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Takuzo Oda* Sekiko Watanabe†

Takashi Nakamura‡ Hideyuki Hidaka**

*Okayama University,

†Okayama University,

‡Okayama University,

**Okayama University,

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Takuzo Oda, Sekiko Watanabe, Takashi Nakamura, and Hideyuki Hidaka

Abstract

Viral nucleoprotein complexes were extracted from nuclei of permissive cells (CV-1) infected with simian virus 40 (SV40) and examined by electron microscopy. SV40 nucleoprotein complexes (SV40 chromatin) showed nucleosomes in linear bead-like arrangements along the extended closed circular DNA. The contour length of the SV40 chromatin was only 1.0-1.8 times shorter than that of viral DNA obtained after deproteinization. The data suggest that the circular DNA in SV40 chromatin can be extended to nearly its full length without detachment of the histone complexes.

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—— BRIEF NOTE ——

SIMIAN VIRUS 40 CHROMATIN SHOWING NUCLEOSOMES IN LINEAR BEAD-LIKE ARRANGEMENTS ALONG EXTENDED CLOSED CIRCULAR DNA

Takuzo ODA, Sekiko WATANABE, Takashi NAKAMURA and Hideyuki HIDAKA

Department of Biochemistry, Cancer Institute, Okayama University Medical School, Okayama 700, Japan

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Abstract. Viral nucleoprotein complexes were extracted from nuclei of permissive cells (CV-1) infected with simian virus 40 (SV40) and examined by electron microscopy. SV40 nucleoprotein complexes (SV40 chromatin) showed nucleosomes in linear bead-like arrangements along the extended closed circular DNA. The contour length of the SV40 chromatin was only 1.0-1.8 times shorter than that of viral DNA obtained after deproteinization. The data suggest that the circular DNA in SV40 chromatin can be extended to nearly its full length without detachment of the histone complexes.

The nucleosome structure of simian virus 40 (SV40) nucleoprotein complexes has recently been demonstrated by electron microscopy and biochemical analyses (1-7). The nucleosome of SV40 nucleoprotein complex (SV40 chromatin) consists of an octameric complex of histones (two each of H2A, H2B, H3 and H4) associated with about 200 base pairs of DNA (1-5). In electron microscopic analysis, SV40 chromatin has been shown to have structural features similar to cellular chromatin and to appear as relaxed circular molecules composed of about 21 nucleosomes with interconnecting filaments of DNA (2-5). The present communication reports on the linear bead-like appearance of nucleosomes in the extended closed circular form of SV40 chromatin.

For preparation of SV40 chromatin, CV-1 cells were infected with a plaque-purified, small plaque type of SV40 (strain 777) at a multiplicity of 50-100 pfu/cell, 24 hr after reaching confluency. The infected cultures were labeled with 5 μCi of [^{3}H]thymidine (48 Ci/mmoles, Radiochemical Centre, Amersham) per plate containing 5 ml of medium from 24 hr post-infection. SV40 chromatin was mostly prepared 48 hr after infection by a modification of the method of Bellard *et al.* (5). The peak fractions of sucrose density gradient containing SV40 chromatin were pooled and dialyzed extensively against 1/10 SSC

(0.015 M NaCl, 0.0015 M sodium citrate) instead of sedimenting the SV40 chromatin onto a glycerol cushion. The SV40 chromatin was prepared for electron microscopy using the method of Dubochet *et al.* (4, 8).

In electron micrographs, the SV40 chromatin appeared as linear bead-like structures in closed circular molecules, as shown in Fig. 1. The length of the linear structures measured about 0.51–0.89 μm (mean, 0.67 μm); hence, the contour length of the closed circular molecules is estimated about 1.02–1.78 μm (mean, 1.34 μm). The number of nucleosomes per one SV40 chromatin was 15–25 (mode, 18). The decreased number of nucleosomes in the present SV40 chromatin may be due to the overlapping or detachment of histone complexes during the process of isolation or preparation for electron microscopy. On shadowed specimens, the nucleosomes measured about 12–16 nm in diameter, and internucleosomal DNA filaments consisting of two double stranded DNA measured 4–6 nm in width, but the true sizes should be much smaller. The overall compaction ratio of DNA in SV40 chromatin was about 1.0–1.8 (mean, 1.3). Therefore, the DNA associated with nucleosomes in the SV40 chromatin seems to be fairly extended.

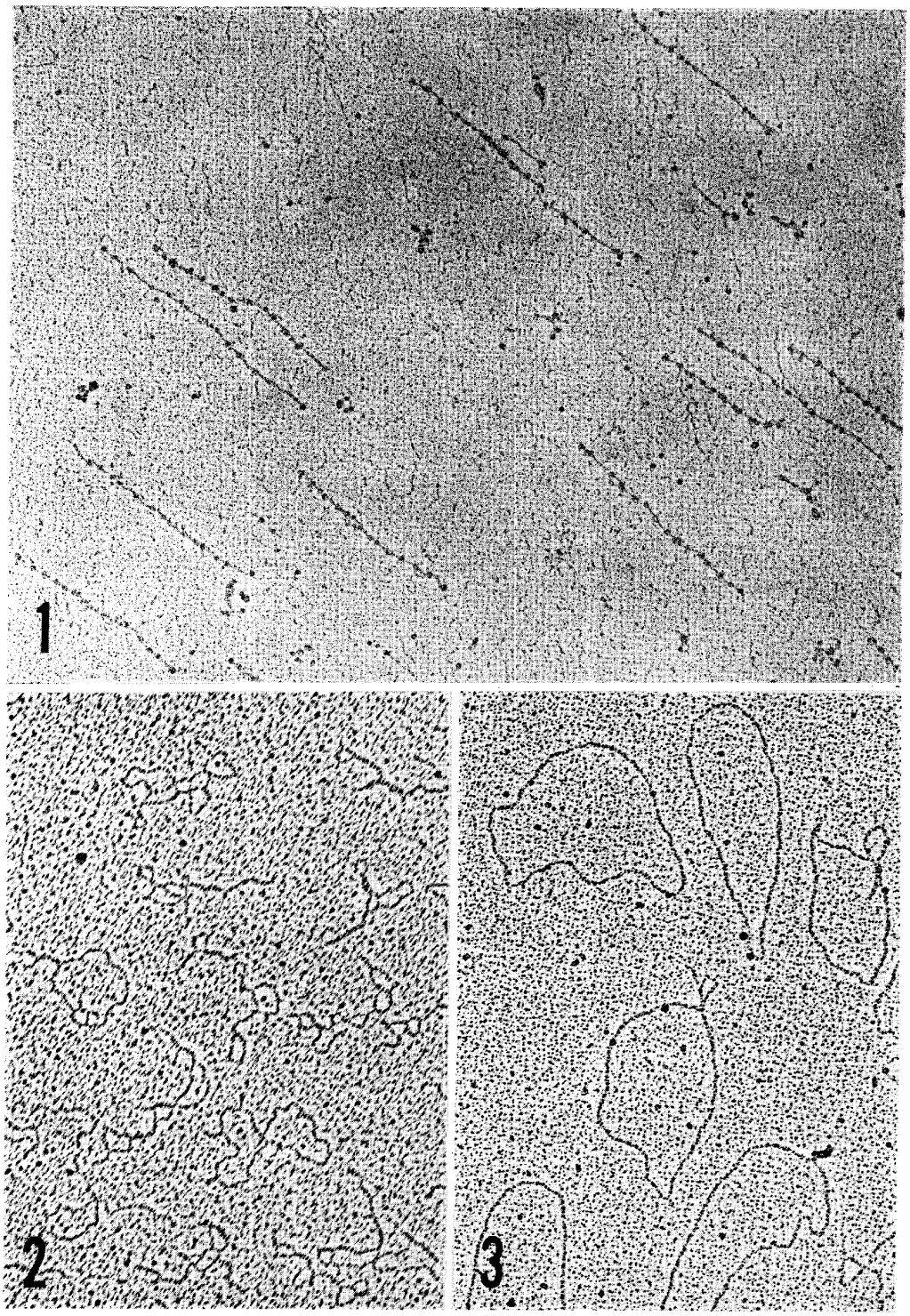
Evidence that the DNA of SV40 chromatin is a closed circular molecule was obtained by electron microscopy of DNA molecules fractionated by 1.4% agarose gel electrophoresis after deproteinization, as shown in Figs. 2 and 3. About 90–95% of SV40 chromatin DNA was separated in twisted closed circular form (form I), about 5–10% in open circular form (form II), and none in linear form (form III). Partially relaxed circular molecules were less frequently observed in the SV40 chromatin preparation. The nucleosome structure of the SV40 chromatin was also confirmed by 1.5% agarose gel electrophoresis of DNA fragments which were obtained by limited digestion of SV40 chromatin with micrococcal nuclease by the method of Noll *et al.* (9). In the nuclease digestion pattern, DNA fragments corresponding to monomer to tetramer nucleosomes were observed.

SV40 chromatin in previous papers (2–5) appeared mostly as relaxed circular molecules. The compaction ratio of the DNA in SV40 chromatin has been reported to be 2.7 (4), 3.1–3.5 (5), and about 7 (1). In such cases, the DNA is

Fig. 1. SV40 chromatin showing nucleosomes in linear bead-like arrangements along extended closed circular DNA. SV40 chromatin was extracted by treatment with 0.25% Triton X-100 from nuclei of SV40-infected CV-1 cells (5), fractionated by 5-20% sucrose density gradient centrifugation, dialysed extensively against 1/10 SSC, diluted with 10mM Tris-HCl-1 mM EDTA, pH 8.1, and prepared for electron microscopy (4, 8). ×50,000.

Fig. 2. Twisted closed circular DNA (form I) obtained by 1.4% agarose gel electrophoresis (12, 13) after extraction of DNA from SV40 chromatin (14) and prepared for electron microscopy (15). ×50,000.

Fig. 3. Open circular DNA (form II) obtained by the same procedures as described in the Fig. 2 legend. ×50,000.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wrapped in a yet undetermined manner around the octameric histone complex (histone core), and the coiling of DNA in a nucleosome is believed to introduce approximately one negative superhelical turn (10). Procedures that remove the histones result in the superhelical configuration only for closed circular DNA (form I) molecules (3, 11). SV40 chromatin in our electron micrographs, however, appeared mostly as extended closed circular molecules, and the compaction ratio was only 1.0–1.8. The structural feature of SV40 chromatin described here was reproduced in repeated preparations. At present we cannot explain the basis for the structural discrepancies between our data and those of other investigators. It may be possible that the SV40 chromatin in the present preparation was artificially extended in the process of isolation and preparation for electron microscopy. Even if this is the case, the demonstrated structure of SV40 chromatin may suggest the presence of strong binding sites and loose association regions in the histone complex with DNA in the nucleosome of SV40 chromatin and that circular DNA in SV40 chromatin can be extended to nearly its full length without detachment of the histone cores. Detail analyses of the present SV40 chromatin are currently in progress.

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