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SV40 virions and viral RNA metabolism are associated with cellular substructures

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Nuclear matrices were prepared by DNase and high salt extraction of SV40-infected epithelial monkey cells. The matrices retain the majority of SV40 virions. This conclusion is based on electron microscopic observations of the occurrence of encapsidated viral DNA that is resistant to DNase digestion and on the analysis of viral proteins by gel electrophoresis. Pulse labeled SV40 RNA is also associated with the nuclear matrix (<15% of the viral RNA is removed by DNase and high salt). Pulse-chase experiments revealed that processing of SV40 RNA takes place on the nuclear matrix and the processed molecules are directly transported to the cytoplasm where they are associated with the cytoskeleton. These results suggest a central role for the nuclear and cytoplasmic substructures in virus assembly and in the biogenesis of viral RNA.

Key words: cytoskeleton/nuclear matrix/SV40 assembly/SV40 RNA metabolism

Introduction

Numerous studies have shown the existence of a subcellular network or cytoskeleton in the cytoplasm of eukaryotic cells (Porter, 1976). It has been suggested that the major cytoskeletal components may determine cell shape and may also function as transducing elements relaying surface signals to the nucleus (Shaper *et al.*, 1979). These filamentous networks of the cell have attracted increasing interest because cell shape is involved in the control of cell proliferation (Benecke *et al.*, 1978; Ben-Ze'ev *et al.*, 1980; Folkman and Moscona, 1978) and differentiation (Gospodorowicz *et al.*, 1978) and because malignant transformation is accompanied by dramatic alterations in cellular architecture (Pollack *et al.*, 1975).

The subnuclear architecture is also characterized by a skeletal network that can be obtained by extracting DNA and histones from nuclei with DNase and high salt (for recent reviews, see Agutter and Richardson, 1980; Berezney, 1979; Shaper *et al.*, 1979). The structural framework of the nucleus, known as the nuclear matrix, is apparently involved in DNA replication (Pardoll *et al.*, 1980), RNA transcription (Faiferman and Pogo, 1975), replication of nuclear viruses (Buckler-White *et al.*, 1980), and nuclear swelling phenomena (Wunderlich and Herlan, 1971).

Here we report on the association of SV40 virions and SV40 RNA metabolism with the filamentous networks of the cell. Using a pulse-chase protocol we showed that newly synthesized viral RNA is quantitatively associated with the nuclear matrix and that the processing and transport of viral RNA take place on the nuclear framework. Transport of the viral nuclear RNA to the cytoplasm appears to proceed from the nuclear matrix to the cytoskeleton. These results suggest that there is a continuity between the nuclear and cytoplasmic skeletal frameworks and that these substrates are involved in RNA metabolism and possibly in controlling gene expression.

Results

Electron microscopic and biochemical characterization of the cytoskeleton and nuclear matrix of SV40-infected cells

To examine the involvement of the cytoplasmic and nuclear networks in SV40 maturation and viral RNA metabolism, we used an extraction scheme that separates the soluble from the structural proteins. First, we exposed SV40-infected cells, late after infection, to Triton X-100 in an appropriate buffer to remove most lipids together with ~50% of the cellular proteins (Ben-Ze'ev *et al.*, 1981). This procedure yields a cytoskeletal framework (Figure 1A). The framework consists of the nucleus packed with virions and bounded by the nuclear lamina and a cytoplasmic filamentous network to which the polyribosomes are attached. The entire structure is bounded by a lamina derived, most probably, from the plasma membrane proteins (Ben-Ze'ev *et al.*, 1979). In the next step, the cytoskeletal networks were separated from the nucleus by homogenization in a DOC/Tween mixture (Penman, 1966). This removes the majority of the cytoplasmic structures without apparent effect on the nucleus (Figure 1B). The detergent-insoluble intermediate filaments co-sediment during all steps of purification with the pellet (see also Figure 2) and they probably hold the 'vacuoles' characteristic of cells supporting SV40 replication (arrows, Figure 1A and B and inset). The involvement of the intermediate filaments with cytoskeletal rearrangements induced by virus infection was also described in poliovirus-infected HeLa cells (Lenk and Penman, 1979).

The nucleus was further extracted with DNase and high salt to remove >95% of the DNA and histones (Herman *et al.*, 1978). The structural framework remaining after the extraction of chromatin is known as the nuclear matrix (Figure 1C and D) and consists of the nuclear lamina and an internal filamentous network that appears here covered by the virions. Since the micrographs are of thin sections, the three-dimensional network of the interior structure is not seen and the amount of internal structure observed depends on where across the nuclear sphere the section was cut.

The association of SV40 with the nuclear matrix is further demonstrated by the experiments summarized in Table I. About 5% of the DNA of uninfected cells is resistant to DNase and high salt. In contrast, ~20% of the DNA of SV40-infected cells survived the same treatment. The much higher proportion of DNase-resistant DNA in infected cells is most likely because of the presence of viral DNA in mature virions that is protected against enzymatic attack. The experiments with tsB and strain 777 of SV40, which is also partly a tsB mutant (Jakobovits *et al.*, 1982), support this notion. When these viruses are grown at the non-permissive temperature, or at the permissive temperature and then shifted up to the restrictive temperature, conditions that cause either a failure of viral DNA encapsidation or disassembly of the

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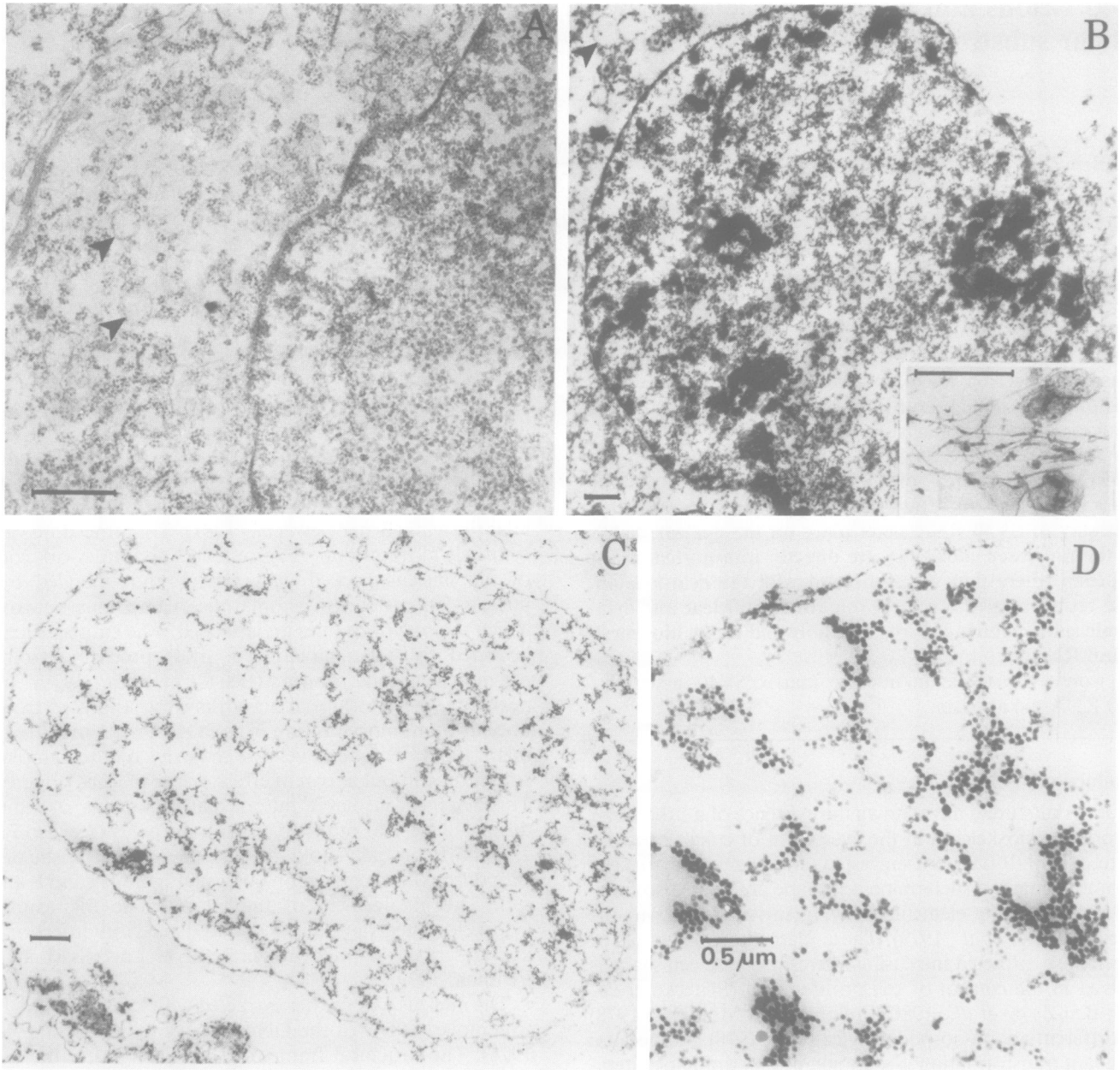

Fig. 1. Electron microscopic characterization of structural frameworks of the cytoplasm and the nucleus of SV40-infected BSC-1 cells at 42 h post-infection. **(A)** Cytoskeleton of SV40-infected BSC-1 cells prepared by Triton X-100 extraction. **(B)** Nuclei after DOC/Tween homogenization of the cytoskeletal framework. **(C)** Nuclear matrix obtained after DNase and salt extraction of nuclei prepared as in **(B)**. **(D)** same as **(C)** at higher magnification. Inset and arrows show 'vacuoles' induced by SV40 infection and surrounded by intermediate filaments co-sedimenting with nuclei. Bars, 0.5 μM.

virus, the percentage of DNase-resistant DNA becomes comparable with that of uninfected cells (Table I). The association of SV40 particles with the nuclear matrix is also shown by the analyses of the proteins, from equivalent amounts of starting material, at each step of the fractionation procedure. Figure 2,I shows the Coomassie blue staining of the gels, and Figure 2,II is an autoradiogram of the same gels, derived from cells labeled for 3 h with [^{3}H]leucine. The virus-specific capsid proteins VP1 and VP3 accumulate in the nucleus (Figure 2C, 2c) and remain associated with the matrix when the chromatin is removed by DNase and salt (Figure 2D–F, 2d–f). 1 M NaCl or 0.5 M $(NH_4)_2SO_4$ are equally effective in removing chromatin following treatment with DNase. The histones remaining with matrices prepared from SV40-infected cells probably represent histones sequestered with the viral DNA in the mature virions, since virtually all histones are solubilized under these conditions from uninfected nuclei (Figure 2C′–F′, 2c′–f′). The intermediate filament proteins (~58 K) cosediment with the nuclear matrices due to their insolubility under the extraction conditions used.

The association of virion assembly with the nuclear matrix was followed in a pulse-chase experiment summarized in Figure 3. SV40-infected cells were pulse labeled with [^{35}S]-methionine for 10 min. The chase was initiated after extensively washing the cells with fresh medium containing unlabeled methionine. The infected cells were then further incubated at 37°C and fractionated at different times into the Triton X-100 soluble fraction, the DOC/Tween homogenate,

the DNase and salt soluble fraction, and the remaining nuclear matrix, as outlined in Figure 2. Equivalent amounts of protein from each fraction were concentrated by ethanol precipitation and analysed by gel electrophoresis. After 10 min of labeling the majority of the viral capsid proteins (VP1 and VP3) were already found in association with the nuclear matrix fraction (Figure 3A). However, the histones were extracted efficiently by DNase and salt and were not observed in the nuclear matrix fraction. At later periods of chase, starting from 1 h (Figure 3E–H) there were no viral capsid proteins in the cytoplasmic fractions, and histones started to appear in association with the nuclear matrix. The histones protected against removal by DNase and salt are most probably those incorporated into the maturing virions which remain associated with the nuclear matrix (Figure 2F,2f). The rate of virus maturation, as estimated by the accumulation of histones on the matrix, was identical to the maturation rate measured by the hypotonic leaching of mature virions from the nuclei followed by sucrose gradient analysis by the technique of Jakobovits and Aloni (1980) (our unpublished results). Thus the association of the virus assembly process and mature SV40 virions with the nuclear matrix appears to be a functional prerequisite and is not a result of artifactual entrapment obtained during preparation of the nuclear matrix.

Based on the above electron microscopic and biochemical analyses, we conclude that the intracellular SV40 virions are associated with the nuclear matrix.

Table I. DNA associated with matrices of uninfected and SV40-infected cells

Cells	Nuclei (c.p.m.)	Matrices (c.p.m.)	Matrices/Nuclei (%)
Uninfected	3 170 600	120 550	3.8
SV40 (777) infected	2 994 600	650 100	21.7
SV40 (tsB) infected at 41°C	3 545 700	159 200	4.4
SV40 (777) infected after shift up to 41°C for 1 h	879 900	51 920	5.9

SV40-infected cells were labeled for 16 h with [methyl-^{3}H]thymidine (50 μCi/plate). At 42 h post-infection nuclei and matrices were prepared from 10^7 cells and aliquots were taken for TCA precipitation. The c.p.m. represent the radioactivity per 10^7 cells in each fraction.

SV40 RNA metabolism is also associated with the nuclear matrix

The synthesis and processing of rapidly labeled viral RNA in the nucleus, its transport to the cytoplasm, and the relation of these processes to the cellular frameworks was followed using an uridine-glucosamine pulse-chase protocol (Ben-Ze'ev *et al.*, 1981; Chiu *et al.*, 1978; Levis and Penman, 1977). Forty-two hours after infection, the cells were pretreated with glucosamine for 60 min and then pulsed for 10 min with [5-^{3}H]uridine. The chase was initiated after washing and incubating the cells in fresh medium containing glucosamine and unlabeled uridine, cytidine, and thymidine. Cytidine and thymidine should be included in the chase to avoid extensive labeling of the viral DNA (A. Ben-Ze'ev and Y. Aloni, in preparation). At different times following the initiation of the chase, the cells were extracted with Triton X-100 and DOC/Tween 40 to prepare nuclei. The nuclei were treated with DNase and salt to remove histones and chromatin, and the DNase high salt soluble nuclear fraction and the nuclear matrix fraction were saved. The RNA was extracted from each fraction and sedimented through sucrose gradients. The virus-specific RNA was identified by hybridization to SV40 DNA filters. Figure 4 shows the profiles of the viral RNA

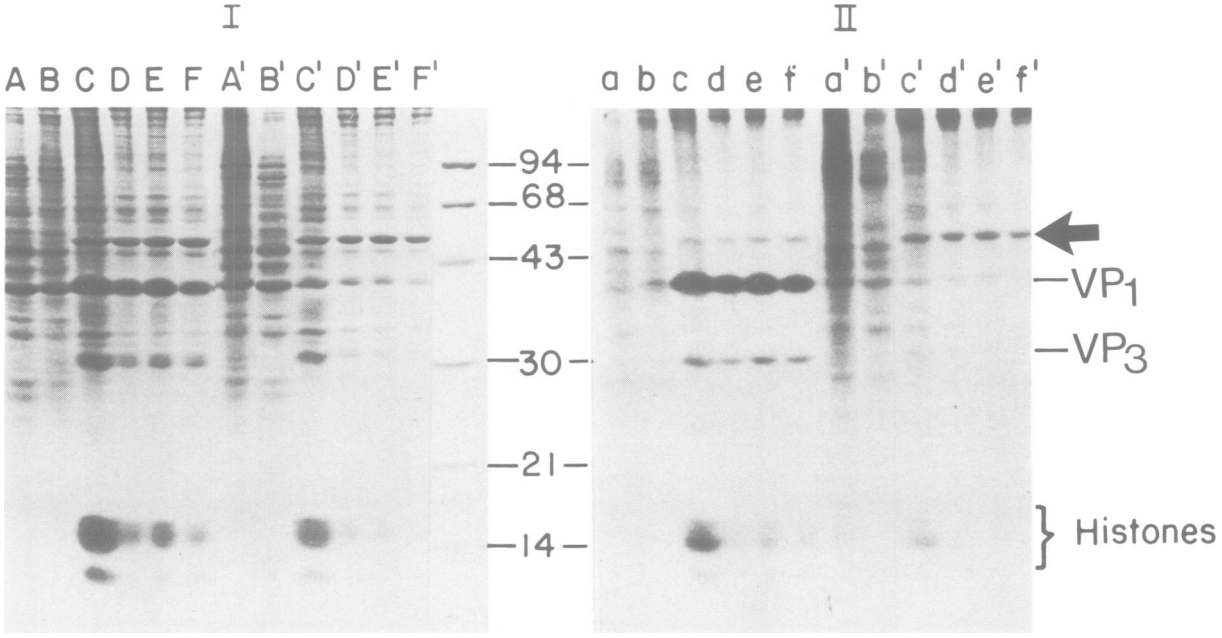

Fig. 2. Polyacrylamide gel electrophoresis of proteins of the various fractions obtained during preparation of subcellular frameworks. SV40-infected and mock-infected BSC-1 cells were labeled at 42 h post-infection for 3 h with [^{3}H]leucine (100 μCi/ml). Equivalent amounts of material from the various fractions were analyzed on 15% acrylamide gels. **(I)** Coomassie blue staining, **(II)** autoradiography of the same lanes as in **I**. **(A)** Triton X-100 soluble material (cytoplasm), **(B)** DOC/Tween solubilized cytoskeleton, **(C)** nuclei obtained after DOC/Tween treatment, **(D)** nuclear matrix obtained with DNase and 1 M NaCl, **(E)** as **(D)** obtained with DNase and 0.3 M $(NH_4)_2SO_4$, **(F)** as **(D)** but with DNase and 0.5 M $(NH_4)_2SO_4$. **A'–F'** the same as **A–F** but of mock-infected cells. **a–f** the autoradiogram of **A–F**, and **a'–f'** the autoradiogram of **A'–F'**. Arrow indicates position of 58-K intermediate filament protein.

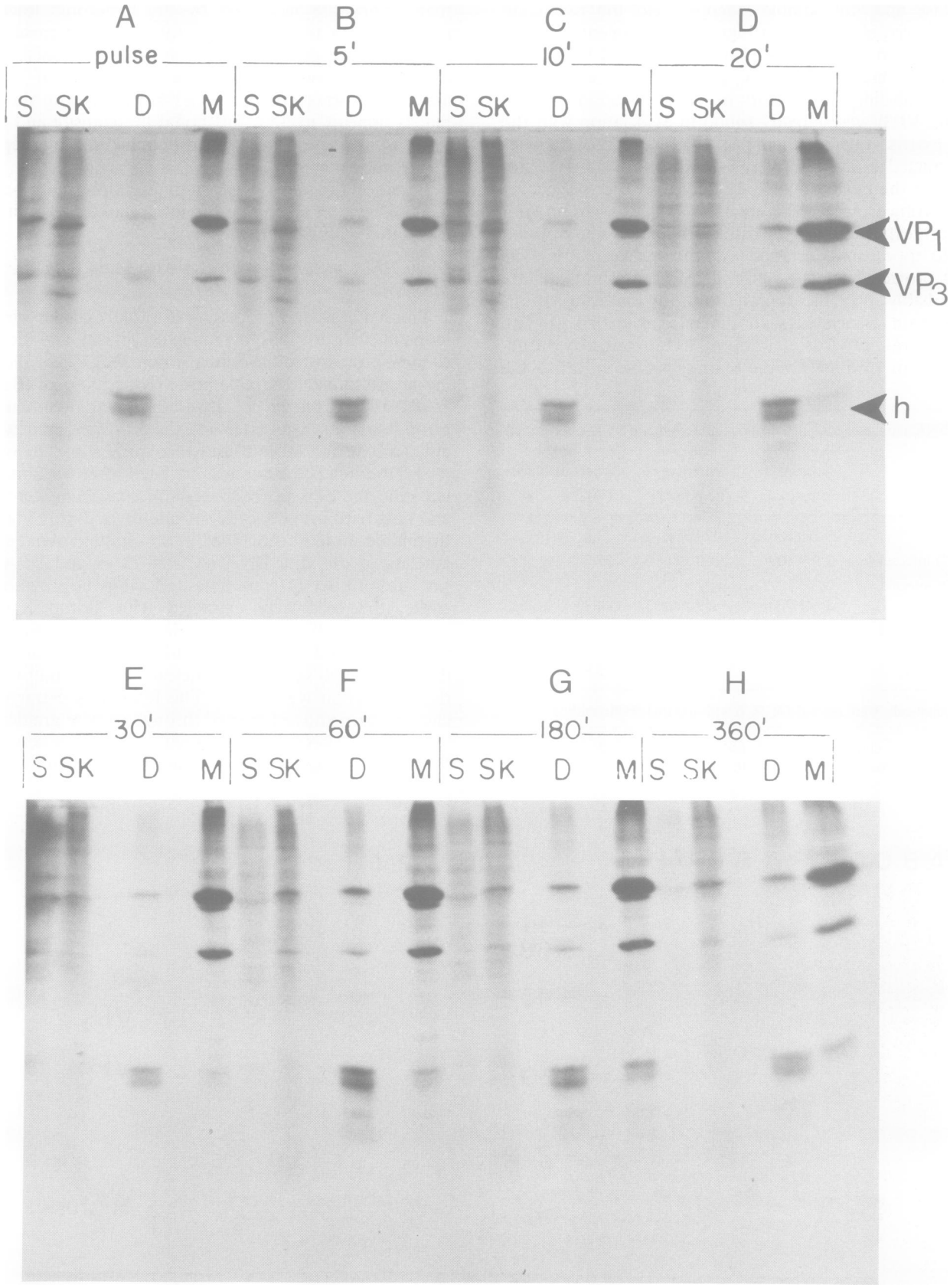

Fig. 3. The assembly of SV40 virions is associated with the nuclear matrix. SV40-infected cells were labelled for 10 min in methionine-deficient medium with 100 μCi [^{35}S]methionine. The chase was initiated by washing the plates with fresh medium and incubating the cells in medium containing unlabeled methionine. At different times after the initiation of the chase, from equal numbers of cells, the following cell fractions were prepared: S – a Trixon X-100 soluble cytoplasmic fraction, SK – a DOC/Tween cytoskeletal fraction, D – a DNase and high salt soluble nuclear extract. M – a nuclear matrix. The proteins were concentrated and analysed on acrylamide gels as described in Materials and methods. **(A)** 10 min [^{35}S]methionine pulse, **(B)** 5 min, **(C)** 10 min, **(D)** 20 min, **(E)** 30 min, **(F)** 60 min, **(G)** 180 min, **(H)** 360 min chase. VP1 and VP3 are the major viral capsid proteins, h – histones.

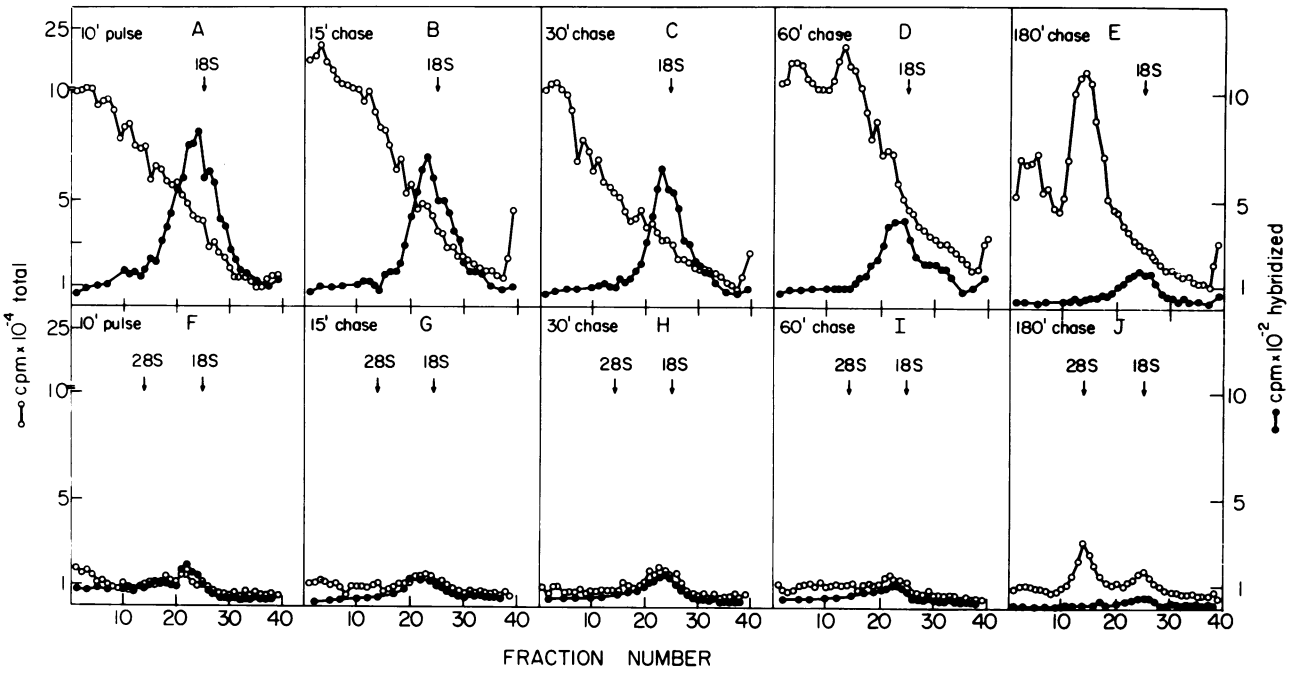

Fig. 4. SV40 RNA is associated with the nuclear matrix during its synthesis and processing in infected cells. At 42 h post-infection, SV40 infected BSC-1 cells were labeled with 200 μCi/ml of [5-^{3}H]uridine for 10 min. A chase was initiated by incubating the cultures with glucosamine and unlabeled uridine, cytidine, and thymidine as described in Materials and methods. At different times during the chase the RNA from the nuclear matrix (**A**–**E**) and the DNase and salt soluble fraction (**F**–**J**) was extracted and analyzed by sedimentation through sucrose gradients. The amount of total RNA was determined by TCA precipitation and one half of each fraction was taken to hybridization with 1 μg of ss SV40 DNA immobilized on filters as described (Laub *et al.*, 1979). ○——○ total RNA, ●——● SV40-specific RNA.

solubilized by DNase and high salt (Figure 4F–4J), after a 10 min pulse and increasing periods of chase. More than 90% of the rapidly labeled viral RNA in the nucleus sedimented with a characteristic major peak around 19S (Aloni, 1974) and was found to be associated with the matrix during the entire chase period. At the end of the 3 h chase, due to the processing and transport of the nuclear RNA to the cytoplasm, there was a 5- to 6-fold decrease of viral RNA in the nucleus in agreement with Chiu *et al.* (1978). During this time only a minor proportion of the viral RNA was found in the DNase and salt-soluble fraction. These results suggest that, in the nucleus, the rapidly labeled viral RNA is associated with the matrix and it remains on this substructure during processing and transport to the cytoplasm. In agreement with previous studies (Faiferman and Pogo, 1975; Herman *et al.*, 1978; Jackson *et al.*, 1981; Long *et al.*, 1979; van Eekelen and van Venrooij, 1981), the majority of the rapidly labeled cellular RNA was found in association with the nuclear matrix.

Newly synthesized viral RNA is transported directly from the nuclear matrix to the cytoskeleton

Figure 5 shows the partitioning of the virus-specific RNA between the structural and soluble fractions of the cytoplasm. The cells were extracted with Triton X-100 and the Triton soluble fraction was removed. The cytoskeleton material was separated from the nuclei by homogenization with DOC/Tween mixture and the nuclear pellet was removed. 19S virus-specific RNA, which appears in the cytoplasm even after a short (10 min) pulse followed by 15 min chase, is found in association with the cytoskeleton (Figure 5A). The majority of the viral RNA is associated with the cytoskeletal fraction upon its emergence from the nucleus and there is almost no detectable viral RNA in the Triton X-100 soluble fraction (Figure 5B). The viral RNA continues to accumulate in the cytoskeletal fraction during the 3 h chase period with the 16S viral RNA becoming the major constituent. The cytoskeleton fraction holds >80% of the virus-specific polyribosomes (Ben-Ze'ev *et al.*, 1981). The Triton X-100 soluble fraction of the cytoplasm continues to contain only a minor part (~15%) of the virus-specific RNA (even after 3 h of chase).

Figure 6 summarizes the distribution of the total virus-specific RNA in the soluble and in the structural fractions of the nucleus and the cytoplasm from equivalent amounts of material from each fraction using the glucosamine-uridine pulse-chase procedure. It appears that the rapidly labeled viral RNA is synthesized and processed in the nucleus in association with the nuclear matrix and is directly transported to the cytoskeleton, where the protein synthetic machinery is located (Ben-Ze'ev *et al.*, 1981). These results suggest that the metabolism and activity of the viral RNA in the cell are intimately associated with cellular substructures.

Discussion

This study demonstrates the association of SV40 virions and viral RNA metabolism with the cellular skeletal networks. Since the metabolism of SV40 RNA is well characterized and utilizes the cellular metabolic machinery, and since viral RNA is produced in abundance late after infection, the lytic interaction of SV40 with monkey cells was chosen as a model system for eukaryotic RNA metabolism. This system enables the use of pulse-chase experiments to follow the fate of newly synthesized viral RNA in the nucleus and its transport to the cytoplasm. We used a cellular fractionation scheme that separates the soluble from the structural components both of the cytoplasm and of the nucleus with relatively little cross contamination. The initial Triton X-100 extraction removes most lipids (~90%), (Ben-Ze'ev *et al.*,

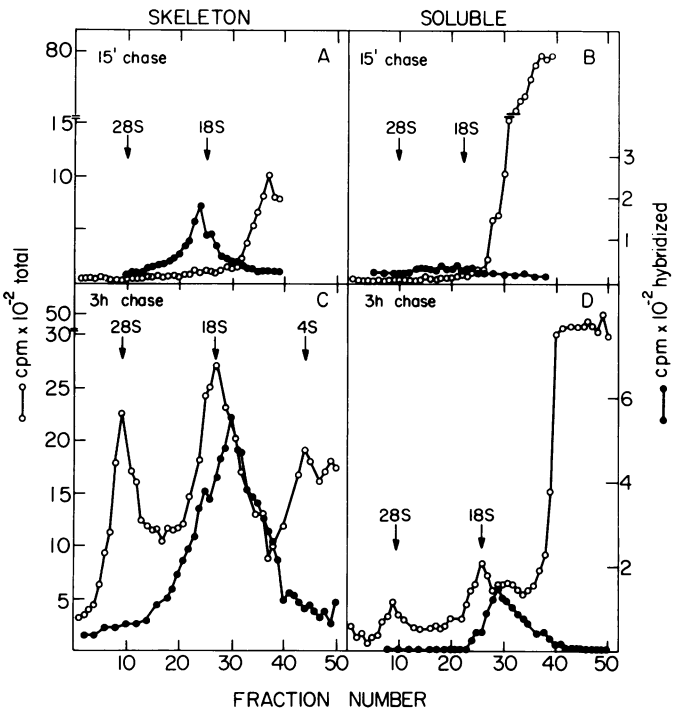

Fig. 5. Newly synthesized cytoplasmic SV40 RNA is associated with the cytoskeleton. SV40-infected BSC-1 cells were pulsed for 10 min with [5-^{3}H]uridine and chased with unlabeled uridine as described in Figure 3. After 15 min chase (**A**,**B**) and 3 h chase (**C**,**D**) the RNA from the Triton X-100 soluble fraction in the cytoplasm (**B**,**D**) and the cytoskeletal fraction obtained with DOC/Tween (**A**,**C**) was extracted and run on 15–30% sucrose gradients as described (Ben-Ze'ev *et al.*, 1981). SV40-specific RNA was determined by hybridizing one half of each fraction with 1 μg of ss SV40 DNA bound to filters as detailed in Materials and methods. ○——○ Total RNA determined by TCA precipitation, ●——● SV40-specific RNA. At each time point 10^7 infected cells were fractionated.

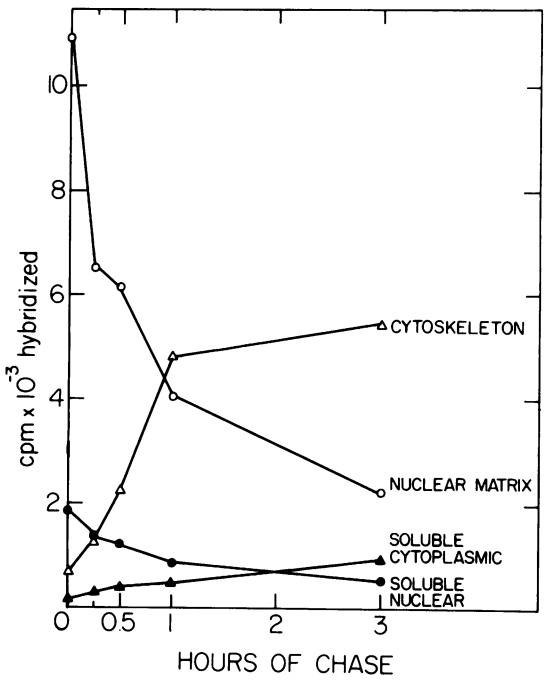

Fig. 6. The synthesis and processing of SV40 RNA is associated with the nuclear matrix and the mature SV40 RNA is transported to the cytoskeleton. SV40-infected BSC-1 cells were pulsed for 10 min with 200 μCi/ml of [5-^{3}H]uridine and chased as described in Figure 3. At each time point, 10^7 BSC-1 cells were fractionated into a Triton X-100 soluble cytoplasmic fraction, a DOC/Tween homogenate that contains the cytoskeletal fraction, a DNase and salt-soluble nuclear fraction, and a nuclear matrix, as described in Materials and methods. The RNA from each fraction was extracted and analyzed on 15–30% sucrose gradients as described (Ben-Ze'ev *et al.*, 1981). One quarter of each fraction across a sucrose gradient was hybridized with 1 μg of ss SV40 DNA bound to filter and a blank filter. The SV40-specific radioactivity is summed across each gradient in each subcellular fraction after 10 min pulse, and after 15 min, 30 min, 60 min, and 180 min of chase. ○——○ SV40 RNA from the nuclear matrix, △——△ SV40 RNA associated with the cytoskeleton, ●——● SV40 RNA removed from nuclei by DNase and 0.5 M $(NH_4)_2SO_4$, ▲——▲ SV40 RNA from the cytoplasm obtained in the initial Triton X-100 lysis.

1979) together with ~50% of the cellular proteins and <15% of the polyribosomes (Ben-Ze'ev *et al.*, 1981). The cytoskeletal network that retains the cellular and viral polyribosomes is efficiently removed by DOC/Tween homogenization as was shown by us (Ben-Ze'ev *et al.*, 1981) and by others (Cervera *et al.*, 1981; Lenk and Penman, 1979; van Venrooij *et al.*, 1981) in a variety of systems. Very little nuclear material is removed during this step, as demonstrated by the complete retention of virus-specific proteins and histones in the nucleus. In addition, we find <1% of pulse-labeled viral DNA removed under these conditions.

To obtain a nuclear matrix, the cells were treated with DNase and high salt, which removes >95% of DNA and all of the histones from uninfected cells. In SV40-infected cells, viral DNA and histones that are sequestered in the mature virions are not removed during this fractionation procedure. The maturation of SV40, namely the encapsidation of the viral minichromosome, could be followed by pulse-chase experiments in which the rate of appearance of mature virions is assayed by the hypotonic leaching of virions from nuclei (Jakobovits and Aloni, 1980). Thus, the assembly of SV40 and the mature virions are associated with the nuclear matrix, and do not appear to result from artifactual entrapments during the preparation of the nuclear matrix. The filamentous nature of the nuclear matrix is obscured by the virions which cover the nuclear matrix. The distribution of the virions in the chromatin-depleted nuclei clearly appears to be non-random and is indicative of the underlying filamentous framework. This network is best visualized by whole mount electron microscopic analysis which emphasizes the nuclear network by providing a three-dimensional view of the matrix (our unpublished results).

The great majority of the pulse-labeled viral RNA in the nucleus is associated with the nuclear matrix. This agrees with earlier reports of the retention of the majority of hnRNA in association with the nuclear matrix (Faiferman and Pogo, 1975; Herman *et al.*, 1978; Jackson *et al.*, 1981; Long *et al.*, 1979; van Eekelen and van Venrooij, 1981). The viral RNA associated with the nuclear matrix is processed and directly transported to the cytoplasm without accumulating in the nucleus in a fraction that can be solubilized by DNase and high salt. The DNase and salt-soluble fraction in the nucleus contains only minimal amounts of viral RNA both after a short pulse or following a prolonged period of chase. About 40–50% of the rapidly labeled nuclear RNA associated with the matrix is transported to the cytoplasm within 1 h and becomes associated with the cytoskeleton. The newly synthesized viral RNA associated with the cytoskeleton is engaged in the formation of virus-specific polysomes, that are quantitatively associated with this framework (Ben-Ze'ev *et al.*, 1981).

The Triton X-100 soluble cytoplasmic fraction contains only a limited portion of the newly synthesized viral RNA but, as shown previously (Ben-Ze'ev *et al.*, 1981), after prolonged periods of chase (8 – 12 h) there is an apparent movement of viral RNA into the soluble fraction to establish an almost equal partitioning between the two subcytoplasmic fractions at steady state. We suggest, therefore, that the cytoskeleton may have a role in controlling viral mRNA activity.

Several recent studies have reported on the selective association of viral mRNA (Cervera *et al.*, 1981; van Venrooij *et al.*, 1981) and of mature virions (Hiller *et al.*, 1981; Lenk and Penman, 1979) with the cytoskeleton, and Jackson *et al.* (1982) have shown that newly synthesized influenza RNA is associated with the nuclear matrix. Taken together, these studies suggest that the cellular networks, both in the nucleus and cytoplasm, play a central role in virus assembly and in localizing RNA metabolism and may have a role in modulating genome activity.

Materials and methods

Cells and virus

BSC-1 cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% calf serum. Confluent cultures were infected with plaque-purified SV40 (strain 777) at 10 – 50 p.f.u./cell in the same medium containing 2% calf serum.

Pulse-chase protocol

SV40-infected cells were treated with 20 mM glucosamine in DMEM containing 2% calf serum for 60 min. The cells were washed twice with fresh medium and labeled for 10 min with 200 μCi/ml of [5-^{3}H]uridine (35.1 Ci/mmol, The Radiochemical Centre, Amersham, UK). Following the pulse, the cells were washed twice with DMEM and the chase was initiated by incubating the cultures with fresh medium containing 20 mM glucosamine, 5 mM uridine, 5 mM cytidine, and 2.5 mM thymidine.

Cell fractionation and RNA extraction

The fractionation into the soluble and cytoskeletal fractions was similar to the procedure described by Ben-Ze'ev *et al.* (1981). Briefly, the cells were washed twice with cold phosphate-buffered saline and once with extraction buffer [50 mM NaCl, 10 mM Hepes, pH 7.4, 2.5 mM $MgCl_2$, 300 mM sucrose, and 1 mM phenylmethyl sulphonyl fluoride (PMSF)]. Extraction was achieved by adding to the washed cells the above buffer with 1% Triton X-100 for 5 min on ice. The Triton X-100 soluble fraction was removed. The cytoskeletons were solubilized by scraping them into DOC/Tween buffer (Penman, 1966) (10 mM NaCl, 10 mM Hepes pH 7.4, 1.5 mM $MgCl_2$, 0.5% deoxycholate, 1% Tween 40, and 1 mM PMSF) followed by homogenization either by pipetting or using a Teflon motor-driven homogenizer. The nuclei were pelleted at 2000 r.p.m. for 2 min at 4°C and the solubilized cytoskeletons removed.

The nuclei were resuspended in 80 μl of a 2 mg/ml DNase I solution containing 0.15 M NaCl, 5 mM $MgCl_2$, 1 mM PMSF. The DNase was previously chromatographed on agarose-5′-uridine-2′(3′) phosphate and further treated with iodoacetate to remove traces of RNase as described (Long *et al.*, 1979; Maxwell *et al.*, 1977). The nuclei were incubated on ice for 30 – 60 min. The nuclear suspension was brought to 0.5 M $(NH_4)_2SO_4$ in 10 mM Hepes pH 7.4 and kept on ice for 5 min. The matrices were then collected by centrifugation at 1000 r.p.m. for 5 min at 4°C. The DNase and salt-soluble fraction was removed and the nuclear matrix was resuspended in SDS buffer (100 mM NaCl, 0.5% SDS 10 mM Tris pH 7.4, 5 mM EDTA). For RNA extraction, SDS was added to all fractions to 0.5% and RNA was extracted with phenol-chloroform and chloroform-isoamyl alcohol as described by Penman (1966). The RNA preparations were denatured with 90% formamide at 37°C for 3 min before sedimentation through 15 – 30% sucrose gradients in SDS buffer in the SW 27.1 rotor at 25 000 r.p.m. for 14 h at 20°C.

RNA-DNA hybridization

Each fraction of a sucrose gradient was brought to 4 x SSC (SSC is 0.15 M NaCl, 0.015 M sodium citrate) and was incubated in a final volume of 250 μl at 68°C for 24 h with 1 μg SV40 single-stranded (ss) DNA immobilized onto 7-mm nitrocellulose filters. After 24 h the filters were washed three times with 2 x SSC, treated with RNase A (10 μg/ml) for 1 h at 22°C, washed again with 2 x SSC, dried, and counted.

Protein gel electrophoresis

Samples for SDS-polyacrylamide gel electrophoresis were concentrated by ethanol precipitation (3 volumes) at −20°C, resuspended in sample buffer (2% SDS, 10% glycerol, 5% β-mercaptoethanol), and analyzed in 15% acrylamide gels according to Laemmli (1970).

Electron microscopy

Samples were fixed with 2% glutaraldehyde in 0.2 M cacodylate buffer, and post-fixed with 0.5% osmium tetroxide in 0.2 M cacodylate buffer. After graded dehydration in ethanol the pellets were embedded in Epon and sectioned. Photographs were taken in a Phillips 300 electron microscope.

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References

Agutter,P.S., and Richardson,J.C.W. (1980) *J. Cell Sci.*, **44**, 395-435.
Aloni,Y. (1974) *Cold Spring Harbor Symp. Quant. Biol.*, **39**, 165-178.
Benecke,B.-J., Ben-Ze'ev,A., and Penman,S. (1981) *Cell*, **14**, 931-939.
Ben-Ze'ev,A., Duerr,A., Solomon,F., and Penman,S. (1979) *Cell*, **17**, 859-865.
Ben-Ze'ev,A., Farmer,S.R., and Penman,S. (1980) *Cell*, **21**, 365-372.
Ben-Ze'ev,A., Horowitz,M., Skolnik,H., Abulafia,R., Laub,O., and Aloni, Y. (1981) *Virology*, **111**, 475-487.
Berezney,R. (1979) in Busch,H. (ed.), *The Cell Nucleus*, Vol. **8**, Academic Press, NY, pp. 413-455.
Buckler-White,A.J., Humphrey,G.W., and Pigiet,V. (1980) *Cell*, **22**, 37-46.
Cervera,M., Dreyfuss,G., and Penman,S. (1981) *Cell*, **23**, 113-120.
Chiu,N.H., Radonovich,M.F., Thoren,M.M., and Salzman,N.P. (1978) *J. Virol.*, **28**, 590-599.
Faiferman,I., and Pogo,A.O. (1975) *Biochemistry (Wash.)*, **14**, 3808-3816.
Folkman,J., and Moscona,A. (1978) *Nature*, **275**, 345-349.
Gospodorowicz,D., Greenburg,G., and Birdwell,C.R. (1978) *Cancer Res.*, **28**, 4155-4171.
Herman,R., Weymouth,L., and Penman,S. (1978) *J. Cell Biol.*, **78**, 663-674.
Hiller,G., Jungwirth,C., and Weber,K. (1981) *Exp. Cell Res.*, **132**, 81-87.
Jackson,D.A., Caton,A.J., McCready,S.J., and Cook,P.R. (1982) *Nature*, **296**, 366-368.
Jackson,D.A., McCready,S.J., and Cook,P.R. (1981) *Nature*, **292**, 552-555.
Jakobovits,E.B., Abulafia,R., and Aloni,Y. (1982) *Virology*, **121**, 95-106.
Jakobovits,E.B., and Aloni,Y. (1980) *Virology*, **102**, 107-118.
Laemmli,U.K. (1970) *Nature*, **227**, 680-685.
Laub,O., Bratosin,S., Horowitz,M., and Aloni,Y. (1979) *Virology*, **92**, 310-323.
Lenk,R., and Penman,S. (1979) *Cell*, **16**, 289-301.
Levis,R., and Penman,S. (1977) *Cell*, **11**, 105-113.
Long,B.H., Huang,C.-Y., and Pogo,A.O. (1979) *Cell*, **18**, 1079-1090.
Maxwell,I.H., Maxwell,F., and Hahn,W.E. (1977) *Nucleic Acids Res.*, **4**, 241-246.
Pardoll,D.M., Vogelstein,B., and Coffey,D.S. (1980) *Cell*, **19**, 527-536.
Penman,S. (1966) *J. Mol. Biol.*, **17**, 117-130.
Pollack,R., Osborn,D.M., and Weber,K. (1975) *Proc. Natl. Acad. Sci. USA*, **72**, 994-998.
Porter,K.R. (1976) in Goldman,R., Pollard,T., and Rosenbaum,J. (eds.), *Cell Motility*, Cold Spring Harbor Laboratory Press, NY, pp. 1-26.
Shaper,J.H., Pardoll,D.M., Kaufmann,S.A., Barrack,E.R., Vogelstein,B., and Coffey,D. (1979) *Adv. Enzyme Regul.*, **17**, 213-248.
van Eekelen,C.A.G., and van Venrooij,W.J. (1981) *J. Cell. Biol.*, **88**, 554-563.
van Venrooij,W.J., Sillekens,P.T.G., van Eekelen,C.A.G., and Reinders,R. (1981) *Exp. Cell Res.*, **135**, 79-91.
Wunderlich,F., and Herlan,G. (1971) *J. Cell Biol.*, **73**, 271-278.