

Nonconservative segregation of parental nucleosomes during simian virus 40 chromosome replication *in vitro*

KAORU SUGASAWA*[†], YUKIO ISHIMI[‡], TOSHIHIKO EKI^{§¶}, JERARD HURWITZ[§], AKIHIKO KIKUCHI[‡],
AND FUMIO HANAOKA*^{||}

*Cellular Physiology Laboratory and [†]Biodesign Research Group, RIKEN Institute, 2-1 Hirosawa, Wako, Saitama 351-01, Japan; [‡]Mitsubishi Kasei Institute of Life Sciences, 11 Minamiooya, Machida, Tokyo 194, Japan; and [§]Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021

Contributed by Jerard Hurwitz, October 21, 1991

ABSTRACT Simian virus 40 chromosomes can be replicated *in vitro* with the same set of purified proteins required for the replication of naked DNA containing the viral origin. With these reconstituted systems, the fate of parental histones during replication was examined *in vitro*. The assembly of nucleosomes on replicating chromosomes was hardly affected by the presence of simultaneously replicating naked DNA competitor, suggesting that replication forks can traverse nucleosomes without the displacement of histones. Moreover, we demonstrate that the nascent nucleosomes were distributed almost equally between the leading and lagging strands. This distributive mode of nucleosome segregation favors the propagation of parental chromatin structures to both daughter cells, which can maintain cellular functions dictated by these structures during cell proliferation.

Eukaryotic gene activation is associated with changes in the structure of chromosomes. This implies that properties leading to the active or inactive state of chromatin must be reproduced after DNA replication to maintain patterns of gene expression during cell proliferation. However, the molecular mechanism involved in the propagation of chromatin structures is unknown. Since histones are metabolized at low rates, almost all of the parental histones are found in daughter chromatin with an equivalent amount of newly synthesized histones. Several *in vivo* studies suggest that conservative segregation of nucleosomes during chromosome replication contributes to the propagation of chromatin structures (1–5), whereas other studies indicate that parental nucleosomes are segregated to both daughter DNA helices (6–11).

What is the fate of parental nucleosomes during DNA replication? Since data from *in vivo* studies have been contradictory, we thought that the replication of chromatin *in vitro* would be more informative. Recently developed cell-free systems for simian virus 40 (SV40) DNA replication have permitted detailed examination of the molecular mechanisms involved in mammalian DNA replication. Although most of these studies have used nucleosome-free DNA as the template, cell-free systems supporting faithful replication of SV40 chromosomes using crude cell extracts have been developed (12–14). By reconstituting these systems with purified components required for SV40 DNA replication (15), we have examined the fate of parental nucleosomes. Here we show that parental histones are not dissociated from the replicating DNA after passage of replication forks. We have also demonstrated that nucleosomes are segregated to daughter DNA strands in a distributive manner and not in the conservative mode. This mode of segregation would favor the maintenance of the parental nucleosome structure in the two functionally identical daughter cells.

MATERIALS AND METHODS

Preparation of Replication Proteins and SV40 Chromosomes. SV40 large tumor antigen was prepared from insect cells (SF9) infected with a recombinant baculovirus (16) as described (17). The other replication proteins, DNA polymerase α (pol α)–primase complex (18), topoisomerase II (18), proliferating-cell nuclear antigen (PCNA) (19), human single-stranded DNA binding protein (also designated RF-A) (20), activator 1 (A1, also designated RF-C) (21), and pol δ (22), were prepared from HeLa cells as described. SV40 chromosomes were prepared as described (14).

Replication Reactions. Reaction mixtures (40 μ l) for the replication of SV40 chromosomes contained 40 mM creatine phosphate-Tris (pH 7.6), 3.5 mM MgCl₂, 0.5 mM dithiothreitol, 2 mM ATP, 200 μ M CTP, 200 μ M GTP, and 200 μ M UTP, 100 μ M dATP, 100 μ M dGTP, and 100 μ M dTTP, 20 μ M [α -³²P]dCTP (\approx 30 cpm/fmol), creatine phosphokinase (1 μ g), bovine serum albumin (12 μ g), SV40 chromosome (100 ng of DNA), SV40 large tumor antigen (0.8 μ g), human single-stranded DNA binding protein (0.2 μ g), topoisomerase II (100 units), and the indicated amounts of pol α –primase complex; pol δ (0.16 unit), A1 (0.06 unit), and PCNA (0.1 μ g) were added as noted. For replication of naked SV40 DNA, 100 ng of SV40 replicative form DNA was substituted for SV40 chromosomes. Reaction mixtures were incubated at 37°C for 2 h and samples were assayed for incorporation of radioactivity into acid-insoluble materials. The remaining replication products were purified and analyzed in 1.5% agarose gels with 30 mM NaOH/1 mM EDTA as described (23).

Micrococcal Nuclease (MNase) Digestion. After incubation, samples of the reaction mixtures were adjusted to 1 mM CaCl₂ and digested at 37°C for 3 min with MNase as indicated. Digestion was terminated by the addition of SDS and EDTA to 0.5% and 10 mM, respectively. The resulting products were electrophoresed through 5% polyacrylamide gels with TBE buffer (23) and autoradiographed. For purification of mononucleosome DNA (MN-DNA), the bands corresponding to MN-DNA, located by autoradiography, were excised from the gels. The MN-DNA was eluted from the gel slices by overnight incubation at 37°C in elution buffer [0.5 M NH₄OAc/10 mM Mg(OAc)₂/1 mM EDTA/0.1% SDS] and then recovered by phenol/chloroform-extraction and ethanol-precipitation.

Hybridization Analysis. Strand-specific M13 DNA probes for SV40 were constructed as described (24). The resulting mp18 SVEC and mp19 SVENC contain the coding and noncoding strands, respectively, in the early transcribed

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: A1, activator 1; MNase, micrococcal nuclease; MN-DNA, mononucleosome DNA; PCNA, proliferating-cell nuclear antigen; pol, DNA polymerase; SV40, simian virus 40.

[¶]Present address: Gene Bank, Tsukuba Life Science Research Center, RIKEN, Koyadai 3-1-1, Tsukuba Science City, Ibaraki 305, Japan.

^{||}To whom reprint requests should be addressed.

region of SV40 DNA, and mp18 SVLNC and mp19 SVLC contain the noncoding and coding strands, respectively, in the late region. To prepare probes specific for pSV01ΔEP (17), the plasmid was cut with *EcoRI* and the resulting longer fragment, which was derived from pBR322 DNA, was inserted into the *EcoRI* site of M13mp18 in both orientations. As a result, mp18 pBR1 and mp18 pBR2 contained the coding and noncoding strands with respect to the ampicillin-resistance gene of pBR, respectively. For hybridization with MN-DNA, purified MN-DNA (≈ 10 ng) and the indicated sets of single-stranded DNA probes (total 5 μ g) were added to 10 μ l of 1 \times standard saline citrate, heated for 5 min at 90°C, and then cooled slowly to 50°C. Unhybridized MN-DNA and MN-DNA hybridized to the added probes were separated by electrophoresis in 1% agarose gels with TAE buffer (23). Filter hybridization analysis was carried out as described (23) by using nitrocellulose membrane filters (BA85) and an apparatus (Minifold II) from Schleicher & Schuell.

RESULTS

SV40 Chromosome Replication with Purified Proteins. Two kinds of reconstituted systems for SV40 DNA replication have been established: one is the "monopolymerase" system containing a single DNA polymerase, pol α (18), and the other is the "dipolymerase" system containing pol α and another DNA polymerase, pol δ , with its accessory proteins, A1 and PCNA (25–27). In the monopolymerase system, both leading and lagging strands are synthesized by pol α , whereas in the dipolymerase system, pol δ plays a major role in synthesis of the leading strand. The dipolymerase system appears to be more physiological, because the involvement of pol δ in eukaryotic DNA replication has been strongly suggested from biochemical studies with the SV40 replication systems (25, 28, 29) and from genetic studies with *Saccharomyces cerevisiae* (30–32).

SV40 chromosomes prepared from infected CV1 cells were incubated in reaction mixtures corresponding to the mono- and dipolymerase systems with various amounts of the pol α -primase complex. The replication products were analyzed by alkaline agarose gel electrophoresis (Fig. 1a). In the monopolymerase system using 0.4 unit of pol α , two main DNA species were synthesized: one was an Okazaki-fragment-like short (≈ 150 bases) DNA and the other was much longer DNA (lane 13). The addition of pol δ with A1 and PCNA caused insignificant changes in the pattern of replication products except for the slight increase in length of the shorter DNA species (lane 16). However, when lower levels of pol α -primase were used, synthesis of the longer DNA species was completely dependent on the presence of pol δ , A1, and PCNA (compare lanes 4 and 8 with lanes 1 and 5, respectively). Parallel analysis by slot-blot hybridization with strand-specific DNA probes revealed that leading strands could be synthesized by high levels of pol α -primase complex alone, whereas pol δ , A1, and PCNA were required with limiting levels of the pol α -primase complex (Fig. 1b). Thus, as shown in the studies on naked SV40 DNA replication, SV40 chromosomes also can be replicated in both the mono- and dipolymerase systems.

Assembly of Replicated DNA into Nucleosomes. To examine the chromatin assembly of nascent DNA produced in these reconstituted systems, replication products were digested with various levels of MNase before deproteinization. When replicated chromosomes were digested and then DNA was separated by nondenaturing polyacrylamide gel electrophoresis, a strong digestion barrier was observed at ≈ 150 base pairs (bp) (Fig. 2 c–f, most obvious with MNase at 3 units/ml). More extensive digestion (with MNase at 10 units/ml) generated one major band of 145 bp and two minor bands of 165 bp and 120 bp. This pattern of digestion is typical for

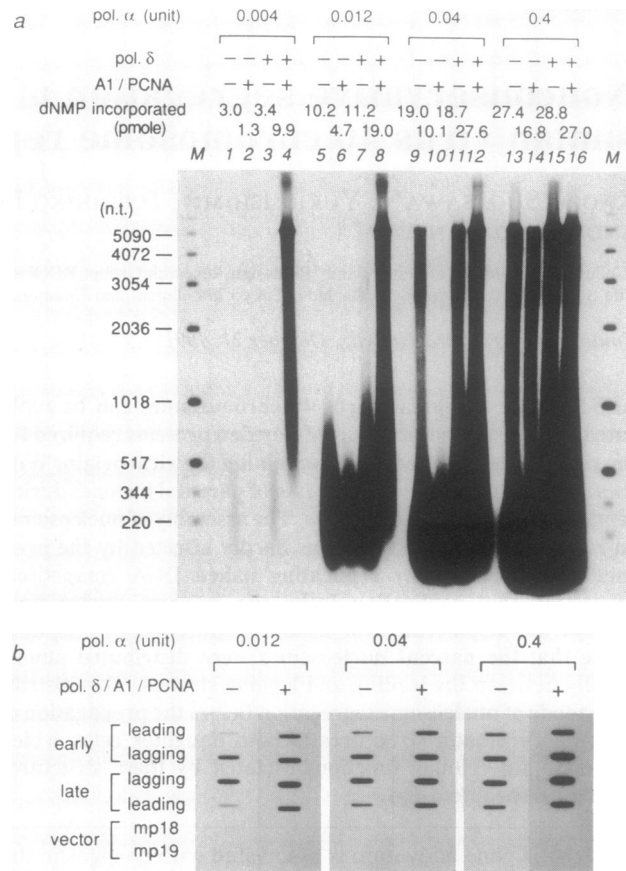


FIG. 1. Replication of SV40 chromosomes with purified proteins. (a) SV40 chromosomes were replicated at 37°C for 2 h in the presence of various amounts of pol α -primase complex as shown at the top. pol δ , A1, and PCNA were added (+) or omitted (-) from the reaction mixtures as indicated above each lane. The replication products that were labeled with 32 P were purified and subjected to alkaline agarose gel electrophoresis and autoradiography. The DNA size markers (lanes M) are a 1-kilobase DNA ladder (BRL) labeled with [α - 32 P]dCTP and the Klenow fragment of pol I. (b) Samples of the products from some reaction mixtures shown in a were digested with *Bam*HI and hybridized to nitrocellulose filters blotted with strand-specific M13 DNA probes (1 μ g per slot) containing the individual strands of the early and late transcribed regions of SV40 DNA. The hybridization signals were constant with more than 0.1 μ g per slot of the probes blotted. nt, Nucleotide(s).

DNA organized into nucleosomes lacking histone H1 (33–35). Although a small amount of dinucleosome DNA was observed after exhaustive digestion, more limited digestion (MNase at 1 unit/ml) produced a smear of DNA products rather than the characteristic ladder-like pattern. These results indicated that SV40 chromosomes replicated in the *in vitro* systems were partially organized into nucleosomes that were not tandemly arranged in a typical regularly spaced manner. These results were unaffected by the presence of pol δ or by changes of the pol α level (compare Fig. 2 c–f). On the other hand, protection of nascent DNA was not observed with the naked DNA template (Fig. 2 a and b), indicating that the nascent nucleosome assembly was specific for chromosome replication.

Competition Between Replicating Chromosomes and Naked DNA for Nucleosome Assembly. Since our purified protein fractions contained no detectable activity for *de novo* nucleosome assembly, the nascent nucleosomes observed in Fig. 2 c–f were probably derived from parental histones, which were originally constituents of the chromosome template. However, other possibilities had to be considered: one was that the chromosome preparations used might contain soluble

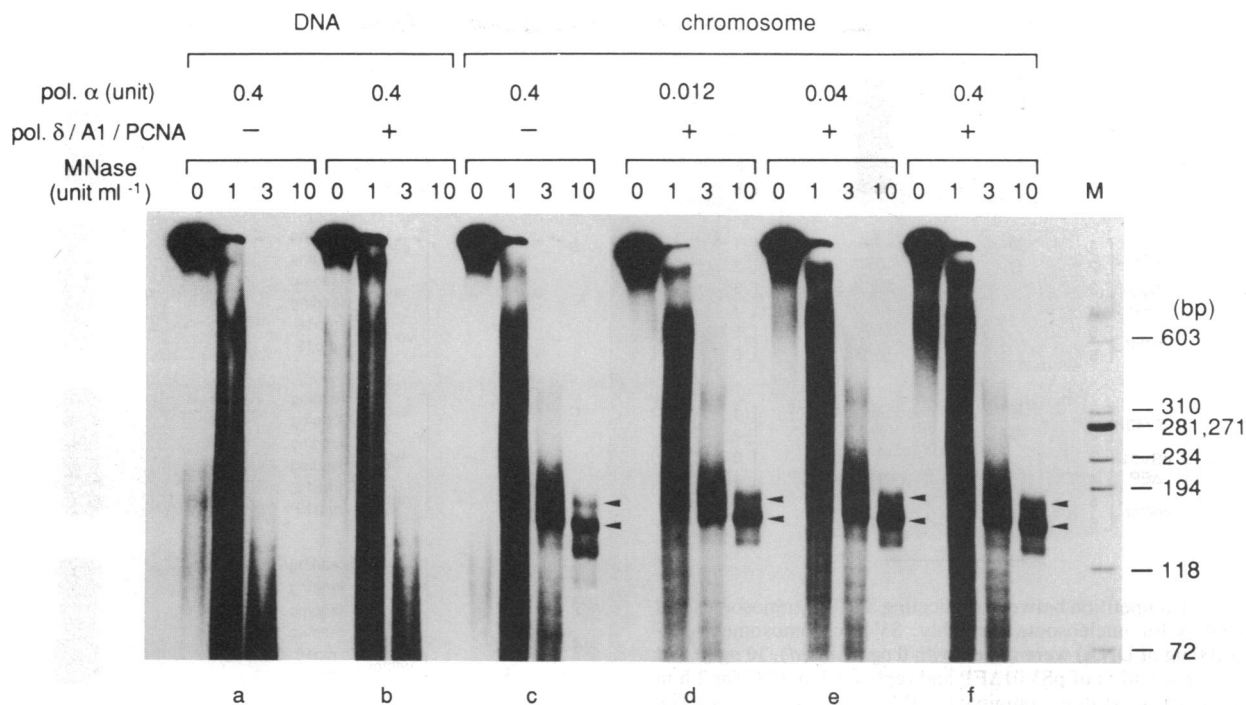


FIG. 2. MNase digestion of replicated SV40 chromosomes. Naked SV40 DNA (*a* and *b*) or SV40 chromosomes (*c*–*f*) were incubated at 37°C for 2 h in the mono- and dipolymerase systems as indicated. Samples of the reaction mixtures were digested at 37°C for 3 min with the indicated final concentrations of MNase. The resulting products were purified, electrophoresed on nondenaturing polyacrylamide gels, and autoradiographed. The DNA size markers are ³²P-end-labeled *Hae* III digests of ϕ X174 replicative form DNA. The positions of DNA bands excised for purification of MN-DNA are shown by arrowheads.

histones available for the nucleosome assembly, and another possibility was that some histones could be transferred from nonreplicating chromosomes to nascent DNA. These possibilities were eliminated by competition experiments. SV40 chromosomes and various amounts of pSV01 Δ EP (a plasmid containing the replication origin of SV40), as a competitor, were mixed and replicated in the dipolymerase system containing 0.012 unit of pol α . After MNase digestion of the labeled products, the resulting MN-DNA corresponding to the bands of 165 bp and 145 bp was purified. To distinguish between nascent nucleosomes on chromosomes and those on competitor DNA, the MN-DNA was heat-denatured and then annealed to a molar excess of single-stranded circular DNA probes bearing sequences specific for either the SV40 chromosome DNA or the competitor DNA. The distribution of nascent nucleosomes between chromosomes and competitor DNA was examined by separating hybridized and unhybridized MN-DNAs by native agarose gel electrophoresis and determining the radioactivity hybridized to the individual probes. If nascent nucleosomes were assembled by some nonspecific mechanisms (i.e., assembly with contaminating soluble histones or histone exchange between DNA helices), they should be distributed randomly on newly synthesized DNA, regardless of chromosomes or competitors.

Slot-blot hybridization analysis showed that, in the presence of increasing levels of competitor, replication of the competitor DNA predominated over chromosome replication (Fig. 3 *d*–*f*). On the other hand, the vast majority of the nascent MN-DNA hybridized to the chromosome-specific DNA probes regardless of the amount of the competitor used (Fig. 3 *a*–*c*). As summarized in Table 1, \approx 90% of the nascent nucleosomes were assembled on the chromosomes even under conditions where the competitor DNA replicated four times more efficiently (i.e., 30 ng of pSV01 Δ EP). Thus, the replicating chromosomes were assembled into nucleosomes with at least a 40-fold preference over the simultaneously replicating naked DNA. Similar results were also obtained

with the monopolymerase system and dipolymerase systems using different levels of pol α -primase (data not shown). From these results, we conclude that nascent nucleosomes were not formed by assembly of contaminating soluble histones or by nonspecific histone exchange. In addition, these results suggest that the parental histones did not completely detach from DNA at replication forks and then rebind to nascent DNA but that they remained physically bound to the template DNA even during the translocation of replication forks through the complex.

Nucleosome Segregation During Chromosome Replication. Based on the results of the competition experiments described above, we measured the ratio of radioactivity recovered as MN-DNA to that incorporated into total replicated DNA in Fig. 2 to assess the percentages of parental histones utilized for the nascent nucleosome assembly. In all reconstituted systems used, 20–25% of the replicated DNA was protected from MNase digestion. If the chromosome template contained one nucleosome per 200 bp and if \approx 150 of 400 bases synthesized (\approx 35%) were protected from MNase action, then at least 60–70% of the parental histones were utilized as octamers for the nascent nucleosome assembly.

We have also examined the mode of segregation of parental nucleosomes between the leading and lagging strands during chromosome replication in the reconstituted systems. For this purpose, SV40 chromosomes alone were replicated in the mono- or dipolymerase system. Nascent MN-DNA was obtained by MNase digestion and samples were hybridized with each of the four strand-specific M13 DNA probes for SV40 DNA. As shown in Fig. 4, the nascent MN-DNAs hybridized to each of the four strands almost equally. These results were hardly affected by the presence of pol δ or by changes in the level of pol α , although the ratio of nucleosome assembly on the leading and lagging strands varied slightly between 1.0 and 1.4. These results suggested that the parental histone octamers were segregated distributively between the

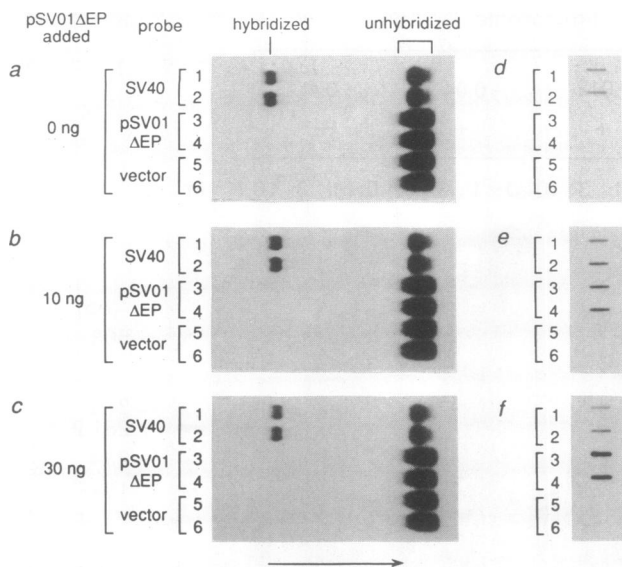


FIG. 3. Competition between replicating SV40 chromosomes and naked DNA for nucleosome assembly. SV40 chromosomes (containing 100 ng of DNA) were mixed with 0 ng (*a* and *d*), 10 ng (*b* and *e*), or 30 ng (*c* and *f*) of pSV01ΔEP and replicated at 37°C for 2 h in the dipolymerase system containing 0.012 unit of pol α . (*a-c*) The reaction mixtures were digested at 37°C for 3 min with MNase at 10 units/ml, and the resulting MN-DNA was purified by gel electrophoresis. Samples of the MN-DNA were denatured and hybridized in solution to single-stranded M13 DNA probes specific for either SV40 DNA or pSV01ΔEP. The hybridized and unhybridized MN-DNAs were separated by native agarose gel electrophoresis and located by autoradiography. The probes used for each lane were as follows. Lanes: 1, mp18 SV4C and mp18 SVLNC (1:1 mixture); 2, mp19 SV4C and mp19 SVLC (1:1 mixture); 3, mp18 pBR1; 4, mp18 pBR2; 5, mp18; 6, mp19. (*d-f*) Samples of the replication products were removed before MNase digestion and hybridized to nitrocellulose filters blotted with the same set of single-stranded DNA probes (1 μ g per slot) as used in *a-c*. The filters were washed, dried, and autoradiographed.

leading and lagging strands during chromosome replication with the purified proteins.

DISCUSSION

In the present study, SV40 chromosomes were replicated with the same set of purified proteins required for the replication of naked SV40 DNA. Cheng and Kelly (13) recently showed that the prebinding of nuclear factor I to its

Table 1. Preferential nucleosome assembly of replicating SV40 chromosomes

pSV01ΔEP added, ng	Relative nucleosome formation* (pSV01ΔEP/chromosome)	Relative DNA synthesis† (pSV01ΔEP/chromosome)
0	—	—
10	0.06	1.17
30	0.12	4.52

*Regions corresponding to hybridized and unhybridized MN-DNAs were excised from each lane of the dried gels shown in Fig. 3 *a-c*, and radioactivity was measured by liquid scintillation counting. The ratios of radioactivity hybridized to the M13 probes for pSV01ΔEP (the sum of lanes 3 and 4) and for chromosomes (the sum of lanes 1 and 2) were calculated for the individual panels.

†Each slot shown in Fig. 3 *d-f* was excised from the nitrocellulose strips and radioactivity was quantified in a liquid scintillation counter. Ratios of radioactivity hybridized to probes specific for pSV01ΔEP (the sum of slots 3 and 4) and chromosome (the sum of slots 1 and 2) were calculated.

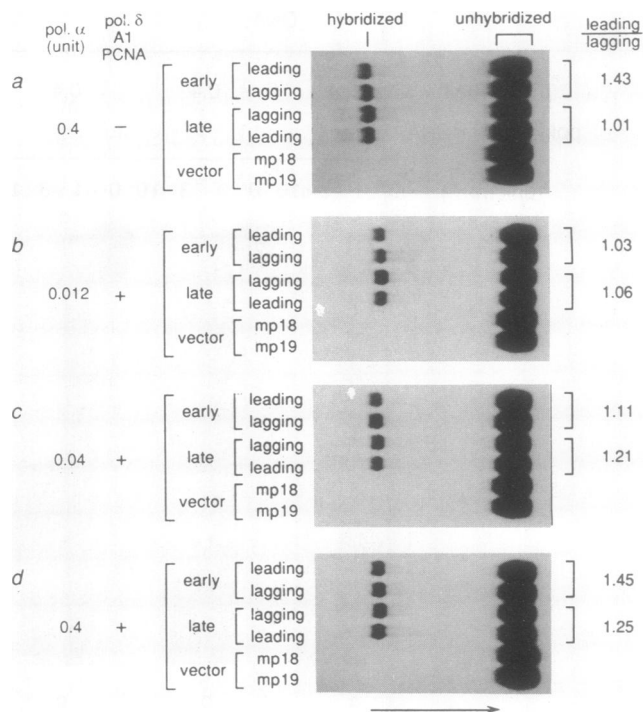


FIG. 4. Segregation of parental histone octamers during SV40 chromosome replication. SV40 chromosomes were replicated at 37°C for 2 h in either the monopolymerase system (*a*) or the dipolymerase systems (*b-d*) containing various amounts of pol α . After MNase digestion (10 units/ml, 37°C, 3 min), the resulting MN-DNA was purified and samples were hybridized in solution to 5 μ g each of the strand-specific M13 probes for SV40 DNA. The hybridized and unhybridized MN-DNAs were separated by native agarose gel electrophoresis and subjected to autoradiography. The percentage of hybridized radioactivity was determined by liquid scintillation counting. The ratio of labeled MN-DNA hybridized to leading and lagging probes was calculated for the early and late sides, respectively, and the results, normalized for the deoxycytidine content of the individual strands, are shown at the right.

DNA binding site situated adjacent to the replication origin of SV40 prevented nucleosomes from covering the origin and increased the template activity of minichromosomes assembled *in vitro* with *Xenopus* oocyte extracts. Since we used SV40 chromosomes prepared from infected cells, the chromosomes that contain a nucleosome-free region around the replication origin may be selectively replicated in our replication system. This would obviate the requirement for a factor to maintain a nucleosome free origin.

Our results suggest that parental histones remain associated with DNA even during passage of replication forks. Similar results were obtained by Bonne-Andrea *et al.* (36), in studies in which an artificial chromosome containing nucleosomes assembled on a circular plasmid DNA containing the M13 origin was replicated with purified T4 proteins. We, on the other hand, have used native SV40 chromosomes as the template and a bidirectional eukaryotic replication system. In spite of the marked differences between the prokaryotic and eukaryotic proteins that constitute the replication fork, histone octamers were not displaced as the replication fork traversed the parental chromosomes. These observations suggest that the retention of the nucleosomes is a property intrinsic to this multiple protein complex. These findings are in keeping with *in vivo* observations. Almost all of parental histones are utilized to form daughter chromatin and newly replicated DNA is rapidly organized into nucleosomes.

Both conservative and nonconservative modes of nucleosome segregation have been reported. In many *in vivo* studies concerned with nucleosome segregation, inhibitors of protein

synthesis, such as cycloheximide and emetine, were used to block *de novo* synthesis of histones. Recently, it was shown that emetine selectively blocked the lagging-strand synthesis and caused an apparent bias in the segregation of nucleosomes to leading strands (37). This effect of protein synthesis inhibitors may explain the controversial results obtained *in vivo*. On the other hand, *in vitro* studies carried out so far agree that parental histone octamers are segregated to both daughter strands. However, Bonne-Andrea *et al.* (36) observed that segregation of histone octamers was biased toward the leading strands, whereas our results indicate that histone octamers were segregated almost equally. This difference may be due to the replication systems used. In prokaryotic replication systems, Okazaki fragments are much longer (>1000 bases) than those in eukaryotes (\approx 200 bases). Therefore, relatively long stretches of single-stranded regions should be present behind prokaryotic replication forks. Because of this, histone octamers may tend to be transferred toward the leading strands at replication forks. Another question is whether the parental histone octamers are segregated to both daughter strands without disruption even when newly synthesized soluble histones can be added to nascent chromatin. Several investigators have suggested that parental histone octamers may not be conserved at replication forks *in vivo* (11, 38–40). The supplementation of the present replication systems with histones and nucleosome assembly factors, such as chromatin assembly factor (41) and nucleosome assembly factor (42), should shed light on this question.

In conclusion, SV40 chromosomes can be replicated with purified proteins without the displacement of parental histone octamers, which are segregated equally between leading and lagging strands. This implies that parental histone octamers are inherited and directly involved in the reconstruction of chromatin structures on both daughter DNA helices. Individual nucleosomes may impart higher-order structures that render chromatin transcriptionally active or inactive, by histone modifications such as acetylation, binding of histone H1 and high mobility group proteins, and positioning on the DNA sequences. If this was the case, the direct inheritance and distributive segregation of parental nucleosomes may be important in the propagation of chromatin structures and the production of two daughter cells that are functionally indistinguishable from each other as well as from their mother cell.

We thank N. Belgado and B. Phillips for their technical assistance. This work was supported by grants from the Ministry of Education, Science and Culture of Japan, by a grant for "Biodesign Research Program" from RIKEN to K.S., and by Grant GM-34559-06 from the National Institutes of Health. J.H. is an American Cancer Society Professor.

- Riley, D. & Weintraub, H. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 328–332.
- Seidman, M. M., Levine, A. J. & Weintraub, H. (1979) *Cell* **18**, 439–450.
- Roufa, D. J. & Marchionni, M. A. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1810–1814.
- Leffak, I. M. (1984) *Nature (London)* **307**, 82–85.
- Handeli, S., Klar, A., Meuth, M. & Cedar, H. (1989) *Cell* **57**, 909–920.
- Pospelov, V. A., Russev, G., Vassilev, L. & Tsanev, R. (1982) *J. Mol. Biol.* **156**, 79–91.
- Russev, G. & Hancock, R. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3143–3147.
- Annunziato, A. T. & Seale, R. L. (1984) *Nucleic Acids Res.* **12**, 6179–6196.
- Cusick, M. E., DePamphilis, M. L. & Wassarman, P. M. (1984) *J. Mol. Biol.* **178**, 249–271.
- Sogo, J. M., Stahl, H., Koller, T. & Knippers, R. (1986) *J. Mol. Biol.* **189**, 189–204.
- Jackson, V. (1988) *Biochemistry* **27**, 2109–2120.
- Decker, R. S., Yamaguchi, M., Possenti, R., Bradley, M. K. & DePamphilis, M. L. (1987) *J. Biol. Chem.* **262**, 10863–10872.
- Cheng, L. & Kelly, T. J. (1989) *Cell* **59**, 541–551.
- Sugasawa, K., Murakami, Y., Miyamoto, N., Hanaoka, F. & Ui, M. (1990) *J. Virol.* **64**, 4820–4829.
- Ishimi, Y., Sugasawa, K., Hanaoka, F. & Kikuchi, A. (1991) *J. Biol. Chem.* **266**, 16141–16148.
- O'Reilly, D. R. & Miller, L. K. (1988) *J. Virol.* **62**, 3109–3119.
- Wobbe, C. R., Dean, F., Weissbach, L. & Hurwitz, J. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5710–5714.
- Ishimi, Y., Claude, A., Bullock, P. & Hurwitz, J. (1988) *J. Biol. Chem.* **263**, 19723–19733.
- Lee, S.-H., Ishimi, Y., Kenny, M. K., Bullock, P., Dean, F. B. & Hurwitz, J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 9469–9473.
- Kenny, M. K., Schlegel, U., Furneaux, H. & Hurwitz, J. (1990) *J. Biol. Chem.* **265**, 7693–7700.
- Lee, S.-H., Kwong, A. D., Pan, Z.-Q. & Hurwitz, J. (1991) *J. Biol. Chem.* **266**, 594–602.
- Lee, S.-H., Eki, T. & Hurwitz, J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7361–7365.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
- Prelich, G. & Stillman, B. (1988) *Cell* **53**, 117–126.
- Lee, S.-H., Kwong, A. D., Ishimi, Y. & Hurwitz, J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4877–4881.
- Wold, M. S., Weinberg, D. H., Virshup, D. M., Li, J. J. & Kelly, T. J. (1989) *J. Biol. Chem.* **264**, 2801–2809.
- Tsurimoto, T., Melendy, T. & Stillman, B. (1990) *Nature (London)* **346**, 534–539.
- Tsurimoto, T. & Stillman, B. (1989) *EMBO J.* **8**, 3883–3889.
- Weinberg, D. H. & Kelly, T. J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 9742–9746.
- Bauer, G. A., Heller, H. M. & Burgers, P. M. J. (1988) *J. Biol. Chem.* **263**, 917–924.
- Boulet, A., Simon, M., Faye, G., Bauer, G. A. & Burgers, P. M. J. (1989) *EMBO J.* **8**, 1849–1854.
- Sitney, K. C., Budd, M. E. & Campbell, J. L. (1989) *Cell* **56**, 599–605.
- Todd, R. D. & Garrard, W. T. (1977) *J. Biol. Chem.* **252**, 4729–4738.
- Albright, S. C., Wiseman, J. M., Lange, R. A. & Garrard, W. T. (1980) *J. Biol. Chem.* **255**, 3673–3684.
- Schlaeger, E.-J. (1982) *Biochemistry* **21**, 3167–3174.
- Bonne-Andrea, C., Wong, M. L. & Alberts, B. M. (1990) *Nature (London)* **343**, 719–726.
- Burhans, W. C., Vassilev, L. T., Wu, J., Sogo, J. M., Nallaseth, F. S. & DePamphilis, M. L. (1991) *EMBO J.* **10**, 4351–4360.
- Senshu, Y., Yamasu, K. & Ohsawa, T. (1985) *Eur. J. Biochem.* **150**, 575–580.
- Jackson, V. (1987) *Biochemistry* **26**, 2315–2324.
- Jackson, V. (1990) *Biochemistry* **29**, 719–731.
- Smith, S. & Stillman, B. W. (1989) *Cell* **58**, 15–25.
- Ishimi, Y., Hirosumi, J., Sato, W., Sugasawa, K., Yokota, S., Hanaoka, F. & Yamada, M. (1984) *Eur. J. Biochem.* **142**, 431–439.