NUCLEOSOMAL STRUCTURE OF SV40 MINICHROMOSOME AS REVEALED BY MICROCOCCAL NUCLEASE ACTION

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1. Introduction

Simian virus 40 (SV40) offers an attractive model system for studies on the nucleosomal organization of eukaryotic chromatin [1–3]. SV40 minichromosome is particularly useful for the analysis of the specific nucleosome arrangement on well-defined coding and regulatory sequences of the SV40 genome [4,5]. It has been suggested that the minichromosome contains less nucleosomes than may be expected from the known SV40 genome size and repeat length. Random irregular spacing of nucleosomes when assayed by micrococcal nuclease digestion has been observed [6]. Another interesting property of the viral minichromosome is the unusual structure involving a certain 'late' stretch of the genome [7–9].

Here, we report results concerning micrococcal nuclease action on a minichromosome preparation assayed in mild physiological conditons. We show that the nucleosome spacing in these conditions is somewhat more regular than that observed in [6]. The differences, however, are sufficient to reconsider some aspects of the minichromosome structure.

We have also analyzed the chromatin structure involving DNA sequences on both sides of the replication origin. Blot hybridization enabled us to demonstrate that a significant portion of the whole free minichromosome population has nucleosomes in these specific regions.

In summary, micrococcal nuclease action on minichromosomes implies both non-random nucleosome location [10] and regular spacing on rather long stretches of the SV40 genome.

2. Materials and methods

CV1 cells and SV40 virus (strain 776) were grown

and propagated as in [11,12]. Minichromosomes were obtained from the nuclei 40–45 h post-infection by extraction in 0.5% NP40, 0.1 M NaCl, 10 mM triethanolamine–HCl (pH 7.8), 0.1 mM Na-EDTA, 0.1 mM PMSF. They were separated from SV40 virions, previrions, and replicative complexes by sucrose gradient centrifugation, they contained 2–3 subfractions as revealed by agarose electrophoresis, the main one representing minichromosomes free of capsid proteins [11,12]. Micrococcal nuclease (EC 3.1.1.1) digestion was performed at 0–4°C (or at 37°C) in 0.1 M NaCl, 5 mM triethanolamine–HCl (pH 7.5) 0.1 mM Na-EDTA, 10% sucrose after $CaCl_2$ addition up to 1 mM. The reaction was terminated by Na-EDTA addition to 10 mM. Purified yeast tRNA was added to 50 μg/ml and digested minichromosomes were concentrated by ethanol precipitation. The pellets were dissolved in 0.1% SDS, 10 mM Tris–acetate (pH 7.5), 1 mM Na-EDTA, 0.01% bromophenol blue, 10% sucrose and released DNA was subjected to conventional agarose gel electrophoresis in 0.1% SDS-containing buffer. Gels were stained with ethidium bromide or blotted; the filters were processed for hybridization as in [13]. *Bgl*I–*Taq*I 'early' and *Bgl*I–*Hpa*II 'late' fragments (508 and 345 basepairs long, respectively) were labeled in vitro with [^{32}P]-NTP by nick translation and used for hybridization. Fragments were sized using SV40 DNA *Hin*dIII digest as a marker. The latter was also used to assay the hybridization probe purity.

3. Results and discussion

SV40 minichromosomes isolated under physiological salt conditions possess their characteristic nucleosomal and supranucleosomal structure. They

sediment at 80 S, contain a full histone set with some non-histone proteins [3,12], and look under the electron microscope like close arrays of nucleosomes [3,14,15]. The time course of micrococcal nuclease digestion is reflected by the changing distribution of the resulting DNA fragments in agarose gels. These gels are particularly useful to reveal the nucleosome spacing since it is possible to resolve up to 15–20 oligomeric bands [6]. It was reported that in contrast to cellular chromatin SV40 minichromosomes (either isolated [16] or inside nuclei [6]) when analyzed at the same stage of digestion yielded lower oligonucleosomal multimers (6–7 vs 18–20). It should be noted, however, that due to the discrete small length of SV40 chromosomes the digestion products' length distribution cannot be directly compared with that obtained for cellular chromatin. Moreover, it may be demonstrated that under conditions of assay (see [6]), the yield of higher oligonucleosomes from cel-

lular chromatin is expected to exceed that of SV40 chromosomes (unpublished).

Fig.1 demonstrates a typical time course of micrococcal nuclease digestion of isolated SV40 minichromosomes. Higher oligomers can be observed only at those stages when a significant part of the molecules is not digested at all (lanes 5,6). Longer runs in 1.2% gels (fig.2) reveal up to 10 multimers (in certain cases >12) corresponding to a repeat length of 195 ± 9 basepairs (as approximated to zero time of digestion). Our results are consistent with those of Shelton et al. [6] (who recently reconsidered the reported value of repeat length according to usual
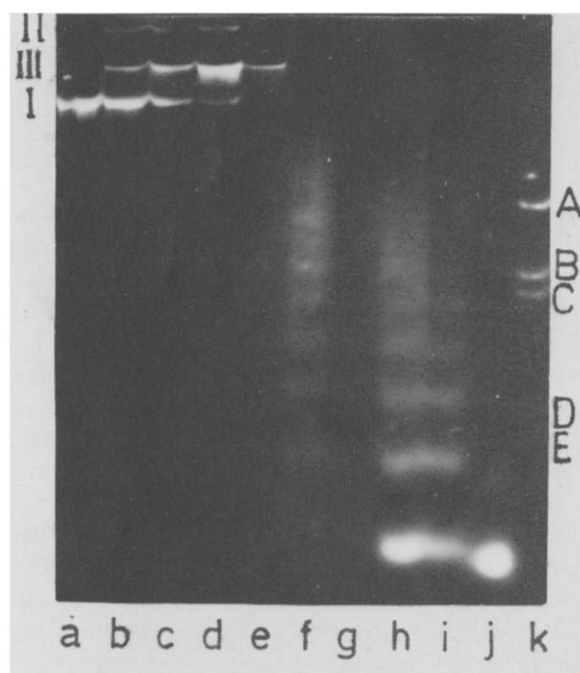

Fig.1. 1.5% agarose DNA gel electrophoresis of SV40 minichromosome digests. Minichromosomes were incubated with micrococcal nuclease (0.2 unit/ml) at 4°C for different times: (a) 10 min without enzyme; (b) 1 min; (c) 2 min; (d) 4 min; (e) 8 min; (f) 15 min; (g) 30 min; (h) 1 h; (i) 2 h; (j) 2 h at 4°C then 10 min at 37°C; (k) HindIII digest of SV40 DNA. Lengths in basepairs: 1762 (A); 1171 (B); 1116 (C); 530 (D); 447 (E); 217 (F). Ethidium bromide staining. I, II, III, supercoiled, nicked and linear SV40 DNA, respectively.
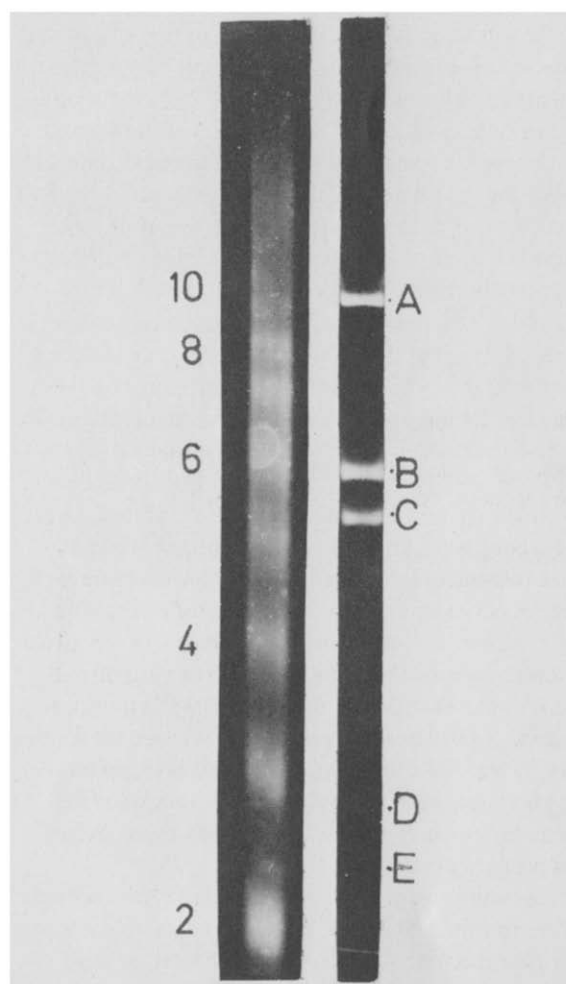

Fig.2. 1.2% agarose DNA gel. (Left) minichromosomes digested at 4°C for 20 min (see fig.1); corresponding nucleosomal DNA oligomers are indicated. (Right) HindIII digest of SV40 DNA (see legend to fig.1).
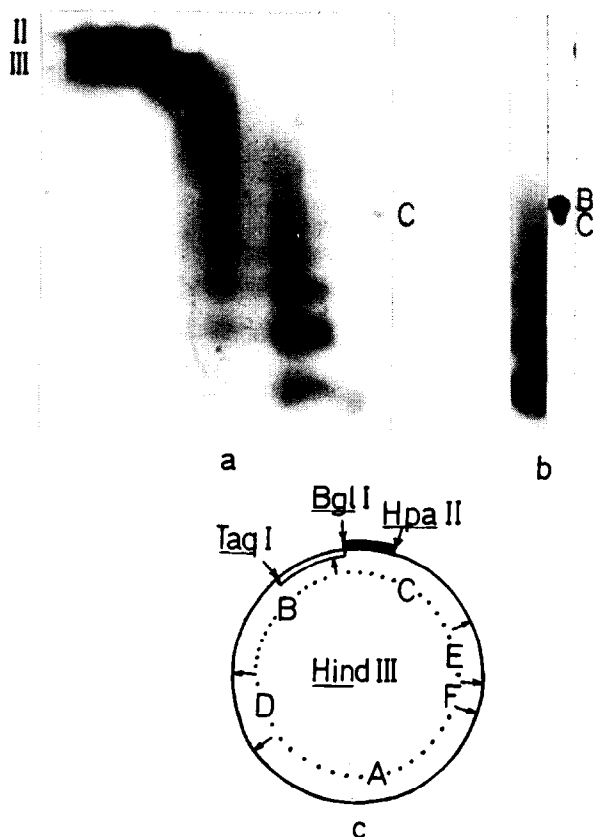
Fig.3. (a) Southern blot-hybridization of DNA gel similar to that shown in fig.1 revealed by autoradiography. ^{32}P-Labeled HpaII–BglI 'late' fragment was used as a hybridization probe; (b) 1 h minichromosome digest at 4°C hybridized to TaqI– BglI ^{32}P-labeled fragment. Hybridization pattern of SV40 DNA HindIII digest is also shown; (c) mutual location of restriction nucleases cleavage sites on SV40 genome map (see [4,5]).

criteria [16]) showing that cellular chromatin gives sharper bands of higher oligomers as compared to minichromosomes. However, the quantitative difference with the results of others is important and implies that another model for nucleosome distribution should be considered (in preparation). In particular, our data indicate that segments of regular nucleosome spacing involve ~1/2 of the initial minichromosome length.

To determine whether all regions of the SV40 genome contain nucleosomes, we hybridized gel-fractionated oligomeric patterns with labelled short fragments of SV40 DNA. TaqI–BglI 'early' (508 basepairs long) and BglI–HpaII 'late' (345 basepairs long) fragments were purified by repeated sucrose gradient centrifugation and used as hybridization probes

(fig.3). Autoradiograms clearly show that the BglI– HpaII fragment efficiently hybridizes to oligonucleosomal and mononucleosomal bands. The same results are valid for the TaqI–BglI fragment (see fig.3 and [10]).

A segment >400 basepairs long, containing the whole BglI–HpaII fragment, is involved in altered chromatin configuration. Among other possibilities, the absence of nucleosomes in this region has been considered. It should be emphasized, however, that SV40 virions were presumably destroyed during minichromosome preparation in [7,9]. Therefore, the question arises as to which particular step of viral chromosome maturation does this structural alteration correspond. Another question concerns the nature of the altered structure. Our results show that, in mild conditions, a significant portion of free viral minichromosomes does contain nucleosomes on both sides of the replication origin. Several explanations should be considered:

(i)   DNase I sensitivity [7] occurs in the nucleosomal structure;

(ii)   Nucleosomes are absent only in a fraction of the whole minichromosome population (in this respect, further fractionation may reveal functionally distinct forms with different nuclease sensitivity);

(iii) The sensitivity is inherent to the chromosome of the assembled virion or to some intermediate structure.

Recent reports published after the completion of our experiments [16–18] support the second possibility. However, the identity of molecules with nucleosome-free gaps under the electron microscope [17,18] and of those specifically cleaved by nucleases remains to be demonstrated.

In conclusion, micrococcal nuclease splits SV40 viral chromosomes even at physiological salt concentration (0.1 M NaCl, 1 mM $CaCl_2$) at 0–4°C. The primary cleavage sites are shown to be non-randomly distributed through the whole SV40 genome and apparently correspond to non-random positions of nucleosomes [10]. In the course of further digestion, oligomeric bands (up to 10–12) appear indicating that DNP stretches long enough to involve ~1/2 of the initial minichromosome fiber are organized in nucleosomes. We have observed unusual spacing between the primary nuclease cuts [10]. If they reflect linker positions, then long regular spacing with a repeat of ~200 basepairs implies the existence of alternative spacing frames rather than unique

positions of nucleosomes. Results on this and other systems support this possibility [19–21]. Mapping of the alternative positions on the whole SV40 mini-chromosome is underway.

Acknowledgement

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