RANDOM LOCATION AND ABSENCE OF MOVEMENT OF THE NUCLEOSOMES ON SV 40 NUCLEOPROTEIN COMPLEX ISOLATED FROM INFECTED CELLS

Chantal Crémisi, Pier Franco Pignatti, and Moshe Yaniv
Département de Biologie moléculaire, Institut Pasteur, 75015 Paris, France.

Received September 23,1976

Abstract.

SV 40 nucleoprotein complexes (NPC) containing viral DNA and cellular histones were extracted from nuclei of permissive cells and treated with either EcoR1 or BamI endonucleases. The fraction of SV 40 linear NPC, monitored by electron microscopy, reached a plateau value of about 15-20% after one hour and no further change occurred during further incubation for 2 hours. Free viral DNA added to the incubation mixture was totally cleaved. During the incubations of NPC and DNA, no redistribution of histones occurred, all the complexes still contained on average 21 nucleosomes and no nucleosomes were generated on the naked viral DNA. Our results suggest a random location and absence of movement of the nucleosomes *in vitro* on SV 40 nucleoprotein complex isolated from infected cells.

Introduction.

Chromatin is visualized by electron microscopy as a chain of spherical particles named ν bodies[1] or nucleosomes[2], interconnected by short DNA filaments. Each nucleosome contains about 200 base pairs of DNA which are bound to the four cellular histones H2A, H2B, H3 and H4[3,4,5,6,7]. Simian virus 40 or polyoma nucleoprotein complexes were isolated both from virions[8,9] and from infected cells[10,11,12] and were found to contain the same four histones[13]. Electron microscopy studies[14,15,16] or digestion experiments with endonucleases[17] have shown that the viral nucleoprotein complexes (NPC) are similar in their structure to cellular chromatin. The viral NPC are visualized as relaxed circles consisting of 21 ± 2 nucleosomes interconnected by thin filaments. The nucleosomes seem to be spaced more and less regularly along the viral DNA[16].

To study the specificity of nucleosome formation, reconstitution experiments were carried out using the four histones complexed with various eukaryotic or prokaryotic DNAs[2,18]. However, this type of experiment is limited, since it can only demonstrate the absence of specificity of histone binding to DNA *in vitro*. The viral nucleoprotein complex, in which the size of the genome, the physical

map of the DNA and the number of nucleosomes which form on the complex are well known, thus provide an excellent model system to study the specificity of nucleosomes formation and their possible movement along the DNA.

Materials and methods.

African green monkey kidney cells (HP8, a subclone of CV1) were infected at a multiplicity of 50 pfu/cell with plaque purified SV 40 virus and labelled with ^3H thymidine at 25 hours post infection. At 30 hours, the cells were collected, the nuclei were isolated and the nucleoprotein complexes extracted as previously described[16].

Endonuclease EcoR1 was purified according to the procedure of Yoshimori (Ph.D. Thesis, Univ. of California, San Francisco, 1971) by chromatography on phosphocellulose and hydroxyapatite columns. No exo or endonuclease contamination could be detected in this preparation. Samples of nucleoprotein complex from sucrose gradients or viral DNA were incubated with a five fold excess of EcoR1 for 3 hours at 37°C in 0.02 M Tris-HCl pH 7.2, 0.1 M NaCl and 0.01 M $MgCl_2$. Endonuclease BamI[19] was a gift from T.A. Bickle. The complex was digested with excess enzyme for 1 hour at 37°C in 0.01 M Tris-HCl pH 7.5, 0.1 M NaCl, 0.007 M $MgCl_2$ and 0.007 M β-mercaptoethanol. The electron microscopy technique described by Dubochet et al.[20] for the observation of DNA or nucleoprotein complexes was applied[16,20].

Results and discussion.

Our electron microscopic studies of both SV 40 and polyoma nucleoprotein complexes have shown that on average 20% of the viral DNA is found in the internucleosomal filaments[16,21]. Since the interconnecting DNA had the same width as deproteinized DNA when analysed under the same experimental conditions, we concluded that it is free of protein. In order to test the possible movement of the nucleosomes along the circular structure, we attempted to cleave the NPC with either endonuclease EcoR1 or BamI, both of which cut SV 40 DNA at a unique site[22]. If the nucleosomes slide during an incubation of 1-3 hours in the digestion buffer, the EcoR1 or BamI cleavage sites should be exposed at one moment to the endonuclease present in excess in the incubation mixture, thus a total conversion of the circular NPC to linear NPC should occur.

The kinetics of cleavage with EcoR1 were monitored by scoring for circular and linear NPC on aliquots prepared for electron microscopy. The number of nucleosomes (21 ± 2) remained constant on both the circular and linear complexes during the incubation (fig. 1 a,b). In each case, a parallel incubation was run in the absence of added endonuclease. Usually from 1% to 10% of NPC were linear in the ini-

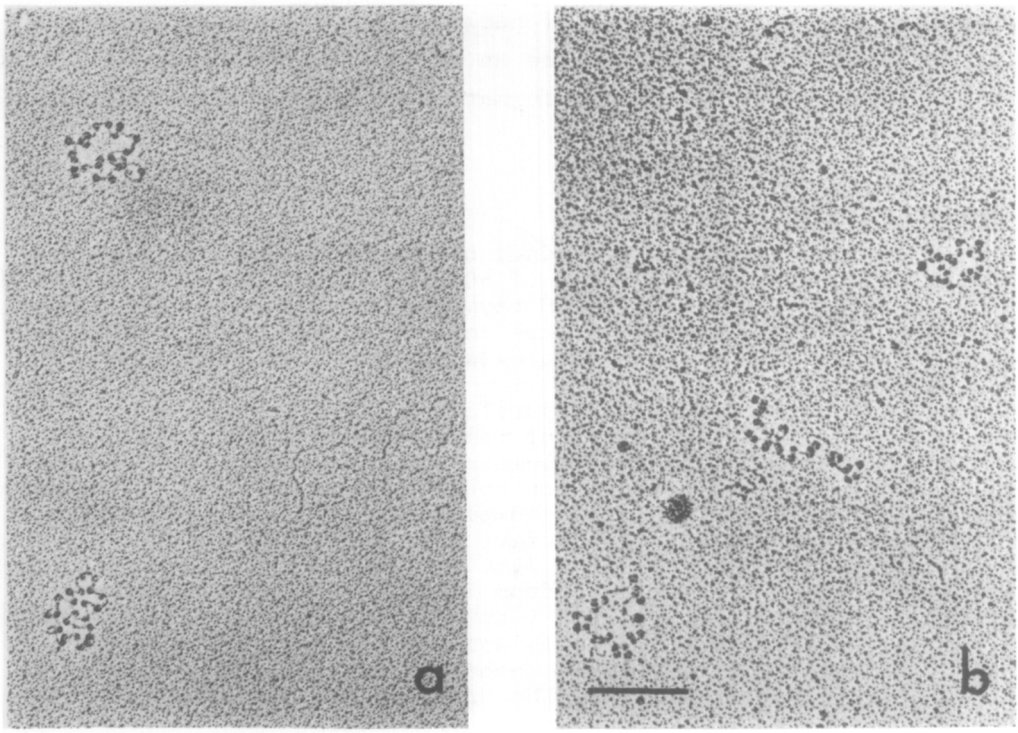

Figure 1. Electron micrographs of SV 40 nucleoprotein complexes before (a) and after (b) treatment by EcoR1. The bar represents 200 nm.

tial preparation and no increase of linear NPC was observed in the absence of endonuclease. As shown in figure 2, the conversion of circular to linear complexes reached a plateau value of about 15% after one hour and no further change occurs during further incubation for 2 hours. When free viral DNA was added to the incubation mixture either in the beginning or after one hour, and the incubation continued for a further hour, the DNA was totally cleaved, showing that excess EcoR1 was present during the incubation and that there is no contamination in the NPC sample able to inhibit or to destroy the EcoR1 activity. Further addition of enzyme did not change the plateau value obtained with the NPC. During these incubations with NPC and free DNA, no redistribution of histones occurred, all the complexes contained on average 21 nucleosomes and no nucleosomes were generated on the naked viral DNA.

Similar results were obtained when the NPC or a mixture of NPC and naked SV 40 DNA were treated with excess BamI : whereas all

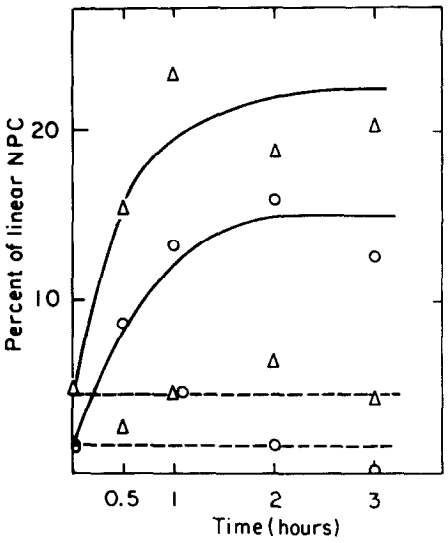

Figure 2. Kinetics of cleavage of SV 40 NPC with EcoR1 : SV 40 NPC from the sucrose gradient peak fractions were incubated in the presence (solid line) or absence (dotted line) of excess EcoR1 in 0.02 M Tris HCl pH 7.2, 0.1 M NaCl and 0.01 M $MgCl_2$ at 37°C. At each time point, an aliquot of the reaction mixture was chilled in ice, and samples were prepared for electron microscopy as described in Materials and Methods. The NPC were scored for linears and circulars independently by two persons (Δ and o - about 100 molecules for each time point). Free DNA added to the incubation mixture after one hour was totally cleaved after another hour. Addition of enzyme at one hour did not change the plateau value obtained with the NPC.

the naked DNA was cleaved only 16% of the complex became linear (table 1).

Treatment of the circular or linear NPC with 1 M NaCl removed all the histones from the viral DNA. Several samples of EcoR1 digested NPC were diluted and scored directly or after treatment with 1 M NaCl. The percentage of linear molecules in the two measurements was in good agreement (see table 1). This comparison excludes the presence of cleaved NPC being held together by histones that could still appear as circles in the microscope. Although the DNA in the nucleosome seem to be on the outside of a protein core [5,6,23], our results show that both EcoR1 or BamI enzymes recognize their cleavage sites only when the DNA is naked and not when it is complexed with the histones in the nucleosomes. It is reasonable to conclude that in the NPC isolated from infected cells, the EcoR1 or

Treatment	Percent of linear NPC		
	control	endonuclease	difference
EcoR1 cleavage	8.7	26.3	17.6
EcoR1 cleavage followed by treatment with 1M NaCl	11.5	33.5	22
BamI cleavage	9	25	16

Table 1. Percent of linear NPC after EcoR1 or BamI treatment. SV 40 NPC from the sucrose gradient peak fraction was incubated with EcoR1 (2 hours) or BamI (1 hour). An aliquot of the EcoR1 reaction mixture was then chilled in ice, while another one was exposed to 1 M NaCl for 30 minutes at 37°C and then chilled in ice. The samples were prepared for electron microscopy as described in Materials and Methods and the complex molecules or free DNA (about 150 molecules for each point) were scored for linears and circulars.

BamI sites are located in the internucleosomal DNA in the sensitive NPC and in the remainder 80% of NPC, they are in the nucleosomes and thus not available for the enzymes. Such a distribution of the EcoR1 or BamI sites is in support of a random location of the nucleosomes along the SV 40 DNA. Non random distribution would either expose the cleavage sites in the internucleosomal DNA in all the complexes or on the contrary protect it in the nucleosomes in all the complexes.

Similar conclusions on the random distribution of the nucleosomes along the SV 40 genome were obtained by Polisky and McCarthy[24] who studied the nucleoprotein complex isolated from purified virions. However, the drastic conditions used by these authors for the isolation of the viral core (pH 10.5) dissociated a fraction of the histones bound to the DNA as it was recently shown to happen by Meinke et al.[13]. The density of their core preparation in CsCl is indicative of a considerable loss of histones. Recent studies by Favre et al.[25] with DNA-histone cores obtained by alkaline dissociation of human or bovine papil loma virus showed that only a small fraction of the complexes contains the entire set of nucleosomes, while the majority lacked a considerable number of nucleosomes. In addition, a possible rearrangement of the histones during the isolation of the core at pH 10,5 cannot be excluded.

Hence, it is not surprising that Polisky and Mc Carthy found that 80% of the complexes were sensitive to EcoR1.

The fraction of cleaved complexes did not vary appreciably when the salt concentration was increased from 0.05 M (19%) to 0.15 M (18%) in the incubation mixture. It appears therefore that no gross change occurs in the size of the internucleosomal DNA in this salt range. These results differ from those of Griffith[14] who observed a condensed structure at 0.15 M NaCl and an open structure at 0.015 M NaCl. However, they agree with those of several other authors who did not observe any differences in chromatin structure in this range of ionic strength[26]. In this study, the DNA seen between the nucleosomes by electron microscopy has been referred to as internucleosomal DNA. It cannot however be excluded that there may be a certain degree of collapsing of the native structure upon air drying of the sample on the grids. In such a case the "internucleosomal DNA" may in fact be more closely associated with the nucleosomes than seen in our images. This "internucleosomal DNA" is sensitive to endonuclease and so is different from the protected DNA/histone core as shown in our experiments.

The origin of replication in SV 40 has been determined[27]. During replication, the ratio of protein to DNA is conserved[11,16,28,29] We can assume that the nucleosomes are dissociated and reformed progressively with the propagation of the replicating forks. The progression of DNA polymerase may involve the dissociation of more than one nucleosome and this would allow more flexibility in the site of formation of the new nucleosomes. This flexibility may be sufficient to finally obtain a random distribution of the nucleosome along the DNA. Otherwise, if during replication the nucleosomes were positioned non randomly, we would have to assume the occurence of nucleosome movement *in vivo* to explain our results.

Acknowledgments.

This work was supported by grants from the Centre National de la Recherche Scientifique and the Jane Coffin Childs Foundation for Medical Research. P.F.P. was supported by a long-term EMBO fellowship. We thank C. Dauguet, D. Cany for technical assistance, C. Maczuka for help in the preparation of the manuscript and G. Butler-Browne for critical reading of the manuscript.

REFERENCES.

1. Olins, A.L. and Olins, D.E. (1974) Science 183, 330-332.
2. Oudet, P., Gross-Bellard, M. and Chambon, P. (1975) Cell 4, 281-300.
3. Rill, R. and Van Holde, K.E. (1973) J.Biol.Chem. 248, 1080-1083.

4. Sahasrabuddhe, C.G. and Van Holde, K.E. (1974) J.Biol.Chem. 249, 152-156.
5. Kornberg, R.D. (1974) Science 184, 868-871.
6. Kornberg, R.D. and Thomas, J.O. (1974) Science 184, 865-868.
7. Noll, M. (1974) Nature 251, 249-251.
8. Huang, E.S., Estes, M.K. and Pagano, J.S. (1972) J.Virol. 9, 923-929.
9. Friedmann, T. and David, D. (1972) J.Virol. 10, 776-782.
10. Green, M.H., Miller, H.I. and Hendler, S. (1971) Proc.Nat.Acad.Sci.Wash. 68, 1032-1036.
11. Seebeck, T. and Weil, R. (1974) J.Virol. 13, 567-576.
12. White, M. and Eason, R. (1971) J.Virol. 8, 363-371.
13. Meinke, W., Hall, M.R. and Goldstein, D.A. (1975) J.Virol. 15, 439-448.
14. Griffith, J.D. (1975) Science 187, 1202-1203.
15. Germond, J.E., Hirt, B., Oudet, P., Gross-Bellard, M. and Chambon, P. (1975) Proc.Nat.Acad.Sci.Wash. 72, 1843-1847.
16. Crémisi, C., Pignatti, P.F., Croissant, O. and Yaniv, M. (1976) J.Virol. 17, 204-211.
17. Louie, A.J. (1974) Cold Spring Harbor Symp.Quant.Biol. 39, 259-266.
18. Steinmetz, M., Streeck, R.E. and Zachau, M.G. (1975) Nature 258, 447-449.
19. Wilson, G.A., Young, F.E. (1975) J.Mol.Biol. 97, 123-125.
20. Dubochet, J., Ducommun, M., Zollinger, M., and Kellenberger, E. (1971) J.Ultrastruct.Res. 35, 147-167.
21. Pignatti, P.F., Crémisi, C., Croissant, O. and Yaniv, M. (1975) FEBS Letters 60, 369-373.
22. Morrow, J.F. and Berg, P. (1972) Proc.Nat.Acad.Sci. Wash. 69, 3365-3369.
23. Baldwin, J.P., Boseley, P.G., Bradbury, E.M. and Ibel, K. (1975) Nature 253, 245-249.
24. Polisky, B. and McCarthy, B. (1975) Proc.Nat.Acad.Sci. Wash. 72, 2895-2899.
25. Favre, M., Croissant, O. and Orth, G. (1975) Proceedings of the 1st Seminar on the Human wart virus, Mérieux, in press.
26. Finch, J.T., Noll, M. and Kornberg, R.D. (1975) Proc.Nat. Acad.Sci. Wash. 72, 3320-3322.
27. Fareed, G.C., Garon, C.F. and Salzman, N.P. (1972) J.Virol. 10, 484-491.
28. Hall, M.R., Meinke, W. and Goldstein, D.A. (1973) J.Virol. 12, 901-908.
29. Goldstein, D.A., Hall, M.R. and Meinke, W. (1973) J.Virol. 12, 887-900.