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Nuclease Sensitivity of Adenovirus Type 2 Chromatin in Lytic Infection

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SUMMARY

We have investigated the sensitivity of adenovirus type 2 naked DNA and chromatin at 5 h and 20 h after infection to digestion by DNase I, micrococcal nuclease and endogenous nuclease between map coordinates 11·3 and 18·0 (*Sma*I-F fragment) using a terminal labelling method. Infected cell nuclei were gently digested with nucleases, DNA was extracted and digested to completion with *Sma*I and the fragments shorter than the *Sma*I-F fragment mapped by hybridization with a 708 base pair probe co-terminal with the *Sma*I-F fragment. Early chromatin contained hypersensitive sites at 16·0 and 14·3. These sites became minor cleavage sites in late chromatin and new hypersensitive sites appeared at 13·5 and 13·0. The change in the location of the hypersensitive sites in the course of infection correlated with the early to late switch in the transcription pattern in this region and the early to late change in the overall structure of adenovirus chromatin.

Nucleases have been widely used as probes to study the organization of eukaryotic and viral chromatin (Mathis *et al.*, 1980; Elgin, 1981; Weintraub & Groudine, 1976; Brown & Weber, 1980; Cremisi, 1981; Daniell *et al.*, 1981; Saragosti *et al.*, 1980; Mirza & Weber, 1982; Scott & Wigmore, 1978). Micrococcal nuclease (MNase) has been particularly useful in deciphering the nucleosomal structure of chromatin. In all these studies it has been tacitly assumed that MNase cleaves DNA at random without regard to nucleotide sequence. This assumption has been challenged recently (Elgin, 1981; Dingwall *et al.*, 1981; Horz & Altenburger, 1981; Keene & Elgin, 1981). We, as others, have made extensive use of MNase to study the structure of adenovirus chromatin (Brown & Weber, 1980; Daniell *et al.*, 1981; Mirza & Weber, 1982; Sergeant *et al.*, 1979; Tate & Philipson, 1979). These studies have shown that during the early phase of infection viral DNA is associated with cellular histones in a structure resembling cellular chromatin, while later progeny molecules are condensed by viral basic proteins into a novel nucleoprotein complex unlike cell chromatin. These studies were conducted by looking at the protected DNA fragments after extensive digestion with MNase. Here we report experiments which examine this shift in viral chromatin structure by a completely different approach, namely by mapping the nuclease sensitive sites after very mild digestion with MNase, DNase I and endogenous nuclease in a defined region of the adenovirus type 2 (Ad2) chromosome. This region is defined by the *Sma*I-F fragment and encompasses the major late promoter and upstream sequences. We mapped specific nuclease-sensitive sites and show that their location changes during the lytic cycle in accordance with the change in viral chromatin structure.

The location of nuclease-sensitive sites was determined at 5 and 20 h after infection using the end-labelling method devised for this purpose by Wu (1980). In all experiments HEp-2 cells were infected with purified Ad2 at multiplicities of 100 p.f.u./cell for the experiments carried out early after infection and 10 p.f.u./cell for those performed late after infection (Mirza & Weber, 1979). The MNase digestion pattern of infected cell nucleoprotein was compared with that of

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purified DNA extracted from similarly infected cells. After digestion with nuclease for increasing periods of time, the total DNA was extracted and digested to completion with *Sma*I. The DNA was electrophoresed in an agarose gel, transferred to nitrocellulose and hybridized with a probe of 708 base pairs (map coordinates 11·3 to 13·2) obtained by subcleaving a *Hin*dIII-C-containing plasmid with *Sma*I and *Acc*I. Because the probe is co-terminal with the left boundary of the *Sma*I-F fragment, the lengths of the labelled subfragments define the distance between the *Sma*I cut and the partial nuclease cuts. Mild digestion revealed a preferential nuclease cleavage site in early chromatin at map position 16·0, which is at the IVa2 promoter or about 150 base pairs upstream from the major late promoter (Fig. 1 *b*, lane 2). Both promoters are active at this time. By contrast, the most sensitive site in late chromatin was at map position 13·0 (Fig. 1 *c*, lane 1). Although the patterns of digestion were very different between early and late chromatin, both were eventually digested at five sites. These sites appeared to be determined by DNA sequence, as they also appeared when naked DNA was extensively digested with MNase (Fig. 1 *a*). However, nucleoprotein was 25 times more resistant to digestion and the unique fragments appeared against a lower background as compared with naked DNA. Furthermore, in DNA all the sites seemed equally sensitive whereas in early chromatin the site at 16·0 was significantly more sensitive than the others (Fig. 1 *b*, lane 2). It is interesting that the five digestion sites were at approximately equal intervals of about 200 to 300 base pairs, which coincides with the distance expected from nucleosome spacing (Mirza & Weber, 1982).

To determine whether the same or different nuclease-sensitive sites are revealed on chromatin by different nucleases, we investigated the sensitivity of chromatin to DNase I and also to endogenous nuclease (McKenna *et al.*, 1981) using the same methods as above. The same region of the Ad2 genome was studied using the *Sma*I–*Acc*I probe.

Digestion of infected cell nuclei with these nucleases gave apparently identical results. Early chromatin was preferentially digested at two sites, namely 14·3 and 16·0 (Fig. 2 *b*, lane 3). A protected region around 13·9 was observed in all early chromatin experiments with DNase I and endogenous nuclease. Naked DNA did not manifest any of these features, being digested non-specifically with the exception of faint and diffuse bands at 16·7, 13·5 and 13·0 (Fig. 2 *a*).

The digestion of late chromatin presented a radically different picture. Both nucleases gave similar digestion patterns (Fig. 2 *c*). Two nuclease-sensitive sites were located at 13·5 and 13·0 as well as a frequently cut region between 14·0 and 14·5. These cleavage sites were also observed with MNase (compare Fig. 1 *c* with Fig. 2 *c*) and contrast sharply with those obtained in early chromatin. In addition to these hypersensitive sites three very faint bands were also always present at 16·0, 16·7 and 17·1. Interestingly, the cleavage site at 16·0 coincides with the location of the IVa2 promoter on the l-strand and is hypersensitive in early but not late chromatin.

During infection of human cells, the human Ad2 genome undergoes several changes in its chromatin structure concomitant with different modes of transcriptional activity. Studies with MNase suggest that the viral genome sheds its core proteins shortly after uncoating and acquires a chromatin structure which resembles that of the cells based on histones in the early phase of infection (Daniell *et al.*, 1981; Sergeant *et al.*, 1979; Tate & Philipson, 1979; Mirza & Weber, 1979). With the onset of DNA replication at 6 to 8 h, this type of chromatin becomes the predominant form up to 16 h and is characteristic of replicating or recently replicated molecules (our unpublished results). As the late phase progresses the bulk of late chromatin assumes a structure similar to the viral core, even in the absence of virus particle assembly (Brown & Weber, 1980; Daniell *et al.*, 1981; Mirza & Weber, 1982). Therefore, the nuclease-sensitive sites observed at 20 h are a consequence of the special features of late viral chromatin. The bulk of this nucleoprotein complex is likely to consist of core-like pre-encapsidation complexes containing the precursor to the viral core protein, PVII, and other viral proteins as well (Weber & Philipson, 1984). How might these complicated and radical changes affect the susceptibility of a specific region of the genome to nucleases? To facilitate comparison of the results, the nuclease sensitivity maps are presented together in Fig. 3. In the case of MNase and to a small extent also in the case of DNase I after extensive digestion, chromatin tends to be cleaved in the same sites as naked DNA. In spite of this preferential cleavage, the kinetics of digestion clearly show the modulating effect of chromatin. The band patterns and the location of hypersensitive

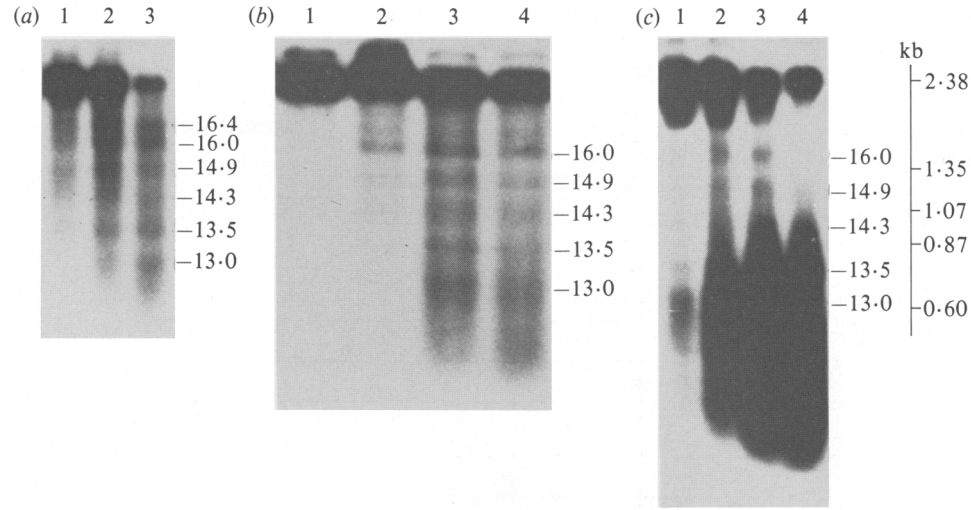

Fig. 1. Specific cleavage of naked adenovirus type 2 DNA and chromatin with MNase. Infected cell nuclei were isolated according to Wu (1980). Nuclei at 1 × 10^8 per ml and DNA at 100 μg/ml were lightly digested with MNase (Worthington) at 25 °C. The reaction was terminated by the addition of EDTA and SDS to 20 mM and 0·5% respectively. The suspension was then digested overnight at 37 °C with 100 μg/ml proteinase K (Merck). The phenol–chloroform-extracted DNA was dialysed against TE buffer (10 mM-Tris–HCl pH 7·5, 1 mM-EDTA) for 2 days. *Sma*I digestions were carried out according to the manufacturer's directions (Bethesda Research Laboratories) at 4 to 5 enzyme units per mg DNA for 4 h. DNA samples were electrophoresed in 1% agarose gels. The DNA was blotted onto nitrocellulose and prehybridization and hybridization were carried out according to Wahl *et al.* (1979). The Ad2 probe (11·3 to 13·2) was obtained by *Sma*I–*Acc*I double digestion of a pBR322 plasmid containing the Ad2 *Hin*dIII-C fragment and isolated from gels by electroelution. Purified fragment was labelled by nick translation to a sp. act. of 2 × 10^8 to 4 × 10^8 c.p.m. per μg DNA. Nick-translated *Hae*III fragments of the replicative form of ϕX174 DNA were used as size markers. (*a*) Purified Ad2 DNA digested with 2 units of MNase/μg DNA for 1 min (lane 1), 3 min (lane 2) or 9 min (lane 3). (*b*) Nuclei digested with MNase 5 h after infection for 0 (lane 1) or 3 min (lanes 2, 3 and 4) with increasing concentrations of enzyme (5, 15 or 50 units). (*c*) Nuclei digested with 50 units of MNase/μg DNA for 0·3 min (lane 1), 1 min (lane 2), 3 min (lane 3) or 9 min (lane 4), at 20 h after infection.

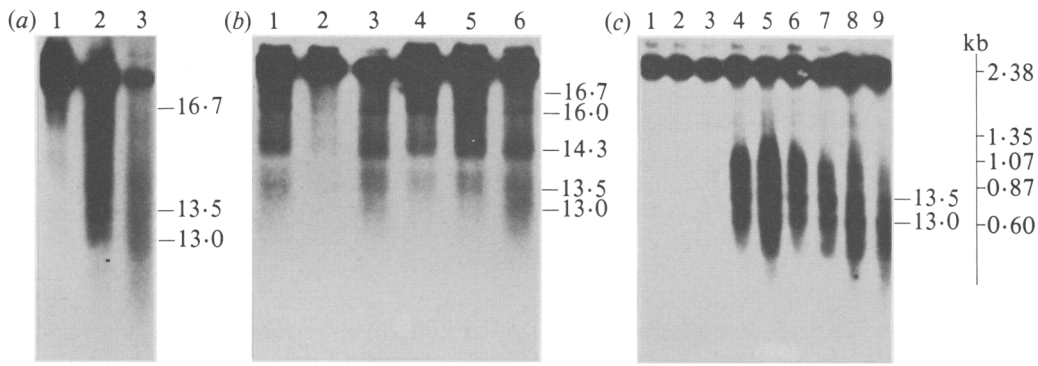

Fig. 2. Specific cleavage of Ad2 DNA and chromatin with DNase I and endogenous nuclease. The experiment was carried out essentially as described in Fig. 1. Cleavage by endogenous nuclease was obtained by simply incubating the nuclei in buffer for increasing periods of time. (*a*) Purified Ad2 DNA was digested with 4 units DNase I for 0·3 min (lane 1), 1 min (lane 2) or 3 min (lane 3). (*b*) Nuclei were digested 5 h after infection by endogenous nuclease for 30 min (lane 1) or 50 units DNase I for 0, 0·3, 1, 2 and 4 min (lanes 2 to 6). (*c*) Nuclei were digested 20 h after infection by endogenous nuclease for 0, 0·3, 1, 3 or 9 min (lanes 1 to 5) or 20 units of DNase I for 0·3, 1, 3 or 9 min (lanes 6 to 9).

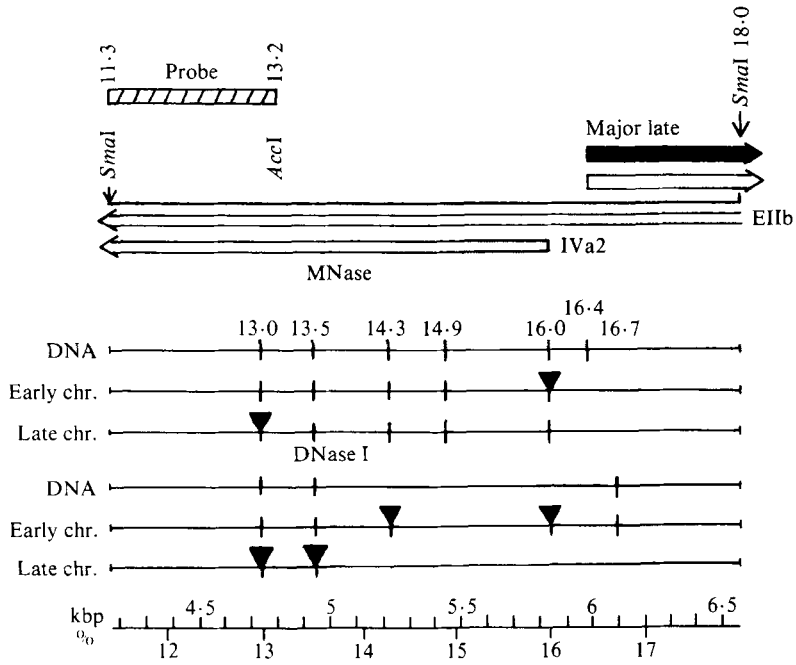

Fig. 3. Summary of results: nuclease sensitivity of Ad2 DNA and chromatin (chr.) early and late after infection. The transcription map of the region studied shows the early RNAs as open arrows and the late RNA as a closed arrow. Major cleavage sites are indicated by closed arrowheads, minor sites by vertical bars.

sites were clearly different in early and late chromatin. This result supports previous reports which demonstrated major differences between early and late chromatin based on simple digestion by MNase (Brown & Weber, 1980; Daniel *et al.*, 1981; Sergeant *et al.*, 1979; Tate & Philipson, 1979). MNase and DNase I revealed a single hypersensitive site at map position 16·0 in early chromatin. DNase I also detects a second site at 14·3 which was not cleaved preferentially by MNase. Therefore, there is one hypersensitive site which is detected by both exogenous nucleases as well as endogenous nuclease at or very near the IVa2 promoter and some 150 base pairs upstream from the major late promoter at 16·4. Larsen & Weintraub (1982) demonstrated a DNase I- and S1 nuclease-hypersensitive site at 16·4 in integrated Ad2 sequences in transformed cells where the major late promoter (16·4) is inactive. They suggested that the hypersensitive site may be related to the potential for hairpin formation in this region. The susceptibility of late chromatin 20 h after infection to these nucleases is dramatically altered. The region around the promoters becomes relatively protected, while the region between 13 and 14 map units becomes the primary site of cleavage. The principal hypersensitive site detected by all nucleases is at 13·0. A second site at 13·5 is digested preferentially only by DNase I and endogenous nuclease.

The change in the location of the hypersensitive sites between early and late chromatin parallels the change in the overall structure of adenovirus chromatin and the accompanying changes in the pattern of transcription. A similar shift in hypersensitive sites during the infection cycle was reported in simian virus 40, which associates with cellular histones throughout infection and does not go through the radical switch from an early to a late chromatin configuration (Cremisi, 1981). As the bulk of late adenovirus chromatin consists of core-like structures, the pattern of nuclease digestion reflects their particular organization rather than the structure of the few transcriptionally active molecules. Therefore, results on late chromatin cannot be interpreted in terms of hypersensitivity related to transcription. It is not possible to say at present whether the hypersensitive sites seen in early chromatin are related to

transcription, because similar sensitive sites were detected all along the viral chromosome without any apparent relationship to promoters (results not shown). A similar conclusion was reached in a report published while this paper was in preparation (Fedor & Daniell, 1983).

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