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Differences in snRNP Localization Between Transformed and Nontransformed Cells

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We have examined the localization of snRNPs in a variety of mammalian cells and have observed differences in the organization of these factors in transformed cells, immortal cells, and cells of defined passage number. Cells of defined passage number exhibit a speckled staining pattern after immunolabeling with anti-Sm, anti-B″, or anti-m_3G antibodies. Furthermore, 2–3% of the cells, in a given population, exhibit labeling of 1 or 2 round coiled bodies in addition to the speckled-labeling pattern. However, transformed cells exhibited 1–4 intensely stained coiled bodies, in 81–99% of the cells, in addition to the speckled-labeling pattern. Immortal cells exhibited 1–4 intensely stained smaller coiled bodies in 4–40% of the cells, in addition to the speckled-labeling pattern. When immortal cells (REF-52) that had been transformed by adenovirus (REF-52Ad5.4) were examined, these cells exhibited an increase in the percentage of cells containing 1 or 2 intensely stained coiled bodies, in addition to the speckled labeling, from 24 to 99%. On the basis of this study, we conclude that the organization of snRNPs within the mammalian cell nucleus is a reflection of the physiology of the cell that may change upon transformation or immortalization.

INTRODUCTION

Several major classes of small nuclear ribonucleoprotein particles (snRNPs) (U1, U2, U4/U6, and U5), as well as other splicing factors have been shown to be involved in the processing of pre-mRNA molecules (for recent reviews see Krainer and Maniatis, 1988; Bindereif and Green, 1990). For most RNA polymerase II transcripts, such processing includes the addition of a 7-methylguanosine cap structure at the 5′ end of the nascent RNA transcripts, hnRNP assembly, splicing, polyadenylation, and the exchange of hnRNP proteins for mRNP proteins (reviewed in Krainer and Maniatis, 1988; Steitz *et al.*, 1988; Bindereif and Green, 1990). Splicing of nuclear pre-mRNA molecules occurs in spliceosomes, macromolecular complexes composed of a pre-mRNA, snRNPs, and other splicing factors (for a review see Krainer and Maniatis, 1988; Bindereif and Green, 1990). Because RNA processing is essential to cellular function, we and others have been interested in identifying the organization of splicing factors in cell nuclei. Localization studies using antibodies directed against a variety of snRNP-specific proteins (Spector *et al.*, 1983; Reuter *et al.*, 1984; Spector, 1984; Nyman *et al.*, 1986; Verheijen *et al.*, 1986; Habets *et al.*, 1989) or the m_3G-cap structure of snRNAs (Reuter *et al.*, 1984) have shown these antigens to be concentrated in a speckled-staining pattern in the interphase nuclei of mammalian cells. In addition, several studies have also shown these splicing factors to be present in coiled bodies (Eliceiri and Ryerse, 1984; Fakan *et al.*, 1984; Raska *et al.*, 1991). However, recent *in situ* hybridization studies with 2′O-methyl or alkyl oligonucleotide probes to various snRNAs, suggest a more restricted distribution of U2, U4/U6, and U5 snRNAs in the nucleoplasm (Carmo-Fonseca *et al.*, 1991a,b). These studies have reported U2, U4/U6, and U5 snRNAs to be concentrated in 3–4 "foci," which correspond to coiled bodies (Raska *et al.*, 1991), with no speckled labeling (Carmo-Fonseca *et al.*, 1991a,b). U1 snRNA was shown to be diffusely distributed throughout the nucleoplasm as well as being present in coiled bodies (Carmo-Fonseca *et al.*, 1991a,b). In addition, peptide antibodies to U2AF showed this protein to be localized to coiled bodies as well as being diffusely distributed in the nucleoplasm (Zamore and Green, 1991). Recently, we have shown U1 and U2 snRNAs to be concentrated in both speckled nuclear regions and in coiled bodies, both of which are labeled with anti-snRNP antibodies (Huang and Spector, 1992), with the same 2′O-alkyl oligonucleotide probes as were

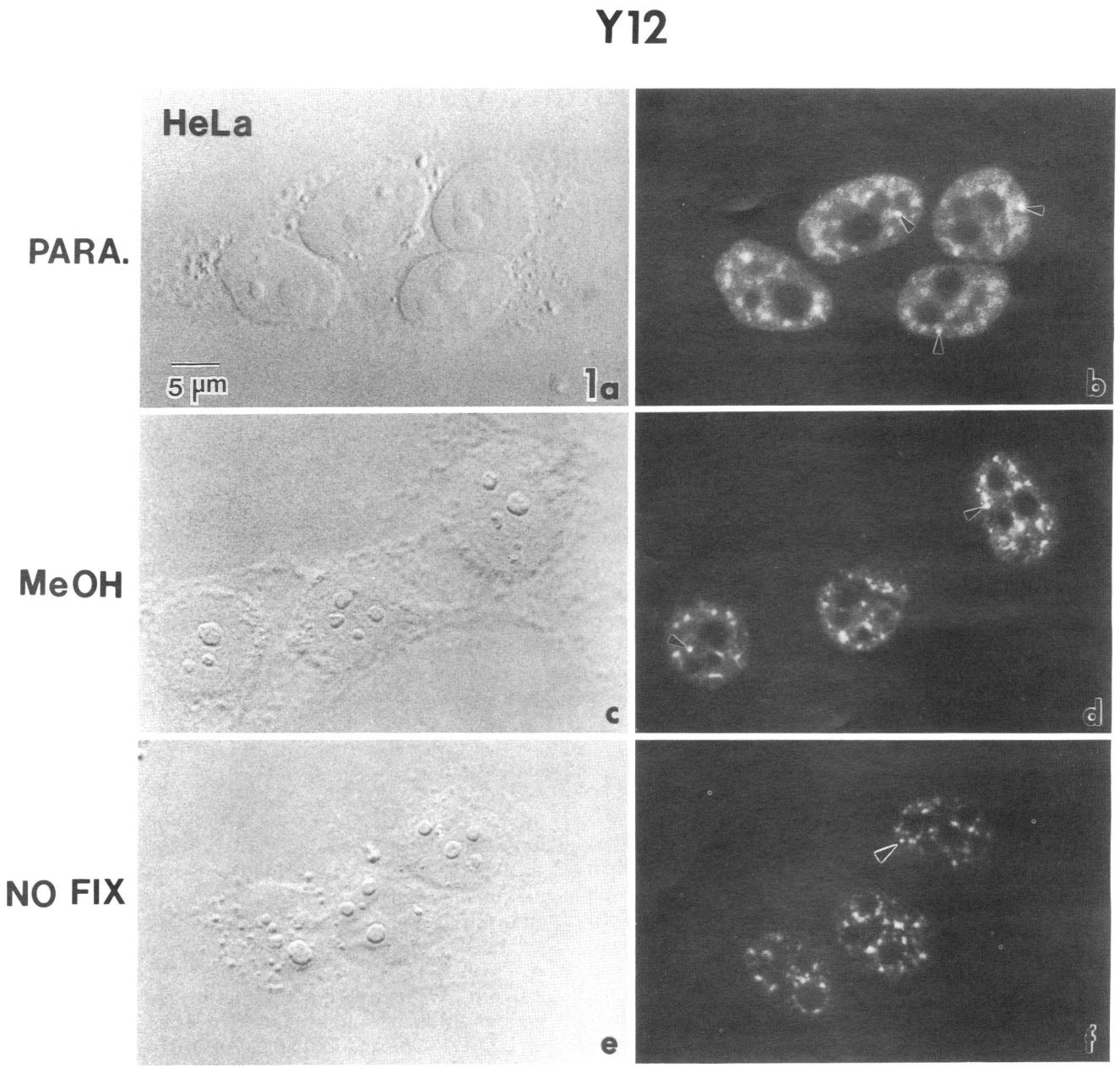

Figure 1. Immunolabeling of HeLa cells with anti-Sm antibodies (Y12) detects snRNPs in a speckled distribution pattern in cells fixed with paraformaldehyde (a and b), methanol (c and d), or unfixed (e and f). One or two brighter, round coiled bodies (arrowheads) are apparent in 81% of the cells.

used in earlier studies. The reported absence of these snRNA species in the speckled nuclear regions in these earlier studies (Carmo-Fonseca *et al.*, 1991a,b) was apparently due to insufficient duration of hybridization with the biotinylated probes. Most recently, Carmo-Fonseca *et al.* (1992) have in fact shown that upon SDS extraction of formaldehyde-fixed cells and subsequent *in situ* hybridization from 1 h to overnight, a colocalization of the U1-, U2-, U4-, U5-, and U6-specific antisense probes and snRNP antigens was observed in speckles as well as in coiled bodies.

In this study, we have investigated the nuclear organization of snRNPs in a variety of mammalian cells by antibody labeling and have observed differences in the organization of these splicing components in transformed cells, immortal cells, and cells of defined passage number. Cells of defined passage number exhibit a

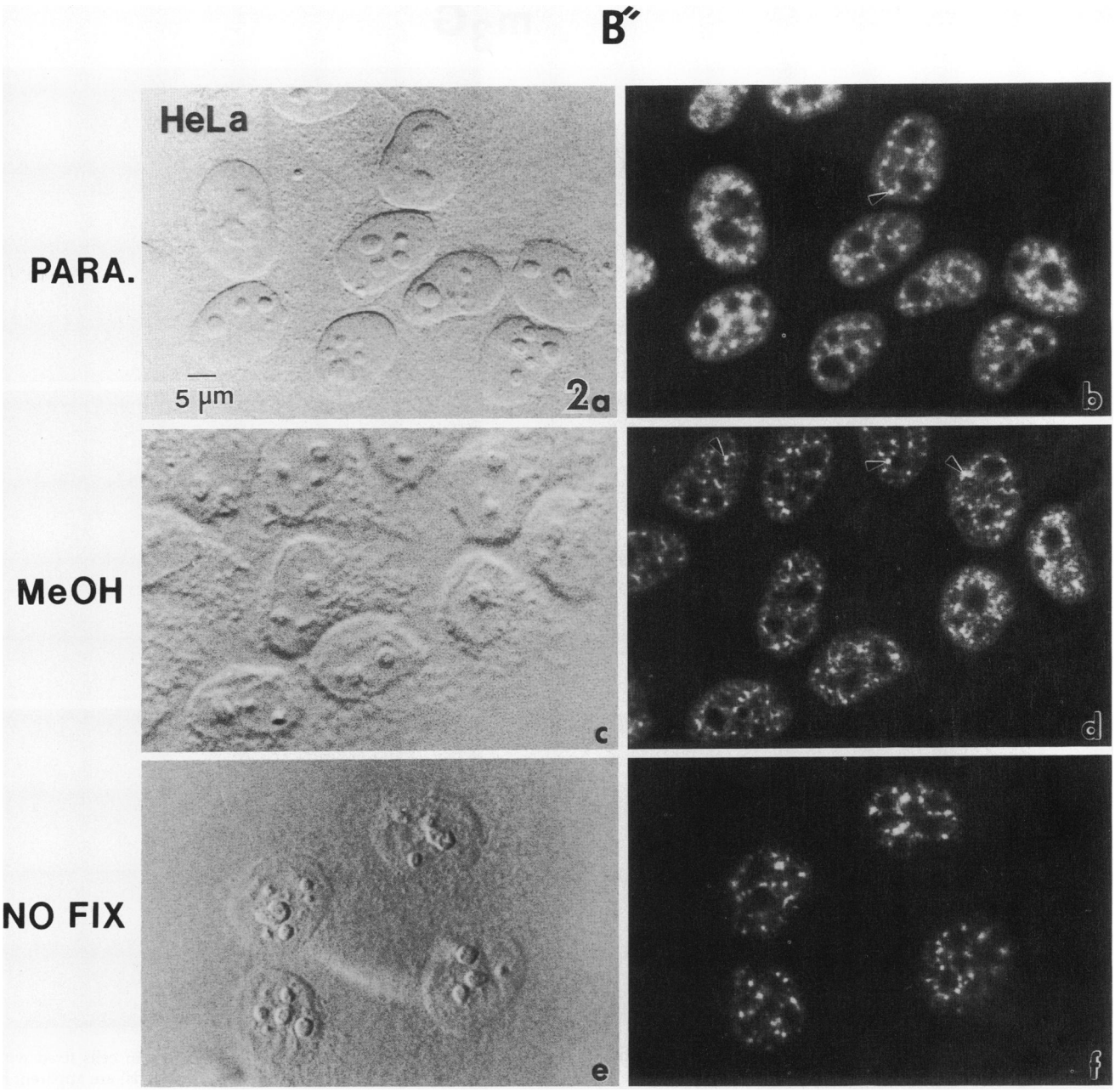

Figure 2. Immunolabeling of HeLa cells with anti-B′ antibodies detects snRNPs in a speckled distribution pattern in cells fixed with paraformaldehyde (a and b), methanol (c and d), or unfixed (e and f). One or two brighter, round coiled bodies (arrowheads) are apparent in 81% of the cells.

speckled-staining pattern. In addition, 2–3% of the cells in a given population exhibited 1 or 2 round immunostained coiled bodies. However, transformed cells exhibited 1–4 large, round intensely stained coiled bodies, in 81–99% of the cells, in addition to the speckled pattern. Immortal cell lines exhibited an intermediate number of cells containing coiled bodies that ranged from 4 to 40%. When immortal cells (REF-52) that had been transformed by adenovirus (REF-52 Ad5.4) were examined, these cells exhibited a significant increase in the percentage of cells containing coiled bodies. On the basis of this study, we conclude that the organization of snRNPs within the mammalian cell nucleus is a reflection of the physiology of the cell, and this organization can change upon transformation or immortalization.

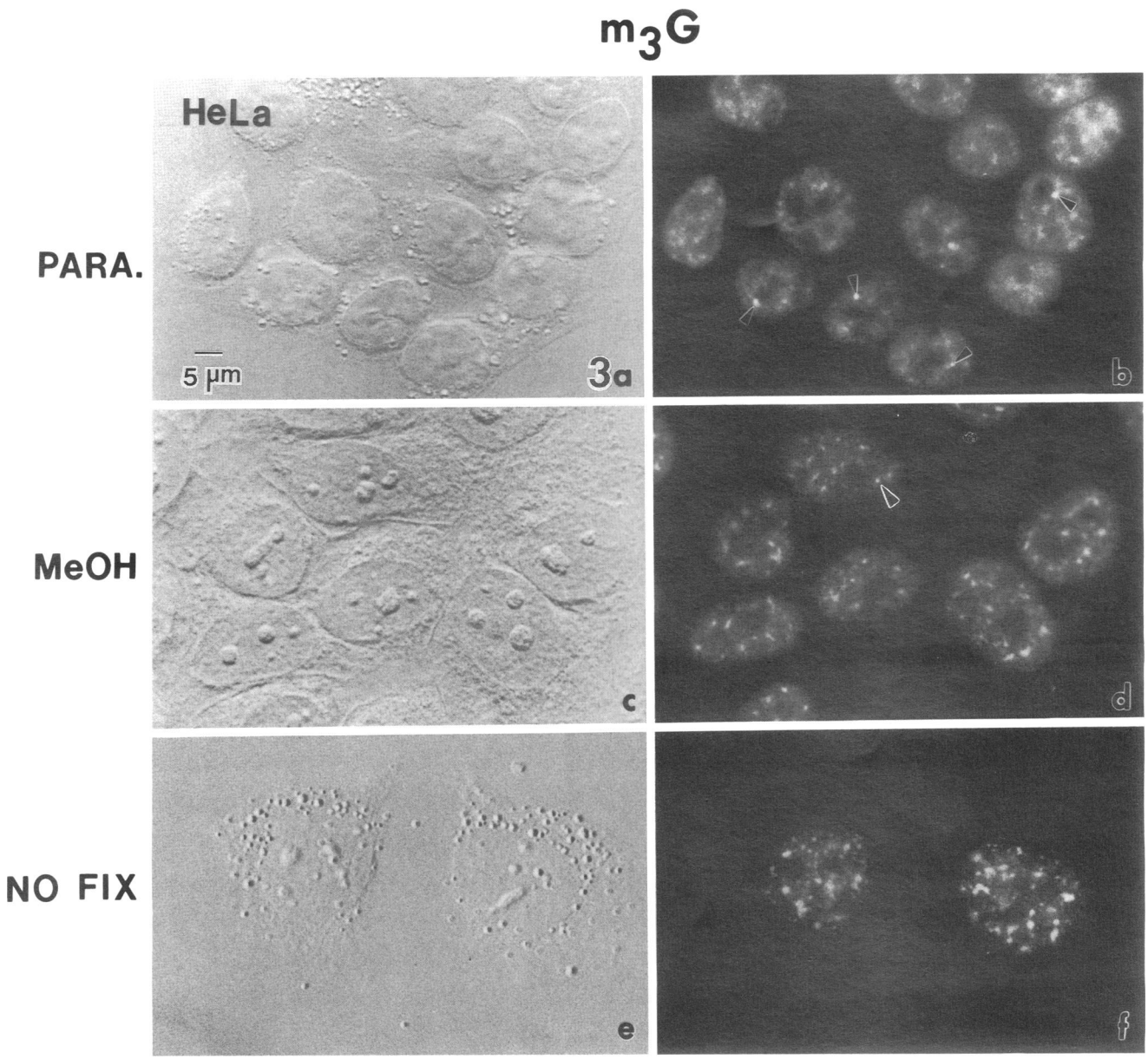

Figure 3. Immunolabeling of HeLa cells with anti-m_3G antibodies detects snRNPs in a speckled distribution pattern in cells fixed with paraformaldehyde (a and b), methanol (c and d), or unfixed (e and f). One or two brighter, round coiled bodies (arrowheads) are apparent in 81% of the cells.

MATERIALS AND METHODS

Cell Culture

Chinese hamster ovary cells (CHO) were cultured on glass coverslips in Ham's F12 medium (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum (FBS), 1% penicillin, and 1% streptomycin. HeLa cells (human epithelial), MRC5 cells (human male lung diploid fibroblasts), WI-38 VA13 cells (human female lung diploid fibroblasts transformed by SV40), NIH 3T3 cells (mouse embryo fibroblasts), REF-52 cells (rat embryo fibroblasts), and REF-52 Ad5.4 cells (rat embryo fibroblasts transformed by adenovirus 5) were cultured in Dulbecco's modified Eagle's medium (GIBCO) supplemented with 10% fetal bovine serum, 1% penicillin, and 1% streptomycin. Detroit 551 cells (human female skin diploid fibroblasts), WI-38 cells (human female lung diploid fibroblasts), and 293 cells (human embryonal kidney cells transformed by adenovirus 5) were grown in Eagle's minimum essential medium (GIBCO) supplemented with 10% fetal bovine serum, 1% penicillin, and 1% streptomycin. All cells were used at ~50% confluency.

Immunofluorescence

Formaldehyde fixed cells were prepared for immunofluorescence microscopy according to previously published procedures (Spector and Smith, 1986). Cells fixed in methanol were done so for 2 min at −20°C.

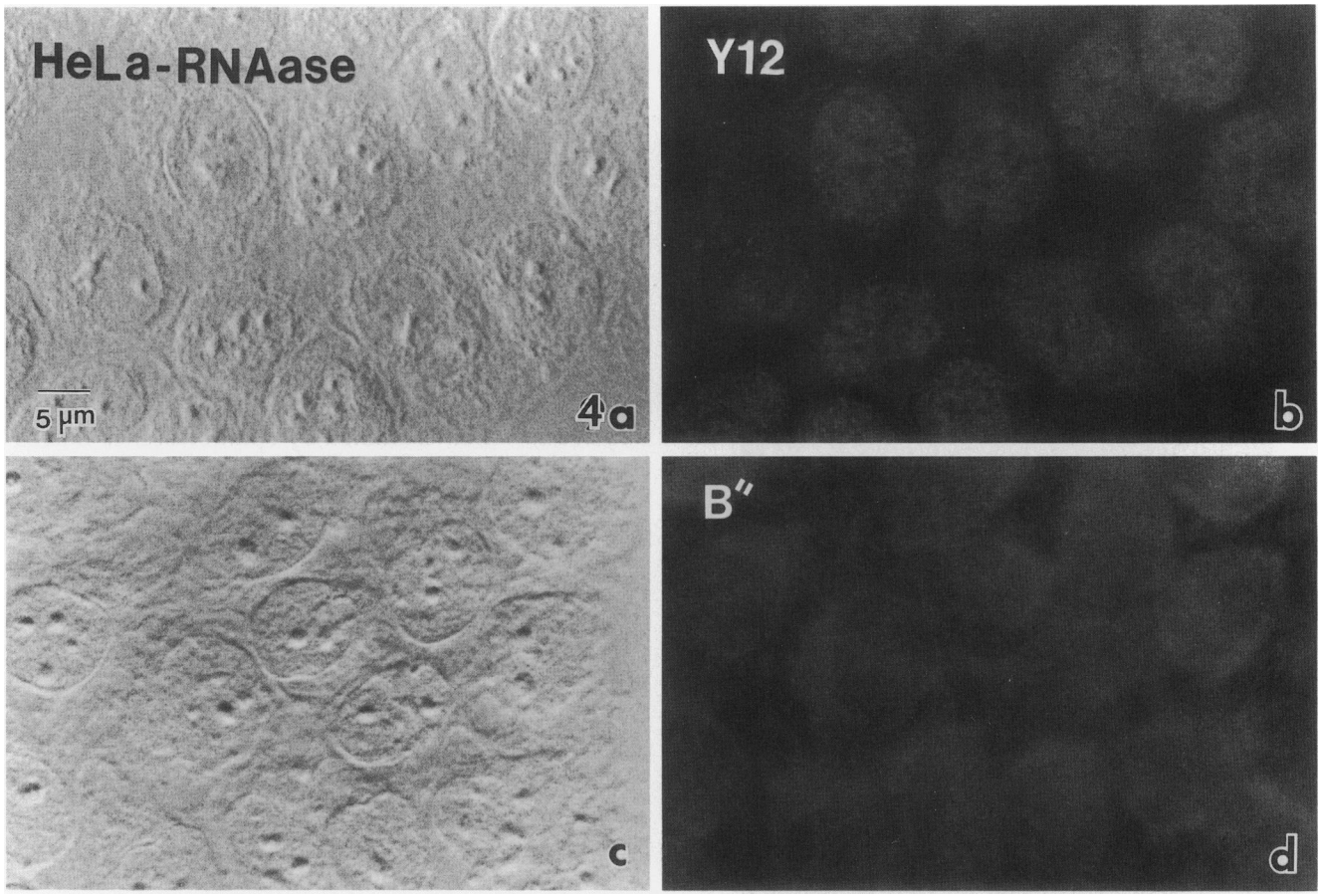

Figure 4. Speckles and coiled bodies are sensitive to RNAase digestion. After RNAase A digestion snRNPs, as visualized by anti-Sm (b) or anti-B″ (d) immunolabeling, are diffusely distributed. The low level of immunolabeling suggests that some snRNPs may have diffused out of the permeabilized cells.

Unfixed cells were permeabilized in 0.2% Triton X-100 in phosphate-buffered saline (PBS, pH 7.3) for 5 min on ice and washed in PBS, 3 × 10 min each, before antibody labeling. Anti-Sm monoclonal antibody ascites (Lerner *et al.*, 1981; Spector and Smith, 1986) were used at a dilution of 1:1000; anti-B″ monoclonal antibody (Habets *et al.*, 1989) tissue culture supernatant was used at a dilution of 1:10; m_3G monoclonal antibody (Krainer, 1988) tissue culture supernatant was used at a dilution of 1:20; anti-coilin rabbit polyclonal antibody (Raska *et al.*, 1991) was used at a dilution of 1:150; and anti-SC-35 monoclonal antibody (Fu and Maniatis, 1990) ascites were used at a dilution of 1:50. Fluorescein isothiocyanate (FITC)- and Texas red-conjugated secondary antibodies (Cappel Laboratories, Cochranville, PA) were used at a dilution of 1:20 for 1 h at 21°C. For statistical analysis of the presence of coiled bodies, cells were grown to 50% confluency, immunostained with anti-coilin antibody (Raska *et al.*, 1991), and three separate counts on three different coverslips of the first 100 cells observed were used.

In Situ Hybridization

Cells were prepared for *in situ* hybridization according to Carmo-Fonseca *et al.* (1991b). Briefly, cells were permeabilized with 0.5% Triton X-100 in cytoskeleton buffer (CSK) buffer (0.1 M NaCl, 0.3 M sucrose, 0.01 M piperazine-*N*,*N*′-bis(2-ethansulfonic acid) [PIPES], pH 6.8, 0.003 M $MgCl_2$) containing 0.001 M phenylmethylsulfonyl fluoride [PMSF] on ice for 3 min. Cells were then fixed in freshly prepared 3.7% formaldehyde in CSK buffer for 10 min at 21°C and washed three times (10 min each) in PBS. After rinsing in 6× SSPE (Sambrook *et al.*, 1989) cells were prehybridized with 6× SSPE, 5× Denhardt's solution, and 0.5 μg/ml of *Escherichia coli* tRNA for 15 min. Cells were subsequently hybridized in the same buffer with 1.2 pmol/μl of biotinylated 2′O-alkyl antisense oligonucleotide probes to U1 or U2 snRNAs for 16–24 h at 21°C. Cells were washed three times (15 min each) in 6× SSPE while shaking and then rinsed with "avidin wash buffer" (0.02 M *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES], pH 7.9, 0.15 M potassium chloride [KCL], 0.05% Tween 20) for 15 min. Hybridization signal was detected by incubating with 2 μg/ml of FITC-conjugated avidin DCS (Vector Labs., Burlingame, CA) in avidin wash buffer containing 1% bovine serum albumin for 30 min at 21°C. Cells were washed in avidin wash buffer (3 × 15 min each). After labeling the hybridization signal with FITC-conjugated avidin, the cells were washed three times (10 min each) in PBS containing 1% normal goat serum (NGS). Cells were then incubated with anti-Sm monoclonal antibody (Lerner *et al.*, 1981) at a dilution of 1:1000 for 1 h and rinsed three times (10 min each) in PBS containing 1% NGS. Cells were then incubated with Texas Red-conjugated goat anti-mouse antibody at a dilution of 1:20 for 1 h followed by three washes in PBS for 10 min each. Cells were washed in PBS (3 × 15 min each) and mounted in 90% glycerol, 10% PBS plus 1 mg/ml of paraphenylenediamine. The final pH was adjusted to 8.0 with 0.5 M carbonate-bicarbonate buffer (pH 9.0). Cells were examined with a Nikon FXA epifluorescence microscope (Melville, NY) equipped with a 60×, 1.4 N.A. objective lens.

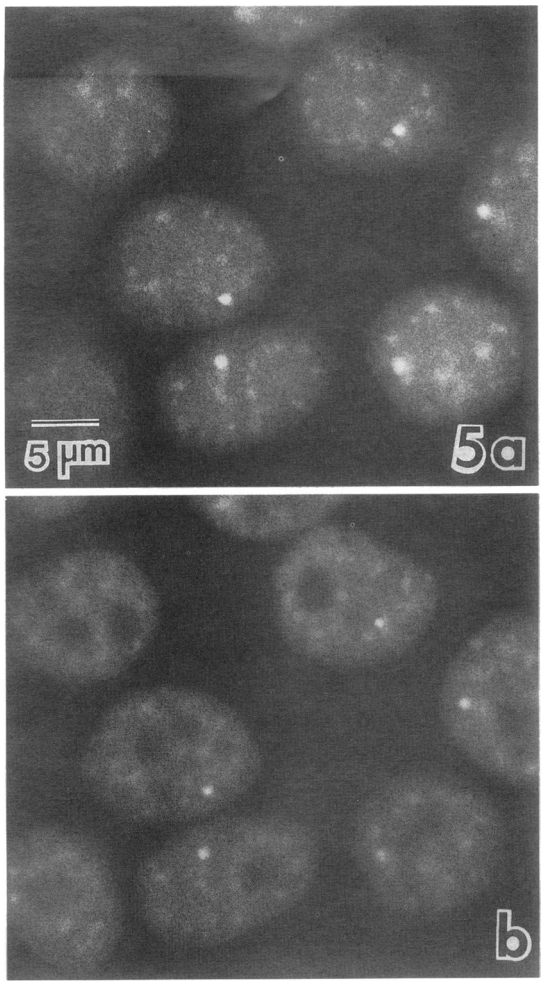

Figure 5. U2 snRNA colocalizes with snRNP-antigens. HeLa cells were hybridized *in situ* with biotinylated oligonucleotide probes complementary to U2 snRNA (a) and were then immunolabeled with anti-Sm monoclonal antibody (b). Oligonucleotide probes (Carmo-Fonseca *et al.*, 1991b) were detected by FITC-conjugated avidin DCS and anti-Sm antibodies were detected by Texas Red conjugated secondary antibodies.

Electron Microscopy of Fluorescently Labeled Cells

To examine a cell by immunofluorescence microscopy and subsequently examine the same cell by electron microscopy, cell samples were prepared for immunofluorescence microscopy as described above except that cell number was reduced so that individual cells or cell clusters could easily be identified. After photographing cells and marking regions of interest on the coverslip with a diamond scribe, the coverslip was floated off the slide, washed three times in PBS (10 min each), and prepared for standard electron microscopy. Cells were fixed in 2% glutaraldehyde in PBS (pH 7.3) for 30 min at 21°C. After washing in PBS (3 × 10 min each), cells were postfixed in 1% OsO_4 in 0.1 M cacodylate buffer (pH 7.3) for 30 min at 21°C. Cells were then washed (3 × 5 min each) in dH_2O, dehydrated in a graded series of ethanol and infiltrated and embedded in PolyBed 812 (PolySciences, Warrington, PA). Thin sections (100 nm) were cut on a Reichert Ultracut E ultramicrotome (Vienna, Austria) with a Diatome diamond knife (Bienne, Switzerland) and were examined with a Hitachi H-7000 transmission electron microscope (Mountain View, CA) operated at 75 kV. Cells previously photographed by immunofluorescence microscopy were identified by comparing characteristic features such as cell position, nuclear shape, and nucleolar number and shape, and identical cells were photographed at the electron microscopic level.

RESULTS

SnRNP Antigens are Localized in Speckles and Coiled Bodies in HeLa Cell Nuclei

HeLa cells were immunolabeled with Y12 monoclonal antibody (Lerner *et al.*, 1981), which recognizes the B, B′, and D antigens common to U1, U2, U4/U6, and U5 snRNPs, or a monoclonal antibody, which recognizes the B″ protein that is specific to U2 snRNPs (Habets *et al.*, 1989). When examined by epifluorescence microscopy, these antigens were concentrated in irregularly shaped nuclear speckles (Figures 1b and 2b), as well as being diffusely distributed throughout the nucleoplasm. In addition, one to three brightly stained round coiled bodies (foci) were observed in 81% of the cells examined (Figures 1b and 2b). Coiled bodies are present in different focal planes from cell-to-cell. The coiled body is distinguished by the presence of the coilin antigen (Raska *et al.*, 1991), its round shape, and intense anti-snRNP immunostaining when compared with speckles, which are irregular in shape, do not contain detectable amounts of coilin, and do not immunostain as intensely as coiled bodies with anti-snRNP antibodies. The presence of snRNP-enriched coiled bodies does not appear to be cell-cycle related because changes in the number of coiled bodies were not observed during the cell cycle (Spector *et al.*, unpublished data). Additionally, serum starved NIH3T3 cells, in the G_0 phase of the cell cycle, exhibited the same number of coiled bodies as logarithmically growing cells. The distribution of snRNPs in a speckled pattern, as well as in coiled bodies, was observed regardless of the fixation method used (formaldehyde [Figures 1b, 2b, and 3b], glutaraldehyde [Spector, unpublished data], methanol [Figures 1d, 2d, and 3d], acetone [Spector, unpublished data]), and even in cells which were not fixed before immunolabeling (Figures 1f, 2f, and 3f). However, in cells that were fixed with methanol or acetone, which both fix by precipitating proteins, or in cells that were not fixed (Figures 1f, 2f, and 3f), the diffuse immunostaining was reduced. It is interesting to note that the extent of reduction of the diffuse staining seems greater in unfixed cells relative to methanol-fixed cells. The distribution of snRNPs detected by the Y12 monoclonal antibody (speckles, coiled bodies, and diffuse) is identical to that detected by antibodies specific to the m_3G cap structure of snRNAs (Figure 3) (Reuter *et al.*, 1984) and the A protein specific to U1 snRNP (Spector *et al.*, unpublished data). The immunolabeling of both speckles and coiled bodies was sensitive to RNAase A digestion (Figure 4). To confirm that snRNP particles, and not just snRNP antigens, were present in the speckles and coiled bodies, *in situ* hy-

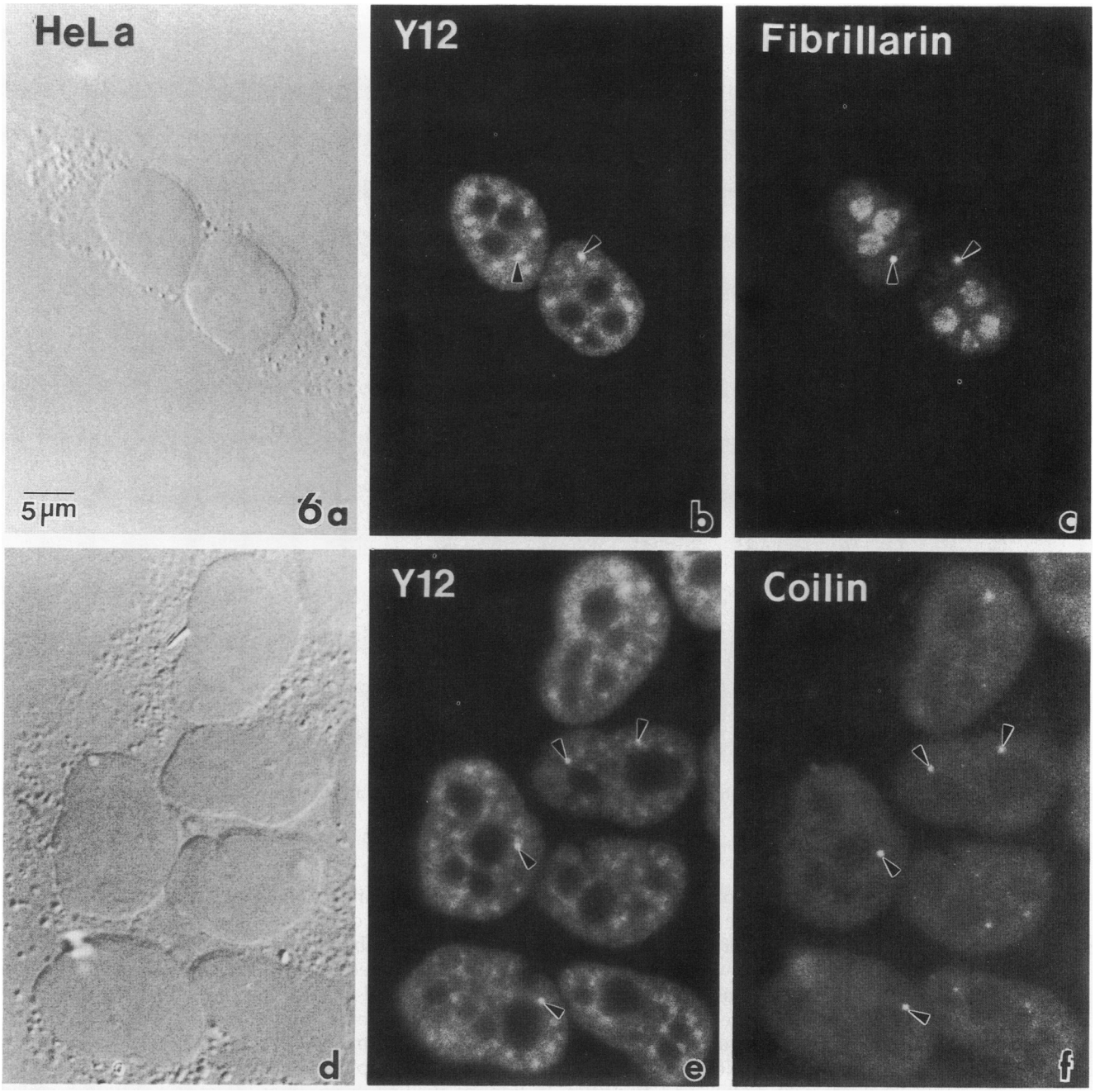

Figure 6. Coiled bodies contain fibrillarin and coilin. HeLa cells were double-labeled with anti-Sm antibody (b) and an antibody, which recognizes the U3 snRNP protein, fibrillarin (c). Both snRNPs and fibrillarin localize to the coiled bodies (arrowheads). HeLa cells were double-labeled with anti-Sm antibody (e) and an antibody which recognizes coilin (Raska *et al.*, 1991), an 80 kD protein associated with coiled bodies (f). Both snRNPs and coilin localize to the coiled bodies (arrowheads).

bridization was performed with a previously characterized (Carmo-Fonseca *et al.*, 1991a,b) biotinylated 2′O-alkyl antisense oligonucleotide probe to U2 snRNA. Cells hybridized with this probe showed U2 snRNA to be concentrated in speckles and coiled bodies which colocalized with the Y12 snRNP-antigen immunostaining pattern (Figure 5). Similar results were obtained for U1 snRNA (Huang and Spector, 1992).

Nuclear "Foci" are Coiled Bodies

To confirm that the previously identified "foci" (Carmo-Fonseca *et al.*, 1991a,b, 1992) are coiled bodies, we

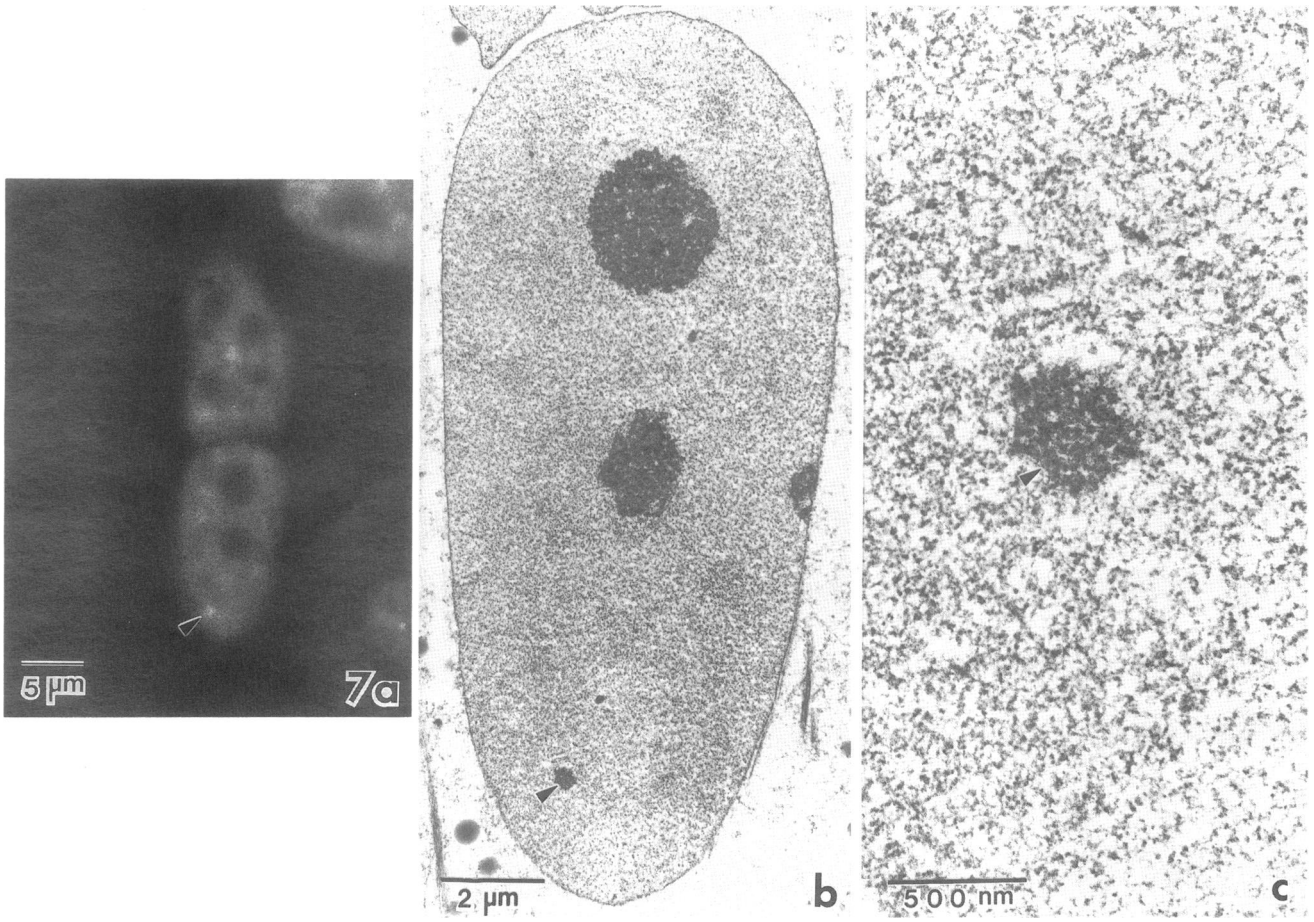

Figure 7. HeLa cells were immunolabeled with anti-Sm monoclonal antibody, photographed and then prepared for electron microscopy. The coiled body shown in (a) by immunofluorescence is identified in the same nucleus by electron microscopy (b and c arrowheads).

evaluated the localization of several other nuclear antigens that were shown to be diagnostic for coiled bodies by Raska *et al.* (1991). To determine if the coiled body represented a portion of a nucleolus, we evaluated the distribution of several nucleolar proteins. We found that fibrillarin, which is associated with the U3 snRNP particle and the fibrillar region of the nucleolus, is also present in coiled bodies (Figure 6c). Nevertheless, several other nucleolar proteins such as B23 and nucleolin are not present in coiled bodies (Spector *et al.*, unpublished data). Antibodies to DNA and the non-snRNP splicing factor SC-35 were also not present in coiled bodies (Carmo-Fonseca *et al.*, 1991a; Raska *et al.*, 1991; Huang and Spector, 1992). However, a recently identified autoantibody to an 80-kDa protein (coilin) associated with coiled bodies (Raska *et al.*, 1991) immunoreacted with the bright-round snRNP enriched structures (Figure 6f), indicating that they represent coiled bodies. To further confirm that the nuclear inclusion previously referred to as a "focus" (Carmo-Fonseca *et al.*, 1991a,b) is a coiled body, we examined the same cell by immunofluorescence and electron microscopy. HeLa cells were immunolabeled with Y12 monoclonal antibody and photographed (Figure 7a). The photographed cell was then prepared for electron microscopy, and the nuclear structure, which corresponded to the immunofluorescent "focus," was identified and confirmed to be a coiled body based on its morphological appearance (Figure 7b and c) and position, as well as its reactivity (Figure 6a) with a coiled body-specific antibody (Raska *et al.*, 1991). In HeLa cells, the coiled body measures ~0.4 μm in diameter and is composed of coiled fibrillar strands (Figure 7c). We confirm that the previously described "foci," which contain snRNPs as well as the U3-specific fibrillarin protein, are coiled bodies.

SnRNPs Localize in Coiled Bodies in a Small Percentage of Cells of Defined Passage

Because HeLa cells are transformed cells with an aneuploid chromosomal composition, snRNP distribution in

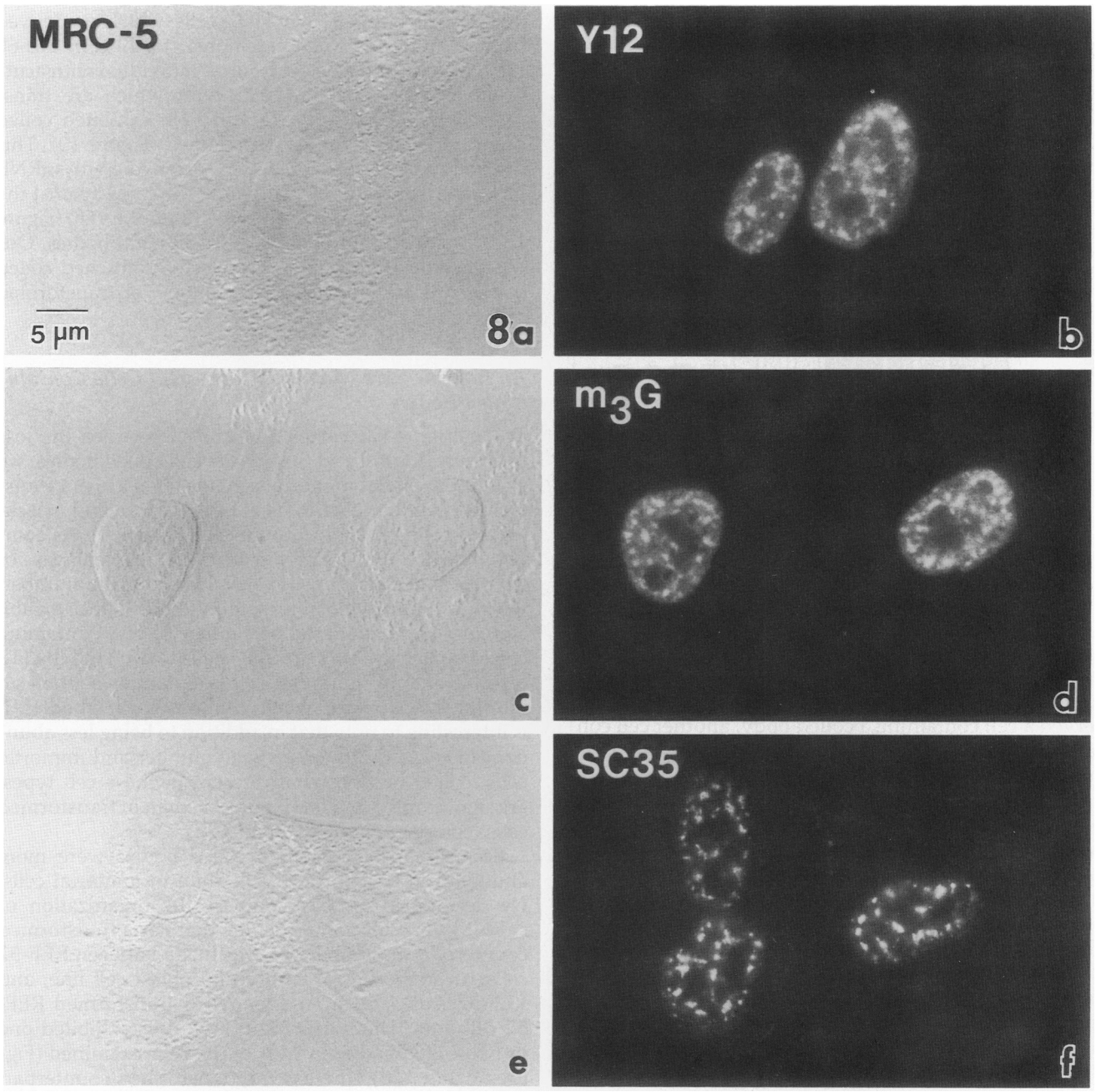

Figure 8. Normal human diploid cells show a similar speckled distribution of snRNPs. MRC-5 cells immunolabeled with Y12 (b) or m_3G (d) antibodies exhibit speckles and diffuse nuclear staining. MRC-5 cells immunolabeled with antibodies against the non-snRNP splicing factor SC-35 (f) also exhibit a speckled staining pattern. Coiled bodies are only observed in 3% of MRC-5 cells stained with Y12 or m_3G antibodies.

coiled bodies may be a reflection of possible altered nuclear organization in response to cellular transformation. To determine whether snRNP-containing coiled bodies are present in all cell types, we examined the distribution of snRNPs in cells of defined passage number that have a diploid karyotype. MRC-5 cells (human male embryonic lung fibroblasts) examined by immunofluorescence microscopy showed that snRNPs localized in a characteristic speckled pattern with some diffuse nuclear staining (Figure 8). The speckled distribution of snRNPs detected by the Y12 monoclonal antibody (Figure 8b) is identical to that detected by antibodies specific to the m_3G cap structure of snRNAs (Figure 8d) or a non-snRNP splicing factor SC-35 (Figure 8f) (Fu and Maniatis, 1990; Spector *et al.*, 1991). However, snRNP-enriched coiled bodies were detected in only 3% of the

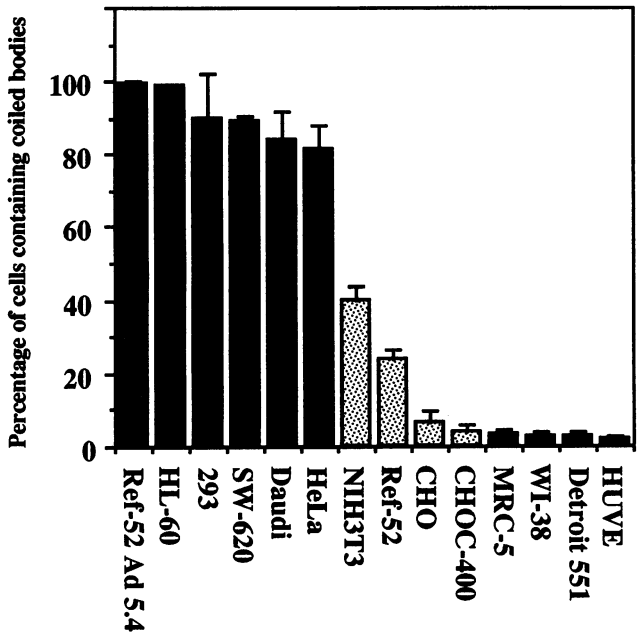

Figure 9. Histogram of the percentage of coiled bodies present in a variety of cell types. Transformed cells (▩), immortal cells (▨), cells of defined passage number (■).

cells in a population of thousands of cells which were examined. It is interesting to note that when one observed a cell containing a coiled body, another cell containing a coiled body was usually in close proximity suggesting that this inclusion is segregated to daughter cells during cell division. To be certain that the low percentage of cells containing coiled bodies in MRC-5 cells was not simply an irregularity of this particular cell type, several other cell types of defined passage number were examined including WI-38 (human female embryonic lung fibroblasts), Detroit 551 (human female skin fibroblasts), and HUVE (human umbilical vein endothelial) cells. In all cases, snRNPs were found to be concentrated in speckles; coiled bodies were observed in 2–3% of the cells examined (Figure 9). Thus we demonstrate that snRNPs localize to coiled bodies in a limited percentage of cells of defined passage number.

SnRNP-Enriched Coiled Bodies are Characteristic of Transformed Cells

Next, we evaluated the distribution of snRNPs in a variety of transformed cell lines (Figure 9) to determine if the presence of snRNPs in coiled bodies is characteristic of these cell types. We examined six cell lines that were either derived from human cancers or were virally transformed (Figure 9). In these cell lines, we found snRNPs to be concentrated in both speckles and coiled bodies. In each cell type examined, snRNPs were present in speckles in 100% of the cells. However, the percentage of cells of a given cell type that contained coiled bodies varied from 81% in HeLa cells, which are derived from a human cervical carcinoma, to 99% in HL-60 cells, which were derived from an individual with acute promyelocytic leukemia. 293 cells, which are transformed by adenovirus E1a and E1b, exhibited coiled bodies in 90% of the cells examined (Figure 10). This distribution was observed with antibodies to snRNP proteins (Figure 10b) or antibodies directed against the m_3G cap structure of the snRNAs (Krainer, 1988) (Figure 10d) and was confirmed by anti-coilin antibodies. Our observations demonstrate that snRNP-enriched coiled bodies are present in higher numbers of transformed cells.

An Intermediate Number of Immortal Cells Contain Coiled Bodies

To determine if a correlation existed between the loss of growth control and the presence of coiled bodies, we examined several immortal cell lines that are not transformed. We found snRNPs to be present in both speckles and coiled bodies in these cells (Figure 9). As compared with the relative consistent percentages of transformed cells or cells of defined passage number, which contain coiled bodies, immortal cell lines exhibit a greater variation in the percentage of cells containing coiled bodies from 4% in CHO cells to 40% in NIH3T3 cells. Therefore, immortal cell populations contain an intermediate number of cells that have coiled bodies. It is interesting to note that in addition to being less abundant in cells of defined passage number and immortal cells, coiled bodies, when present in these cell types, are significantly smaller (Figure 11) than in transformed cells (compare Figures 10 and 11).

Because snRNP-enriched coiled bodies were more abundant in transformed cells than in immortal cells, we decided to directly compare the organization of snRNPs in an immortal cell line that has a transformed counterpart. A comparison was made between REF-52 cells, an immortal rat embryo fibroblast cell line, and REF-52 Ad5.4 cells (an adenovirus transformed REF-52 cell line). The immortal REF-52 cells exhibited one or two coiled bodies in 24% of the cells examined (Figures 9 and 12b) whereas the transformed counterpart exhibited one or two coiled bodies in 99% of the cells examined (Figures 9 and 12d). Therefore, the transformed phenotype results in a change in the distribution of snRNPs.

DISCUSSION

In this study, we have identified differences in the organization of snRNPs in transformed cells, immortal cells, and cells of defined passage number. SnRNPs are concentrated in a speckled nuclear pattern in all cells examined by immunofluorescence microscopy. However, in transformed cells, snRNPs are also concentrated

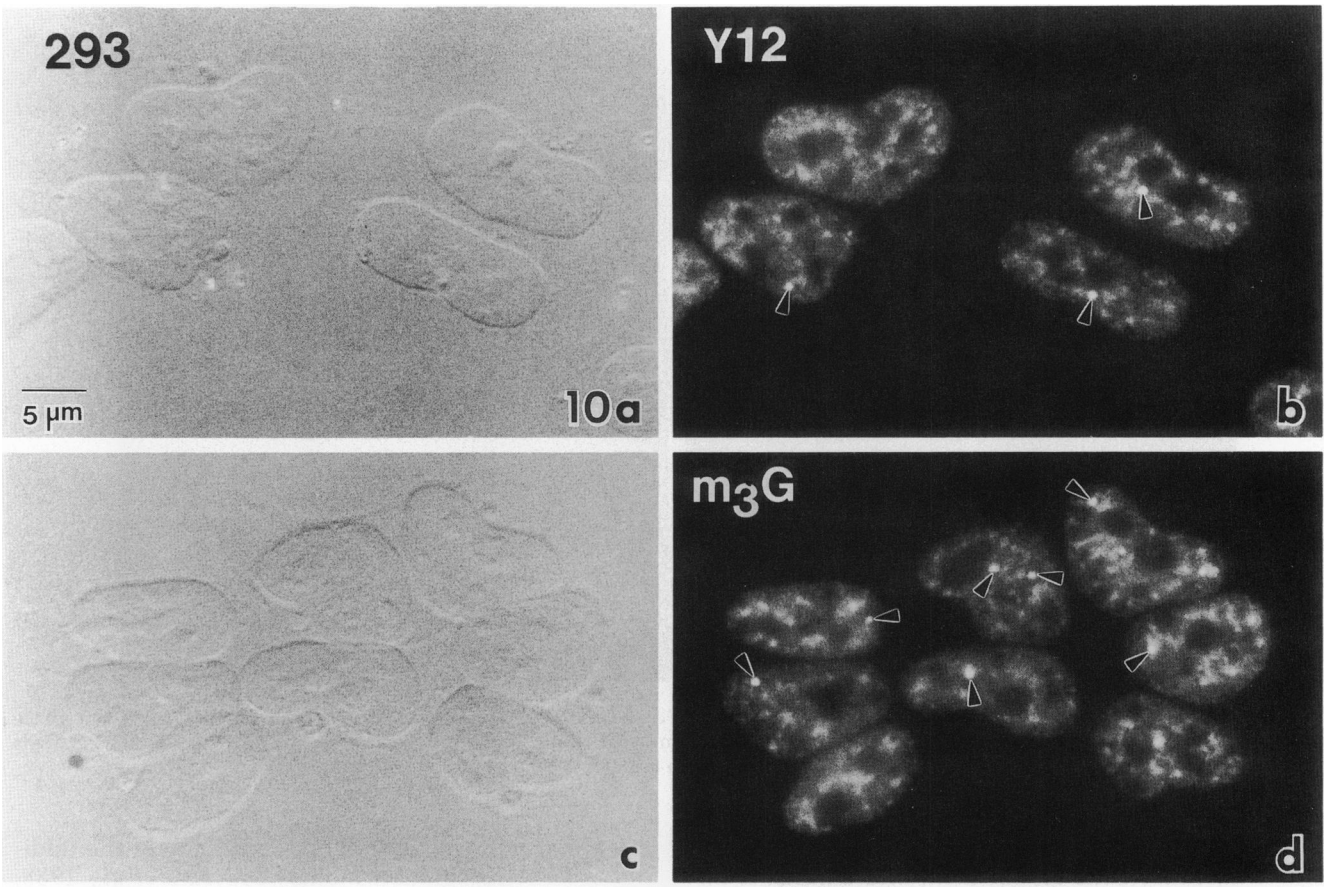

Figure 10. Other transformed cells exhibit speckles and coiled bodies when labeled with anti-snRNP antibodies. 293 cells immunolabeled with anti-Sm antibodies (b) or anti-m_3G (d) antibodies exhibit speckles and diffuse nuclear staining. One to four large, round coiled bodies were observed in 90% of the cells examined.

in structures that have previously been called foci (Leser *et al.*, 1989; Carmo-Fonseca *et al.*, 1991a,b), coiled bodies (Fakan *et al.*, 1984; Raska *et al.*, 1991), or Sm clusters (Eliceiri and Ryerse, 1984). Coiled bodies are found in a low percentage (2–3%) of cells with defined passage number, but are more abundant in transformed cells (81–99%), and are present in intermediate numbers of immortal cells (4–40%). Therefore, we have found a direct correlation between the percentage of cells containing coiled bodies and transformation. On the basis of these findings, we propose that the presence of coiled bodies is a reflection of cell physiology.

An association of snRNPs with coiled bodies has been demonstrated directly or indirectly in several previous studies. Fakan *et al.* (1984) examined the distribution of snRNPs in sections of mouse and rat liver. In addition to labeling interchromatin granules and perichromatin fibrils, which are components of the speckled immunostaining pattern (Spector *et al.*, 1991), snRNP-specific antibodies were shown to immunolabel-coiled bodies in these tissue sections (Fakan *et al.*, 1984). Our findings are not inconsistent with this study because liver represents a tissue with the potential to divide and regenerate and is a metabolically active tissue, which is extensively involved in specific functions including detoxification processes. In a second study, Eliceiri and Ryerse (1984) immunolabeled HeLa cells with anti-Sm antibodies and described staining of a novel intranuclear structure. Examination of the micrographs in their paper suggests that the structure they observed were coiled bodies. In two studies at the light microscopic level, Leser *et al.* (1989) described bright foci in HeLa cells, which were immunolabeled with anti-Sm antibodies, and Carmo-Fonseca *et al.* (1991a,b) described snRNAs to be present in foci. On the basis of studies by Raska *et al.* (1991), Carmo-Fonseca *et al.* (1992), and the present study, it appears that these bright foci are coiled bodies.

Coiled bodies were first identified at the light microscopic level in 1903 by Ramon y Cajal who called them "accessory bodies" in neuronal cells (Cajal, 1903). The correlation of the light microscopic designation of the "accessory body" with the electron microscopic designation of the coiled body (Monneron and Bernhard,

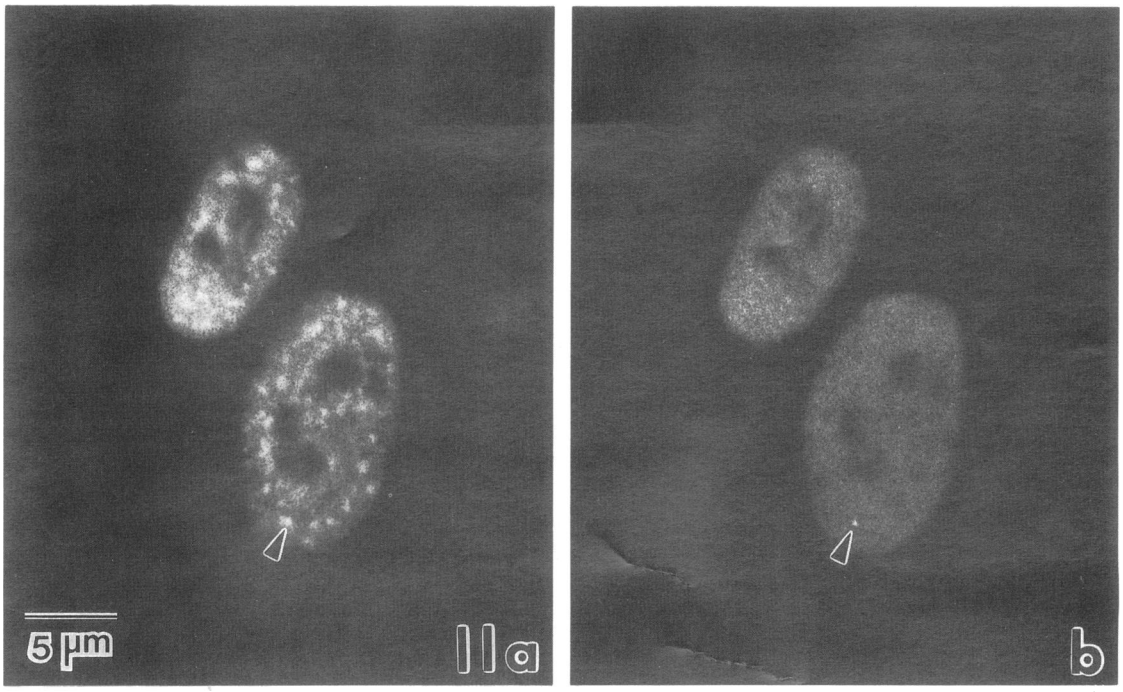

Figure 11. Detroit 551 cells (normal human diploid fibroblasts) exhibit coiled bodies (arrowhead) in 2% of the cells. The size and fluorescence intensity of the coiled body in these cells is significantly reduced as compared with coiled bodies of transformed cells (Figs. 6 and 11). Cells were double-labeled with anti-Sm antibody (a) and an antibody, which recognizes coilin (b).

1969) was suggested by Hardin *et al.* (1969) and later demonstrated by Seite *et al.* (1982) and Lafarga *et al.* (1983). These structures are generally round and measure 0.5–1.0 µm in diameter and consist of coiled fibrillar strands (Monneron and Bernhard, 1969; Moreno Diaz de la Espina *et al.*, 1982). Cytochemical staining of ribonucleoproteins by the EDTA regressive method (Bernhard, 1969) has demonstrated that coiled bodies contain RNPs (Monneron and Bernhard, 1969). In addition, coiled bodies have been shown to contain orthophosphate ions and acid phosphatase activity (Moreno Diaz de la Espina *et al.*, 1982). However, DNA (Monneron and Bernhard, 1969) and newly transcribed RNA (Moreno Diaz de la Espina *et al.*, 1980) have not been identified in these structures. A relationship between coiled bodies and nucleoli has been suggested because these structures are often found in close proximity (Hardin *et al.*, 1969; Monneron and Bernhard, 1969; Seite *et al.*, 1982; Schultz, 1989). Furthermore, Lafarga *et al.* (1991) have recently shown an increase in the number of coiled bodies in supraoptic neurons of the rat after stimulation of nucleolar transcription.

Recently, Raska *et al.* (1991) have identified a novel protein that is localized to the coiled body. The 80-kDa autoantigen, called p80-coilin, has been localized by sera from individuals with various autoimmune disorders (Raska *et al.*, 1990, 1991; Andrade *et al.*, 1991). Partial cDNA clones coding for the p80-coilin antigen have been characterized (Andrade *et al.*, 1991). The sequence of these clones did not reveal any of the traditional RNA-recognition motifs (Dreyfuss *et al.*, 1988; Chan *et al.*, 1989). However, the protein contains two stretches rich in arginine and lysine, which could be involved in RNA binding (Zamore *et al.*, 1990). p80-coilin is the first antigen identified to be restricted to the coiled body. Several other nuclear antigens have been localized to the coiled body in addition to being present in other nuclear regions; these include DNA topoisomerase I and fibrillarin as well as snRNPs (Raska *et al.*, 1991). Other nuclear components such as DNA, nucleolin, nucleolar protein B23, 5S rRNP, hnRNP L protein, and SC-35 have not been detected in coiled bodies (Raska *et al.*, 1991; Huang and Spector, 1992). Using the p80-coilin antibody, Raska *et al.* (1991) determined that the number of coiled bodies varies among different cell types. However, cycling mammalian cells usually contain less than six coiled bodies per nucleus. In frozen sections from rat and mouse brain, a frequent association between coiled bodies and nucleoli was observed (Raska *et al.*, 1991). Furthermore, at the cytochemical level, Seite *et al.* (1982) and Raska *et al.* (1990) have shown that coiled bodies are stained by the nucleolar-organizing-region (NOR) silver staining technique in a way similar to the fibrillar regions of the nucleolus. The association between coiled bodies and nucleoli was investigated further in cycling cells which were treated with actinomycin D or 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) at a dose which results in

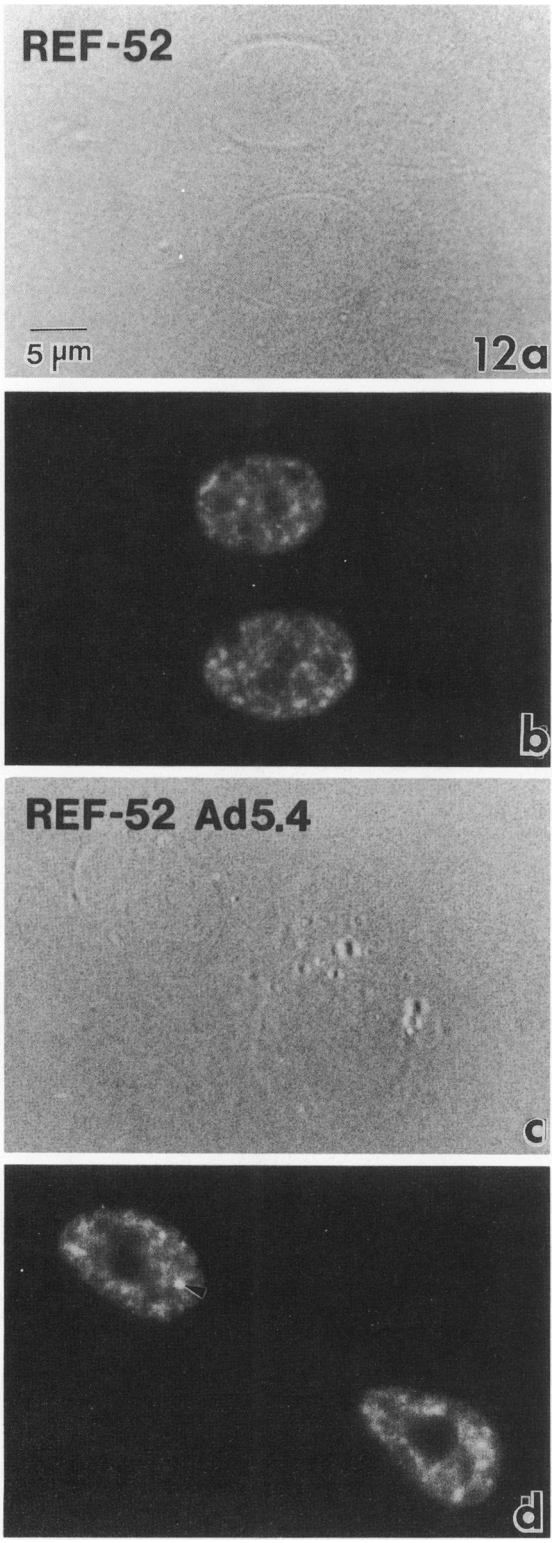

Figure 12. REF-52, an immortal-cell line exhibits one or two small coiled bodies in 24% of the cells examined. REF-52 cells transformed by the adenovirus E1a and E1b proteins exhibit one or two large coiled bodies (d) in 99% of the cells examined.

nucleolar segregation. These studies demonstrated that when nucleoli segregate, the p80-coilin antigen colocalizes with fibrillarin-positive nucleolar components. Fibrillarin is a 34-kDa antigen associated with the nucleolar U3 snRNP particle (Lischwe *et al.*, 1985; Ochs *et al.*, 1985; Tyc and Steitz, 1989), which is involved in pre-rRNA processing (Kass *et al.*, 1990). In cells incubated with lower concentrations of actinomycin D (0.02 μg/ml), nucleoli did not segregate. However, p80-coilin was observed to be associated with paranucleolar structures similar to what was observed in primary neuron cultures, which had received no drug treatment. On the basis of these data, it was suggested that coiled bodies may be involved in the processing, transport, and storage of nucleolar metabolites (Raska *et al.*, 1990). However, the presence of other nuclear components not involved in nucleolar function has suggested a more general role for this nuclear organelle (Raska *et al.*, 1991).

This study is the first to directly correlate an increase in the number of cells containing coiled bodies in a given cell population with the transformed phenotype. On the basis of the presence of snRNAs in coiled bodies, it was suggested that these structures may play a role in pre-mRNA processing (Carmo-Fonseca *et al.*, 1991a,b). However, the fact that snRNP-enriched coiled bodies are not present in all cells of a given population, which still must process pre-mRNA, suggests that this nuclear inclusion probably does not play a central role in pre-mRNA splicing. Furthermore, the lack of [^{3}H]-uridine incorporation (Moreno Diaz de la Espina *et al.*, 1980), hnRNP proteins (Carmo-Fonseca *et al.*, 1991a; Raska *et al.*, 1991), or the essential non-snRNP splicing factor SC-35 (Carmo-Fonseca *et al.*, 1991a; Huang and Spector, 1992) in coiled bodies significantly reduces the possibility that these inclusions are involved in pre-mRNA splicing. It is unclear at this time whether the presence of coiled bodies is the result of changes in cell physiology, including cellular transformation, or if their presence contributes to the maintenance of this altered physiology. Future studies aimed at isolating and biochemically characterizing these interesting nuclear inclusions are sure to shed light on their function(s).

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