Chromatin Structure: Deduced from a Minichromosome

Abstract. *Cells lytically infected with simian virus 40 contain viral DNA in the form of very small chromosomes (minichromosomes) segmented into 100-Å lengths, each segment containing about 200 base pairs of DNA. This form resembles that of eukaryotic chromosomes and is consistent with the model of chromatin structure proposed recently.*

The interphase chromosome (chromatin) contains an equal mass of DNA and histone protein (*1*). The way in which histones interact with DNA to generate nucleoprotein fibers many times the diameter of the bare duplex is not established. Bram and Ris (*2*) have shown that chromatin prepared for electron microscopy by the most gentle means and maintained in physiologic salt solution is visualized as a network of fibers 80 to 120 Å in diameter (unit fibers). In a recent model of chromatin structure, R. Kornberg (*3*) proposed that the unit fiber is a flexibly jointed chain of repeating units 100 Å in length and estimated that the length of duplex DNA contained in a given length of unit fiber (packing ratio) would be 6.8 : 1. During the course of my studies of a novel chromatin, I have been able to directly measure a unit fiber packing ratio of $7 \pm 0.5 : 1$ and visualize the unit fiber as a chain of 100-Å repeating units joined by short flexible bridges. Comparison of my results with the model may provide another step in understanding the structure of chromosomes.

After permissive monkey cell cultures are infected with simian virus 40 (SV40), a pool of circular viral DNA molecules accumulates in the nucleus of the infected cell. The SV40 DNA can be isolated as a nucleoprotein complex with a sedimentation coefficient between 50 and 60*S* (*4, 5*). More than 95 percent of the proteins in this SV40 nucleoprotein complex are removed by treatment with 1*M* NaCl (*4, 6*) and are identified as cellular histones (*6, 7*). The histones are present in a 1 : 1 weight ratio with the viral DNA as revealed by fixation and equilibrium density sedimentation (*5, 6*). This ratio, as well as the nearly equal proportion of arginine-rich and slightly lysine-rich histones (*8*) is typical of eukaryotic chromatin. In earlier electron microscopic studies this SV40 complex was visualized as a short DNA-protein fiber loop very similar in diameter and appearance to the unit fiber (*6*). The structure, nuclear location, and stoichiometry of histones in this SV40 complex suggest that it is a very small chromosome, here termed a minichromosome.

SV40 minichromosomes were prepared (*9*) by modifications of the Bonner procedure for isolating interphase chromatin (*10*), including the use of sodium bisulfite to inhibit histone protease (*11*), and maintenance of physiologic salt concentrations (0.15*M* NaCl) to preserve the native structure of the unit fiber.

Purified minichromosomes were fixed (Fig. 1) and visualized by high resolution electron microscopy (*12*). Loops of a fiber 110 Å in diameter and 2100 Å in length were observed. These were very similar in appearance to unit fibers extracted from uninfected monkey cells. Loops were either open circles or circles twisted about themselves (Fig. 1). The defined dimensions of the SV40 minichromosome and the DNA contained in it allowed a direct measurement of the packing ratio in the unit fiber; the value was $7 \pm 0.5 : 1$ (Table 1).

Table 1. Dimensions of the SV40 minichromosome. These values were determined by electron microscopy as described in the legend to Fig. 1. Diameters have been corrected for the fractional increase by deposited tungsten. The bridges separating the beads in the samples prepared in solutions of low salt concentration were measured from bead edge to bead edge. An average value of 145 Å for the bridge length can be derived from the difference in contour lengths in native and beaded samples. The total duplex DNA within the beads can be calculated by subtracting from the total duplex length (14,800 Å) the sum of the bridge lengths (134 Å × 21) to obtain 12,000 Å. From this value an estimate of 170 base pairs per bead was derived. The packing ratio was calculated by dividing the contour lengths of deproteinized DNA by that of the native minichromosome. Samples for the packing ratio determination were prepared under identical conditions.

Quantity measured	Value (Å)	Measurements (No.)
Native (in 0.15M NaCl)		
Contour width	105 ± 10	50
Contour length	2,070 ± 125	50
Beaded (in 0.015M NaCl)		
Contour length	5,140 ± 560	13
Bead diameter	109 ± 7	63
Bridge diameter	20 ± 5	22
Bridge length	134 ± 28	55
[Beads per circle	21 ± 1*	41]
Deproteinized (relaxed, protein-free SV40 DNA)		
Contour width	20 ± 4	50
Contour length	14,800 ± 370	50

* Absolute number.

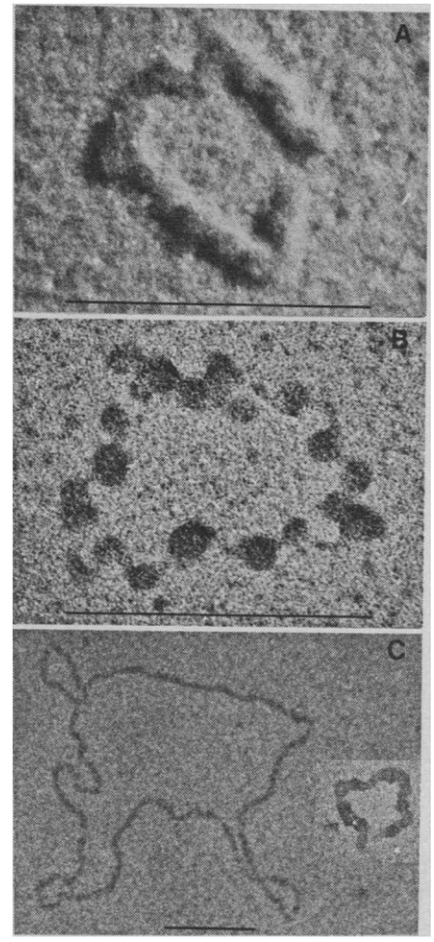

Fig. 1. SV40 minichromosomes. High resolution electron micrographs of the SV40 minichromosome in its native (A), beaded (B), and deproteinized (C) state. The example of the native state was taken in the single side-band strioscopy (*20*) mode to accentuate the appearance of repeating units along the fiber. The example of the beaded state was prepared by diluting native minichromosomes tenfold with distilled water for 1 minute and fixing with glutaraldehyde (to 0.1 percent) for 15 minutes at 20°C. Minichromosomes were deproteinized (*21*) and relaxed by mild x-ray treatment. The bars represent 1000 Å, the inset in (C) compares a native minichromosome at an equal magnification with the DNA. Samples were visualized as described (*12*). The samples were absorbed onto thin carbon films, washed, dehydrated, and stained by vacuum tungsten decoration. The magnification at each step was calibrated against a known diffraction grating. Measurement of the 23.5-Å repeat in tobacco mosaic virus by this calibration was within 3 percent of the value obtained from x-ray studies. For these measurements a Philips EM 300 electron microscope was operated in the direct transmission mode under the standardized conditions described in the operation manual. Measurements were made directly on the 35-mm micrographs, with an optical device coupled to a computer (*22*). The increase in diameters due to deposited tungsten was estimated from the fractional increase in the diameters of M13 phage and tobacco mosaic virus.

A tenfold decrease in ionic strength caused the compact unit fiber to relax into a flexible string of 21 beads, each 110 Å in diameter. The beads were joined by bridges roughly 20 Å in diameter and 130 Å long.

These measurements indicate that when the DNA of the minichromosome is complexed with histone proteins, the DNA is condensed sevenfold to form a 2100-Å length of the typical unit fiber. This condensation appears to occur in blocks, each block or bead containing 170 base pairs of DNA and each bridge about 40. At physiologic salt concentrations these 21 beads condense sequentially to form a 2100-Å loop, and a repeat of roughly 100 Å can be seen along the fiber axis (Fig. 1). In solutions of low salt concentration the beads are exposed uniformly spaced about the circle. The short bridges have a diameter close to that of the bare duplex and constitute less than 20 percent of the total DNA. Thus, most of the histone protein must reside within the beads.

In a study of water-swollen nuclei by electron microscopy, linear arrays of 70-Å spherical bodies were reported (*13*). Although smaller, they would appear to be related to the beads described here. A 100- to 110-Å repeat along the axis of oriented chromatin fibers has been detected by x-ray crystallography and is dependent on the integrity of the histone-DNA complex and lost on stretching of the chromatin (*14*). It seems likely that the repeating unit corresponds to the 110-Å blocks. Digestion of chromatin fibers with certain nucleases has revealed sensitive regions spaced roughly 200 base pairs apart (*15*). These bridges described here are separated by about 200 base pairs and should correspond to the nuclease sensitive regions. Indeed, nuclease digestion of the analogous polyoma nucleoprotein complex has yielded fragments of DNA consisting of 200 base pairs (*16*).

This work has exploited a defined natural chromatin to measure the unit fiber packing ratio and to verify other key predictions of the Kornberg model. The encapsidation of the SV40 minichromosome (*17*) illustrates the great flexibility of the unit fiber.

DNA in the unit fiber exists in two states revealed by electron microscopy. Bead DNA is highly condensed and masked from the action of a duplex-specific nuclease (*15*). Bridge DNA is flexible, less condensed, and cleaved by this nuclease. The SV40 minichromosome provides a unique structure for studying the factors that determine bead and bridge (*18*) formation and for ascertaining whether they are specified by the DNA sequences they contain (*19*).

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9. SV40 minichromosomes were purified in the following manner. CV-1 monkey cell cultures were harvested 36 hours after a high multiplicity infection with SV40 RH911. Nuclei were purified by differential centrifugation in a buffer containing 0.15*M* NaCl, 10 m*M* tris · HCl (*p*H 7.4), and 10 m*M* $NaHSO_3$. The nuclei were then suspended in buffer (including 0.25 percent Triton X100) and incubated for 5 minutes at 37°C. The SV40 minichromosomes contained in this extract were purified by sucrose velocity sedimentation in this buffer (*5*).
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17. The most tightly twisted forms of the SV40 minichromosome loop are roughly spherical particles 400 Å in diameter which are capable of fitting within the virus capsid. It is possible that the flexible nature of the bridge regions provides a mechanism for the generation of more condensed nucleoprotein fibers during metaphase.
18. Electron micrographs of the beaded forms show open, relaxed contours. Deproteinization, however, frees tightly supercoiled DNA molecules, suggesting that the duplex is partially melted, primarily within the beads, releaving roughly 42 supertwists.
19. This may be approached by investigating the bead or bridge location of various restriction nuclease sites or by studying the reannealing behavior of the nuclease-generated 200 base pair fragments.
20. Single side-band strioscopy is a term to denote the partial darkfield condition; a conical darkfield aperture in the condenser system and an off-centered objective aperture were used.
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Preparation of Native Chromatin and Damage Caused by Shearing

Abstract. *Chromatin prepared by a method involving limited nuclease digestion contains the same repeating structure as chromatin in the nucleus, whereas chromatin prepared by conventional methods involving shear does not.*

The finding of a tetramer of the arginine-rich histones (*1*) has led to a model of chromatin structure based on a repeating unit about 100 Å in diameter, comprising eight histone molecules surrounded by 200 base pairs of DNA (*2*). One line of support for this model comes from studies of the digestion of chromatin by nucleases. The DNA from rat liver nuclei digested with an endogenous nuclease gave a regular pattern of bands on polyacrylamide gels corresponding to multiples of a unit size (*3*). This same pattern of bands was observed when nuclei were digested with purified micrococcal nuclease, and the unit size was shown to be about 205 base pairs (*4*).

Since the nuclease digestion studies were done on whole nuclei, whereas the model involves only histones and DNA, the question arose whether chromatin extracted from the nuclei would give similar digestion results. We found that, when extracted by conventional methods, it does not. We now show that this result is due to damage caused by shearing, and we describe a method of extracting chromatin whereby the native structure is better preserved.

The first requirement in most studies on chromatin is to obtain the material in homogeneous solution. In the simplest of conventional methods, nuclei are lysed in hypotonic buffer, and the viscous mass of chromatin is converted to a homogeneous solution by shearing in a motor-driven homogenizer (*5*). In our alternative to this procedure, the chromatin is first cut into large fragments by digesting the nuclei with micrococcal nuclease and then the nuclei are gently lysed in hypotonic buffer, giving a homogeneous solution; we refer to this as the nuclease method.