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Parental adenovirus DNA accumulates in nucleosome-like structures in infected cells

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ABSTRACT

Micrococcal-nuclease digestion of adenovirus 2(ad 2) infected HeLa cell nuclei early after infection has been used to investigate the nucleoprotein nature of parental viral DNA. Viral DNA is more susceptible to nuclease digestion than cellular DNA. The pattern of digestion products changes as digestion proceeds from an indistinct pattern 1 hour post infection(pi) to a nucleosome-like pattern at 6 hours pi. The major differences between viral and cellular nucleoprotein products were i) a subnucleosome fraction from viral DNA and ii) the repeat size of DNA in viral nucleosomes was 165 base pairs and in cellular nucleosomes, 195 base pairs. Up to 50% viral DNA in nuclei 6 hours pi seems to be in nucleosome-like structures. Such patterns are not seen on digestion of partially-uncoated virus or isolated cores.

INTRODUCTION

The pattern of transcription of the adenovirus genome changes dramatically during the course of infection of cultured human cells. At early times, messenger RNA has been shown to be transcribed from four regions of the genome (1,2), each region having at least one promoter (3). Following the onset of DNA replication, greater than 90% of the viral messenger RNA is transcribed from a single major promoter at map position 16.3 (4). The reason for this switch in promoter specificity is unknown. Both early and late mRNAs are tran -scribed by cellular RNA polymerase II (5,6), and no major changes seem to occur in the nature of the enzyme between early and late times to account for the switch in specificity of promoter binding. This does not rule out the possibility that there may be as yet unidentified virus-coded proteins involved in determining promoter specificity.

An alternative explanation for the change in the pattern of transcription between early and late times is that specificity is conferred by the template and not by the RNA polymerase. The nature of the template can be considered at two levels, that of the nucleic acid (single versus doublestranded) and that of any possible DNA-binding proteins which may associate with it. We

have therefore made an attempt to define the nucleoprotein nature of the viral tempate early after infection.

Adenovirus DNA inside the infecting particle is associated with at least three species of viral-coded proteins. Two of these proteins, polypeptides V and VII, of respective molecular weights 48,000 and 18,500 daltons, are highly basic in nature (7). The third protein seems to be specifically associated with the molecular ends of the DNA (8).

During the initial stages of viral infection, partially uncoated virions, probably lacking penton and fibre proteins which are situated at the vertices of the capsid, are transported to the nucleus where the nucleoprotein core alone is thought to enter via the nuclear pores (10).

Previous results from DNAase I digestion studies suggested that the viral DNA, on entry into the nucleus, is released from its core proteins (11), a finding which should be reconsidered in the light of what is now known about protein-DNA interactions in chromatin. Even if the DNA is completely uncoated, it is unlikely that such a highly-charged molecule will exist free in the nucleus. Therefore the question arises as to whether the DNA could associate with cellular histones after decoating.

The strategy employed in this study was to infect cells with ^{32}P-labelled ad 2 and to probe for changes in the nucleoprotein nature of the viral DNA during the early phase of infection using micrococcal nuclease. The products of digestion of cellular chromatin by this enzyme have been extensively studied, and have led to our current concept of the subunit organisation of eukaryotic chromatin. The basic unit of chromatin, the nucleosome, contains after extensive micrococcal nuclease digestion, 140 base pairs of DNA and two each of the four histones H2A, H2B, H3 and H4 (reviewed ref. 12). The presence of these nucleosomes in infected, digested nuclei provides an internal control against which can be judged changes that occur in the viral nucleoprotein structure during infection.

The results of these experiments show that the viral nucleoprotein structure changes slowly throughout the early phase of infection towards a pattern which is similar to that of the cellular chromatin.

MATERIALS AND METHODS

^{32}P virus, pentonless virus and core preparation

^{32}P ad 2 was prepared and purified as described by Johansson et al. (13). The specific activity of the virus was between 1 and 6 x 10^6 cpm per µg

equivalent of viral DNA. The particle: infectivity ratio of such preparations was 15-30 particles per fluorescent focus-forming unit (FFU) when freshly prepared. Such virus was used within 1 week of preparation. Pentonless virus and pyridine core were prepared by the method of Prage et al. (14).

Infection of cells and preparation of nuclei

HeLa-S3 spinner cells at 3 x 10^5/ml in Eagles spinner medium with 7% calf serum were prelabelled with 1 μCi/ml of ^{3}H-methyl thymidine (40-60 Ci/mM) for 16 hours. For infection, cells were concentrated to 1 x 10^7/ml in fresh spinner medium, virus was added at multiplicities of infection (moi) ranging from 200 to 2000 particles per cell and allowed to adsorb for 1 hour. Spinner medium with 5% calf serum was added to decrease the cell concentration to 4 x 10^5/ml. Samples were withdrawn, at the times indicated in the text, into ice-cold phosphate-buffered saline (PBS). Cells were washed three times with PBS and then resuspended at approximately 1 x 10^7/ml in 10 mM Tris-HCl pH 7.9, 1mM $CaCl_2$, 0.3M sucrose and 0.5% NP40. The cell suspension was vortexed for 15 seconds, allowed to stand on ice for 15 minutes, revortexed and spun at 2000g for 10 minutes to pellet nuclei. The nuclei were washed once with the same buffer, then resuspended in 10mM Tris-HCl pH 7.9, 0.1mM $CaCl_2$ and 0.3M sucrose. The nuclei were counted and controlled for absence of cytoplasmic tags by phase contrast microscopy. Finally, nuclei were washed once more and resuspended in the low calcium buffer at a concentration of approximately 4 x 10^7/ml.

Micrococcal nuclease digestion

Nuclei, prepared as described above, were digested with 15 units of micrococcal nuclease (P.L. Biochemicals Inc.) per ml of nuclei at 37°C. Digestions were stopped by the addition of EDTA to 20 mM final concentration and rapid chilling on ice. At this stage samples were analysed for acid-soluble products by perchloric acid (PCA) precipitation. The nuclear pellet was then removed by centrifugation at 2000g for 10 minutes at 4°C and the supernatant was further analysed.

Pentonless virus and pyridine core in 10 mM Tris-HCl pH 7.9, 0.1mM $CaCl_2$ were digested at a concentration of 0.5 OD_{260} units/ml with 5 units micrococcal nuclease/ml for various times at 37°C. Reactions were stopped as with nuclear digestions.

Isokinetic sucrose gradient centrifugation

The nuclear supernatant obtained after micrococcal nuclease digestion of infected cell nuclei was analysed by isokinetic sucrose gradient centrifugation

(15). The gradient parameters were: meniscus sucrose concentration 21.4% (^{W}/v); reservoir sucrose concentration 40.4% (^{W}/v); mixing volume 12.6 ml; gradient volume 11.5 ml, in 10mM Tris-HCl pH 7.9, 5mM EDTA. Gradients were spun in a Beckman SW40 rotor at 200,000g for 30 minutes at 4^{o}C. The top fractions from these gradients were dialysed against the gradient buffer, then reanalysed for nucleosomal structures on a second gradient whose parameters were: meniscus concentration 5.1% (^{W}/v); reservoir concentration 30.5% (^{W}/v); mixing volume 10.3 ml; gradient volume 11.5ml. These gradients were spun in the SW40 rotor at 150,000g for 16 hours at 4^{o}C.

Extraction of DNA and Gel electrophoresis

DNA was extracted by the method of Pettersson and Sambrook (16). After ethanol precipitation, samples were solubilised in 10mM Tris-HCl pH 7.9, 1 mM EDTA and analysed by slab gel electrophoresis (20 x 16 x 0.15) on either 1.4% agarose or 6% polyacrylamide gels (29gms acrylamide: 1gm bisacrylamide) with 90mM Tris-borate pH 8.3, 1 mM EDTA buffer. The running buffer was half-strength gel buffer.

Restriction enzymes and Hybridization

Restriction enzymes were purified as described (17). Ad 2 cleaved with Sma I or Hind III was transfered to cellulose nitrate membrane filters by the method of Southern (18) and ^{32}P parental viral DNA was hybridized to such blotted DNA as described in (17).

RESULTS

Characterisation of parental ^{32}P adenovirus in infected nuclei

^{32}P ad 2 attached rapidly to HeLa cells and maximal numbers of virus particles reached the nucleus by between 1 and 2 hours pi (not shown). ^{32}P- parental virus DNA was found to be intact up to 6 hours pi by agarose gel electrophoresis after extraction from nuclei (not shown), in confirmation of previously published results (19).

The kinetics of digestion of parental DNA by micrococcal nuclease in the cell nucleus were investigated at various times early after infection. Figure 1 shows that the rate of digestion of parental DNA is faster than that of cellular DNA prelabelled with ^{3}H thymidine. At 1 hour pi, parental virus DNA is rendered acid-soluble initially 25% faster than cellular DNA, whilst later pi the initial rate increase is nearly 100%. Purified ad 2 DNA in the presence of uninfected HeLa cell nuclei is digested 5 times faster than cellular DNA.

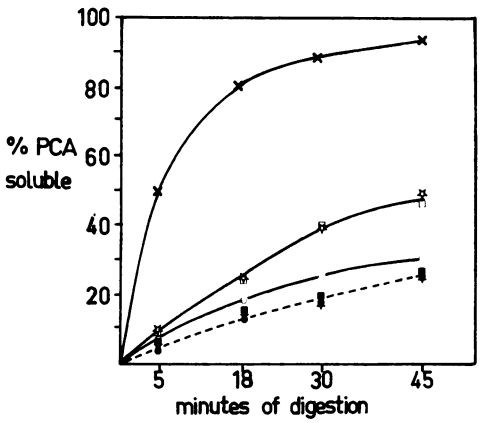

Figure 1. Kinetics of micrococcal nuclease digestion of parental ad 2 DNA in HeLa cell nuclei. Nuclei were prepared at 1hr (○/●), 3hrs (☆/★) and 6 hrs (□/■) pi, digested and percent PCA solubility was determined. Open symbols- viral DNA; closed symbols- cellular DNA. x- digestion of deproteinised viral DNA.

In order to analyse the nucleoprotein products released from infected nuclei after digestion, the nuclear supernatant was centrifuged through sucrose gradients under conditions where intact virus particles will sediment to the middle of the gradient. Figure 2A shows that when nuclei were isolated 1 hour pi and digested briefly, a large peak of ^{32}P material sedimented at about 600S relative to the virus marker (750S). There was a smaller peak cosedimenting with the ^{3}H cellular label up to 250S and a minor peak at about 900S. There was no cellular material sedimenting faster than

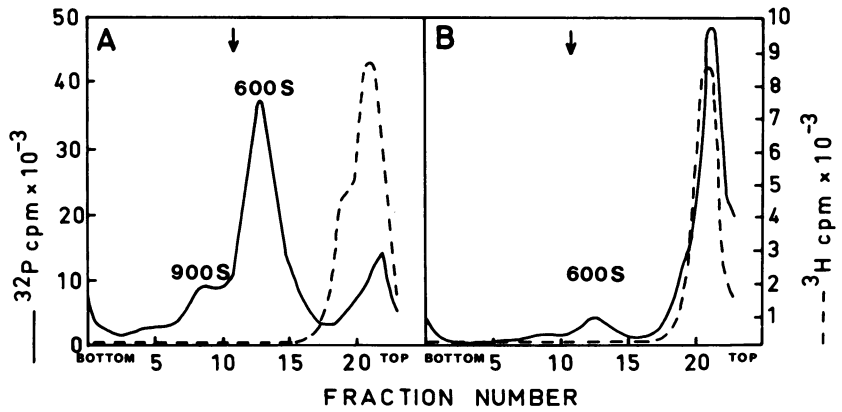

Figure 2. Isokinetic sucrose gradient analysis of ad 2 (——) and cellular (----) nucleoproteins released after 1 minutes micrococcal nuclease digestion of infected nuclei. A- 1 hours pi; B- 3 hours pi. Arrow- position of marker virus.

300S. The same pattern is seen at 3 hours pi, although there is much less label in the 600 and 900S peaks relative to the material at lower S values. Following longer periods of digestion, the percent ^{32}P material in the 600S peak was greatly reduced at both times until it represented approximately 1% of the solubilised material. The other 99% of radioactivity remained in the upper fractions of the gradient (not shown).

From consideration of size alone, the 600S material may represent partially uncoated virus released from the nuclear membrane during nuclease digestion. If this material was fixed and banded in a CsCl gradient, it had a buoyant density fractionally less than marker virus (not shown) as would be expected if digestion of the virus particles had caused the loss of some DNA. This material was not analysed further.

Viral DNA in nucleosome-like structures

The next step was to reanalyse the material sedimenting between 0 and 300S on a second isokinetic sucrose gradient, to study in particular those structures sedimenting up to 35S, corresponding to the size range of cellular mono-, di- and trinucleosomes. Figure 3 illustrates the profiles obtained from a kinetic experiment, digesting infected nuclei, taken at 1 and 3 hours pi, for increasing periods of time. The major viral nucleoprotein component evident after brief digestion of nuclei collected 1 hour pi is a 3.5S subnucleosome. Also there is material cosedimenting with the mononucleosome (monomer) peak of cellular chromatin. The identity of the cellular nucleoprotein peaks was confirmed by extraction and separation of DNA by polyacrylamide gel electrophoresis to display the typical ladder pattern of approximately 200, 400, 600 base pairs etc as described later.

If the sucrose gradient profiles for 1 and 3 hours pi are compared, certain differences are apparent. At 3 hours pi, the first component appearing cosediments with the cellular monomer and, as digestion proceeds, a peak appears in the subnucleosomal region. Radioactivity in this peak increases until, after long digestion, it is the major component in the gradient. In the region of the larger nucleoprotein structures, the distribution of ^{32}P viral material more closely resembles the pattern seen with the cellular nucleosomes than was the case at 1 hour pi. At 6 and 8 hours after infection (not shown), the profiles very closely follow those seen at 3 hours except that the proportion of radioactivity in the viral subnucleosome is lower relative to the monomer peak.

Characterisation of viral DNA from nucleosomes

DNA was isolated from the nucleoprotein components less than 300 S after

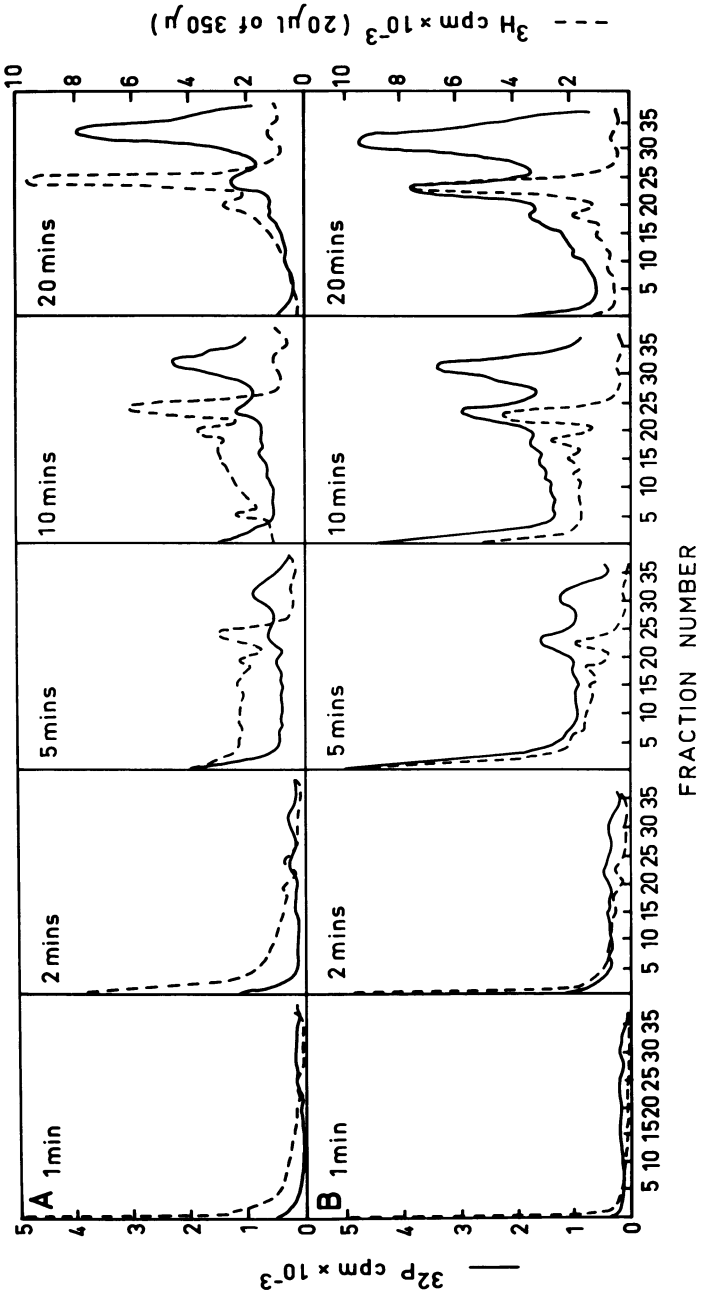

Figure 3 Isokinetic sucrose gradient analysis of nucleoprotein structures released from infected nuclei 1 hour (A) and 3 hours (B) pi by micrococcalnuclease digestion. Parental virus DNA (——) ; cellular DNA (---). Sedimentation from right to left. Subnucleosomes peak in fractions 31 and 32; cellular and viral monomers in fractions 20-25; dimers in fractions 17-19.

separation on sucrose gradients and its size distribution analysed by gel electrophoresis. Figure 4 compares the ethidium bromide-stained gel pattern obtained, indicating the size of the cellular protected DNA fragments, with an autoradiograph of the same gel to display the protected viral DNA fragments after varying times of digestion of nuclei isolated 1,3 and 6 hours pi. The digestion time used in these experiments were relatively long and therefore the higher oligomers are not seen since they are digested further. The monomer bands, however, have been sized and the protected DNA fragment, both viral and cellular, is 145 base pairs at limit digestion,which agrees well with previously reported values for the core nucleosome of 140 base pairs. Also, there is a second monomer species in both viral and cellular digests of 185 base pairs, the relative proportions of the two monomer species being the same in both cases. The existence of at least two monomer

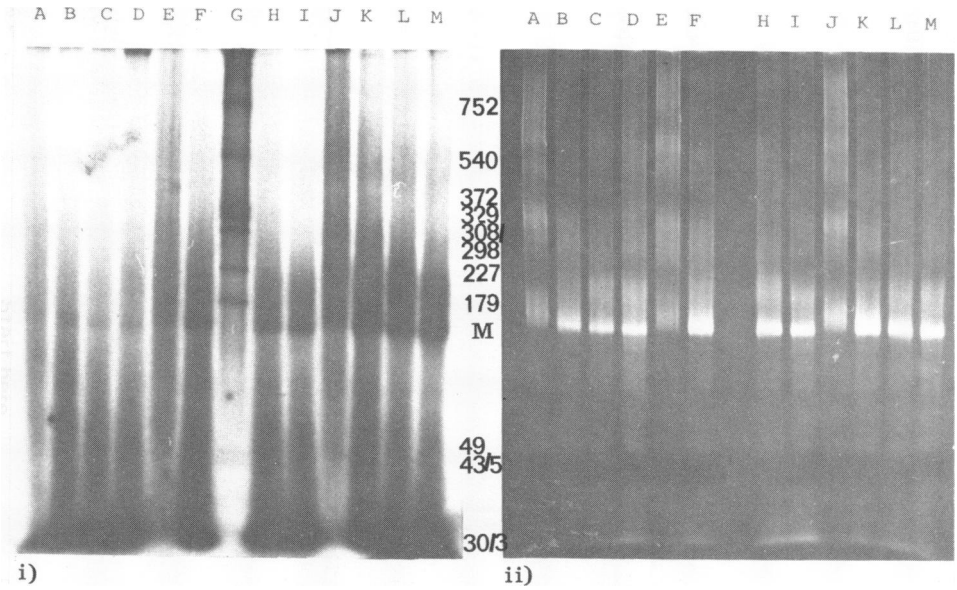

Figure 4. Size fractionation of DNA in nuclease-solubilised material. Nuclei from cells infected with ^{32}P ad 2 were isolated 1hr pi (tracks A,B,C,D), 3hrs pi (E,F,H,I) and 6 hrs pi (J,K,L,M) and digested with micrococcal nuclease for 5 mins (A,E,J), 20 mins (B,F,K), 30 mins (C,H,L) or 60 mins (D,I,M). DNA was extracted from the nuclease-solubilised material and run on a 6% polyacrylamide gel. i) autoradiograph of gel showing ^{32}P viral DNA ii) ethidium bromide stained pattern of same gel showing cellular DNA. Size marker (track G) is ^{32}P SV 40-Hae III restriction enzyme digest-sizes shown in base pairs. M- position of monomer band.

species at limit digest has been reported previously (20). In addition, subnucleosomal DNA bands can be seen in both viral and cellular digests. On 12% polyacrylamide gels (not shown), the major subnucleosomal viral DNA species was found to contain 20 to 30 base pairs DNA. DNA extracted from the subnucleosomal regions of sucrose gradients as in figure 3 contains predominantly these small-sized DNA fragments. The distribution of radioactivity in the viral monomer and subnucleosomal DNA bands seen on these gels reflects the change in peak heights seen on the sucrose gradients, i.e. an increase in monomer length DNA relative to subnucleosomal length DNA as infection proceeds.

In order to examine the viral and cellular oligomers, separate shorter digests were performed. Analysis of protected DNA fragments on 1.4% agarose gels followed by densitometry produced the patterns seen in figure 5. By comparison with a ØX174 DNA-HaeIII restriction enzyme cleavage as size marker (not shown), the sizes of the oligomers could be calculated, and, knowing the size of DNA protected by the core nucleosome, it was possible to calculate the minimum size of DNA linking each unit. The most striking feature of this analysis is that the repeating unit for the viral nucleoprotein is shorter than that for the cellular chromatin, being very close to 165 base pairs for the virus and 195 base pairs for the cellular chromatin. Therefore there may be only 20 base pairs linker DNA between viral nucleosomes, compared with 50 base pairs for cellular chromatin.

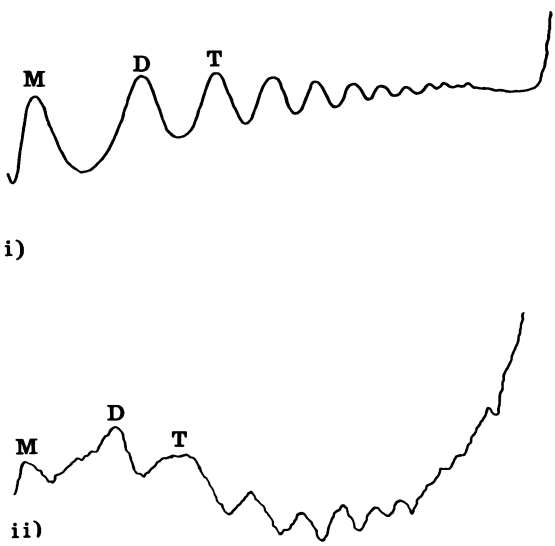

Figure 5. Densitometer tracing of 1.4% agarose gel to display oligomeric pattern of i) cellular and ii) ^{32}P viral micrococcal nuclease-protected DNA fragments. M-monomer; D-dimer; T-trimer i) tracing of ethidium bromide -stained gel and ii) tracing of autoradiograph of same gel.

These procedures analyse only those structures which are released from the nuclei following digestion and it could be argued that they are not a representative fraction of the overall viral nucleoprotein structures. That this is not the case has been established by extracting DNA from the pelleted material following nuclease digestion of infected nuclei and by analysing on gels (not shown). A very similar pattern to that obtained with the nuclease-solubilised material is obtained except that there is a higher proportion of larger molecular weight DNA fragments as was expected. Particularly at 1 hour pi there are considerable amounts of ^{32}PDNA at the top of the gel which presumably reflect the presence of partially uncoated virions as described earlier. Estimation of radioactivity in the monomer and subnucleosomal viral DNA bands on gels analysing both nuclease-released material and nuclear pellets leads to the conclusion that at least 50% of the viral DNA is in a nucleosomal-type configuration at 3 and 6 hours pi.

Reconstitution of nucleosomes resulting from micrococcal nuclease digestion

In order to rule out the argument that the viral DNA digestion patterns are a result of reassociation of cellular histones with the viral DNA during the course of digestion, control experiments were carried out. ^{32}P deproteinised viral DNA was nuclease- digested in the presence of uninfected HeLa cell nuclei and the products of digestion were analysed by sucrose gradient centrifugation and polyacrylamide gel electrophoresis (figure 6). ^{32}P DNA was found to sediment as a mononucleosome by sucrose gradient analysis and this was confirmed by extracting DNA from this peak and subsequent polyacrylamide gel analysis. However, oligomers higher than trimers were never seen. By repeating these experiments under a variety of conditions of low ionic strength and varying Ca^{++} concentrations, the maximal percentage of ^{32}P DNA in these reconstituted nucleosomes was 10%. Since, at 3 and 6 hours pi, the viral nucleosome structures can be estimated to include at least 50% of the viral DNA, it is believed that, although reconstitution of cellular histone with viral DNA may account in part for the nucleosomal structures seen, it is not sufficient to account for all such structures, nor to cause the observed higher oligomer structures.

Micrococcal nuclease digestion of adenovirus particles and core structures

Corden et al. (9) have investigated the pattern of micrococcal nuclease digestion of ad DNA in particles and they interpret their data as showing that the DNA has a chromatin-like organisation. If this were the case, then it might be concluded that the protection patterns seen early after infection are a consequence of the retention of viral proteins to form a core struc-

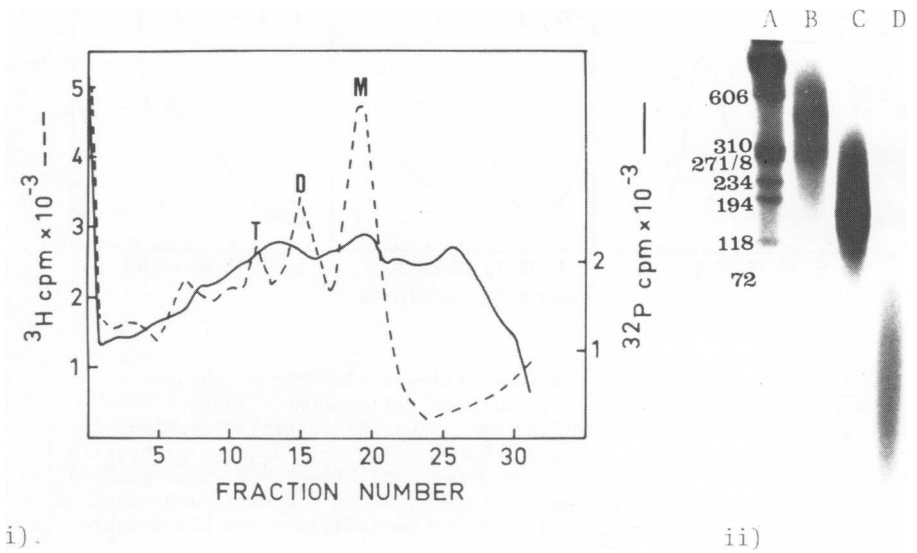

Figure 6. ^{32}P Ad2 DNA was added to ^{3}H uninfected nuclei in proportions equivalent to 1000 genomes per nucleus. Digestion was carried out as described for infected nuclei except that 0.025mM $CaCl_2$ was used. After 20 minutes digestion (7.0% ^{32}P PCA soluble; 2.6% ^{3}H PCA soluble), nuclease-released material (100% ^{32}P; 4.3% ^{3}H material) was analysed on i) isokinetic sucrose gradients to display nucleosomal structures. ii) fractions 13, 14 (track B), 19,20 (track C) and 25,26 (track D) from sucrose gradient pooled, DNA extracted and run on 8% polyacrylamide gel with ØX174-HaeIII restriction enzyme digest (track A) as size marker (sizes shown in base pairs).

ture in the nuclei. Therefore, the pattern of micrococcal nuclease digestion of isolated viral cores was reinvestigated to see whether the patterns obtained reflected those seen in the infected cell. Pentonless virus particles, to represent partially uncoated virus, were included in these experiments. Numerous attempts were unsuccessful in showing any chromatin-like pattern of digestion with viral cores. The products of digestion were analysed by sucrose gradient centrifugation and results are shown in figure 7. Nucleoprotein structures were released from virus particles and sedimented in a position between that of viral monomer and subnucleosome in a parallel gradient. Viral cores gave rise to structures slightly smaller than these which in turn were rapidly degraded. DNA analysis (not shown) did not indicate any chromatin-like repeating structure, the pattern being very similar to that seen on digestion of nuclei isolated 1 hour pi (see figure 4, tracks

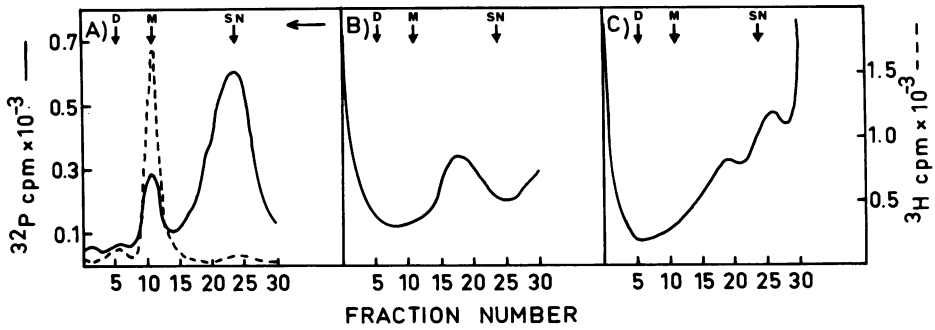

Figure 7. Isokinetic sucrose gradient analysis (200000g x 21hours) of nucleosomal structures released by micrococcal nuclease digestion from pentonless virions (B) and pyridine cores (C). A) Control digestion of ^{32}P virus infected nuclei isolated 3 hours pi. (---) ^{3}H cellular profile showing position of monomers (M) and dimers (D). (——) ^{32}P virus profile showing position of subnucleosomal peak (SN). 27.5% ^{32}P viral and 12.5% ^{3}H cellular material PCA soluble. B) 0.9% ^{32}P pentonless virus PCA soluble. C) 2.8% ^{32}P pyridine core PCA soluble.

A,B,C,D). Therefore it was concluded that the chromatin-like patterns seen in the infected cell were not arising from viral core structures.

Genomic distribution of nucleosome-like structures

The most important question to consider, following the identification of parental viral DNA in nucleosomal structures in the infected cell nuclei, is the possible role of such structures in expression of the viral genome. It has been reported (21,22) that very brief micrococcal nuclease digestion of nuclei selectively releases transcriptionally active gene sequences from eukaryotic chromatin. The experiments reported here were a modification of such procedures and therefore it was expected that those viral DNA sequences present in solubilised nucleosomes may show an over-representation of genome regions known to be active early after infection (1,2). DNA was extracted from the nucleosome fractions, and from the nuclear pellets remaining after nuclease digestion, for comparison, and hybridized to Southern blot strips (18) of ad2 DNA cleaved with SmaI and HindIII restriction enzymes (figure 8). It is evident that sequences from the complete genome are represented in both fractions. However it was noted that fragments from early regions (eg. SmaI-J) appeared to hybridize more radioactivity from the released nucleosomal fraction than from the nuclear pellet. Conversely, fragments containing sequences thought to be "silent" at early times after infection (see HindIII-J and SmaI-

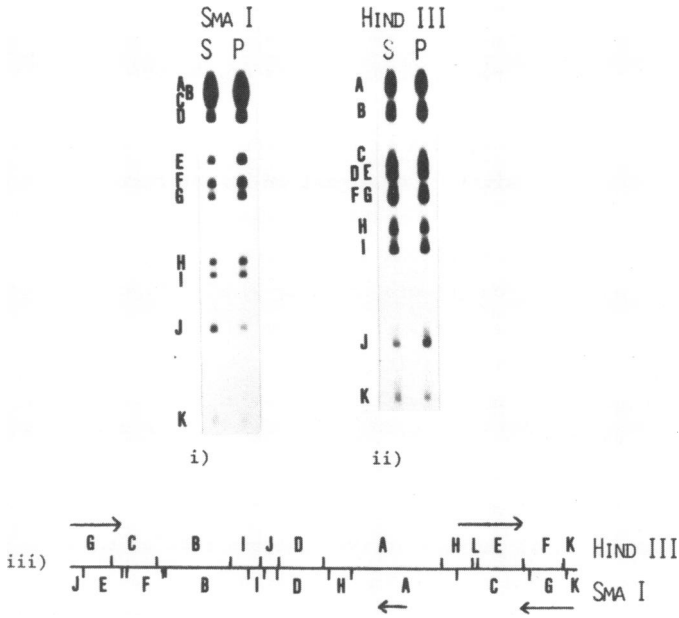

Figure 8. i) and ii) Hybridization of ^{32}P viral DNA fragments, prepared after micrococcal nuclease digestion of ^{32}P viral-infected nuclei isolated 3 hours pi and fractionation into nuclease-released (S) and pelleted (P) material with i) SmaI-ad2 DNA and ii) HindIII-ad2 DNA Southern blot strips. iii) Map of cleavage sites for restriction endonucleases HindIII and SmaI on ad2 DNA. Regions and direction of early RNA transcription shown by solid arrows (ref.2).

H and I) appeared to hybridize less radioactivity from the nucleosomal fraction.

DISCUSSION

An experimental approach, to follow the fate of infecting adenovirus particles in HeLa cell nuclei, has been described. The object of this investigation was to determine whether parental DNA remains associated with its own core proteins or whether these basic proteins are replaced by cellular histones.

Parental viral DNA within the nucleus is micrococcal nuclease-digested faster than cellular chromatin at all times during the early phase of infection. However, very early, ie. around 1hour pi, the rate of viral DNA digestion is only marginally faster than that of cellular DNA. At this time, much

of the viral DNA associating with the nucleus is in structures behaving as partially uncoated particles. The slower rate of viral DNA digestion may therefore depend on the decreased accessibility of the parental viral DNA within such particles.

At least 50% of the parental DNA is in nucleosome-like structures (including subnucleosomes), but kinetics of digestion do not follow those of cellular chromatin. There are several possible explanations for this phenomenon. The stepwise transfer of parental DNA from virions via core structures into a nucleosome-like configuration appears to occur slowly (figure 3) and, if these intermediates are more susceptible to the nuclease, this would result in an increase in nuclease sensitivity of the total viral DNA. It is not ruled out, although highly unlikely, that the viral DNA could exist free of basic protein in the nucleus and this could again contribute to the faster kinetics of digestion. A second explanation for the increased nuclease sensitivity of parental viral DNA could be that, although the DNA is in a nucleosomal configuration, it is more active in transcription than the bulk chromatin. From published data it seems likely that nucleosomes are present in actively transcribed regions of cellular chromatin (reviewed 25) and methods have been developed to study active genes based on their increased susceptibility to attack by micrococcal nuclease (21,22).

Sucrose gradient analysis of low molecular weight material released from nuclei after micrococcal nuclease digestion showed the presence of a subnucleosomal fraction which was the major species seen at very early times. The proportion of viral DNA within this peak remained constant during the early phase whereas the fraction of radioactivity in the mono- and oligomers increased. Such a subnucleosomal fraction is seen with cellular chromatin although it represents a minor fraction of the radioactivity on the gradient. Fractions from this region of the gradient do not bind significantly to cellulose nitrate filters (not shown), indicating that it probably contains pieces of double-stranded DNA which are not associated with protein. Analysis of the DNA on gels revealed its size to be about 20 base pairs. Micrococcal nuclease digestion of pentonless virus particles and isolated viral cores did not result in either monomer or subnucleosomal particles as seen in the infected cell. Therefore, subnucleosomes are probably not the products of digestion of partially uncoated virus particles or cores released into the nuclei.

Analysis of DNA present in the low molecular weight fractions by gel electrophoresis confirms the results seen by sucrose gradient analysis. The 145 base pair size of the monomer at limit digestion is in good agreement with

published values for cellular chromatin (26,27) as is the 50 base pair size of the linker DNA for HeLa cell chromatin (28). The linker DNA between viral nucleosomes is probably shorter, being about 20 base pairs. It has been suggested (29,30) that the length of the linker DNA in eukaryotic chromatin is determined by histone H1. Therefore, it may be suggested that histone H1 is either not associating with viral DNA, or that it is functionally replaced by another protein.

Digestion of deproteinised viral DNA in the presence of uninfected nuclei showed that a small, but reproducible, fraction of viral DNA accumulated as monomer, dimer and even trimer. Such reconstitution may be expected to occur considering recently published data which has identified nuclear factors which enhance nucleosome formation in vitro and probably in vivo (31). We believe that the accumulation of nucleosome-like particles of parental virus DNA in infected nuclei was not due wholly to reconstitution during incubation for three reasons. i) no more than 10% deproteinised viral DNA could be reconstituted into nucleosomes during digestion. ii) Higher oligomers were never seen with deproteinised DNA whereas they were visible, up to at least 6-mers, in parental viral DNA in the infected cell. iii) The change of digestion patterns of viral DNA in the infected cell during the early phase is difficult to reconcile with a reconstitution phenomenon.

The nucleosome-like structures may possibly reflect viral integration into the cellular genome. However, it would appear that a minor fraction of infecting viral genomes may integrate during the first hours after infection (32) and this would not account for the observed digestion patterns.

Throughout this discussion we have avoided the statement that the proteins conferring the nucleosome-like pattern on the viral DNA are, indeed, cellular histones. We have attempted to characterize the proteins associated with the viral DNA but, since the viral nucleosomes behave almost identically to cellular nucleosomes, it is virtually impossible to purify viral nucleosomes sufficiently to obtain an unambiguous answer. We can only state that, since the behaviour of viral and cellular nucleoproteins is so similar, and since similar results are not obtained with isolated viral cores, the proteins associating with the viral DNA most probably are cellular histones.

If cellular histones are associating with viral DNA upon entry into the nucleus, it is pertinent to consider the origin of these histones. Histone synthesis is closely coupled to cellular DNA synthesis and both decrease drastically with the onset of the late phase of adenovirus infection. It is generally thought that a large pool of free histone does not exist in normal

cells but there is evidence that a small quantity of free histone can be detected in certain cells (33). This pool is not large enough for cellular DNA synthesis but would easily meet the requirement for associating with up to 1000 infecting adenoviral genomes on a 1:1 weight ratio basis. Alternatively, if de novo histone synthesis were required, then the viral infectious cycle should be linked to the cell cycle. There is conflicting evidence on this points (34,35) and it is unclear whether virus must infect during the S phase in the cell cycle to establish infection.

The digestion pattern seen at 1 hour pi should reflect the nuclease digestion pattern that would be obtained from free adenoviral core or partially uncoated virus since, at this time, uncoating is apparently incomplete. There is one report to show that a nucleosomal-like DNA pattern can be seen on digestion of virus particles (9), although this pattern is not clearly seen on digestion of isolated viral cores. Numerous unsuccessful attempts have been made to repeat these observations with virus particles and cores (36, unpublished observations). Also, X-ray diffraction and circular dichroism studies on isolated viral cores do not show typical chromatin patterns (36). Such studies indicated that the repeating unit of the viral core is smaller and less compacted that the cellular nucleosome.

The simplest model to explain why the virus should exchange its own basic protein for cellular histones can be reached by consideration of the various roles these proteins play. Late in infection, an efficient packaging mechanism must be developed to remove DNA molecules from transcribing and replicating pools. This is achieved by a greater affinity of pVII, the precursor to polypeptide VII (37), for viral DNA than histone and the highly compacted structure so formed. On reinfection, the viral DNA should be readily available for transcription, hence the cleavage of pVII to VII, with a lower binding affinity for viral DNA to facilitate uncoating. Since the viral DNA uses the cell's transcriptional machinery, it may be advantageous for the virus to achieve a configuration as close to that of cellular chromatin as possible, at least in the early stages of infection. We are currently investigating those viral nucleoprotein structures which are being actively transcribed.

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