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NON-RANDOM CLEAVAGE OF SV40 DNA IN THE COMPACT MINICHROMOSOME AND FREE IN SOLUTION BY MICROCOCCAL NUCLEASE

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SUMMARY

The distribution of the primary cleavage sites produced by micrococcal nuclease on SV40 DNA in the compact minichromosome was analysed. Minichromosomes purified by improved method were digested in mild conditions in order to minimize possible changes in nucleosomal and supranucleosomal arrangement. The primary cuts were found to be non-randomly and unevenly distributed through the whole SV40 genome and those located in the "early" half of SV40 DNA were mapped. Some of them were also preferentially attacked on the naked DNA treated under the same conditions. Possible relation of the results to the nucleosomal organization of the compact minichromosome is discussed.

INTRODUCTION

The specificity of nucleosome arrangement with respect to DNA sequence was analysed by different approaches (I,2,3,4,5,6). A small natural minichromosome of papova viruses seemed to be an attractive model for such investigation. SV40 genome 5226 (or 5245) base pairs long was studied in detail and completely sequenced (7,8,9). SV40 native chromosome was shown to be organized at nucleosomal and supranucleosomal levels (I0,II,I2,I3).

First, it was found that several single-cut restriction enzymes (introducing one double stranded cut in covalently closed SV40 DNA) cleaved only about 30% of DNA molecules in minichromosome preparation (as a limit digest). As a result random distribution of nucleosomes and the absence of their rearrangement in the course of digestion were suggested. Later Beard detected that nucleosomes could slide under physiological salt and temperature conditions (I4). Ponder and Crawford studied the location of Bam HI and Eco RI sites in the core obtained from the limit digest of SV40 and polyoma minichromosomes by micrococcal nuclease and found them to be non-randomly distributed and spaced by multiples of I0 base pairs (I5). Another kind of specificity was observed recently by several authors showing a small part of the genome to be readily available for DNAase I and site-specific nuclease digestion of the minichromosomes(I6,I7,I8,I9).

In our work we used micrococcal nuclease known to cut DNA mainly in linker region of folded nucleosomal fiber. Our preparation of minichromosomes was purified from disrupted virions and differed in many respects from those

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used elsewhere (II,I2,I5,I6,I8). The digestion was carried out at lower temperature which supposedly minimized the rearrangements at nucleosomal level.

We found the sites of the primary nuclease attack to be non-randomly and unevenly distributed along the whole SV40 DNA of the compact minichromosome. Unexpectedly, free SV40 DNA digested under the same conditions was also specifically cleaved. Although a part of the primary cleavage sites on the naked DNA appeared to be accessible for nuclease in the compact minichromosome, the overall distribution was markedly affected by the presence of nucleosomes or by further folding of the nucleosomal fiber. Possible relation of these results to nucleosomal organization of the compact minichromosome is considered in Discussion.

MATERIALS AND METHODS

The details of growth, infection and labeling conditions were described (20,2I). SV40 strain 776 was used. Nuclei were isolated and extracted 36 hrs post infection by improved technique (2I). Minichromosomes sedimenting at 80-90 S were significantly purified from the products of virion disintegration (as judged by agarose gel electrophoresis of minichromosomes, ref. 2I), they contained mostly cellular histones and looked under electron microscope like compact particles of different configurations (20,2I). Minichromosomes were digested at 30-50 ug/ml by I-2 unit/ml of micrococcal nuclease (EC 3.I.4.7, Worthington) at 2-4°C in the sucrose gradient buffer (i.e. 0.I M NaCl, 5 mM triethanolamine-HCl, pH 7.5, 0.I mM Na-EDTA, 5-I0% sucrose) after $CaCl_2$ addition up to I mM. The reaction was stopped by Na-EDTA when 30-50% of supercoiled DNA was transformed into linear form III DNA. Free SV40 DNA I purified from Hirt supernatant by CsCl-dye density centrifugation was digested under identical conditions. DNA was isolated from the digests after RNAase and Pronase treatment by phenol-chloroform procedure. Restriction with Bgl I,Bam HI was performed in I0 mM Tris-HCl, pH 7.5, I0 mM $MgCl_2$, I0 mM 2-mercaptoethanol at 37°C; that with Eco RI (37°C) or Taq I (65°C) was performed in the same buffer but containing 50 mM NaCl. The small "early" Bgl I - Taq I fragment ($\sim$500 base pairs, sedimented at $\sim$ 8 S) was separated from larger one by two consequent sucrose gradient centrifugations, highly labeled with ^{32}P by nick-translation (22) and used for hybridization. DNA samples were analysed by agarose gel electrophoresis (23). The bands were trasferred onto nitrocellulose filters as described by Southern and hybridized (24,25) with labeled Bgl-Taq fragment. The purity of the fragment was confirmed by hybridization pattern with fractionated digest of SV40 DNA with Hind III. Autoradiographs were obtained on NS-5T film (Kodak) exposed for I0-60 hrs.

RESULTS

Specific cleavage of SV40 DNA in the compact minichromosome. To determine the sites of the primary attack of SV40 minichromosome by micrococcal nuclease the following scheme was elaborated (Fig. I). Minichromosomes in the compact form were isolated from infected CV-I monkey cells. The experimental conditions were selected (2I) under which the contamination of free minichromosomes by the products of virion degradation was low. Immediately after the isolation SV40 chromosomes were digested at 2-4°C by micrococcal nuclease. The course of the digestion was monitored by DNA electrophoresis,and the transition of DNA from form I to linear form III and then to oligonucleosomal and

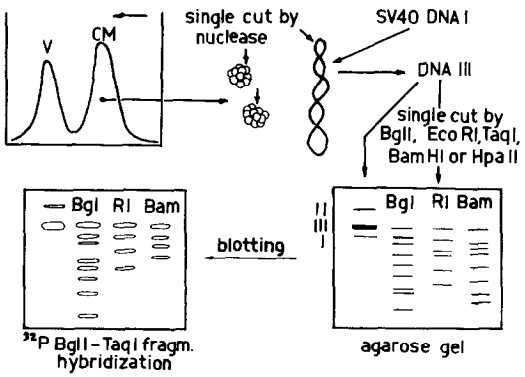

Figure I. The scheme of the experiment (details see in Methods). V - SV40 virions; CM - compact minichromosomes. DNA I, II and III correspond respectively to supercoiled, nicked and linear SV40 DNA.

mononucleosomal DNA fragments was followed. The stage was chosen when about a half of DNA was converted into full length linear molecules. Another half was represented by covalently closed DNA (forms I and II) and about 10-15% of shorter fragments. DNA isolated from the digests was redigested with different single-cut restriction enzymes. In the most of experiments Bgl I cleaving SV40 DNA close to the replication origin was used. A number of discrete bands could be seen on the stained gels after restriction (Fig. 2) demonstrating a non-random distribution of the primary nuclease cuts on SV40 DNA. Eco RI and Bam HI used for additional analysis gave band pattern differing from that of Bgl I.

The sizing of resulting DNA fragments was determined on the basis of their mobility in gels (Hind III digest of SV40 DNA was used as a marker). However, at this stage it was not possible to localize the cuts on SV40 DNA map unless the polarity of the fragments is known. To determine the latter fractionated DNA bands after restriction were transferred onto nitrocellulose filter and hybridized with highly labeled DNA segment adjacent to Bgl I restriction site. In this work Bgl I - Taq I 500 base pairs long fragment was used. It could hybridize only to the fragments directed from Bgl I site to the "early" part of the genome (Fig. 3). It is obvious from Fig. 2 that only a part of DNA bands has bound the label.

Experiments of this type enable us to map all primary cleavage sites. In the present work, however, we have succeeded in mapping unambiguously only those located in the "early" region and occasionally some sites in the "late" one. For completion of the analysis one has to use a fragment adjacent to Bgl I site but in the "late" region and fragments adjacent to Bam HI site. This work is in progress now.

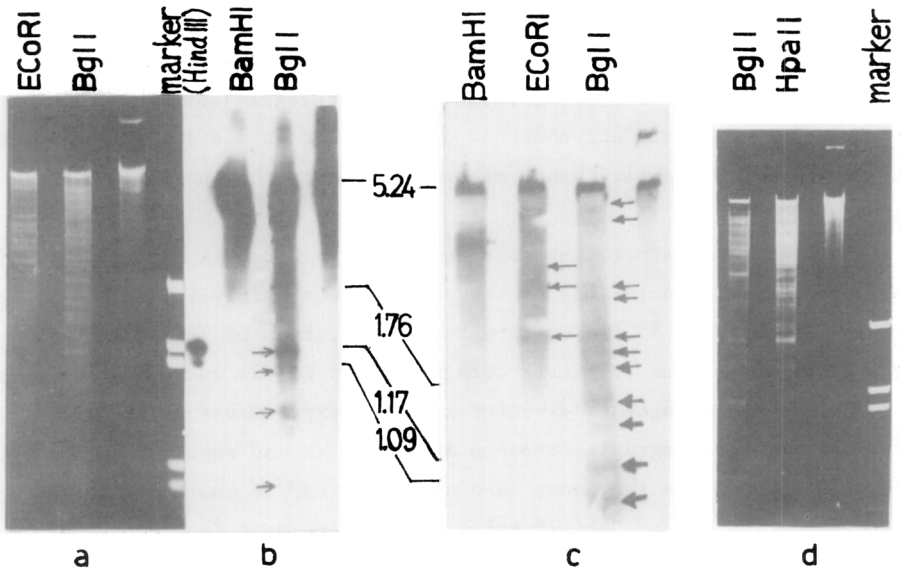

Figure 2. Agarose gel electrophoresis of DNA fragments after micrococcal nuclease treatment of compact minichromosomes or free SV40 DNA followed by redigestion with single-cut restriction enzymes.
(a) DNA from compact minichromosomes cleaved on average once by nuclease (right) and after redigestion with Bgl I or Eco RI (left). I.6% gel, ethidium bromide staining.
(b) Example of overexposed autoradiograph (Southern image of I.6% gel) after hybridization with labeled Bgl I - Taq I "early" fragment. From left to right: SV40 DNA Hind III digest (note, that only B and C fragments bound labeled DNA) the same DNA preparation from minichromosomes primarily cleaved by nuclease and redigested with Bam HI or Bgl I; the same but before redigestion (right panel). Short fragments in Bgl slot can be detected.
(c) The same preparations as in (a) and (b). From right to left: minichromosome DNA preparation before redigestion or redigested with Bgl I, Eco RI and Bam HI. Example of the autoradiograph (I.I% gel). The positions of SV40 Hind III fragments in kilobases are indicated.
(d) Purified SV40 DNA I digested by micrococcal nuclease as described in Methods (second from right) and redigested with Bgl I or Hpa II. The "early" fragments were ~300 pairs shorter after Bgl I (as compared to Hpa II) redigestion (see Fig. 3).

Mapping of the primary cleavage sites. The following data were exploited for mapping. First, the sizes of the fragments obtained after redigestion with Bgl I, Eco RI or Bam HI were estimated. For the majority of the fragments additive complementary ones (comprising in sum the full length of SV40 DNA) could be found. Second, the sizes of the fragments hybridized with Bgl-Taq

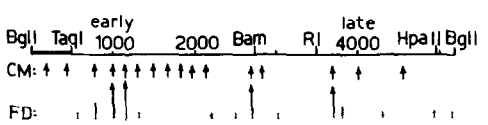

Figure 3. The map of the primary cleavage sites produced by mild micrococcal nuclease digestion of the compact minichromosomes (CM) and free SV40 DNA(FD). Only "main" cuts were located for the naked DNA with the aid of Bgl I and Hpa II redigestion (see Fig. 2d).

segment were determined. The bands on autoradiographs coincided with certain ethidium bromide stained fragments. Only one of the two complementary bands bound the label. In addition, autoradiography revealed some new bands of rather short DNA fragments not observed on stained gels due to low amount of DNA while larger complementary bands migrated close to the top of gels in poorly resolved region.

Combining the information obtained from stained gels and from autoradiographs we constructed the map of the primary cuts mainly in the "early" part of SV40 genome (Fig. 3). To confirm the localization of the mapped sites we used the sizing of the fragments obtained after Eco RI and Bam HI treatment. In most cases the fragments expected from the experiments with Bgl I were found. On the autoradiographs obtained after Eco RI and especially after Bam HI redigestion short DNA fragments were not detected. It could be explained by distant location of the "early" Bgl I - Taq I fragment from Eco RI and Bam HI restriction sites. On the other hand, some new cuts in the "late" region and close to the replication origin were mapped using these autoradiographs. Figure 3 shows the preliminary map of the primary nuclease cleavage sites.

The following conclusions could be drawn. First, the shortest distance between two neighbouring abundant sites was about 140-150 base pairs. The resolution on our gels was enough to discriminate between two bands differing by 30-50 base pairs (for fragments shorter than 3000-3500 pairs), thus, the cuts located closer than 120-140 pairs to each other could not be missed. Second, the bands were somewhat broader than those containing restriction fragments. Probably, the cuts were not precisely located but rather scattered in the regions in few dozens of base pairs (see Discussion). Third, the mapped sites were distributed unevenly, several clusters could be seen.

Specific cleavage of the naked SV40 DNA by micrococcal nuclease. When experiments similar to those described above were performed with purified SV40 DNA, a strong specificity of the first double stranded cleavage was observed. Discrete DNA fragments could be occasionally seen on stained gels at different stages of DNA digestion with micrococcal nuclease, however, they were buried under "continuous" band distribution when more than one cleavage was introduced. Alternatively, when those DNA molecules which were cleaved only once were redigested with restriction nucleases, a number of distinct bands could be detected. The band pattern differed from that described for the minichromosomes (many sites were cleaved with approximately equal probability) in two respects: (i) there were several "main" bands, part of them corresponding to those observed in the case of minichromosomes, and a few dozens (possibly, up to 100) of much weaker bands; (ii) certain bands clearly resolved for DNA from minichromosomes were not detected (or were too weak) for the naked DNA.

The specificity of the primary cleavage of SV40 DNA by micrococcal nuclease did not appear to depend on the superhelical state of DNA. At least a part of "main" bands (particularly, those 2000-3000 pairs long revealed after Eco RI redigestion) could be observed if digestion with micrococcal nuclease followed the restriction.

DISCUSSION

The non-random distribution of micrococcal nuclease primary cleavages on DNA in the compact SV40 minichromosome has been demonstrated. Several explanations of this phenomenon should be considered. Firstly, SV40 nucleoprotein complexes are heterogeneous (20,21,26) and may well have different accessibility to nuclease attack. To minimize the effect of this factor we performed our experiments with homogeneous fraction of the compact minichromosomes free of the products of virion disintegration. The second possibility is that the cleavage specificity is entirely determined by DNA sequence. In favor of this explanation are the results, showing a non-random cleavage pattern of the naked DNA. Micrococcal nuclease is known to attack primarily the DNA segments located in the spacers of nucleosomal fiber. However, if nucleosomes are randomly distributed (or can slide), the occasional occurrence of "sensitive" DNA sequences within spacers would lead to specific cutting similar to that of free DNA (see also ref. 6). Although this explanation cannot be excluded we consider it to be applicable for the "main" cleavages of the naked DNA, while the cuts observed only for the compact minichromosome are suggested to reflect the nucleosomal organization. Thus, the third possibility, that nucleosomes are distributed non-randomly around certain sites of the genome seems also be reasonable. Additionly, the distances between two neighbouring sites exceed the DNA content of the core particle, and the number of sites mapped in the "early" half of the genome is compatible with known overall number of nucleosomes. One may suggest that nucleosomes are located at several alternative positions differing by a dozen of base pairs around the primary cleavage sites.

Non-random distribution of nucleosomes observed even after limit nuclease digestion (15) in the conditions favouring their sliding (14) surely does not imply site-specific interactions of the histone octamer with DNA. Some sequence-specific proteins may direct to certain extent the location of nucleosomes on the neighbouring DNA segments. A part of the genome in the vicinity of the replication origin in papova viruses may be an example of such regions (10,27,28). On the other hand, the supranucleosomal structure may result in uneven distribution of nucleosomes. For example, the suggested dodecahedrical organization of the compact minichromosome before encasidation (12, 13,29) predicts clustering of 3-4 nucleosomes with different spacers.

Finally, sliding may play an important role in the mode of nucleosome arrangement. Recent results on the core particle structure (see e.g. ref.30, 31) suggest that DNA-histone contacts in nucleosomes are multiple, almost equivalent and have the periodicity of the DNA double helix. The sliding of nucleosomes under conditions favouring the binding of the histone octamer may occur by stepwise replacement of the DNA-histone contacts, twist rigidity of the short free DNA segments determining the same periodicity of the newly formed contacts. This means that DNA slides in caterpillar-like fashion with the step equal to (or multiple of) approximately 10 pairs. Non-random location of nucleosomes and such mechanism of nucleosome sliding in the vicinity of the sites readily cleavable by micrococcal nuclease (see above) may give also another explanation to the results of Ponder and Crawford (15).

When this manuscript was ready for publication, a paper appeared (32), where essentially the same approach was used. However,our results differ from those described (32) in several significant points: (i) in our hands compact SV40 minichromosomes may be digested to oligo- and mononucleosomal fragments at 2-4°C in 0.1 M NaCl; (ii) in fact, a part of the sites found to be preferentially attacked in minichromosomes (32) is also available for specific cleavage on the naked DNA (Fig. 3); (iii) no preferential cleavage by micrococcal nuclease at the origin of replication is observed (absence of strong 1700 pairs long fragment after Eco RI redigestion in Fig. 2 above and in Fig. 3 from ref.32). These discrepancies may be explained either by possible contamination by partially disrupted virions (32) or by the differences in nuclease digestion procedure.

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