Histone Octamer Dissociation Is Not Required for in Vitro Replication of Simian Virus 40 Minichromosomes*

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Replication of chromosomal templates requires the passage of the replication machinery through nucleosomally organized DNA. To gain further insights into these processes we have used chromatin that was reconstituted with dimethyl suberimidate-cross-linked histone octamers as template in the SV40 in vitro replication system. By supercoiling analysis we found that cross-linked histone octamers were reconstituted with the same kinetic and efficiency as control octamers. Minichromosomes with cross-linked nucleosomes were completely replicated, although the efficiency of replication was lower compared with control chromatin. Analysis of the chromatin structure of the replicated DNA revealed that the cross-linked octamer is transferred to the daughter strands. Thus, our data imply that histone octamer dissociation is not a prerequisite for the passage of the replication machinery and the transfer of the parental nucleosomes.

Replication of eukaryotic genomes involves both the duplication of DNA and the assembly into chromatin. Formation of chromatin during replication consists of two distinct reactions: the transfer of parental nucleosomes from the unreplicated DNA to the daughter strands and the assembly of new nucleosomes from newly synthesized histones (reviewed in Refs. 1–3).

The assembly of new nucleosomes during DNA replication is mediated by chromatin assembly factor-1, a heterotrimeric protein originally purified from human nuclear cell extracts (4). Chromatin assembly factor-1 mediates the first step of nucleosome assembly, namely the deposition of newly synthesized, posttranslationally modified H3/H4 tetramers onto the replicating DNA. This reaction is dependent on ongoing DNA replication (5) and as shown recently this process is mediated by the proliferating cell nuclear antigen, a DNA polymerase clamp. Proliferating cell nuclear antigen binds directly to p150, the largest subunit of chromatin assembly factor-1, and the two proteins colocalize at sites of DNA replication in cells (6). In a second step, which appears to be independent of ongoing DNA replication, two histone H2A/H2B dimers are deposited onto the H3/H4 tetramer precursor particle to complete the full nucleosome core (5).

The mechanism of the transfer of parental nucleosomes to the replicated DNA has been the subject of intense research. Experiments with reconstituted chromatin (7) or with SV40 minichromosomes (8,9) led to the conclusion that the parental nucleosomes remain associated with the DNA during the passage of the replication machinery. However, this association must be weak because a large excess of competitor DNA can cause the dissociation of histones during replication, indicating that histones/nucleosomes remain only loosely associated with the DNA during passage of the replication fork (10). In fact, electron microscopic examination of replicating SV40 minichromosomes revealed that replication forks move up to the next prefork nucleosome and that new nucleosomes appear on the replicated daughter strands at average distances of about 250 nucleotides behind the fork (11). Based on these observations, Randall and Kelly (9) suggested that advancing replication forks release positively charged amino acid side chains in histones of the prefork nucleosome. Releasing positively charged amino acids then immediately gain contact with newly synthesized DNA in daughter strands. Removal of the amino-terminal histone domains by trypsin treatment of SV40 minichromosomes revealed, however, that tailless nucleosomes are efficiently transferred to the daughter strands excluding a participation of the basic histone domains in nucleosome transfer (12).

By using SV40 minichromosomes photoreacted with psoralen under moderately destabilizing conditions, it has been shown that on the average two nucleosomes are destabilized in front of the replication fork (13). This seems to be caused by a dissociation of histone H1 from one to two nucleosomes immediately in front of the fork. In addition the size of the last prefork nucleosome indicates a dissociation of one or two H2A/H2B dimers (13). This is consistent with in vivo data, showing that nucleosomes dissociate into H2A/H2B dimers and H3/H4 tetramers (14,15). Thus it was shown that H3/H4 tetramers selectively deposit on newly replicated DNA, whereas new H2A/H2B dimers associate with either new or old H3/H4 tetramers (14,15). These conclusions were supported by in vitro replication studies (5,10,16,17) and biochemical and electron microscopic analysis of SV40 minichromosomes replicated under cell-free conditions. These studies revealed that a subnucleosomal particle, most probably an H3/H4 tetramer, is the transferred unit (10), which is complemented in a second step by the association of H2A/H2B dimers.

To investigate whether the disruption of histone octamers is necessary for the passage of the replication machinery we compared chromatin, reconstituted with either control or with cross-linked histone octamers, as substrates in the SV40 in vitro replication system. We found that histone octamer cross-linking does not impede the passage of the replication machinery and the transfer of the parental nucleosomes to the daughter strands.

EXPERIMENTAL PROCEDURES

Preparation of Histone Octamers—Nucleosome core particles and histone octamers were prepared from HeLa nuclei as described (18). Cross-linked histone octamers were prepared according to O’Neill et al.
Chromatin Replication and Histone Dissociation

RESULTS

Chromatin Reconstitution with Cross-linked Histone Octamers—Histone octamers were purified from HeLa nuclei; one half served as the untreated control, whereas the second half was cross-linked with dimethyl suberimidate. As demonstrated before by circular dichroism spectra, DNase I digestion, electron microscopy, and DNA supercoiling assays cross-linked histone octamers have properties that are similar to those of native control octamers (33). To check the efficiency of dimethyl suberimidate cross-linking, control and cross-linked octamers were analyzed by SDS-PAGE (Fig. 1, input). The cross-linked histone octamer migrates with an apparent molecular mass of ~100,000 dalton, with no detectable contamination of lower molecular mass components. By using salt gradient dialysis we then reconstituted SV40 DNA into chromatin with control and cross-linked histone octamers. Following reconstitution the integrity of the cross-linked histone octamer was confirmed by SDS-PAGE (Fig. 1, chromatin), where we detected only one band with ~100,000 dalton and no dissociated free histones.

The effect of histone octamer cross-linking on the efficiency of chromatin assembly was then examined by DNA supercoiling analysis (Fig. 2). As one nucleosome introduces one constrained negative supercoil into closed circular DNA templates (34), the change in DNA linking number can be taken as a measure of nucleosome cores assembled on DNA templates from control and cross-linked octamers. SV40 DNA was reconstituted by salt gradient dialysis with increasing amounts of control and cross-linked histone octamers. Nonconstrained supercoils were relaxed by topoisomerase I, the DNA was purified and investigated by agarose gel electrophoresis (Fig. 2). We found that relaxed form II DNA is converted into a topoisomer ladder and subsequently to form I DNA by the addition of increasing amounts of histone octamers. No difference in the efficiency and kinetic of nucleosome assembly could be detected between control and cross-linked octamers, indicating that both templates carry the same number of nucleosomes. We have also investigated the reconstitution products by micrococcal nuclease digestion and obtained very similar digestion products with the familiar ladder of mono-, di-, and trimeric nucleosomal DNA size (data not shown, see Refs. 9, 24, and 31).

1 The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; MNase, micrococcal nuclease; bp, base pairs.
DNA weight ratios are indicated at the top, deproteinized, and separated on a 0.8% agarose gel. The histone to control and cross-linked histones were relaxed with DNA topoisomerase. SV40 DNA and chromatin reconstituted with increasing amounts of superhelical form I DNA are indicated on the left. Histone marker; HM, histone marker; HMW, high molecular weight marker. The molecular masses are indicated in kDa.

Histone Octamer Dissociation Is Not Required for Passage of the Replication Machinery—To see whether the dissociation of histone octamers is necessary for a passage of the replication machinery through nucleosomally organized DNA we used chromatin, reconstituted with control and cross-linked histones, as templates in the SV40 in vitro replication system (Fig. 3). Chromosomal DNA was incubated for 2 h in the presence of the SV40 T-Ag and [α-32P]dATP. Replication products were purified and analyzed by agarose gel electrophoresis and autoradiography (Fig. 3A). The incorporation of radioactive nucleotides was determined by trichloroacetic acid precipitation (Fig. 3B). Protein-free DNA replicates very efficiently in this system. However, the replication efficiency of chromatin was reduced, and the extent of reduction depended on the amounts of nucleosomes. As demonstrated before, the decreased template activity of reconstituted chromatin is because of the packaging of the origin sequences into nucleosomes, which renders the DNA inaccessible to the SV40 T-Ag and thus inactive for replication (10, 24, 35). Comparison of the replication efficiencies of templates with control and cross-linked histone octamers revealed that the efficiency is more drastically reduced with templates containing cross-linked histones. This could be seen both from the autoradiographic signal (Fig. 3A) and the incorporated nucleotides (Fig. 3B). Importantly however, we detected completely replicated form I DNA with molecules containing cross-linked histone octamers, indicating that histone dissociation is not necessary for the passage of the replication machinery.

To further investigate the reasons for the reduced replication efficiency of templates containing cross-linked octamers and to discriminate between an effect on the initiation or elongation of replication we performed pulse-chase experiments. To this end initiation complexes were formed in the presence of the SV40 T-Ag, dialyzed cytosolic S100 replication extract and ATP/Mg2+ using protein-free DNA and chromatin reconstituted with control and cross-linked octamers (histone:DNA weight ratio 1:1.2) as template. During this preincubation step no T-Ag-dependent DNA synthesis was measured (data not shown). DNA elongation was initiated by the addition of ribonucleoside triphosphates, dGTP and dCTP, to preincubated reactions and pulse labeled with [α-32P]dATP. After 30 s, dTTP was added, and the elongation reactions were chased with an excess of unlabeled dATP (Fig. 4). The products were then analyzed under denaturing conditions by alkaline gel electrophoresis (Fig. 4A) and the incorporated nucleotides were determined by trichloroacetic acid precipitation (Fig. 4B). The average lengths of the replication products were evaluated by densitometric scanning of the autoradiograms (Fig. 4C). This kind of pulse-chase analysis allows two different measure-
ments: (i) the intensities of the autoradiographic signal and the efficiencies of nucleotide incorporation correspond to the fraction of minichromosomes participating in replication and (ii) comparison of the lengths of the replication products gives the rate of DNA chain elongation. We found a 3-fold difference in the initiation rate between control and cross-linked octamers as visualized by the autoradiographic signal (Fig. 4A) and the efficiency of nucleotide incorporation (Fig. 4B). However, after 4 min of chase we measured only a 1.3-fold difference in the product lengths between control and cross-linked chromatin (Fig. 4C). This supports the conclusion that the reduced replication efficiency of templates containing cross-linked octamers is mainly because of a reduced initiation rate.

We have shown recently that topoisomerase I and II have a stimulatory effect on the initiation of chromatin replication (31). Higher amounts of topoisomerases seem to be necessary for the removal of topological links in chromatin compared with protein-free DNA. In addition nucleosomal organization is a critical determinant for the amount of topoisomerases needed for efficient replication (31, 36). Therefore cross-linked histone octamers might represent a stronger barrier for supercoil diffusion compared with control octamers, requiring a higher amount of topoisomerases for efficient replication. To test this assumption the preinitiation complex was formed in the absence or presence of additional topoisomerases (31). We found that both templates were stimulated by the addition of topoisomerases, the difference in the number of initiation events between control and cross-linked templates, however, remained constant (data not shown).

Another possibility for the reduced replication efficiency of templates containing cross-linked octamers could be a lower accessibility for the SV40 T-Ag to the origin sequences. To investigate the efficiency of T-Ag binding we used digestion experiments with micrococcal nuclease (MNase) (Fig. 5). To release and quantitate all DNA fragments bound by T-Ag the chromatin was extensively digested with MNase. The MNase protection pattern at specific sites was revealed by Southern blotting and hybridization with oligonucleotides complementary to the origin sequences. Upon extensive digestion with MNase a subnucleosomal fragment of around 80 bp became evident. This fragment consisted of origin DNA protected from MNase digestion by bound T-Ag. As recently shown (31), this fragment is protected by T-Ag against MNase digestion on protein-free DNA. Importantly, the same amount of DNA was protected by T-Ag in templates containing control and cross-linked histone octamers.
linked histone octamers, indicating that cross-linked histone octamers do not restrict the access of T-Ag to their binding sites.

Transfer of Parental Nucleosomes to the Replicated Daughter Molecules Is Not Impeded by Octamer Cross-linking—The precise mechanism of parental nucleosome transfer is still not completely understood. To determine whether histone octamer dissociation is necessary for the transfer of the parental nucleosomes, we investigated the chromatin structure of the daughter strands of templates containing control or cross-linked histone octamers by micrococcal nuclease digestion of the replicated daughter strands. Because of the absence of histatin assembly factor-1 chromatin assembly from newly synthesized histones should not occur in this system (5). However, some free histone H2A/H2B dimers do exist in the extract and may confound the analysis of nucleosomes on replicated DNA. Therefore the extracts were depleted using SV40 DNA coupled to paramagnetic beads (29). To enable an efficient removal of H2A/H2B dimers, the coupled DNA was preassembled with purified H3/H4 tetramers (27, 28). Depletion of S100 replication extracts was achieved by two consecutive incubations with the coupled beads. Bound proteins were eluted from the beads, precipitated with trichloroacetic acid, and separated on an 18% SDS-PAGE. Input, 1-μg beads preassembled with H3/H4 tetramers. M, histone marker. B, the replicated material was digested with micrococcal nuclease and stopped after 1, 5, and 20 min. Purified DNA was analyzed on a 1.3% Tris-glycine-agarose gel and autoradiography. As marker a 32P-labeled 123-bp ladder was used. The sizes are indicated in bp.
replication. In fact cross-linked histone octamers could represent a more stringent barrier for supercoil diffusion than untreated control octamers. However, additional topoisomerases stimulated the replication of cross-linked and control minichromosomes to the same extent. A third possibility is that cross-linking of histone octamers might inhibit interactions with other as yet unidentified proteins necessary for efficient initiation of replication. There could be chaperone-like proteins that facilitate transient detachment of histones from the DNA template during replication and re-assembly onto DNA daughter strands after replication fork passage. A candidate protein template during replication and re-assembly onto DNA daughter strands after replication fork passage. A candidate protein could be a factor termed FACT recently purified from human cell nuclei that facilitates efficient transcript elongation through nucleosomally organized DNA (41, 42). Indeed, the finding that the yeast counterpart of FACT (Spt16/pob3) interacts specifically with DNA polymerase α (43) suggests a role for FACT in chromatin replication and histone displacement.

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REFERENCES

1. Krude, T. (1995) Curr. Biol. 5, 1232–1234
2. Gruss, C., and Knippers, R. (1996) in Progress In Nucleic Acid Research and Molecular Biology (Cohn, W. E., and Moldave, K., eds) Vol. 52, pp. 337–365, Academic Press, San Diego
3. Krude, T. (1999) Eur. J. Biochem. 263, 1–5
4. Smith, S., and Stillman, B. (1989) Cell 58, 15–25
5. Smith, S., and Stillman, B. (1991) EMBO J. 10, 971–980
6. Shibahara, K., and Stillman, B. (1999) Cell 96, 575–585
7. Bonne-Andrea, C., Wong, M. L., and Alberts, B. M. (1999) Nature 343, 719–726
8. Krude, T., and Knippers, R. (1991) Mol. Cell. Biol. 11, 6257–6267
9. Randall, S. K., and Kelly, T. J. (1992) J. Biol. Chem. 267, 14259–14265
10. Gruss, C., Wu, J., Koller, T., and Sogo, J. M. (1993) EMBO J. 12, 4533–4545
11. Sogo, J. M., Stahl, H., Koller, T., and Knippers, R. (1986) J. Mol. Biol. 189, 189–204
12. Quintini, G., Treuner, K., Gruss, C., and Knippers, R. (1996) Mol. Cell. Biol. 16, 2888–2897
13. Gasser, R., Koller, T., and Sogo, J. M. (1996) J. Mol. Biol. 258, 224–239
14. Jackson, V. (1987) Biochemistry 26, 2315–2325
15. Jackson, V. (1990) Biochemistry 29, 719–731
16. Fotedar, R., and Roberts, J. M. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 6459–6463
17. Almouzni, G., Clark, D. J., Mechali, M., and Wolffe, A. P. (1990) EMBO J. 9, 5767–5774
18. O’Neill, T. E., Roberge, M., and Bradbury, E. M. (1992) J. Mol. Biol. 223, 67–78
19. O’Neill, T. E., Smith, J. G., and Bradbury, E. M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6203–6207
20. Hirt, B. (1967) J. Mol. Biol. 26, 365–369
21. Lanford, R. E. (1986) Virology 167, 72–81
22. Simanis, V., and Lane, D. P. (1985) Virology 144, 88–100
23. Stillman, B. W., and Gluzman, Y. (1985) Mol. Cell. Biol. 5, 2051–2060
24. Alexiadis, V., Varga-Weisz, P. D., Bente, E., Becker, P. B., and Gruss, C. (1998) EMBO J. 17, 13428–13438
25. Glikin, G. C., Ruberti, I., and Worcel, A. (1984) Cell 37, 33–41
26. Shimamura, A., Tremethick, D., and Worcel, A. (1988) Mol. Cell. Biol. 8, 4257–4269
27. Sopp, W., and Worcel, A. (1990) J. Biol. Chem. 265, 9357–9365
28. Zucker, K., and Worcel, A. (1990) J. Biol. Chem. 265, 14487–14496
29. Sandaltzopoulos, R., Blank, T., and Becker, P. (1994) EMBO J. 13, 373–379
30. Fotedar, A., Cannella, D., Fitzgerald, P., Rousselle, T., Gupta, S., Davie, M., and Fotedar, R. (1996) J. Biol. Chem. 271, 31627–31637
31. Halmer, L., Vestner, B., and Gruss, C. (1998) J. Biol. Chem. 274, 34792–34798
32. Varga-Weisz, P. D., Blank, T. A., and Becker, P. B. (1995) EMBO J. 14, 2209–2216
33. Stein, A., Bina-Stein, M., and Simpson, R. T. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 2780–2784
34. Germond, J. E., Hirt, B. E., Oudet, P., Gross-Bellard, M., and Champon, P. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 1843–1847
35. Cheng, L., and Kelly, T. J. (1989) Cell 59, 541–551
36. Halmer, L., and Gruss, C. (1997) Mol. Cell. Biol. 17, 2624–2630
37. Waga, S., and Stillman, B. (1998) Annu. Rev. Biochem. 67, 721–751
38. Stein, A., Whitlock, J. P., Jr., and Bina, M. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 5000–5004
39. Leffak, I. M., Grainger, R., and Weintraub, H. (1977) Cell 12, 837–845
40. Leffak, I. M. (1984) Nature 307, 82–85
41. Orphanides, G., LeRoy, G., C.-H., C., Luse, D. S., and Reinberg, D. (1998) Cell 92, 105–116
42. LeRoy, G., Orphanides, G., Lane, W. S., and Reinberg, D. (1998) Science 282, 1900–1904
43. Wittmeyer, J., Joss, L., and Formosa, T. (1999) Biochemistry 38, 8961–8971
44. Germond, J. E., Bellard, H., Oudet, P., and Champon, P. (1976) Nucleic Acids Res. 3, 3179–3192
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