RANDOM CLEAVAGE OF INTRANUCLEAR HERPES SIMPLEX VIRUS DNA BY MICROCOCCAL NUCLEASE

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1. Introduction

Biochemical and electron microscopic evidence indicates that the basic structure of eukaryotic chromatin consists of repeated subunits, termed nubodies or nucleosomes, containing ~200 base pairs of DNA complexed to a histone octamer [1]. A similar structure has been shown to exist for some DNA viruses replicating in the nuclei of infected cells. Thus, SV40 DNA has been shown to be associated with cellular histones in a structure similar to cellular chromatin [2]. During the replication of larger DNA viruses, belonging to the adenovirus and herpesvirus groups, the synthesis of cellular proteins is inhibited and the virions appear to lack cellular histones [3]. However, analysis of micrococcal nuclease digestion products has shown that adenovirus DNA is in a repeating-unit chromatin-like structure in the nuclei of infected cells and in the virions [4].

Herpesviruses are another group of large DNA viruses replicating in the nucleus. In the present work, we have used micrococcal nuclease as a probe for analysing the structure of the nucleoprotein of herpes simplex virus in the nuclei of infected cells.

Advantage was taken of the fact that, owing to the inhibition of cellular DNA synthesis late in infection, radioactive label is preferentially incorporated into viral DNA. Thus, viral DNA-containing structures can be studied in their intranuclear state, by following the fate of radioactive DNA during digestion of nuclei isolated from cells labelled late in infection.

2. Materials and methods

2.1. *Cells and virus*

RL cells, an established cell line from rat liver [5], were infected with herpes simplex virus type 1, strain A44 [6], at a multiplicity of infection of 10. DNA was labelled with [^{3}H]thymidine (CEA).

2.2. *Isolation of nuclei and digestion with micrococcal nuclease*

Cell monolayers were washed twice with PBS and scraped with a rubber policeman. Cells were pelleted by centrifugation, resuspended in Tris–HCl 10 mM (pH 7.4), $CaCl_2$ 1 mM, sucrose 10% (w/w) and lysed by the addition of Nonidet P40 to 0.5%. The nuclei were pelleted and washed once in digestion buffer (Tris–HCl 1 mM (pH 8.5), $CaCl_2$ 0.1 mM, sucrose 10%) and resuspended in digestion buffer at 500–800 μg DNA/ml (estimated by A_{260}). The nuclei were incubated with micrococcal nuclease (Worthington) (10 μg/ml) for the times indicated. Digestions were terminated by the addition of EDTA to 2 mM and SDS to 1%, and a fraction of each sample was applied directly to the gel after addition of bromophenol blue to 0.1%.

2.3. *Polyacrylamide gel electrophoresis*

Micrococcal nuclease digests were electrophoresed in 2.6% polyacrylamide gels with a 30:1.725 acrylamide:bisacrylamide ratio in Loening's buffer [7] containing 6 M urea. Reservoirs contained Loening's

buffer only. Gels were run in an 11 cm long slab apparatus (gel thickness 1.5 mm) at 15–40 V at room temperature. Electrophoresis was stopped when the bromophenol blue markers were near the bottom of the gel. Gels were processed for fluorography as in [8], and exposed to Kodak RP Royal 'X-Omat' films at −70°C.

3. Results and discussion

Infected cells were labelled with [^{3}H]thymidine for 1 h at 6–7 h post-infection. At least 80% of labelled DNA was viral as shown by CsCl equilibrium centrifugation. The nuclei were isolated and subjected to limited digestion with micrococcal nuclease. Control mock-infected cells were labelled with [^{3}H]thymidine for 1 h and nuclei were prepared and digested under the same conditions. The size of DNA fragments was examined by electrophoresis in 2.6% polyacrylamide gels followed by fluorography (fig.1). In contrast to the repeat subunit pattern obtained with uninfected cell chromatin, viral DNA appeared to be digested into fragments of heterogeneous size, with a gradual shift towards smaller sizes with increasing digestion times. Under the conditions of limited digestion used here, cellular DNA was not cleaved into fragments smaller than the mononucleosomal band. No similar nuclease-resistant subunit was seen in viral DNA. Under conditions of extensive digestion (not shown) which converted all cellular DNA into mononucleosomal and subnucleosomal fragments, viral DNA fragments migrated out of the gel.

That the random pattern of cleavage seen in fig.1 is specific for viral DNA is demonstrated by the experiment shown in fig.2. Cellular DNA was labelled with [^{3}H]thymidine before infection. Infection was allowed to proceed for 7 h and the nuclei were digested with micrococcal nuclease. Figure 2 shows that the cellular chromatin of infected cells was digested in the same fashion as uninfected cell chromatin. This eliminates the possibility that an endonuclease present in infected cells cleaves DNA non-specifically inside nucleosomes.

Micrococcal nuclease cleaves the internucleosomal regions of cellular DNA preferentially, while intranucleosomal DNA is protected by its association with histones [1]. Since the synthesis of cellular proteins is arrested during the replication of herpesviruses [3], viral DNA is presumably not associated with cellular histones. Little is known of the intranuclear state of

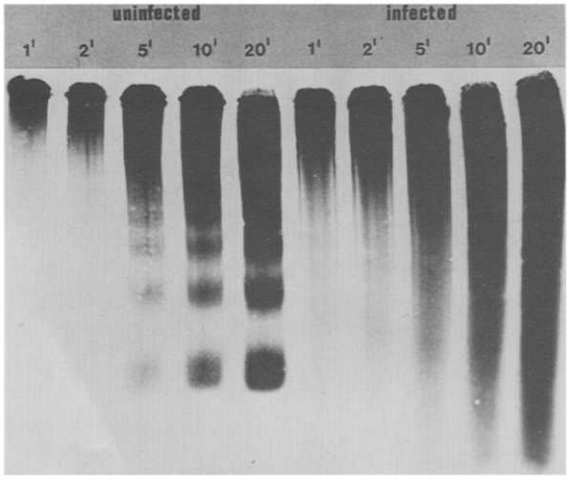

Fig.1. Limited digestion of cellular and viral DNA in nuclei by micrococcal nuclease. Cells were labelled for 1 h at 6–7 h post-infection or mock-infection. Nuclei were isolated immediately after labelling and digested for the times indicated on top of each slot. Digests were then electrophoresed and fluorographed.

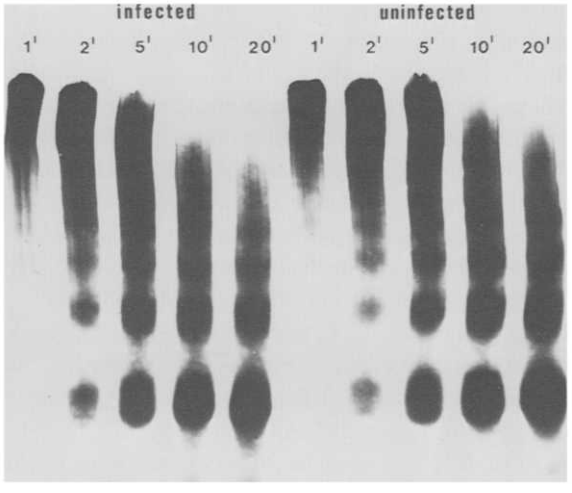

Fig.2. Limited micrococcal nuclease digestion of prelabelled cellular DNA in infected and uninfected nuclei. Cells were labelled for 9 h immediately before infection. Nuclei were isolated 7 h after infection and digested for the times indicated on top of each slot. Electrophoresis and fluorography were as in fig.1.

viral DNA and its possible association with cellular or virus-induced proteins. The striking difference which we have observed between the patterns of digestion of viral and cellular DNA by micrococcal nucleases indicates that the intranuclear state of herpesvirus DNA, in contrast to other DNA viruses [2,4], is different from that of cellular chromatin. The heterogeneous size of digestion fragments suggests that nucleosome-like repeat subunits are either absent from the 'chromatin' of herpesviruses or, if present, differ from cellular nucleosomes by failing to protect DNA against micrococcal nuclease attack.

Acknowledgements

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