The Ordered Assembly of the φX174-type Primosome

II. PRESERVATION OF PRIMOSOME COMPOSITION FROM ASSEMBLY THROUGH REPLICATION*

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Gel filtration chromatography was used to isolate both preprimosomal and primosomal complexes formed on single-stranded DNA-binding protein-coated φX174 DNA by the combination of PriA, PriB, PriC, DnaT, DnaB, DnaC, and DnaG. The presence and relative amounts of primosomal proteins in these complexes were determined by Western blotting. Protein-DNA complexes isolated (i) after assembly in the presence of 10 μM ATP, (ii) after preprimosome movement in the presence of 1 mM ATP, (iii) after priming in the presence of the four ribonucleoside triphosphates, or (iv) after complementary strand DNA replication in the presence of the DNA polymerase III holoenzyme all had the same protein composition; preprimosomes contained PriA, PriB, PriC, DnaT, and DnaB, whereas primosomes included DnaG. The stable association of DnaG with the protein-DNA complex could be attributed partially to its ability to remain bound to the primers synthesized. In the absence of PriC, the efficiencies of priming and replication were reduced by one-third and one-half, respectively, even though PriC was not required for the formation of stable protein-DNA complexes on a 304-nucleotide-long single strand of DNA containing a primosome assembly site (Ng, J. Y., and Marians, K. J. (1996) J. Biol. Chem. 271, 15642–15648). We hypothesize that maintenance of the primosome on the replicated DNA may provide a mechanism to allow primosomes to participate in the resolution of recombination intermediates and intermediates formed during double strand break repair by permitting the re-establishment of a replication fork.

Although the φX174-type primosome was discovered over 20 years ago (1, 2) because of its involvement in bacteriophage DNA replication, its functions in normal cellular processes have only recently begun to surface (3–8). In order to expand our understanding of the cellular roles played by this multi-enzymatic unwinding and priming replication machine, we have examined the mechanisms of primosome assembly and the subsequent enzymatic reactions catalyzed by it in detail.

There are three main stages in the replication cycle of φX174 DNA (9). The first stage is the conversion of the viral single-stranded circular (ss(c)) DNA to the duplex replicative form (ss(c) → RF) initiated by the φX primosome (1, 2). Seven primosomal proteins, PriA, PriB, PriC, DnaB, DnaC, DnaG, and DnaT, are required for the assembly of a primosome at the primosome assembly assembly site (PAS) on a single-stranded DNA-binding protein (SSB)-coated φX174 phage DNA (1, 2, 10–13). This multi-enzyme complex can translocate in both the 3' → 5' and 5' → 3' directions along single-stranded DNA (10) and is able to utilize ribonucleoside triphosphates to synthesize multiple primers at random sites along the viral DNA (2, 10, 15, 16). Chain elongation by the DNA polymerase III holoenzyme (pol III HE) then converts the single-stranded viral genome to RFII DNA.

The first (17) and third (18) articles in this series address the mechanism of, and the role of PriB in, the assembly of the φX-type primosome, respectively. We focus here on the composition of the primosome as it makes the transition from assembly to replication. Earlier studies on primosome composition produced incomplete and, at times, contradictory information with respect to the presence of PriB, PriC, and DnaT (12, 19, 20). There were also conflicting proposals as to when some of the primosomal proteins left the protein-DNA complex (21).

We approached these issues by isolating, using Bio-Gel A-50m gel filtration chromatography, primosomal complexes at each of the distinct steps during φX ss(c) → RF DNA replication. A sensitive, physical detection technique was employed to identify the primosomal proteins present in each complex. We present here the composition and relative stoichiometry of the φX-type primosome as it proceeds from assembly through translocation, priming, and replication.

MATERIALS AND METHODS

Reagents, Enzymes, Primosomal Proteins, and Antibodies

[1H]TTP, [α-32P]CTP, [α-32P]dATP, and extended chemiluminescence (ECL). Western blotting reagents were purchased from Amersham Corp. Immobilon-P transfer membrane was from Millipore Corp. DNA polymerase I was from Boehringer Mannheim. Polyclonal antisera against PriA, PriB, PriC, DnaB, DnaC, DnaG, and DnaT were raised in rabbit. Bio-Gel A-50m agarose and goat anti-rabbit IgG conjugated to horseradish peroxidase were from Bio-Rad. Primosomal proteins were purified from overexpression strains as described (22). SSB was purified according to Minden and Marians (23), and pol III HE and DnA (which reconstitute the pol III HE) were purified as described (24).

DNAss

φX174 ss(c) DNA was prepared by an established procedure (25). φX RF I DNA was prepared as described (26). Labeled primed φX ss(c) DNA was prepared by first performing priming reactions under identical conditions as described under “Complex Formation.” The reaction was terminated by the addition of EDTA to 20 mM. Proteins were removed by phenol-chloroform extraction, and the primed template was recovered by ethanol precipitation. The extent of primer synthesis was determined by measuring acid-precipitable radioactivity. Labeled φX RF II DNA was prepared by nick translation using DNA polymerase I, dNTPs, and [α-32P]dATP, followed by phenol-chloroform extraction and ethanol precipitation.
Isolation of Protein-DNA Complexes

Preprimosomal and primosomal complexes were isolated from the successive stages of the complementary strand synthesis reaction as follows.

Preprimosome—Reaction mixtures (50 µl) containing buffer A (50 mM Hepes-KOH (pH 8.0), 10 mM MgOAc, 10 mM diethiothreitol, 50 mM potassium glutamate, and 0.1 mg/ml bovine serum albumin), 16 nm φX ss(c) DNA, 4.2 µM SSB, 48 µM PriA (omitted as indicated in the legend to Fig. 1), 12 nM DnaB, 20 nM DnaC, 11 nM DnaG, 60 nM DnaT, and either 10 µM (for static preprimosomes) or 1 mM (for mobile preprimosomes) ATP were incubated at 30 °C for 8 min.

Primosome—Complex formation was carried out under the same conditions as described for preprimosome formation except that 160 nM DnaG and 1 mM ATP were added to the reaction mixture.

Post-priming Complex—Priming reactions were performed as per priming reactions except that 100 µM each of CTP, GTP, and UTP, and 0.4 µM [α-32P]CTP were also included in the reaction mixture.

Post-cellulose chromatography—For replication reactions, 2 mM ATP, 100 µM each of CTP, GTP, UTP, and the dNTPs, 67 nM [α-32P]dATP, and 80 nM pol III HE were added to the reaction mixture described for preprimosome formation.

Primase-primer Interaction—Reaction mixtures (50 µl) containing buffer A, 4.2 µM SSB, 160 nM DnaG, and either 16 nM φX ss(c) DNA or labeled primed φX ss(c) DNA were incubated at 30 °C for 8 min. No ATP was present in the reaction mixtures.

Preprimosome-RF II DNA Interaction—Reaction mixtures (50 µl) containing buffer A, 16 nM labeled φX RF II DNA, 4.2 µM SSB, 48 µM PriA, 48 µM PriB, 96 µM PriC, 96 µM DnaB, 144 nM DnaC, 160 nM DnaG, 48 nM DnaT, and 2 mM ATP were incubated at 30 °C for 8 min.

Gel Filtration—Reaction mixtures were loaded onto a Bio-Gel A-50m column (100–200 mesh) (0.6 × 19 cm) that had been equilibrated with buffer A. The column was developed at 0.1 ml/min with the same buffer. Ten micromolar ATP was present in the column buffer except in the case of isolation of the primase-primed template complexes. Fractions (120 µl) were collected and analyzed as described below.

DNA Detection—Unlabeled φX ss(c) DNA in the fractions was assayed using the φX174 ss(c) → RF DNA replication reaction. Nineteen microliters of every other fraction were mixed with 3 µl each of concentrated reaction mixture and protein mixture and incubated at 30 °C for 10 min. The reactions were stopped by the addition of 0.1 ml of 0.2 M sodium pyrophosphate, 0.1 ml of 1 mg/ml heat-denatured salmon sperm DNA (as carrier), and 4 ml of 5% trichloroacetic acid. The amount of radioactivity present in acid-insoluble material was then determined by liquid scintillation spectrometry. Replication reaction mixtures containing buffer A, 2 mM ATP, 100 µM each of CTP, GTP, and UTP, 40 µM each of dATP, dCTP, dGTP, and [α-32P]dATP, 48 µM PriA, 3 nM PriB, 12 nM PriC, 12 nM DnaB, 20 nM DnaC, 11 nM DnaG, 60 nM DnaT, 4.2 µM SSB, and 10 nM pol III HE. 32P-labeled φX DNA was detected and quantitated by direct counting of the radioactivity present in each fraction.

Protein Detection—Primosomal proteins were detected by precipitating 20–120 µl of individual or pooled fractions with an equal volume of 10% trichloroacetic acid at 0 °C for 20 min. The precipitate was vacuum-dried after centrifugation and removal of the supernatant and resuspended in 8 µl of protein dye (125 mM Tris-HCl (pH 6.8), 25 mM diethiothreitol, 0.5% SDS, 0.025% bromphenol blue, and 5% glycerol). SDS-PAGE was then performed through either 10 or 12% polyacrylamide gels. ECL-Western blotting was then carried out according to Ng and Marians (17).

Replication Product Analysis

An aliquot (150 µl) of the excluded pool collected from the replication complex isolation procedure was subjected to phenol-chloroform extraction. The DNA products were recovered by ethanol precipitation and analyzed by electrophoresis through a 1% agarose gel using 50 mM Tris-HCl (pH 7.9), 40 mM NaOAc, and 1 mM EDTA as the electrophoresis buffer. Following ethidium bromide staining and photography to determine the efficiency of template utilization, the gel was dried, and the replication products were visualized using a Fuji BAS1000 phosphor imager. An autoradiogram of the dried gel was also obtained.

RESULTS

Formation of the Preprimosomes Is Dependent on the Presence of PriA—In order to ensure that proteins co-eluting with the φX DNA in the excluded fractions during Bio-Gel A-50m gel filtration were truly components of primosomal complexes, we conducted parallel experiments to isolate and analyze preprimosomes in the presence or absence of PriA. All previous data (1, 2, 27, 28), including our observations in the accompanying article (17), indicate that PriA is the specificity factor during primosome assembly. In its absence, there are no specific interactions between the other primosomal proteins and the PAS.

After complex formation and isolation as described under “Materials and Methods,” unlabeled φX DNA was detected using the φX174 ss(c) → RF DNA replication reaction; in addition, 80 µl of every other fraction were used for protein detection as described under “Materials and Methods.” The elution profiles of the φX DNA and primosomal proteins in the presence and absence of PriA are shown in Fig. 1. In both cases, the free proteins were well resolved from their complexed forms. Free PriA and PriB could not be detected in these experiments, no matter the amount that was present in the reaction mixture that was loaded on the column. This was true if these proteins were chromatographed through the column in the absence of DNA and all the other primosomal proteins. We assume that free PriA and PriB bound irreversibly to the column matrix even though there was a high concentration of bovine serum albumin present in the elution buffer. Binding of these proteins to the DNA apparently prevented this nonspecific association with the column matrix.

In the presence of PriA, four other proteins, PriB, PriC, DnaB, and DnaT, were detected in the excluded fractions containing the DNA along with PriA, whereas in its absence, only PriB was found to be associated with the SSB-coated DNA. Therefore, PriA is required for the formation of the preprimosome. Preprimosomes isolated in this manner were active for replication if supplemented with DnaG and the pol III HE. We routinely recovered 80% of the replication activity (data not shown). The interaction between PriB and SSB-coated φX DNA observed here was not surprising, because similar findings have been reported before (20). This association is likely to be mediated by protein-protein interactions between PriB and SSB, because no PriB-PAS interaction could be demonstrated by our gel mobility shift assay described in an accompanying article (17).

All subsequent protein detection was performed by analyzing two pooled fractions, an excluded pool (DNA pool) and an included pool (free protein pool).

Stationary and Mobile Preprimosomes Have the Same Composition—Previous studies from our laboratory showed that translocation of the preprimosome along SSB-coated single-stranded DNA required much higher concentrations of ATP...
than assembly (10). The composition of a stationary and a mobile preprimosome was therefore compared by determining the composition of the protein-DNA complexes formed in the presence of high (1 mM) and low (10 μM) concentrations of ATP as described under "Materials and Methods." Aliquots (100 μl) of each excluded pool were tested for the presence of the preprimosomal proteins. It is evident from Fig. 2 that there was no compositional difference between preprimosomes formed under the two conditions specified. PriA, PriB, PriC, DnaB, and DnaT were present in both preprimosomes, although more protein-DNA complexes may have been formed in the presence of the higher concentration of ATP.

The Composition of the Preprimosome Is Not Affected by the Addition of Primase—The next step in the φX ss(c) → RF DNA replication cycle is the transient formation of the primosome via the association of primase with the preprimosome. Primosomes were assembled in the presence of PriA, PriB, PriC, DnaB, DnaC, DnaT, and DnaG and isolated and analyzed as described under "Materials and Methods." Aliquots (100 μl) of the excluded and included pools were used for protein detection. Again, PriA, PriB, PriC, DnaB, and DnaT remained in the complex (Fig. 3). Thus, the presence of DnaG did not alter the composition of the preprimosome. Interestingly, our complex isolation and detection protocol was sufficiently sensitive to capture the transient interaction between primase and the preprimosome, because a trace amount of DnaG was also detected in the excluded pool (Fig. 3, lane 10).

Primer Synthesis Does Not Change the Composition of the Preprimosome—Priming reactions were carried out in the presence of [α-32P]CTP as described under "Materials and Methods." Aliquots (30–120 μl) of the excluded and included pools were used for primer detection. In the priming reaction, approximately 66 pmol of ribonucleotides were incorporated into acid-insoluble product. Assuming an average primer length of 30 nucleotides (15), this represents about three primers synthesized per φX DNA template. Aliquots (30–120 μl) of the excluded and included pools were tested for primosomal proteins. No compositional change was found after priming. PriA, PriB, PriC, DnaB, and DnaT were all present in the protein-DNA complex (Fig. 4).

DnaG Binds to Primers Synthesized on φX DNA—A higher amount of DnaG was present in the excluded pool after primer synthesis (Fig. 4, lane 10) compared with in the absence of primer synthesis (Fig. 4, lane 5). This enhanced interaction could be a result of (i) primase binding to newly synthesized primers or (ii) a stronger interaction between DnaG and the preprimosome during primer synthesis. Because the composition and the relative stoichiometry (see below) of the proteins present in the preprimosome remained the same before and after priming, a major rearrangement within the preprimosome during priming resulting in an altered affinity for primase seemed unlikely. In addition, the protein-protein interaction that attracts primase to the preprimosome does not require primer synthesis (29). Thus, we examined whether a primase-primer interaction could be detected in the absence of the other primosomal proteins.

DnaG was incubated with primed, SSB-coated φX ss(c) DNA. The protein-DNA complex isolated in the excluded volume of
the column was analyzed as described under "Materials and Methods." ATP was omitted from both the reaction mixture and the elution buffer to ensure that any observed primase binding was the result of the enzyme binding to previously synthesized primers and not the result of de novo primer synthesis. As a control, SSB-coated \( \phi X \) ss(c) DNA was used in place of the primed DNA. Aliquots (30 and 75 \( \mu l \)) of the included and excluded pools, respectively, were tested for the presence of DnaG. DnaG was not detected in the \( \phi X \) ss(c) DNA pool (Fig. 5, lane 1), whereas it was clearly present in the primed DNA pool (Fig. 5, lane 2), suggesting that DnaG possesses an affinity for primers synthesized on the \( \phi X \) DNA, and probably for oligoribonucleotides in general. This may account for the DnaG detected in the excluded column fractions after primer synthesis.

The Composition of the Primosome Remains Unchanged After Replication—The final step in the \( \phi X \) ss(c) → RF DNA replication reaction, disregarding ligation and supercoiling, is nascent chain elongation. The primosome should no longer be required once primers are made. To investigate the fate of the primosome after chain elongation, we isolated the protein-DNA complexes after DNA replication in the presence of the pol III HE and [\( \alpha ^{32}P \)]dATP as described under "Materials and Methods." About 4140 pmol of deoxyribonucleotides were incorporated into acid-insoluble product in the replication reaction. Even so, the interaction between the preprimosome and DNA replication products was present as RF II DNA, indicating that most of the single-stranded template DNA had been converted to duplex form.

Because we could not attribute the difference in relative stoichiometry to the efficiency of template utilization, we assessed whether the remaining preprimosomes were binding nonspecifically to the RF II DNA. Primosomal proteins were incubated with labeled \( \phi X \) RF II DNA, and any protein-DNA complexes formed were purified and analyzed as described under "Materials and Methods." Aliquots (30–120 \( \mu l \)) of the excluded and included pools were used for protein detection as in previous experiments. Only very low levels of the primosomal proteins were detected in the excluded pool (Fig. 8 and Table I). Thus, the residual association of preprimosomes with the \( \phi X \) RF II DNA appeared to be specific.

Relative Stoichiometries—The relative stoichiometries of primosomal proteins present in protein-DNA complexes purified by gel filtration chromatography are listed in Table I. They were determined based on one of two analytical techniques. PriC, DnaB, DnaC, DnaT, and DnaG were determined based on the percentage of total protein (bound plus free) that was found to be present in the excluded fractions. This formulation could not be used for PriA and PriB, because the free protein bound irreversibly to the column and could not be recovered. The amounts of these two proteins present in the excluded pools were determined by comparison with a standard curve constructed from known amounts of these proteins present on the same gels used for the ECL-Western analyses. In all cases, the estimates of amounts of primosomal proteins were made under conditions where the ECL response was linear with respect to both standard protein concentrations and exposure time to the x-ray film.

PriA, PriB, PriC, DnaB, and DnaT were found to form the preprimosome complex on the DNA. These proteins remained in the complex from assembly, through translocation, priming, and replication. The number of primosomes on each DNA template stayed relatively stable throughout the \( \phi X \) ss(c) → RF DNA replication reaction, decreasing by one-half after replication. Even so, the interaction between the preprimosome and
the replicated DNA appears to be specific, because the affinities of the primosomal proteins for \( \alpha X \) RF II DNA were rather low.

Each preprimosome seemed to contain two monomers of PriA (the simplest interpretation of the stoichiometry of 3, given the gel shift results reported in the first article in this series (17)), two dimers of PriB, a monomer of PriC, and a hexamer of DnaB. At the moment, we cannot distinguish whether DnaT exists as a monomer in the preprimosome or only one in three preprimosomes contains a trimer (the reported native form in solution (21)) of DnaT.

Although DnaC was detected in the excluded pools of some of the purified complexes (Figs. 2, 3, 4, and 6), it is not considered a stable component of the preprimosome, because its relative stoichiometry was quite low. The ratio of DnaC monomer to DnaB hexamer in the DnaB-DnaC complex is 6:1. Thus, we would have to interpret the observed ratio of DnaC to DnaB in the preprimosome as indicating preferential association of DnaC with one DnaB protomer in the hexamer. We consider this unlikely. Instead, it is possible that a small amount of DnaC coeluted with the \( \alpha X \) DNA as a result of a residual interaction between free DnaC and DnaB in the protein-DNA complexes.

We could also detect transient interactions between DnaG and the preprimosome and found that DnaG could bind primers in the absence of oligoribonucleotide synthesis. The higher relative stoichiometry of DnaG after priming may reflect these combined interactions, whereas the observed decrease of its relative stoichiometry after replication may reflect a loss of the primase-primer interaction.

**DISCUSSION**

Seven primosomal proteins are required for the formation and full activity of the \( \alpha X \) primsome during the first stage of the viral replication cycle. The same \( \alpha X \) primsome is capable of supporting the progression of the replication fork (24) and is required for certain types of recombination-dependent repair and replication (3–8).

In the accompanying reports (17, 18), we assessed the mechanism of primsome assembly and the role of PriB in the process. Here we have investigated the fate of the preprimosome during the different stages of the replication process that could be defined enzymatically: (i) assembly of the preprimosome, (ii) translocation of the preprimosome along the SSB-coated single-stranded DNA, (iii) formation of the primsome and primer synthesis, and (iv) nascent chain elongation. We found that the preprimosome was composed of PriA, PriB, PriC, DnaT, and SnaB and that it was remarkably stable. Its composition did not alter during successive stages of the replication process, and it remained associated with the DNA even after replication was complete.

Earlier studies agreed on the conservation of PriA and DnaB throughout the stages of complementary strand synthesis (12, 19–21). On the other hand, there has been no evidence supporting the presence of either PriC or DnaC in the primsome (12, 19). Previous reports on the presence of the remaining primosomal proteins in the primsome were not consistent.

Depending on the experiments conducted, PriB was reported to be either present in (12, 20) or absent from (19) the preprimosome. Most previous studies also did not detect DnaT in the primsome (12, 20), although one reported that DnaT was present in the complex prior to chain elongation (21).

We attribute the confusing results reported in earlier studies to the fact that they all relied on indirect means to detect the proteins present in the protein-DNA complexes and thus must be considered somewhat unreliable. The study reported here utilized complex isolation and direct physical detection of the proteins present. The results of our analyses are summarized in the table below.

**Table I:** Relative stoichiometries of primosomal proteins present in various protein-DNA complexes

| Complex | Preprimosome | Primosome | Primase + ss(c) DNA | Primase + primed DNA | Postpriming | Postreplication | RF II DNA |
|---------|--------------|-----------|---------------------|----------------------|-------------|----------------|----------|
| PriA (monomer) | 3.4 | 3.3 | NA* | NA | 3.0 | 2.1 | 0.47 |
| PriB (dimer) | 2.1 | 2.4 | NA | NA | 2.7 | 1.6 | 0.21 |
| DnaT (trimer) | 0.33 | 0.33 | NA | NA | 0.33 | 0.18 | 0.05 |
| PriC (monomer) | 1.1 | 1.2 | NA | NA | 1.0 | 0.5 | 0.30 |
| DnaB (hexamer) | 1.2 | 1.5 | NA | NA | 1.3 | 0.57 | 0.04 |
| DnaC (monomer) | 0.6 | 0.9 | NA | NA | 1.68 | 1.5 | 0.12 |
| DnaG (monomer) | NA | 0.34 | <0.05 | 0.29 | 1.0 | 0.44 | <0.05 |

*NA, not applicable.

**Fig. 8.** Binding of primosomal proteins to RF II DNA.

The \( \alpha X174 \) template DNA was incubated with the primosomal proteins, and protein-DNA complexes were isolated as described under “Materials and Methods.” Analysis was as in the legend to Fig. 2.
in Fig. 9 and discussed below.

One preprimosome, composed of one monomer of PriC and DnaT, two monomers of PriA (although it is possible that this protein dimerizes on the DNA (17)), two dimers of PriB, and one hexamer of DnaB assembles at the PAS on SSB-coated $\phi X$ ss(c) DNA. The preprimosome can travel in either direction along the DNA by hydrolyzing ATP (10). When it transiently interacts with DnaG, priming occurs. The pol III HE then converts the primed $\phi X$ DNA into its duplex form by elongating the primers synthesized. Throughout the replication process, one preprimosome seems to associate with one $\phi X$ DNA template, and no rearrangement of preprimosome composition takes place.

Our analysis confirms the presence of PriA and DnaB in the preprimosome. Their roles are clearly to produce a mobile preprimosome and to initiate priming by DnaG. DnaC is not a component of the preprimosome, and its participation in the reaction probably ends once DnaB is loaded onto the protein-DNA complex. It has been reported that excess DnaC can inhibit DnaB movement on the DNA (31). It seems likely that this results from a transient interaction between DnaC in solution and DnaB in a protein-DNA complex. In addition to being a component of the preprimosome, PriB also binds to the SSB-coated DNA by itself. This may facilitate primosome assembly by bringing PriB closer to PriA. In an accompanying article, PriB was found to stabilize PriA on DNA by reducing its off rate during preprimosome assembly (18). This same function is probably required to maintain the integrity of the preprimosome throughout complementary strand synthesis. DnaT was found to be a stable component of the preprimosome and, contrary to previous claims (21), remained so even after chain elongation. DnaT is generally thought to facilitate DnaB binding to DNA, and, in high concentration, was observed to form a complex with PriA on the DNA (18). It therefore may act as the anchor that holds the two opposing helicases together in the preprimosome.

DnaT has been determined to be a trimer in solution (21). We found its relative stoichiometry to be one monomer per protein-DNA complex. Thus, there is either one monomer of DnaT in each preprimosome or one trimer of DnaT every three preprimosomes. We find the first scenario more likely because all, rather than one-third of, the DNA templates were replicated. This suggests that the DnaT trimers break down into its monomeric units upon primosome assembly. Additional investigation will be required to assess this possibility.

The role of PriC in preprimosome function has always been obscure. We could not detect any effect of PriC on preprimosome assembly by gel shift analysis using a 304-nucleotide-long single-stranded DNA fragment containing a PAS (17). Therefore, it was surprising to find PriC present in the preprimosome throughout the $\phi X$ ss(c) → RF DNA replication reaction. We assessed the issue of PriC dependence by repeating all complex formation, isolation, and analysis reported here in the absence of PriC. We found a 2–3-fold reduction in the efficiencies of priming and replication, and a slight decrease in the number of preprimosomes formed (data not shown). Thus, it is possible that PriC contributes to the overall stability of the preprimosomal complex and that this contribution only becomes apparent under the more stringent demands of complex isolation by gel filtration chromatography, where the caging effect (32) that acts to stabilize less stable complexes during gel shift analysis is not pronounced.

Even though there is no obvious reason for the preprimosome to remain associated with the replicated $\phi X$ RF II DNA, only one-half of the preprimosomes present initially on the template disassembled after replication. It is unclear where, on the RF II DNA, the residual preprimosomes are bound. One possibility is at the preprimosome assembly site. Although this might be predicted to result in a gap in the nascent complementary strand after replication, it is unlikely that this gap would have been detected by the native gel electrophoretic analysis used here.

It is certainly possible that the maintenance of the preprimosome on the replicated DNA is peculiar to the $\phi X$ life cycle. The disposition of the preprimosome relative to the polymerase on the DNA is different from that expected at a replication fork. Thus, the normal mechanisms that operate to remove the replication apparatus from the DNA may not be operative here. Consonant with this, Low et al. (19) suggested that the preprimosome was conserved for the repeated initiation of complementary strands at the subsequent stage during the viral life cycle of RF multiplication.

On the other hand, recent evidence suggests that even a preprimosome formed into a proper replication fork can be maintained on the DNA after a double-stranded plasmid DNA template is completely replicated (33). If these preprimosomes become uncoupled from the polymerase, they may track through the double-stranded DNA as a transient unwinding bubble. Recent genetic studies implicate PriA, and thus, presumably, the preprimosome, as functioning to help resolve homologous recombination intermediates (4, 8) and intermediates arising during double-strand break repair (8, 34) by catalyzing the assembly of a replication fork at or very close to a Holliday junction. Whereas these models are attractive, they fail to account for the dearth of PAS sequences on the Escherichia coli chromosome (14, 35).

Movement of preprimosomes through the DNA may provide a way to deliver them to sites where their action is required but where there is no PAS available. Thus, in this scenario, the $\phi X$ preprimosome could be viewed as a type of chromosomal repair sentinel.

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