the ethidium bromide-stained gel is shown, with the positions of plasmid and phage DNA restriction fragments indicated. The molecular size scale on the left was generated from the migration of XbaI fragments of T4 dC DNA (Kutter and Ruger, 1983).
amber mutations in genes involved in DNA synthesis.

The five additional phage strains tested each contained amber mutations in specific genes involved in DNA metabolism. The total amount of phage DNA recovered from each was much reduced in infections of the suppressor-free host AB1 (Fig. 3, compare lanes A and B), indicating that the amber mutations resulted in reduced phage genomic DNA replication. (The gene 52 mutant was only marginally defective in host AB1 (sup); 55 for unknown reasons.) In addition, plasmid pKK467 DNA replication was completely blocked as the result of a usx mutation (lane 3B) and dramatically reduced by mutations in genes 46, 59, 39, and 52 (lanes 4B, 5B, 6B, and 7B, respectively). Since all of these same gene products have been implicated in phage secondary initiation, we conclude that non-origin plasmid replication requires the same or a very similar set of phage proteins. It should be noted that mutations in genes usx, 46, and 59 did not reduce the replication of a tertiary origin-containing plasmid, demonstrating two distinct modes of plasmid replication (see Kreuzer et al., 1988). A possible model to explain the slight recovery of replicated plasmid DNA in the 46, 59, 39, and 52 infections is presented under “Discussion.”

Evidence against Passive Replication of Non-origin Plasmids—The evidence presented above demonstrates that non-origin plasmid replication requires at least some of the same proteins as phage secondary initiation. However, since these same gene products are also involved in recombination, it could be argued that plasmid replication is passive. Once a plasmid DNA molecule becomes integrated into the phage genome by homologous reciprocal recombination, it should be replicated passively whenever that phage DNA molecule undergoes replication initiated by any mechanism (see Mattson et al., 1983a). The results of Mattson et al. (1983b) and Selick et al. (1988) demonstrate that plasmids do integrate into the T4 genome.

If the passive replication model is correct, the replicated plasmid DNA should exist in a simple integrated form, with integrated plasmid DNA surrounded by T4 genomic DNA. In control experiments, we have observed that the bulk of the circular plasmid molecules remain monomeric after T4 infection (data not shown). Therefore, tandem copies of concatemeric plasmid should result mainly from iterative recombination events between free plasmid molecules and phage DNA. Since simple integration of a monomeric plasmid (with an insert of about 1 kb) occurs in only 1–4% of progeny phage (Selick et al., 1988; Kreuzer et al., 1988), such iterative events should be quite infrequent.

An alternative model for non-origin plasmid replication is that it results from active initiation events on the plasmid DNA molecule. In this model, the structure of the replicated plasmid DNA would depend on the exact mode of replication. For example, if replication occurred by the rolling-circle mode, then long concatemeric plasmid DNA would result. Thus, the structure of the replicated plasmid might allow a distinction between passive and active replication.

We examined the structure of replicated plasmid DNA by the procedure outlined in Fig. 3. Restriction enzyme NdeI cleaves modified T4 genomic DNA into a number of fragments, the largest of which is approximately 10 kb. Plasmid derivatives lacking NdeI restriction sites were first constructed so that any plasmid concatamers would be resistant to NdeI cleavage. After an infection of plasmid-bearing E. coli, phage lysates were prepared, and packaged DNA from

### Table I

| Plasmid     | Map coordinates of insert | Replication<sup>a</sup> | Replication<sup>b</sup> |
|-------------|---------------------------|--------------------------|--------------------------|
|             | kb                        | usx<sup>Y</sup>         | usx<sup>Y</sup>         |
| pKK221      | 2.85–5.55                 | +                        | –                        |
| pKK435      | 3.35–4.90                 | +                        | –                        |
| pKK1150     | 8.00–10.60                | +                        | –                        |
| pKK921      | 16.19–17.98               | +                        | –                        |
| pMNH61      | 17.98–20.06               | +                        | –                        |
| pJK671      | 28.95–34.00               | +                        | –                        |
| pKK427      | 80.30–91.30               | +                        | –                        |
| pKK207      | 80.80–82.70               | +                        | –                        |
| pKK405      | 82.70–86.55               | +                        | –                        |
| pKK1103     | 90.10–95.70               | +                        | –                        |
| pKK415      | 99.90–103.11              | +                        | –                        |
| pKK119      | 102.10–105.76             | +                        | –                        |
| pKK032      | 105.76–108.10             | +                        | –                        |
| pKK211      | 108.10–111.60             | +                        | –                        |
| pKK202      | 111.60–117.65             | +                        | +                        |
| pKK405      | 113.10–114.50             | +                        | +                        |
| pKK401      | 114.50–118.10             | +                        | –                        |
| pKK408      | 118.10–121.20             | +                        | –                        |
| pKK204      | 125.70–127.20             | +                        | –                        |
| pKK025      | 150.75–151.85             | +                        | –                        |
| pKK1119     | 152.60–154.75             | +                        | –                        |
| pKK404      | 163.39–157.01             | +                        | –                        |
| pKK026      | 154.78–157.85             | +                        | –                        |
| pKK419      | 157.01–157.81             | +                        | –                        |
| HH870       | 165.56–0.43               | +                        | –                        |

<sup>a</sup>The replication of each plasmid was tested after infection by T4 I/S (usx<sup>Y</sup>) or T4 KK608 (usx<sup>Y</sup>), essentially as described in the legend to Fig. 2. The total amounts of replicated plasmid DNA from various infections were quite different, and no attempt to quantitate the differences of those represented by plus signs was made. All T4 genomic DNA map coordinates are from Kutter and Rüger (1983). The HindIII restriction site at 118.10 kb does not appear in the map of Kutter and Rüger (1983), but was previously detected by Kreuzer and Alberts (1986).

<sup>b</sup>Recombination-dependent Plasmid Replication. E. coli MCS1 (sup+; lanes A) or AB1 (sup; lanes B) bearing non-origin plasmid pKK467 was infected with various T4 strains and incubated for 1 h at 37°C. Total DNA samples were then prepared and digested with SapI and HaeIII, and the resulting fragments were separated by agarose gel electrophoresis. The infecting phage strains were T4 I/S (lanes 1), T4 KK608 (lanes 2), T4 I/S usx<sup>Y</sup> (lanes 3), T4 I/S 46<sup>Y</sup> (lanes 4), T4 I/S 59<sup>Y</sup> (lanes 5), T4 I/S 39<sup>Y</sup> (lanes 6), and T4 I/S 52<sup>Y</sup> (lanes 7). Each of the latter five T4 strains is isogenic with T4 I/S, except for the indicated amber mutation (see Kreuzer et al., 1988). A photograph of the ethidium bromide-stained gel is shown, with the positions of plasmid and phage DNA fragments indicated. The molecular size scale on the left was generated from the migration of XbaI fragments of T4 dC/DNA (Kutter and Rüger, 1983).

![Fig. 3. In vivo test of the protein requirements for non-origin plasmid replication. E. coli MCS1 (sup+) lanes A) or AB1 (sup; lanes B) bearing non-origin plasmid pKK467 was infected with various T4 strains and incubated for 1 h at 37°C. Total DNA samples were then prepared and digested with SapI and HaeIII, and the resulting fragments were separated by agarose gel electrophoresis. The infecting phage strains were T4 I/S (lanes 1), T4 KK608 (lanes 2), T4 I/S usx<sup>Y</sup> (lanes 3), T4 I/S 46<sup>Y</sup> (lanes 4), T4 I/S 59<sup>Y</sup> (lanes 5), T4 I/S 39<sup>Y</sup> (lanes 6), and T4 I/S 52<sup>Y</sup> (lanes 7). Each of the latter five T4 strains is isogenic with T4 I/S, except for the indicated amber mutation (see Kreuzer et al., 1988). A photograph of the ethidium bromide-stained gel is shown, with the positions of plasmid and phage DNA fragments indicated. The molecular size scale on the left was generated from the migration of XbaI fragments of T4 dC/DNA (Kutter and Rüger, 1983).]
phage particles was purified and digested with \textit{NdeI}.

DNA is packaged by the headful mechanism during T4 infection, with approximately 170 kb inserted into each phage particle. By the passive replication model described above, one or a few copies of the plasmid DNA would be surrounded by phage genomic DNA in the packaged DNA samples. Based on the locations of \textit{NdeI} sites in this region of the phage genome and the roughly 4.5-kb size of the relevant plasmids, a simple integrant with one copy of plasmid should generate an \textit{NdeI} fragment of about 8 kb, double (tandem) integrants should produce fragments of about 12.5 kb, etc. Based on the active replication model described above, plasmid concatemers might be expected to include molecules of 170 kb without even a single \textit{NdeI} site. This would require the headful packaging of plasmid DNA from a precursor molecule comprised of more than 35 tandem copies of plasmid sequence (see Fig. 4).

In order to distinguish between molecules in this size range, the technique of inverting-field gel electrophoresis was employed (Carle et al., 1986). The phage genomic DNA (visualized by ethidium bromide staining) in all samples formed the expected set of \textit{NdeI} restriction fragments, with the largest being about 10 kb in size (data not shown). Plasmid DNA was visualized by performing Southern hybridization, with radioactively labeled vector (plasmid pBR322) as the probe.

We began our analysis with several tertiary origin-containing plasmids since a completely independent procedure has already been used to demonstrate the existence of long plasmid concatemers after tertiary origin plasmid replication (Kreuzer and Alberts, 1986). A major product of replication of each of the tertiary origin plasmids co-migrated with the native T4 DNA (170 kb) marker (Fig. 5, lanes 1A–3B). The 170-kb plasmid DNA represents fully concatemeric plasmid with no \textit{NdeI} sites since DNA fragments of the same size were observed without restriction digestion (data not shown). A trail of fragments between the 15- and 170-kb markers may include integrated forms of plasmid, plasmid DNA concatemers that have undergone recombination with the phage genome, and/or molecules that suffered a random breakage event during the manipulations. A low level of such fragments was observed even without restriction enzyme treatment (data not shown), indicating that some random breakage had occurred. DNA fragments between 10 and about 50 kb in size were not well resolved by the inverting-field gel electrophoresis (see markers in Fig. 5); and this may account, in part, for the relatively large amount of plasmid DNA in that small region of the gel. The most important conclusion is that the majority of the packaged tertiary origin plasmid DNA is in the form of plasmid concatemers containing greater than 35 tandem copies of plasmid sequence.

When the same analysis was performed with several plasmid derivatives that did not contain tertiary origins, a 170-kb plasmid DNA fragment was again observed, but only in the \textit{uvsY}+ infections (Fig. 5, lanes 5A, 6A, and 7A). The large replicated product was absent from the DNA samples prepared after T4 \textit{uvsY}– infections (lanes 5B, 6B, and 7B) and also from samples prepared after infection of cells containing only the pBR322 vector (lanes 4A and 4B). The ratio of smaller plasmid DNA fragments to the 170-kb DNA was higher for the non-origin plasmids than for the tertiary origin

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**Fig. 4. Test to distinguish between active and passive plasmid DNA replication.** The result of active plasmid replication with a rolling-circle intermediate is diagramed on the left. Phage genomic DNA is indicated by the filled lines, and plasmid vector sequence by the open lines. The small dashes below the DNA molecules (bottom) indicate the numerous \textit{NdeI} sites in the T4 genome (not drawn to scale). Each plasmid used for this test of active initiation was devoid of \textit{NdeI} sites.

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plasmids. In fact, DNA fragments smaller than 170 kb appear to comprise the majority of the replicated plasmid pKK467 DNA (lane 7A). These fragments are clearly larger than the fragments expected from integrated monomeric plasmid and presumably are composed of a variable number of tandem concatemeric plasmids linked to phage genomic DNA. These results indicate that the replicated non-origin plasmid DNA had undergone frequent recombination with the phage genome, perhaps, in part, during the initiation of plasmid DNA replication (see "Discussion"). In general, simple monomeric plasmid integrants were not visualized, with the possible exception of a faint band with an estimated size of 8 kb in the uswY+ infection of cells bearing pKK467 (lane 7A). Although digestion by restriction enzyme HaeIII was not included in this experiment, the pattern of fragments was essentially identical when that enzyme was included (data not shown). Therefore, the long plasmid concatemers did not, in general, contain even a single copy of nonreplicated plasmid DNA. Nonreplicated DNA lacks the T4-specified cytosine modifications and, consequently, would have been cleaved by HaeIII into small (less than 1 kb) fragments. This analysis demonstrates that non-origin plasmid replication generates at least some packaged concatemeric DNA molecules containing about 35 tandem copies of plasmid sequence and many more molecules with an intermediate number of tandem plasmids. Such molecules cannot be explained by the passive replication model described above.

**DISCUSSION**

The process of secondary initiation of bacteriophage T4 DNA synthesis is most intriguing because of its apparent linkage to recombination and DNA repair. The majority of late DNA synthesis during T4 infection is dependent on a collection of genes whose products are also involved in recombination. These proteins include the T4-encoded synthase (gpwuX), an exonuclease involved in recombination (gp46/47), the T4-induced type II topoisomerase (gp39/52/60), and at least two other proteins of unknown function (gpwuY and gp59) (see Mosig, 1983; Bernstein and Wallace, 1983). Moreover, secondary initiation is intimately related to the process of recombinational repair of damaged DNA since many of these same gene products are deficient in the repair of UV-damaged phage DNA (see Bernstein and Wallace, 1983).

Replication of non-origin plasmids after T4 infection mimics phage secondary initiation in many respects. The products of T4 genes uswX, uswY, 59, 46/47, and 39/52/60 are each required for both secondary initiation and non-origin plasmid replication. In addition, both processes are inhibited by the presence of a λ phage in the infected cell (Mosig et al., 1984; Kreuzer and Menkens, 1987). Finally, secondary initiation of phage genomic DNA has been proposed to occur in a homology-dependent, but DNA sequence-independent, manner (Mosig, 1983; also see below). Likewise, homology to the phage genome is the only apparent DNA sequence requirement for non-origin plasmid replication. An assortment of recombinant plasmids that encompass about 35% of the T4 genome exhibited gpwuY-dependent replication, whereas the plasmid vector alone was incapable of significant replication (Figs. 1 and 2 and Table I). Mattson et al. (1983a) also demonstrated that non-origin plasmid replication was blocked when the infecting phage carried a deletion of the DNA segment carried on the plasmid.

In their initial study of the fate of recombinant plasmids after T4 infection, Mattson et al. (1983a) suggested that non-origin plasmid replication could be explained by a passive mechanism. Once a plasmid molecule becomes integrated into the phage genome by homologous recombination, replication initiated anywhere on the phage DNA molecule should passively replicate the integrated plasmid sequence. The observation that non-origin plasmids replicated into long concatemeric products (Fig. 5) cannot be explained by this passive replication model. Therefore, we propose that at least some of the replicated non-origin plasmid DNA arises from active initiation events catalyzed by proteins involved in phage genomic secondary initiation. Since the integration of monomeric plasmids into the phage genome does occur (Mattson et al., 1983b; Selick et al., 1988), passive replication may account for a subset of the replicated plasmid DNA. This could provide an explanation for the finding that T4 uswX and uswY mutants produced no detectable replicated plasmid DNA, whereas gene 46, 59, 39, and 52 mutants each produced a small background (Fig. 3). Perhaps the uswX and uswY mutants are deficient in both plasmid integration and secondary initiation, whereas the latter four mutants are deficient in only secondary initiation. According to this model, all plasmid replication in the gene 46, 59, 39, and 52 mutant infections is of the passive variety. We are currently attempting to measure plasmid integration and passive plasmid replication during infections by each of these mutants to test this interpretation.
One significant difference between our conclusions and previously published experiments is that we infer a gp46/47 requirement for non-origin plasmid replication, whereas Mattson et al. (1983a) do not. In that study, plasmid replication was detected only at early times in the gene 46 mutant, whereas secondary initiation occurs predominantly at late times of infection. Perhaps the early, gene 46-independent plasmid replication represents the background of passive replication resulting from plasmid integration, and not the active initiation by the secondary mechanism.

The focus of this study has been to develop a simple model system to study the mechanism of phage T4 secondary initiation and the significance of its coupling to recombination. Two mechanisms have already been proposed to explain the relationship between secondary initiation and recombination. Mosig (1983) has developed a model involving the conversion of recombination intermediates into replication forks (also see Kozinski, 1983). One particular pathway proposed for the conversion of recombination intermediates also solves the problem of complete replication of the linear T4 genome. Whenever a replication fork reaches the end of a linear genome, the 3'-end of the parental DNA molecule is presumably left in a single-stranded form as a consequence of the priming mechanism and the polarity of T4 DNA polymerase (see Watson, 1972). This single-stranded end of T4 DNA is postulated to invade a homologous region of the same or a different DNA molecule and thereby create a unique recombination intermediate. Secondary initiation is then thought to occur by the utilization of the invading 3'-end as a primer for leading strand synthesis on the invaded DNA molecule (Mosig, 1983). This general model for secondary initiation could also be extended to include the conversion of other kinds of recombination intermediates into replication forks.

In the context of the Mosig (1983) model, the requirement for homology between plasmid and phage genomes (Fig. 1B; also see Mattson et al., 1983a) raises an interesting mechanismatic question. Why is it that two plasmid DNA molecules cannot undergo the coupled recombination/replication reaction? Since pBR322 and other ColEl-derived plasmids maintain a relatively high copy number (Clewell and Helinski, 1972), it might be expected that even vector alone could replicate by this mechanism. One attractive answer to this question is that only the invasion of a single-stranded 3'-end can trigger non-origin plasmid replication. The only significant source of single-stranded 3'-ends during a T4 infection may be the unreplicated ends of the linear T4 genome. Since the T4 genome is circularly permuted, any plasmid with homology to phage DNA could accept invasion at some frequency.

A different model for secondary initiation was postulated based on the properties of a novel in vitro recombination-dependent replication reaction (Formosa and Alberts, 1986). The most radical aspect of this second model is that it involves conservative rather than semiconservative DNA replication. The model is based on the properties of in vitro DNA replication reactions that include a duplex DNA template, a homologous single-stranded fragment, the T4-encoded nuclease (gpwusX), and certain other replication proteins. In such in vitro reactions, the single-stranded fragment acts as a primer for DNA synthesis, invading and then utilizing the duplex DNA molecule as template. As DNA synthesis proceeds, the primer/product strand is displaced from its association with the template, as occurs for product RNA molecules during transcription. This leads to the conservative nature of this model for secondary initiation. Another important feature of the model is that the replication complex is postulated to “jump” between different template molecules, permitting, for example, the by-pass of a pyrimidine dimer in one particular template molecule. A jump between two template molecules could result in the generation of a “recombinant” progeny molecule, implying a very intimate linkage between replication and recombination. Since the conservative in vitro DNA synthesis reaction is initiated by invasion of a homologous 3'-end, certain aspects of the models of Mosig (1983) and Formosa and Alberts (1986) are not mutually exclusive.

The in vivo model system for secondary initiation described here should be quite useful in testing these (and other) models for recombination-dependent replication. The evidence presented in this study places certain restrictions on any proposed mechanism. For example, the data argue against the requirement for any specific extensive nucleotide sequence in secondary initiation since a large number of different recombinant plasmids replicated in a gpwusY-dependent fashion. Furthermore, as few as 200 base pairs of homology between plasmid and phage DNAs are sufficient for non-origin plasmid replication (Fig. 5, lane 5A). The requirement for homology between the plasmid and phage genomes is consistent with both models of secondary initiation described above. The production of concatemeric plasmid products may provide a clue to the mechanism of secondary initiation. The conservative in vitro DNA synthesis summarized above resulted in long concatemeric DNA products whereas, circular DNA, and these products were apparently generated by a “rolling-bubble” rather than a “rolling-circle” mechanism (Formosa and Alberts, 1986). It is therefore important to determine whether rolling circles or rolling bubbles generate the concatemeric products of in vivo plasmid replication. Regardless of the mechanism that is found to be correct, an understanding of phage T4 secondary initiation will certainly have important implications for the general processes of DNA replication, recombination, and repair.

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