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Anchorage of Adenoviral RNAs to Clusters of Interchromatin Granules

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Previous in situ hybridization experiments have revealed that clusters of interchromatin granules in adenovirus type 5 infected HeLa cells contain not only spliceosome components but also significant amounts of viral RNA and poly(A)$^+$ RNA molecules whereas nonpolyadenylated viral RNA molecules are present within the still enigmatic viral compact rings. To determine the levels of association of the viral RNA molecules with cellular clusters of interchromatin granules and viral compact rings, we investigated the effects of a cell extraction technique on these structures. The spreading apart of the nucleoproteins by exposure of infected cells to a detergent-containing hypotonic solution, which exclusively preserves structurally linked components, resulted in the persistence within the clusters of interchromatin granules of U1 snRNA, U2 snRNA, viral RNA, and poly(A)$^+$ RNA. These data clearly reveal that, in addition to the well-known strong binding of spliceosome components within the clusters of interchromatin granules, there also is an anchorage of viral RNA and messenger RNA molecules to these structures, which suggests functional relationships. Taken together, the data indicate that the clusters of interchromatin granules might be the sites of accumulation and retention of those cell and viral messenger RNA molecules that are transiently stored in the nucleus before their degradation or their transport to the cytoplasm. In addition, the firm binding of nonpolyadenylated viral RNA to the viral compact rings suggests a role for these structures in the transient storage of the nonused portions of the viral primary late transcripts.

Adenoviral RNAs   Interchromatin granules   Cell extraction   Adenovirus type 5

THE infection of HeLa cells with adenovirus type 5 (Ad5) induces extensive structural changes in nuclear organization, which have been studied in detail (11,13,20,22,23,25,31,50,53,54). Recently, the in situ hybridization technique at the ultrastructural level has been decisive in determining the relationships between certain virus-induced nuclear structures and the synthetic activities of the viral genomes. It clearly demonstrated the segregation of viral DNA molecules into distinct compartments, which is caused by and varies with the level of involvement of the viral DNA in its replication process (11,38,39). For example, at the initiation of the replicative phase of viral genomes, both viral single-stranded (ss) and double-stranded (ds) DNA molecules are intermingled into small fibrillar areas of the nucleoplasm designated the early replicative sites. The subsequent intense replicative activity of the viral genomes induces the segregation of the viral molecules into two concentric compartments. One compartment, which has the shape of a pleomorphic, compact fibrillar mass, is the site of accumulation of the viral ssDNA molecules before they undergo replication. The other compartment, named the peripheral replicative zone, has the aspect of a loose fibrillogranular network and consists of replicating viral dsDNA. Finally, as the replicative activity of viral DNA diminishes later in infection, a third compartment appears in the center of the nucleus that consists of an accumulation of viral genomes in a resting state.

In agreement with studies at the molecular level demonstrating that transcription of viral genomes

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may occur concomitantly with their replication (7,8,24,51), we also found that transcription and replication of viral DNA occur in the same intranuclear structure, that is, in the early replicative site during the early stage of nuclear modification and then, subsequently, in the peripheral replicative zone during the intermediate and late stages of nuclear modifications (41,42). It must be emphasized that transcribing genomes are homogeneously distributed within the entire fibrillogranular network of the peripheral replicative zone whereas viral replication increases gradually in intensity at decreasing distances from the enclosed viral ssDNA accumulation sites (3). Therefore, the site of viral transcription colocalizes with the site of moderate replication, a finding that contrasts with recent light microscope studies (32) that detected replication only adjacent to the viral ssDNA accumulation sites. The resolution and sensitivity limits of the fluorescence microscope could explain this discrepancy.

The mechanisms that govern the synthesis of host and adenoviral messenger RNA (mRNA) have certain similarities. Both undergo posttranscriptional events that include cleavages of large RNA precursors into several individual mRNAs and polyadenylation of their 3′ terminus prior to the migration of mature mRNA from the nucleus to the cytoplasm (21,27–29). Splicing of viral transcripts requires the presence of spliceosomes of cellular origin that always are present at the transcription site and in large amounts within the clusters of interchromatin granules (12,32,34,56). The latter, which are constituents of the nuclear matrix (2,44), become rearranged in adenovirus-infected nuclei to become located exclusively at the periphery of the viral inclusion body and to be juxtaposed to the compartment involved in viral genome transcription (10,18,34,56). In addition, significant amounts of viral RNA molecules are present within compact rings, still enigmatic intranuclear viral structures that are often adjacent to the clusters of interchromatin granules and in which a few spliceosome components, but not poly(A) tails, are present (34).

Numerous studies have demonstrated the association of adenoviral DNA with the nuclear matrix of infected cells (15,19,21,45,47,55,57), which suggests that the nuclear matrix may be an important site of adenoviral DNA replication and transcription and of virus assembly as well. The above data were obtained from such nuclear matrices isolated by a variety of techniques, all of which include extraction of cells or nuclei by high salt solutions. The electron microscope identification of such nuclear matrices is difficult because the extractive treatment induces marked aggregation of the residual structures. In the past, however, we developed a cell extraction technique and visualized decondensed chromatin fibers in situ within slightly swollen interphase nuclei. This procedure, which preserves only strongly associated components, led to an in situ study of the organization of transcription complexes in DNA-rich nuclear matrices (33,37). Clusters of interchromatin granules in noninfected cells also are preserved but they are indistinguishable structurally from the surrounding nucleoprotein fibers. Only their labeling with specific probes allows their identification under these conditions (35).

In this study we examine the effects of a swelling and spreading apart of the components of the intranuclear structures involved in viral RNA synthesis. Determination of the intranuclear distribution of poly(A)$^+$ RNA was undertaken to identify mature RNAs. Spliceosome components were identified by detection of their snRNAs (U1 and U2 snRNAs) and proteins. The results were compared with the intranuclear distribution of viral RNA and viral DNA. We found that the loosening procedure did not disturb the topological distributions of viral RNAs, poly(A)$^+$ RNAs, and snRNAs, and clearly revealed their strong binding within the virus-modified clusters of interchromatin granules.

MATERIALS AND METHODS

Cells and Viruses

HeLa cells near confluence were infected with 5–10 plaque formation units of adenovirus type 5 (Ad5) per cell for 30 min. After infection, the cells were cultured for 17 h, at 37°C, and in an atmosphere of 5% CO_2, with Eagle's minimum essential medium supplemented with 5% calf serum.

Fixation and Embedding

For the observation of loosened material, cell cultures were rapidly rinsed in distilled water adjusted to pH 8.5 with 0.1 M borate buffer (37). They then were covered for 1 h at 4°C with 6 ml of a 1% formaldehyde aqueous solution containing 0.4% Photo flo (Kodak-Pathé, Chalon, France) and 0.1 M sucrose, pH 8.5. During the 1-h loosening treatment, the cells were scraped and centrifuged. Methanol dehydratation of the resulting translucent pellets and their infiltration with Lowicryl K4M (Chemische Werke Lowi, Waldkrai-

burg, Germany) were performed according to Roth (43). Polymerization was carried out for 5 days at −30°C under long wavelength UV light (Philips fluorescence tubes TL 6W). Ultrathin sections were collected on formvar-carbon-coated gold grids (200 mesh).

In Situ Hybridization

For detecting either viral RNA or viral DNA, we used a genomic Ad5 dsDNA probe (purchased from Enzo Biochemicals Inc, New York, USA) with a fragment size ranging between 50 and 500 base pairs. For detecting poly(A)$^+$ RNA, we used a poly(dT) probe that consisted of a synthetic polynucleotide with an average length of 174 nt (Pharmacia P-L Biochemicals Inc., Milwaukee, WI, USA). The U1 and U2 snRNAs were detected by using U1 and U2 human DNA probes, respectively.

Hybridizations were performed on undigested sections except for specific labeling of viral RNA and viral DNA. For the detection of viral RNA, the sections always were digested for 1 h at 37°C with 1 mg/ml DNase 1 (Worthington Biochemical Corp., Freehold, NJ, USA) in 10 mM Tris-HCl, pH 7.3, containing 5 mM $MgCl_2$, 2% RNasin ribonuclease inhibitor (Promega, Madison, WI, USA), and 2 mM dithiothreitol. This DNase digestion was performed to suppress hybridization of the probe with the viral ssDNA molecules of the sections. For the localization of viral DNA, the sections always were digested with 1 mg/ml RNase A (from bovine pancreas, BDH Biochemicals, Poole, UK) in 10 mM Tris-HCl, pH 7.3, for 1 h at 37°C, prior to a 4-min 0.5 N NaOH treatment. The latter was performed to denaturate the host and viral double-stranded DNA of the sections.

To localize viral RNA, U1 RNA, U2 RNA, and poly(A) RNA by electron microscope in situ hybridization, gold grids bearing sections of loosened cells were floated on 1–2-μl drops of hybridization solutions as previously described (38,41, 48,49). Hybridizations with each hybridization solution were performed in a wet chamber for 3.5 h at either 37°C for the detection of viral RNA and poly(A)$^+$ RNA or 64°C for the detection of U1 and U2 snRNAs. Hybrids formed at the surface of the Lowicryl sections were revealed by incubating the grids for 30 min on 5-μl drops of anti-biotin antibody conjugated to gold particles, 10 nm in diameter (Biocell Research Lab., Cardiff, UK), diluted 1/25 in PBS. Grids were observed after a 10-min staining with 5% aqueous uranyl acetate. For controls, some sections were treated with RNase A prior to the hybridization step.

To localize viral DNA, gold grids bearing RNase-NaOH pretreated sections of loosened cells were floated on 1–2-μl drops of the viral probe-containing hybridization solution for 1.5 h at 37°C. Hybrids were subsequently revealed by direct immunogold labeling.

Immunocytochemistry

Lowicryl sections of loosened cells were incubated for 30 min at room temperature in the presence of either 72-kDa polyclonal antibody diluted 1/100 in PBS (36,38) or anti-Sm Y12 monoclonal antibody diluted 1/25 in PBS (34) to identify structures containing viral ssDNA and cellular snRNP, respectively. After 15 min of washing in PBS, the grids were floated for 30 min on 1/50 dilution of goat anti-mouse IgG conjugated to gold particles, 10 nm in diameter (Biocell Research Lab.). After a final PBS wash, the grids were rapidly rinsed in a jet of distilled water, air-dried, and stained for 10 min with uranyl acetate.

Double Labeling

For simultaneous detection of structures containing U1 RNA and snRNP proteins, grids bearing untreated sections of loosened cells were incubated successively over drops of U1 DNA probe, anti-Sm monoclonal antibody Y12, and finally over a cocktail of gold-labeled anti-biotin antibody and goat anti-mouse IgG, 10 and 5 nm in diameter, respectively.

RESULTS

Effects of the Cell Extraction Technique on the Intranuclear Distribution of Viral DNA Molecules in Infected Nuclei

All three of the main steps of intranuclear modifications, which can be observed in HeLa cell cultures infected with adenovirus type 5 for 17 h because of the high asynchrony of the infectious cycle, are still recognizable after the loosening procedure (4). The latter did not allow random dispersion of the different types of viral DNA. Following in situ hybridization of the viral DNA probe on RNase-NaOH pretreated Lowicryl sections of loosened infected cells to detect viral DNA molecules, gold particles were localized exclusively over the dissociated viral region whatever the stage of the infectious cycle (Fig. 1a, b). The viral ssDNA accumulation sites, which were present within the viral region at intermediate (Fig. 1b) and late stages of nuclear modifications, also were

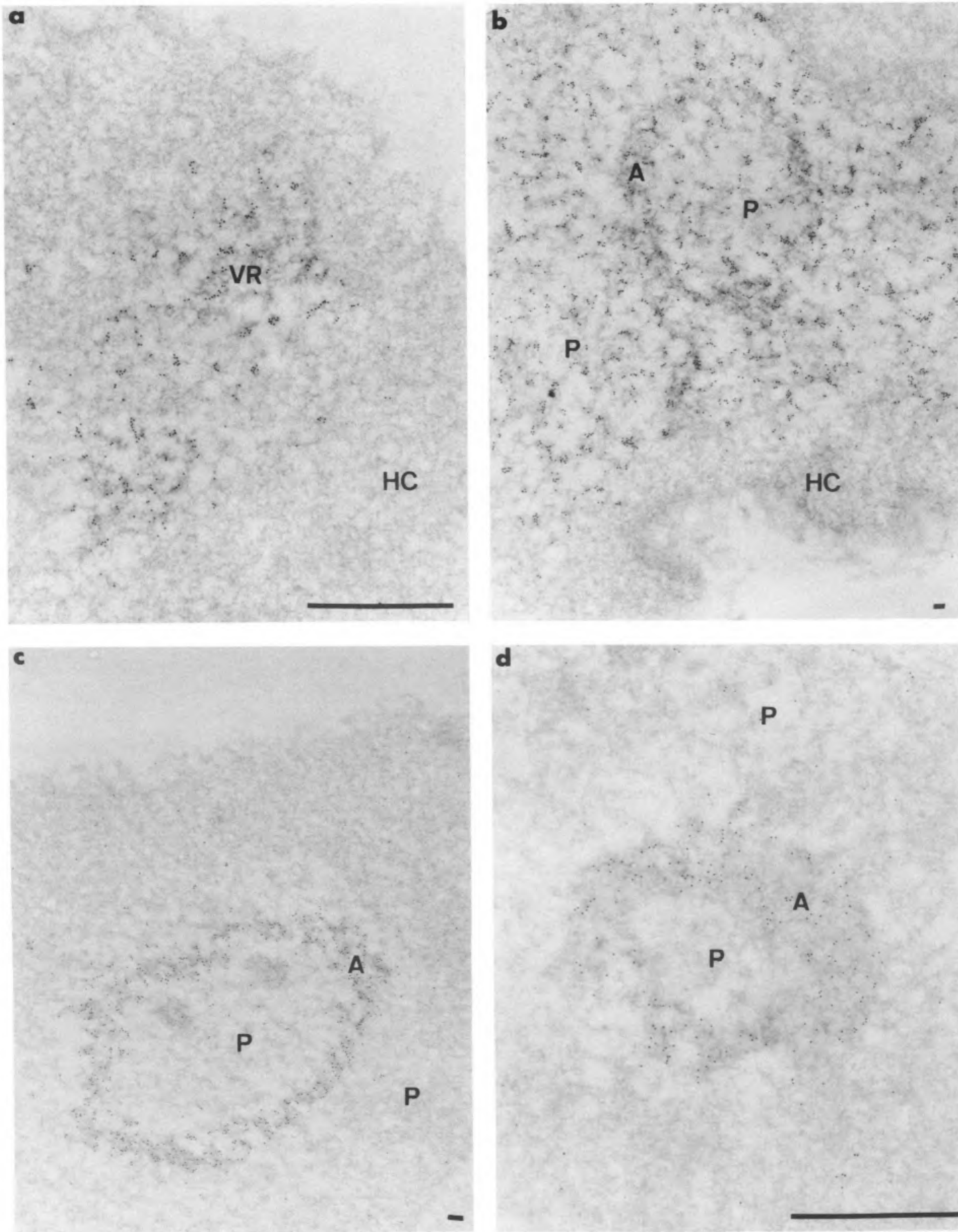

FIG. 1. Intranuclear distribution of adenoviral DNA (a, b) and single-stranded DNA-binding 72-kDa protein (c, d) following cell extraction technique. Bars represent 1 μm. (a, b) In situ hybridization of a viral probe. Gold particles that localize viral DNA are restricted to the loosened viral region without any labeling of the surrounding host chromatin fibers (HC). (a) Early stage of nuclear modification. The small viral region (VR), named the early replicative site, consists of intermingled fibrils. (b) Intermediate stage of nuclear modification. A ring-shaped structure, named viral single-stranded DNA accumulation site (A), is present in the viral region. Gold particles are homogeneously distributed over this structure and the surrounding fibrillogranular network of the peripheral replicative zone (P). (c, d) Immunogold localization of the viral single-stranded 72-kDa protein. Intermediate stage of nuclear transformation. The ring-shaped viral single-stranded DNA accumulation sites (A) are intensely labeled whatever the plane of the section, perpendicular (c) or tangential (d). P: peripheral replicative zone.

labeled. The occasional ring-shaped configuration of these poorly dissociated structures was preserved (Fig. 1b).

Following immunogold detection of the viral ssDNA binding 72-kDa protein, gold particles were almost entirely confined to the poorly dissociated viral ssDNA accumulation sites that sometimes assumed the shape of a ring (Fig. 1c, d). Taken together, the data indicate that the cell extraction technique preserves the specific topological distributions of the different kinds of viral DNA, dsDNA, and ssDNA, as well as the association of the viral ssDNA molecules with their associated viral 72-kDa protein.

Effects of the Cell Extraction Technique on the Intranuclear Distribution of Viral RNA Molecules in Infected Nuclei

Following in situ hybridization of the viral DNA probe on DNase-digested Lowicryl sections of loosened infected cells, gold particles that labeled viral RNA were localized exclusively over the poorly dissociated early replicative sites of slightly virus-modified nuclei (Fig. 2). Later in infection, at the intermediate stage of nuclear modification, only the dissociated fibrillogranular network of the peripheral replicative zone was labeled. The adjacent poorly dissociated viral ssDNA accumulation sites were not labeled (Fig. 3). Still later, gold particles were found over three structures that occasionally were contiguous, the dissociated fibrillogranular network (Fig. 4a), the clusters of interchromatin granules (Table 1, Fig. 4a), and the viral compact rings (Table 2, Fig. 4a, b). The electron opacity of the viral compact rings was markedly reduced following loosening treatment but their ring-shape was preserved. The low salt treatment revealed the fibrillar organization of the ring that, in conventionlly fixed cells, seemed devoid of internal structure (13,42). As described for unloosened infected nuclei (42), the labeling of the viral compact rings was highly variable (Fig. 4a, b) and viral RNA molecules were detected within the ring but not within the central translucent core (Fig. 4b).

No labeling was obtained when the probe was applied on sections of loosened infected cells digested with RNase and DNase prior to hybridization.

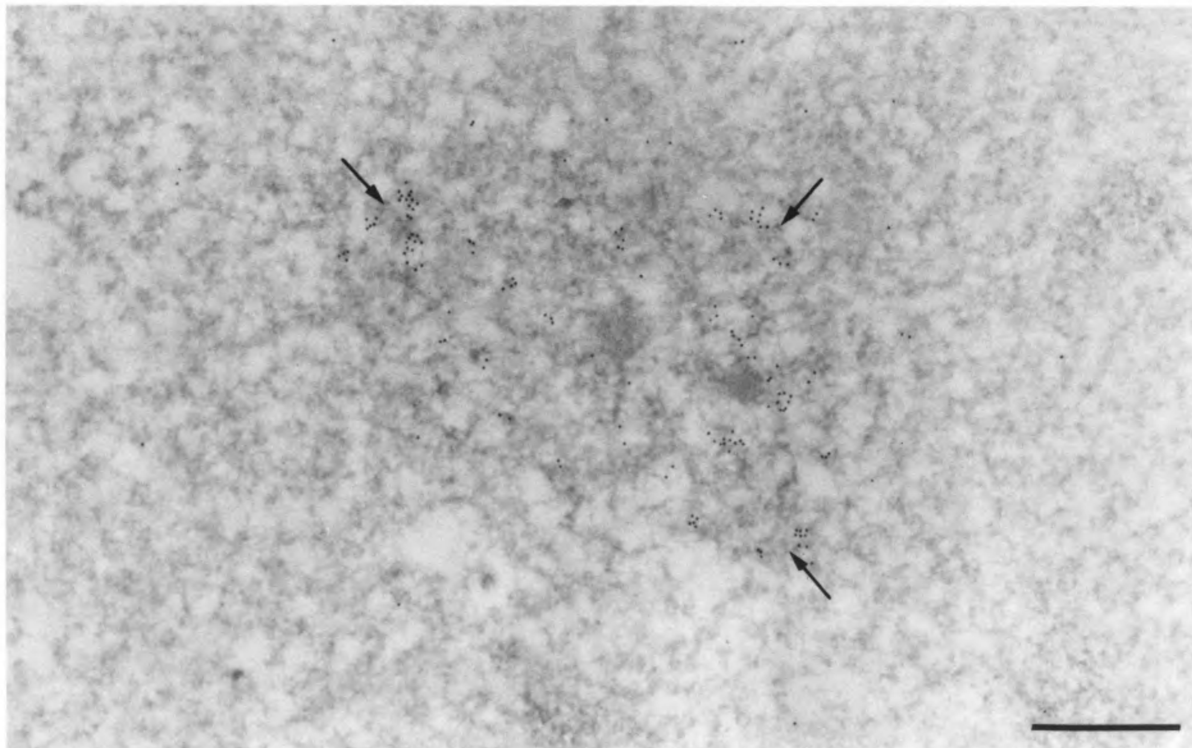

FIG. 2. Intranuclear distribution of adenoviral RNA following cell extraction technique. In situ hybridization of a biotinylated viral DNA probe. Bar represents 0.5 μm. Early stage of nuclear modification. Gold particles are restricted to a limited area of the nucleoplasm (arrows), which is the partially loosened early replicative site. The surrounding loosened nucleoproteins are entirely devoid of labeling because of their cellular origin.

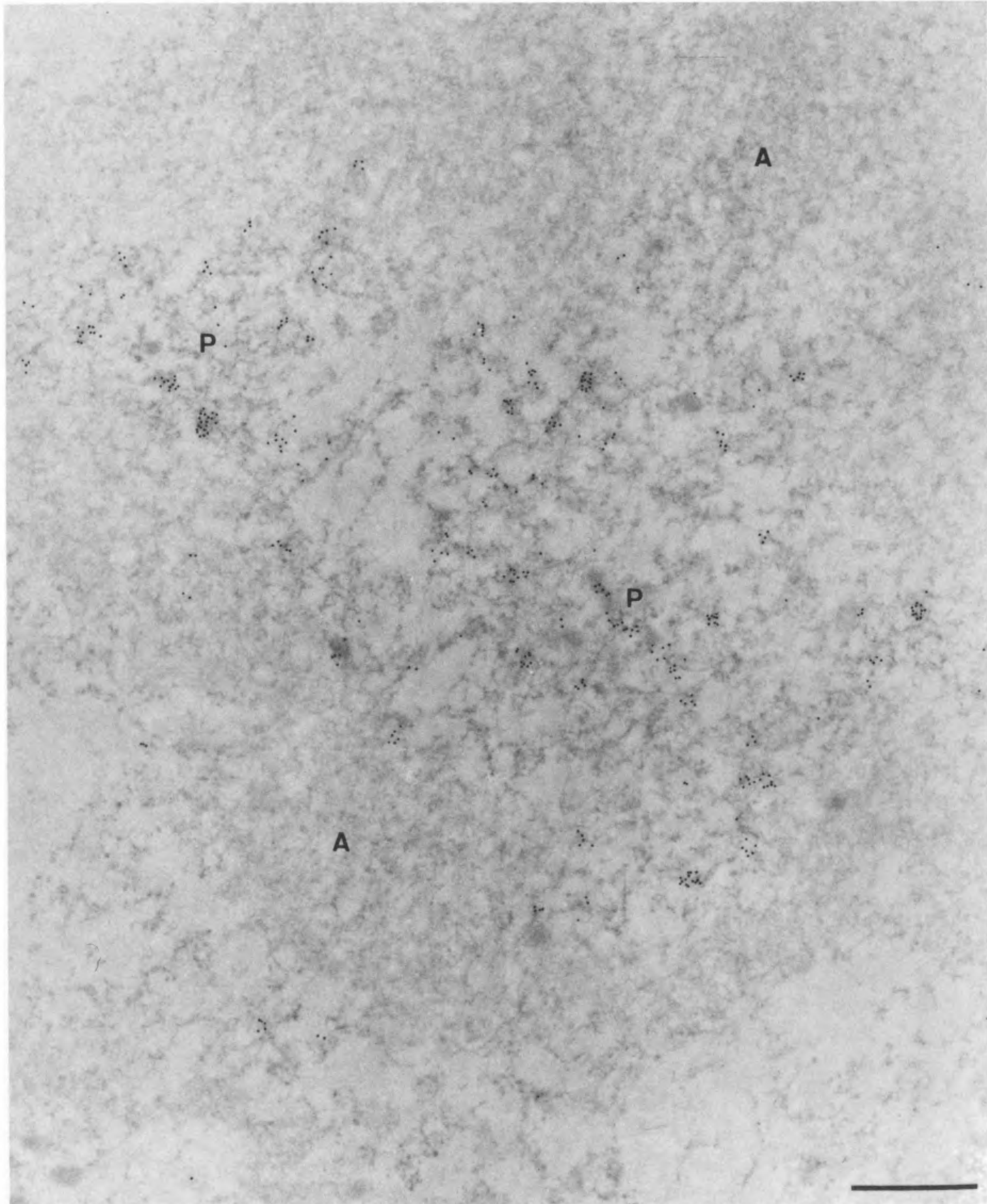

FIG. 3. Intranuclear distribution of adenoviral RNA following cell extraction technique. In situ hybridization of a biotinylated viral DNA probe. Bar represents 0.5 μm. Intermediate stage of nuclear modification. Gold particles are exclusively localized over the loosened components of the peripheral replicative zone (P) whereas the poorly dissociated viral single-stranded DNA accumulation site (A) is entirely devoid of labeling.

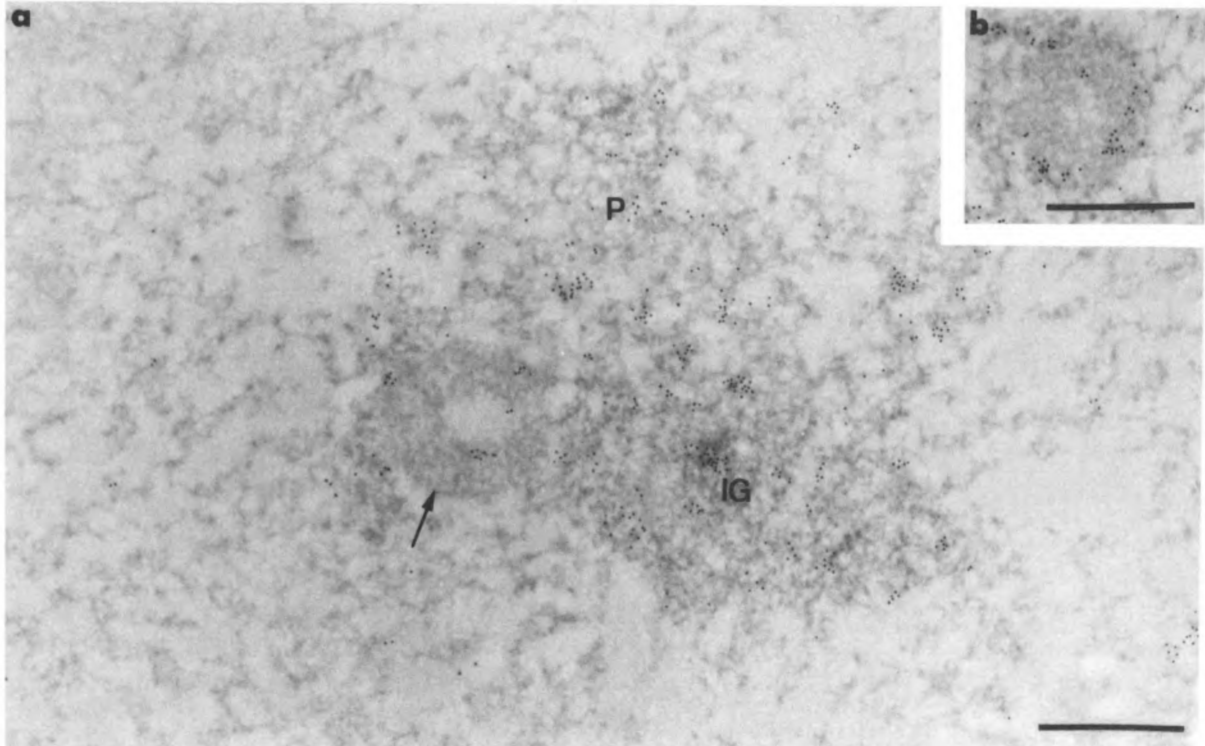

FIG. 4. Intranuclear distribution of adenoviral RNA following cell extraction technique. In situ hybridization of a biotinylated viral DNA probe. Bars represent 0.5 μm. Late stage of nuclear modification. (a) Gold particles are numerous over two structures, the cell clusters of interchromatin granules (IG) and the viral partially loosened peripheral replicative zone (P). The contiguous viral compact ring (arrow), the fibrillar configuration of which is revealed by the hypotonic treatment of cells, is poorly labeled. (b) The labeling of the compact ring is much more intense than in (a).

Effects of the Cell Extraction Technique on the Intranulear Distribution of U1 and U2 snRNA Molecules

After using the U1 and U2 DNA probes on undigested sections of loosened infected cells, the clusters of interchromatin granules were the most intensely labeled structures whatever the stage of the infectious cycle (Table 1, Figs. 5a and 6a). Gold particles also were present over the dissociated early replicative sites of slightly infected nuclei and over the fibrillogranular network of nuclei more advanced in infection. In addition, gold particles were present over some, but not all, compact rings (Table 2, Figs. 5b and 6b).

No labeling was observed on sections digested with RNase prior to hybridization whatever the probe used.

TABLE 1
LABELING DENSITIES OVER THE CLUSTERS OF INTERCHROMATIN GRANULES IN INTENSELY ADENOVIRUS-MODIFIED NUCLEI

Probe	Kind of Labeled Nucleic Acid	Loosened Material	Unloosened Material
Viral DNA	Viral RNA	110 ± 45	50 ± 5*
U1 DNA	U1 snRNA	102 ± 38	43 ± 12†
U2 DNA	U2 snRNA	92 ± 33	19 ± 8†
poly(dT)	poly(A)$^+$ RNA	64 ± 12	21 ± 7†

The gold particle density was expressed as number of particles per μm^2. Number of individual measurements was 15.

*Unpublished values from Puvion-Dutilleul et al. (42).

†Published values from Puvion-Dutilleul et al. (34).

TABLE 2
QUANTITATIVE ANALYSES OF THE GOLD PARTICLES LOCATED OVER THE ADENOVIRUS-INDUCED COMPACT RINGS

Probe	Kind of Labeled Nucleic Acid	Loosened Material	Unloosened Material
Viral DNA	Viral DNA	0 to 16	0 to 13*
U1 DNA	U1 snRNA	0 to 13	0 to 5†
U2 DNA	U2 snRNA	0 to 12	0 to 5†
poly (dT)	poly $(A)^+$ RNA	0	0†

The labeling over the compact rings was highly variable. By reason of the very homogeneous size of these structures, the extreme values for each probe are reported. Number of individual measurements was 10.

*Unpublished values from Puvion-Dutilleul et al. (42).

†Published values from Puvion-Dutilleul et al. (34).

Effects of the Cell Extraction Technique on the Intranuclear Distribution of snRNP Proteins

After using anti-Sm monoclonal antibody Y12 on sections of loosened infected cells, gold particles that localized snRNP proteins accumulated over the clusters of interchromatin granules and were rare over the contiguous viral structures (Fig. 7a). Following concomitant detection of U1 RNAs by in situ hybridization and snRNP proteins by immunocytochemistry, both 10 and 5 nm gold particles were observed over the clusters of interchromatin granules (Fig. 7b), which indicates that the extraction treatment preserves both the nucleic acid and proteinaceous component of spliceosomes.

Effects of the Cell Extraction Technique on the Intranuclear Distribution of Poly(A)$^+$ RNA

After using a poly(dT) probe on undigested sections of loosened infected cells, gold particles were present mainly over the clusters of interchromatin granules whatever the stage of infection, that is, in seemingly unaltered nuclei (Fig. 8a) as well as in slightly or intensely modified nuclei (Table 1, Fig. 8b). In addition, a few gold particles were randomly dispersed over the dissociated fibrillogranular network of the peripheral replicative zone (Fig. 8b). The compact rings were entirely devoid of gold particles (Table 2). A RNase digestion of sections prior to hybridization abolished the labeling.

DISCUSSION

In previous article we have described the results of studies identifying those intranuclear structures that are involved in RNA processing and their rearrangements in response to adenovirus and herpes simplex virus infection (5,34,48). During these studies we revealed for the first time that the clusters of interchromatin granules, which are the major site of accumulation of spliceosome components, also might be involved in the transient storage and/or degradation of mature RNA molecules (34,49). In addition, we presented evidence that the composition in snRNPs of the three accumulation sites for spliceosome components in normal cells, the coiled bodies, the clusters of interchromatin granules, and their associated zones, was not affected by exposure to media of high and low ionic strength, which suggests that snRNPs are integral components of these nuclear structures (35). In the present investigation we extend our earlier observations with evidence that the associations of viral RNAs, poly(A)$^+$ RNAs, and snRNPs (snRNAs and their associated proteins) with clusters of interchromatin granules and viral transcription sites of adenovirus type 5-infected nuclei are stable, that is, not disrupted by experimentally induced loosening of nucleoproteins.

In the nuclei of infected cells, viral transcription is associated only with specific regions that also are the sites of viral genome replication (38,39,42). These are the early replicative sites, which are the earliest viral structures detectable after viral genome replication is initiated, and the peripheral replicative zones, which make up one component of the subsequent complex group of viral replicative foci. The persistence of viral RNAs within these two sequentially developed structures after treatment of infected cells with a detergent-containing hypotonic solution emphasizes the strong anchorage of growing transcripts on active genes. Indeed, this treatment is known to induce the dissociation of weak interactions because of the great dilution of the soluble compo-

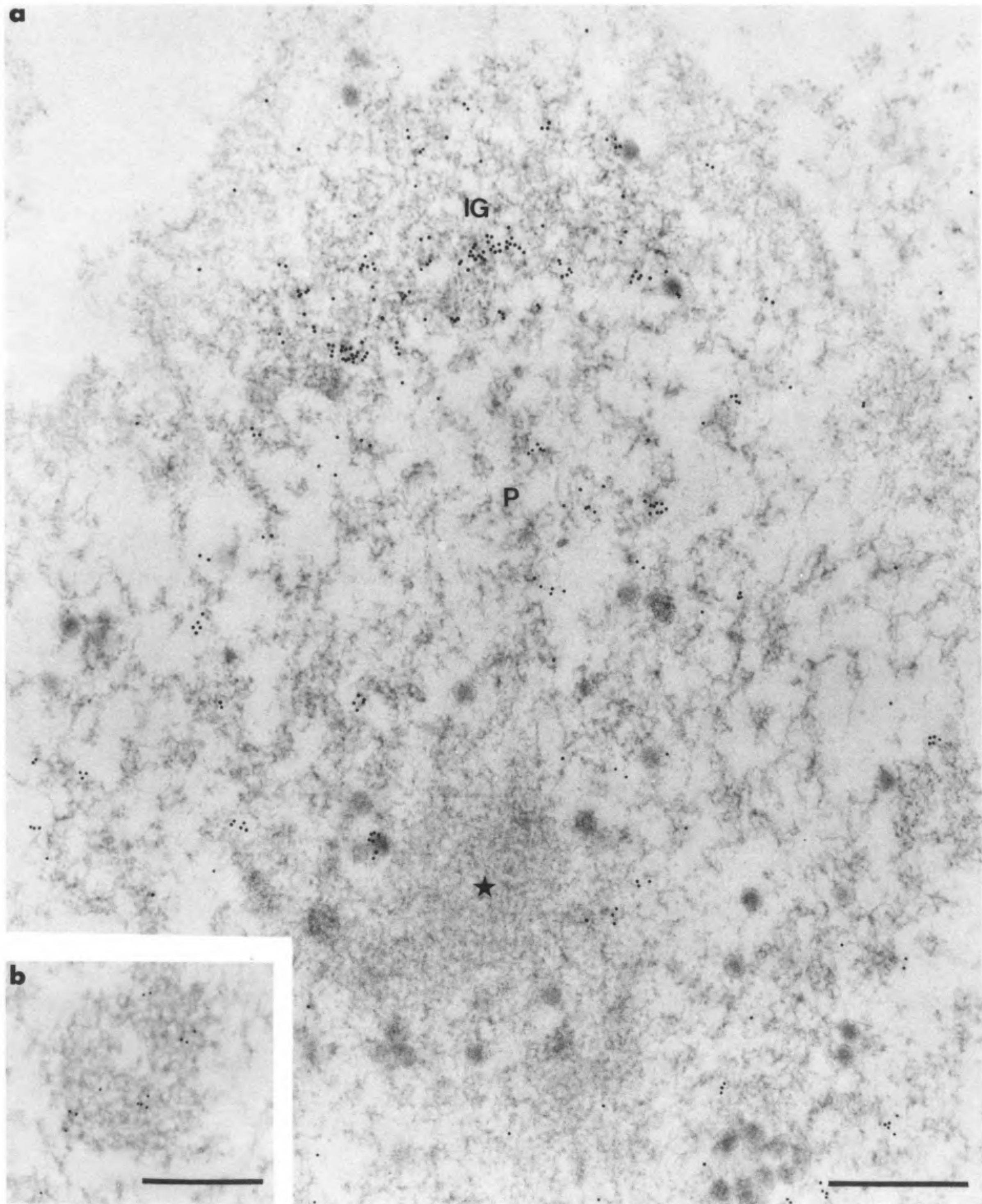

FIG. 5. Intranuclear distribution of snRNA molecules following cell extraction technique. Late stages of virus-induced intranuclear modifications. Bars represent 0.5 μm. In situ hybridization with biotinylated human U1 DNA probe. (a) Gold particles that localize U1 RNAs accumulate over the large cluster of interchromatin granules (IG) and are present to a lesser extent over the adjacent partially loosened peripheral replicative zone (P). The viral genome storage site (star) is devoid of labeling. (b) The fibrillar component of the virus-induced compact ring is labeled.

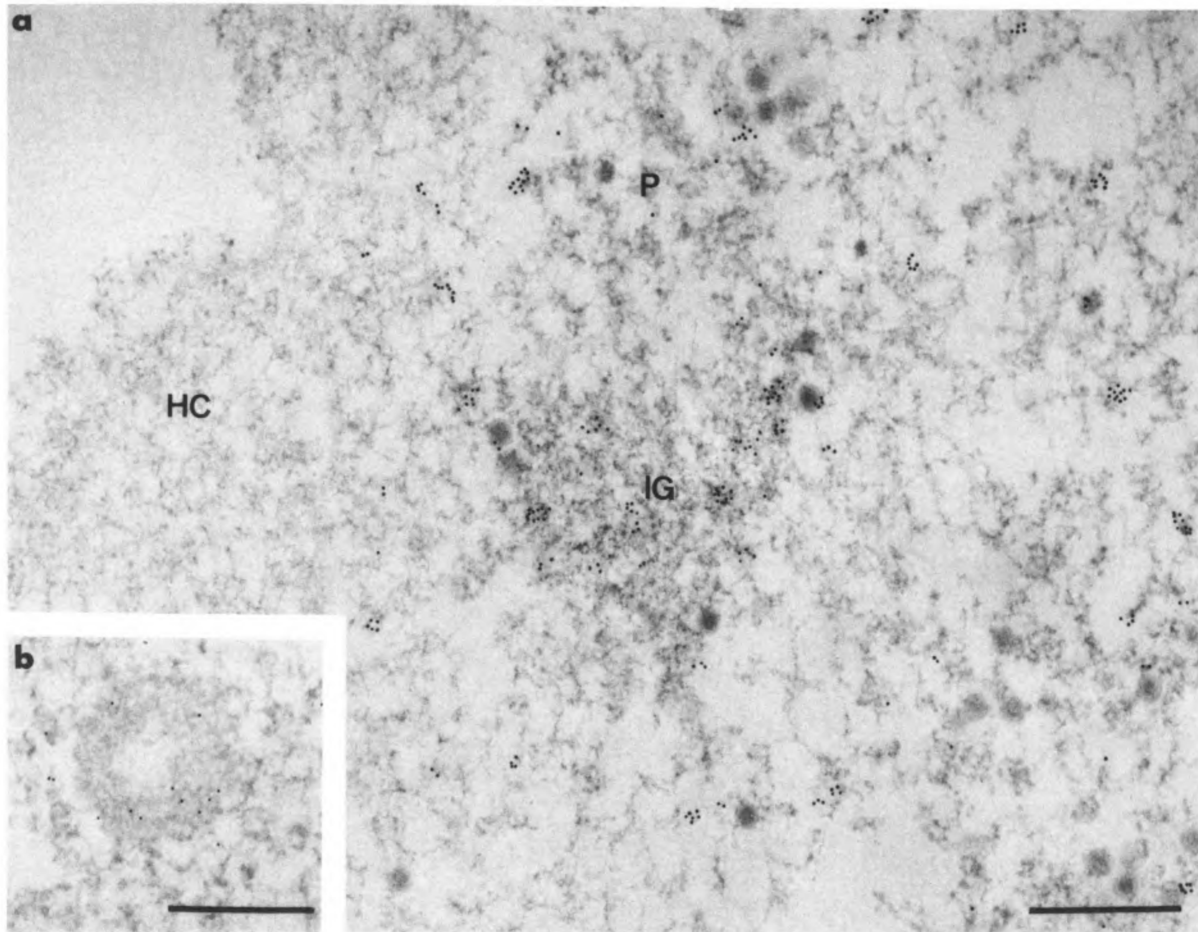

FIG. 6. Intranuclear distribution of snRNA molecules following cell extraction technique. Late stages of virus-induced intranuclear modifications. Bars represent 0.5 μm. In situ hybridization with biotinylated human U2 DNA probe. (a) The large cluster of interchromatin granules (IG) is intensely labeled whereas the contiguous partially loosened peripheral replicative zone (P) is poorly labeled. (b) Gold particles are present over the fibrillar component of the virus-induced compact ring.

nents whereas strong associations are preserved. The conservation of viral RNA molecules within the viral transcription site following the loosening procedure is in good agreement with the observation of viral transcripts still attached to the viral genomes following more extensive dispersal of nuclear contents (7,8,24). In addition, the preservation of the topological distribution of RNA transcripts in loosened infected cells indicates that intranuclear development of adenovirus requires a precise spatial organization and also agrees with the strong attachment of transcribing genomes to the nuclear matrix (15,45).

The U1 and U2 snRNAs, which are components of the cellular spliceosomes, also are present within the viral compartments described above and also are related to viral transcription. The results obtained herein by thin sectioning of dissociated nucleoproteins indicate that snRNAs are firmly attached to growing viral transcripts. This is in complete agreement with results obtained at the molecular level, which detected snRNP antigens on transcripts still attached to the DNA matrix (14) and affirmed that some regions of the viral and cellular transcripts had loop arrangements and that a few viral transcripts were cleaved during their development (6–9,30,52), strongly suggesting that splicing is a cotranscriptional event. In addition, following loosening of nuclear constituents, the clusters of interchromatin granules that are accumulation sites for spliceosome components were labeled with U1 and U2 DNA probes and anti-Sm monoclonal antibody Y12, which indicates that the spliceosomes persist without changes in their snRNP contents in the loosened nuclei. However, the partial unfolding of sn-

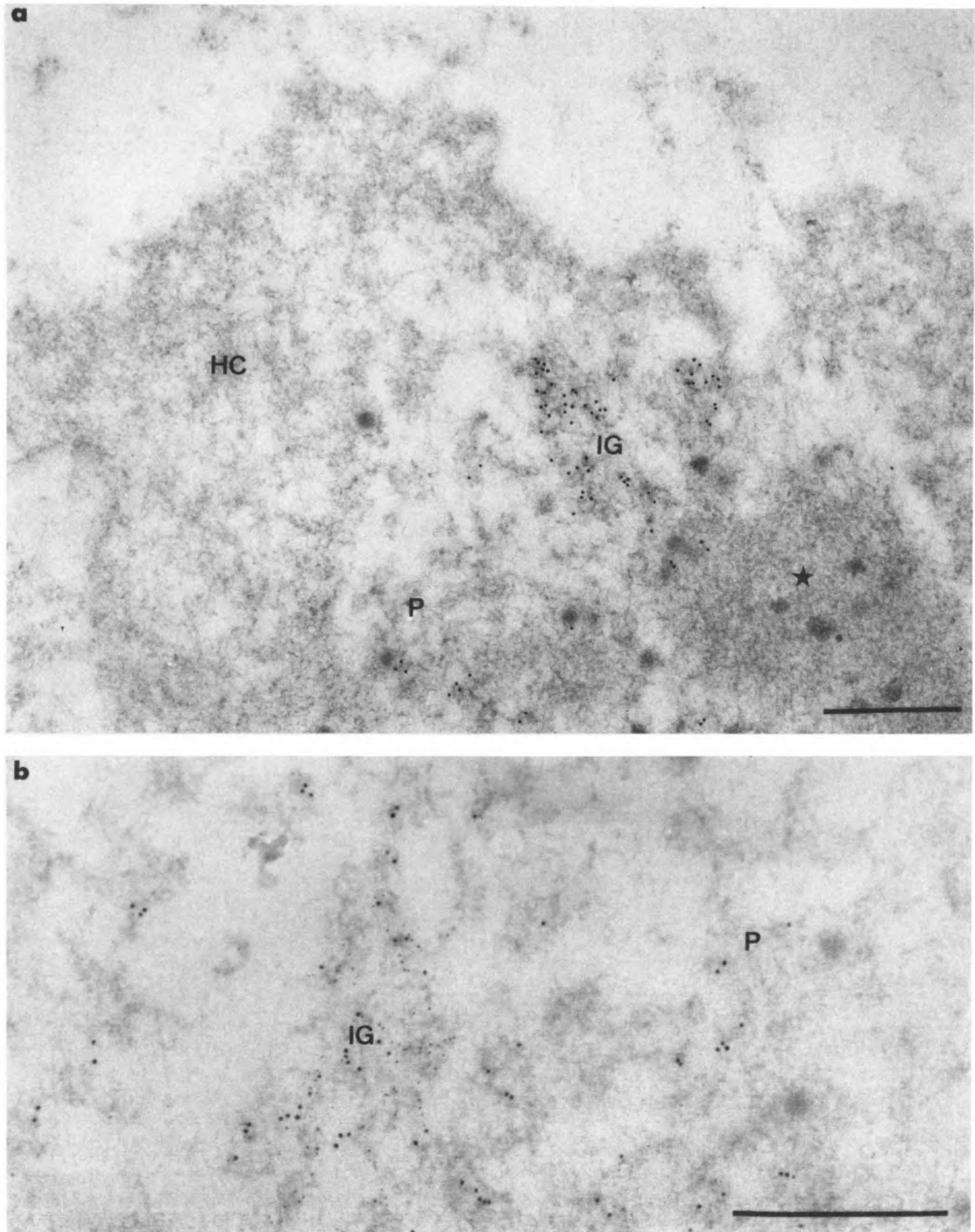

FIG. 7. Intranuclear localization of snRNP proteins with (b) or without (a) concomitant detection of U1 snRNA. Late stages of nuclear transformation. Bars represent 0.5 μm. (a) Immmunogold detection of snRNP proteins using anti-Sm monoclonal antibody Y12. Gold particles accumulate over the cluster of interchromatin granules (IG). They are scattered over the adjacent peripheral replicative zone (P) and absent over the more distant viral genome storage site (star). (b) Concomitant detection of snRNP proteins and U1 snRNA molecules. The snRNP proteins are revealed by immunogold labeling with specific antibody and 5-nm gold particles whereas the U1 snRNA molecules are revealed by in situ hybridization with the specific probe and 10-nm gold particles. The cluster of interchromatin granules (IG) is decorated by the two sizes of gold particles.

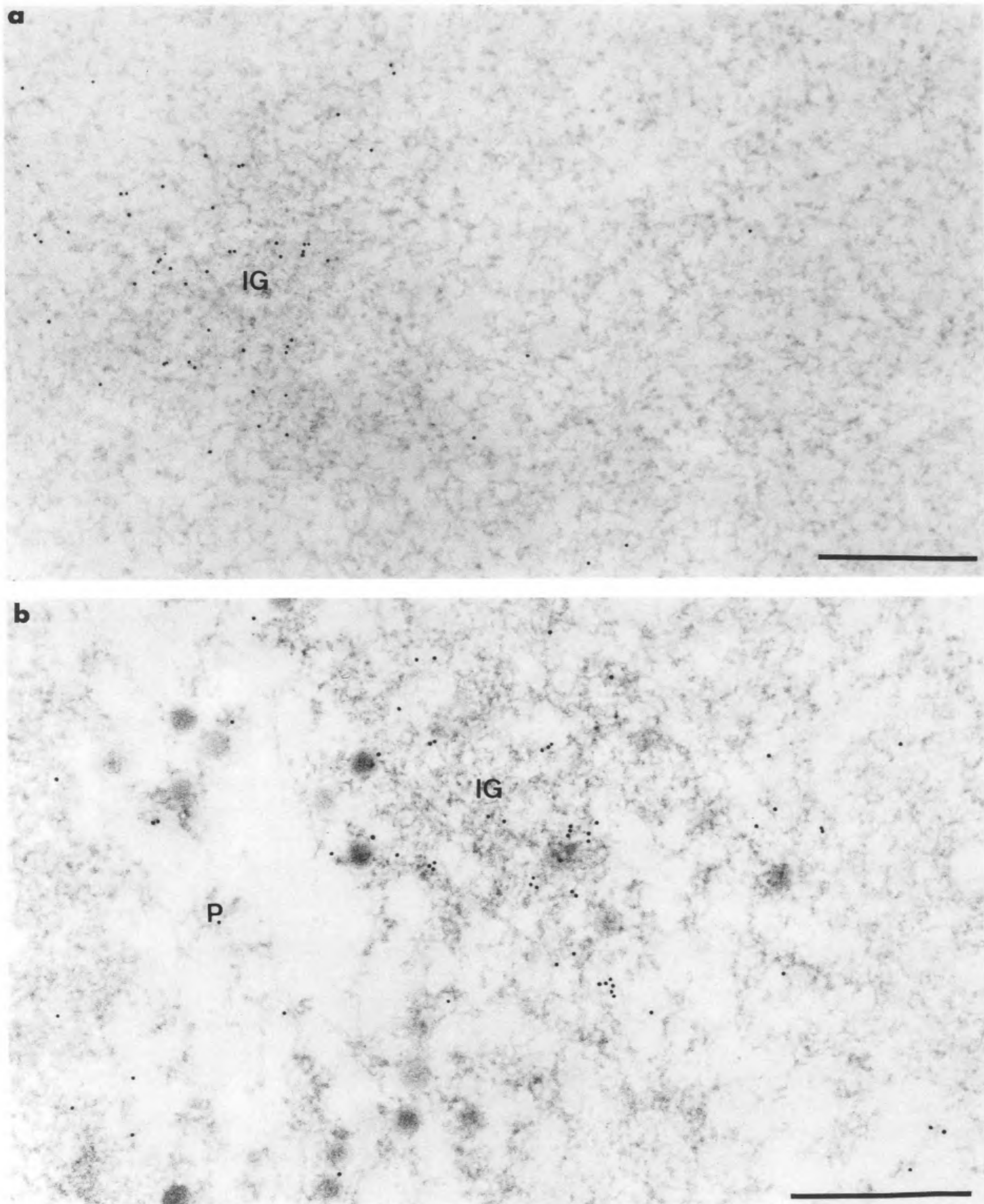

FIG. 8. Intranuclear distribution of poly(A)$^+$ RNA molecules following cell extraction technique. Adenovirus-infected HeLa cells. In situ hybridization with a biotinylated poly(dT) probe. Bars represent 0.5 μm. (a) Part of a seemingly unmodified nucleus. Gold particles accumulate over the cluster of interchromatin granules (IG) and a few are widely scattered over the surrounding loosened nucleoproteins. (b) Part of an intensely virus-modified nucleus. Gold particles are numerous over the cluster of interchromatin granules (IG) and rarer over the surrounding widely spread peripheral replicative zone (P).

RNP molecules experimentally induced by the detergent–hypotonic treatment markedly improves the accessibility of the snRNAs to their specific probes compared to unloosened material (Tables 1 and 2).

A particularly important finding of this study is the observation that the association of viral RNA and poly(A)$^+$ RNA molecules with virus-modified clusters of interchromatin granules was not disrupted by the hypotonic shock and detergent treatment employed in the loosening procedure. It must be pointed out that polyadenylated molecules might comprise viral and cellular messenger RNAs (16,46). It cannot be excluded, however, that unspliced viral transcripts might be transiently stored in the clusters of interchromatin granules to be degradated. The firm anchorage of viral RNAs and poly(A)$^+$ RNAs throughout the clusters of interchromatin granules, even after spreading of the nucleoproteins, indicates that these RNA molecules and clusters of interchromatin granules are structurally linked, and strongly suggests that such associations imply functional relationships. Clusters of interchromatin granule-associated RNA molecules, which cannot be easily removed under our experimental conditions, may represent those messenger RNA molecules that are stored in the nucleus before their transport to the cytoplasm. The anchorage of individual messenger RNA to the clusters of interchromatin granules, however, is of short duration, usually no more than a few hours, as revealed by pulse-chase autoradiographic studies performed on infected cells (42). It must be mentioned, however, that following other approaches (light microscope in situ hybridization and preembedding electron microscope in situ hybridization) Huang et al. (17) found that the population of poly(A)$^+$ RNA molecules in clusters of interchromatin granules of normal cells was stable.

Our observation of the strong binding of viral RNAs and snRNAs within the virus-induced compact rings after loosening treatment while poly(A)$^+$ RNA molecules remain absent (34) suggests that these structures, which contain nonpolyadenylated viral RNA, are a storage and/or degradation site for the cleaved portions of the primary late transcripts resulting from differential poly(A)$^+$ site selection at the posttranscriptional step (1,26).

In conclusion, the results of electron microscopic in situ hybridization of poly(dT) probe, U1 and U2 DNA probes, and viral DNA probe performed on loosened chromatins of adenovirus-infected cells demonstrate a stability in the distribution of the viral transcription sites and also in the accumulation sites of splicing components under experimental conditions that spread apart the nucleoproteins and only preserves functionally relevant associations. Therefore, the results support the concept of a strong binding of viral genomes to the nuclear matrix and a firm attachment of spliceosome components to the interchromatin granules, which are themselves constituents of the nuclear matrix. The data also reveal the anchorage of mRNA molecules to clusters of interchromatin granules and reinforce our previous assumption (34,49) that these structures might play a crucial role in the regulation of the export of mRNA from the nucleus into the cytoplasm.

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