p80-coilin: a component of coiled bodies and interchromatin granule-associated zones

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SUMMARY

We investigated at the electron microscope level the fate of the three intranuclear structures known to accumulate snRNPs, and which correspond to the punctuate immunofluorescent staining pattern (the coiled bodies, the clusters of interchromatin granules and the interchromatin granule-associated zones) after exposure to either a low salt medium which induces a loosening and partial spreading of nucleoprotein fibers or a high ionic strength salt medium and subsequent DNAse I digestion, in order to obtain DNA-depleted nuclear matrices. The loosened clusters of interchromatin granules and the coiled bodies could no longer be distinguished from surrounding nucleoprotein fibers solely by their structure, but constituents of the clusters of interchromatin granules could be detected by in situ hybridization with both U1 and U2 DNA probes, and constituents of the coiled bodies were detectable mainly with the U2 DNA probe. The interchromatin granule-associated zones, the electron-opacity and compactness of which were preserved despite the loosening treatment, remained labeled with the U1 DNA probe only. In DNA-depleted nuclear matrices, the snRNA content of the coiled bodies, the clusters of interchromatin granules and their associated zones, which were all easily recognizable within the residual nuclear ribonucleoprotein network, was unmodified. The data indicate, therefore, that the loosening procedure as well as the high salt extraction procedure preserve the snRNA content of all three spliceosome component-accumulation sites and reveal that interchromatin granule-associated zones are elements of the nuclear matrix. The p80-coilin content of coiled bodies was also preserved whatever the salt treatment used. An intriguing new finding is the detection of abundant p80-coilin within the interchromatin granule-associated zones, both before and after either low or high salt treatment of cells. Therefore, p80-coilin is an integral constituent of the interchromatin granule-associated zones.

Key words: coiled body, electron microscope, HeLa cell, in situ hybridization, interchromatin granule, interchromatin granule-associated zone, mild loosening procedure, nuclear matrix, p80-coilin, spliceosome, U1 RNA, U2 RNA

INTRODUCTION

The components of the cell nucleus involved in transcription and splicing have been clearly identified by immunocytochemical and in situ hybridization procedures designed for electron microscopy. It was clearly shown that the lower-definition speckled pattern observed by fluorescence microscopy following specific detection of spliceosome components (Carmo-Fonseca et al., 1991, 1992; Huang and Spector, 1992; Lamond and Carmo-Fonseca, 1993a,b) corresponds to perichromatin fibrils and to three different, well-defined structures, i.e. the clusters of interchromatin granules, the interchromatin granule-associated zones and the coiled bodies (Visa et al., 1993a). Although all three structures accumulate spliceosome components, they differ in their specific contents. U1 snRNAs are present in large amounts in the interchromatin granule-associated zones but are much rarer in the coiled bodies. Conversely, U2 snRNAs are absent in the former and accumulate in considerable amounts in the latter. Both snRNAs accumulate within the clusters of interchromatin granules (Spector, 1993; Visa et al., 1993a). As already mentioned (Visa et al., 1993a), the interchromatin granule-associated zones, might be equivalent to the A snurposomes described in amphibian germinal vesicles by the Gall’s group (Gall, 1991; Wu et al., 1991). On the other hand, the very low level of labeling of the clusters of interchromatin granules on electron microscope autoradiographs following labeling of cells with radioactive uridine (Fakan, 1978; Fakan and Puvion, 1980; Puvion and Moyne, 1981), as well as the total absence of DNA within these structures (Thiry, 1993; Visa et al., 1993a), exclude the possibility that they could be sites of transcription and active splicing. Instead, it was proposed that they are more likely to be concerned with pre- and/or post-splicing events such as the final maturation or storage of snRNP particles, assembly of spliceosomes and/or intron degradation (Lamond et al., 1990; Visa et al., 1993a,b). How-
ever, the permanent presence of poly(A)+ RNA in clusters of interchromatin granules (Visa et al., 1993b) and, at least in certain metabolic conditions, of ribosomal RNA (Puvion et al., 1984b) suggests that they also could be sites of some sorting and/or degradation of mature RNA molecules. Concerning coiled bodies, recent results have shown that this type of nuclear body could also be multifunctional and related to pre- and/or post-splicing events and to unknown nucleolus-associated functions (Brasch and Ochs, 1992; Lamond and Carmona-Fonseca, 1993b; Malatesta et al., 1994). In fact, it is now generally accepted that the perichromatin fibrils represent the main morphological substrate of splicing (Nash et al., 1975; Fakan et al., 1976; Puvion and Mayone, 1978; Puvion and Viron, 1981; Gallinaro et al., 1983). These fibrils form at transcription sites, contain nascent RNA (Nash et al., 1975; Fakan et al., 1976; Puvion and Mayone, 1978; Puvion and Viron, 1981; Fakan, 1994) and are associated with hnRNP core proteins and spliceosome components (Fakan et al., 1984; Puvion et al., 1984a; Visa et al., 1993a). This is consistent with the detection of snRNP antigens on transcripts which are still attached to the DNA matrix as seen in studies of spread transcription complexes (Fakan et al., 1986) and in observations indicating that splicing may occur co-transcriptionally (Beyer and Osheim, 1988; Wu et al., 1991).

Several years ago, we developed a new fixation procedure which helped to bridge the gap between the molecular organization of chromatin-RNP complexes in vitro after spreading of the nuclear contents, on the one hand, and the more compact in situ structures observed in thin sections of routinely fixed cells, on the other hand (Puvion-Dutilleul and Puvion, 1980; Puvion-Dutilleul, 1983). This method, adapted from Miller’s spreading technique (Miller and Beatty, 1969), consists of preparation of cells in a detergent-containing hypotonic fixative solution, which induces only a mild loosening of the chromatin on the accumulation sites of spliceosome components (Fakan et al., 1984) and in observations indicating that splicing may occur co-transcriptionally (Beyer and Osheim, 1988). For electron microscope study, HeLa cells (5×10^5 cells per 5 cm plastic culture dish) were grown at 37°C, in the presence of 5% CO₂, in Eagle’s minimum essential medium supplemented with 5% calf serum. Twenty-four hours later, when cells were near confluence, they were processed for electron microscope study.

For the observation of unloosened nucleoproteins, cell cultures were fixed in situ, at 4°C, in 4% formaldehyde (Merck, Darmstadt, Germany) in 0.1 M Sörensen phosphate buffer, pH 7.3. During the 1 hour fixation, the cells were detached from the culture dish and centrifuged. The pellets were dehydrated in increasing concentrations of methanol and embedded in Lowicryl K4M (Chemische Werke Lowi, Waldkraiburg, Germany). Polymerization was carried out for 5 days at −30°C under long wavelength UV light (Philips fluorescence tubes TL 6W) (Roth, 1989). Ultrathin sections were collected on Formvar/carbon-coated gold grids (200 mesh).

For the observation of loosened nucleoproteins, culture medium was removed and cell cultures were exposed for about 5 seconds to distilled water adjusted to pH 8.5 with 0.1 M borate buffer (Puvion-Dutilleul and Puvion, 1980). The cells were then covered for 1 hour with 6 ml of 1% formaldehyde aqueous solution containing 0.4% Photo flo (a commercial wetting agent containing Triton X-100; Kodak-Pathé, Chalon, France), and 0.1 M sucrose. This solution was previously adjusted to pH 8.5 with the borate buffer. During the 1 hour loosening treatment, the cells were sprayed and centrifuged. The resulting translucent pellets were dehydrated in methanol and embedded in Lowicryl as described above for pellets of unloosened cells.

Nuclear matrices of HeLa cells were kindly provided by F. Harper (Villejuif, France). They were prepared from cells still attached to the plastic culture dishes as previously described (Puvion et al., 1988) according to a slight modification of the method of Buckler-White et al. (1980). Briefly, cultures were treated for 3 minutes at 4°C in TMS buffer (0.25 M sucrose, 5 mM MgSO₄, 50 mM Tris-HCl, pH 7.4 containing 1% Triton X-100 prior to being incubated for 30 minutes at 37°C in TMS buffer containing 50 μg/ml DNase I (pancreatic DNase I type IV, Sigma St Louis, MO, USA). After washing with 10 mM Tris-HCl buffer, pH 7.4, plus 0.2 M MgSO₄, cultures were treated for 2× 15 minutes with a high salt buffer (2 M NaCl, 0.2 mM MgSO₄, 10 mM Tris-HCl, pH 7.4) and washed in 10 mM Tris-HCl buffer added with 0.2 mM MgSO₄ and fixed with formaldehyde. During fixation, matrices were detached from their substratum and centrifuged. The resulting pellets were dehydrated in methanol and embedded in Lowicryl K4M as above.

For optical study, cells were grown on glass coverslips. Some cultures were fixed with cold methanol-acetone (3:7, v/v) for 5 minutes at −20°C and air-dried, whereas others were fixed for 20 minutes with 4% formaldehyde in PBS, washed for 15 minutes in PBS, incubated for 15 minutes in 0.3% Triton X-100 in PBS and washed again in PBS prior to being used for immunolabeling and in situ hybridization.

**Immunodetection of p80-coilin**

For in situ detection at the ultrastructural level of structures contain-
ing p80-coilin, grids bearing Lowicryl sections of HeLa cells, with or without loosening of the nucleoproteins, and nuclear matrices were incubated for 30 minutes at room temperature on a 10 μl drop of rabbit serum R288 containing polyclonal antibodies against recombinant p80-coilin (Andrade et al., 1993), diluted 1/50 in PBS. After 15 minutes washing in PBS, the grids were floated for 30 minutes on a 10 μl drop of 1/25 dilution of goat anti-rabbit IgG-conjugated to gold particles, 10 nm in diameter (Biocell Research Laboratories, Cardiff, UK). After a final PBS wash, the grids were rapidly rinsed in a jet of distilled water, air-dried and stained for 10 minutes with 5% aqueous uranyl acetate. For controls, a 1/10 dilution of normal rabbit serum was used instead of serum R288. Grids were observed after a 10-minute uranyl acetate staining.

For immunofluorescence detection of p80-coilin, drops of 10 μl of serum R288 and normal rabbit serum, both diluted 1/50 in PBS, were deposited at the center of the coverslips bearing either methanol-acetone-fixed cells or formaldehyde-Triton-treated cells. Incubations were performed for 30 minutes at 37 °C in a wet chamber. After three washings in PBS, the coverslips were incubated for 30 minutes at room temperature in rhodamine (TRITC)-conjugated goat anti-rabbit IgG (Nordic Immunology, Tilburg, The Netherlands), 1/50 in PBS. Following 3×5 min washings in PBS, the coverslips were finally rinsed in distilled water and mounted in glycerol-PBS. Samples were observed using an Axiophot microscope (Carl Zeiss, Oberkochen, Germany) equipped with epifluorescence.

Localization of U1 and U2 snRNAs by in situ hybridization

The two biotinylated probes used in this study were kindly provided by J. P. Bachellerie and have been described in our previous publications (Visa et al., 1993a; Puvion-Dutilleul et al., 1994). Briefly, the U1 DNA probe (kindly provided by J. E. Dahlberg) corresponds to a 260 bp BgIII fragment of human DNA encompassing the entire U1 RNA coding region, which was cloned into the vector pSP64. The U2 DNA probe (kindly provided by T. Pederson) corresponds to the pSPU2 fragment of human DNA that encodes the entire U2 RNA coding sequence plus 12 3′-flanking nucleotides and was cloned into the vector pSP64. Both DNA probes were biotinylated by nick-translation of the whole plasmid using biotinylated dATP (biont 14- dATP, Bethesda Research Lab., Bethesda, MD/USA).

In order to localize U1 and U2 snRNAs by electron microscope in situ hybridization, gold grids bearing sections of formaldehyde-fixed cells, loosened nucleoproteins or nuclear matrices were floated on 1-2 μl of hybridization solutions containing 50% deionized formamide, 10% dextran sulphate, 2× SSC buffer (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate), 10 μg/ml biotinylated probe, and 400 μg/ml competitor Escherichia coli DNA. The hybridization solutions were treated for 4 minutes in boiling water in order to denature the double-stranded DNA (dsDNA) molecules and rapidly chilled prior to use. Hybridizations with each hybridization solution were performed in a wet chamber for 3.5 hours at 64 °C, i.e. in experimental conditions which were established in our previous studies (Visa et al., 1993a).

Hybrids formed at the surface of the Lowicryl sections were revealed by incubating the grids for 30 minutes on a 5 μl drop 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 minute staining with 5% aqueous uranyl acetate.

We verified that a protease pre-treatment of sections (0.2 mg/ml protease from Streptomyces griseus type V1; Sigma, St Louis, MO, USA) in distilled water for 15 minutes at 37 °C did not markedly improve the accessibility of targets to the probes. In addition, the specific activity of the labeling was attested by the total absence of labeling following an RNase treatment of sections prior to hybridization (1 mg/ml RNase A from bovine pancreas; BDH Biochemicals, Poole, UK) in 10 mM Tris-HCl, pH 7.3, for 1 hour at 37 °C).

For the detection of U1 and U2 snRNA at the optical level, coverslips previously treated with formaldehyde-Triton were cut up into little squares (less than 10 mm side). These were floated for 90 minutes at 64°C on 2 μl hybridization solutions containing either U1 or U2 biotinylated DNA probe, rapidly rinsed over 20 μl drops of cold PBS and transferred over drops of BSA (5% in PBS) for 3 minutes as previously described (Besse and Puvion-Dutilleul, 1994). A rabbit anti-biotin IgG antibody (1/75 in PBS) (Enzo Biochem, New York, NY, USA) was used as the primary antibody. A rhodamine TRITC-conjugated goat anti-rabbit IgG (Nordic) diluted 1/50 in PBS was used as the secondary antibody. Samples were mounted in glycerol-PBS and observed using an Axiophot microscope.

RESULTS

Immunogold localization of p80-coilin

In conventionally formaldehyde-fixed HeLa cells, gold particles which localized p80-coilin accumulated mainly over the coiled bodies, 0.5 to 1 μm in diameter (Fig. 1A). Higher magnifications clearly revealed the tight association of the label with the coiled threads of about 20 nm in diameter and the total absence of labeling in the interstices between the threads (Fig. 1B). In addition, cross-sections of the coiled threads had the appearance of rings surrounding electron-translucent cores, which suggests that coiled bodies might consist either of hollow threads or of threads composed of more than one constituent. Gold particles also were present over compact fibrillar masses, which we named the interchromatin granule-associated zones (Visa et al., 1993a) (Fig. 1A,C). These latter structures were sparsely and irregularly labeled. The contiguous clusters of interchromatin granules were always devoid of labeling. A few gold particles were dispersed over the surrounding nucleoplasm.

In loosened nucleoproteins following fixation of the cells in a hypotonic solution of formaldehyde, the nuclei were swollen and the chromatin separated into threads which filled the nucleoplasm. The chromatin fibers of Lowicryl-embedded material are about 12-15 nm wide; that is, thinner than those in Epon-embedded material (20-25 nm wide; Puvion-Dutilleul and Puvion, 1980). These fibers were punctuated irregularly by granules which had grossly the same diameter as the fibers and corresponded to chromatin fibers seen in cross-section. The clusters of interchromatin granules and the coiled bodies were no longer identifiable by morphological criteria because their fibers were thinner and resembled those of the surrounding spread nucleoprotein fibers. Only the interchromatin granule-associated zones, the compact fibrillar organization of which was preserved by the loosening treatment, were detectable, and they could be identified even in the absence of specific labeling. They also could be decorated by gold particles (Fig. 2A) whereas adjacent areas which might correspond to the clusters of interchromatin granules were entirely devoid of labeling.

Gold particles bound to p80-coilin also were abundant over roughly spherical areas, 0.5 to 1 μm in diameter, which consisted of 12 nm thick coiled filaments (Fig. 2B). These labeled areas were coiled bodies, the diameter of the filaments having been markedly decreased under the loosening conditions. Once again, labeling was associated exclusively with the filaments of the coiled bodies whereas the surrounding nuclear material was decorated with only a few randomly dispersed gold particles.

In nuclear matrices following both DNase and high salt extractions prior to fixation, the clusters of interchromatin granules and their associated zones were not modified by the treat-
ments and were easily identifiable among the residual ribonucleoprotein network. Coiled bodies, which were recognizable by their round shape, consisted of filaments enclosed within a homogeneous, moderately electron-opaque matrix. The immunogold method revealed an intense labeling restricted to the coiled bodies (Fig. 2C) and the interchromatin granule-asso-

Fig. 1. Immunogold labeling of Lowicryl sections of formaldehyde-fixed HeLa cells using anti-p80 coilin antibody. Uranyl acetate staining. (A) Low magnification of a part of a nucleus. Gold particles mainly accumulate over the coiled body (cb) and the two interchromatin granule-associated zones (stars) whereas the clusters of interchromatin granules (ig) are devoid of labeling. A few gold particles are scattered over the surrounding nucleoplasm. (B) Higher magnification of a coiled body (cb), which shows the exclusive labeling of its filaments, about 20 nm in diameter, without labeling of the interstitial spaces. The arrow points to a ring-shaped configuration of a cross-sectioned filament. (C) Higher magnification of an interchromatin granule-associated zone (star) which shows an intense although uneven labeling. Bars, 0.5 µm.
associated zones whereas their still adjacent clusters of interchromatin granules were entirely devoid of labeling (Fig. 2D).

No labeling was obtained when normal rabbit serum was used instead of serum R288.

**Immunofluorescence localization of p80-coilin**
Since electron microscopic detection of p80-coilin by the use of serum R288 revealed the presence of the protein not only in the coiled bodies but also in the interchromatin granule-assoc-
associated zones, we have reinvestigated the intranuclear distribution of p80-coilin at the optical level. Following either methanol-acetone fixation or formaldehyde-Triton treatment of cells, we observed that serum R288 gave 1 to 6 fluorescent spots in most of the nuclei whatever the fixative used (Fig. 3A,C). About 10% of nuclei also displayed elongated fluorescent material which had the appearance of short filaments (Fig. 3B,D).

**Localization of U1 and U2 RNA by electron microscope in situ hybridization**

Denatured biotinylated U1 and U2 DNA probes were applied to sections of unloosened and loosened HeLa cells and nuclear matrices in order to hybridize them to complementary RNA sequences, and the hybrids were subsequently revealed by direct immunogold labeling. Data with conventionally fixed cells were previously reported (Visa et al., 1993a); therefore, the scope of the present study was restricted to the distribution of U1 and U2 snRNAs in loosened nucleoproteins and nuclear matrices.

As previously reported (Visa et al., 1993a), after hybridization of U1 and U2 DNA probes to sections of cells conventionally fixed with formaldehyde, gold particles were found over the perichromatin fibrils and the clusters of interchromatin granules. The interchromatin granule-associated zones were labeled with the U1 DNA probe only. The coiled bodies were labeled intensely with the U2 DNA probe and only slightly with the U1 DNA probe.

Following mild loosening of nuclear constituents, the interchromatin granule-associated zones, which were easily identifiable among the loosened chromatin fibers, were labeled with the U1 DNA probe (Fig. 4A) but were entirely devoid of labeling by the U2 DNA probe. Both probes, however, labeled areas of irregular shape which consisted of granules, 13 to 15 nm in diameter, inter-connected by low contrasted filaments of about 5 nm in width, thereby forming a continuous and slack network. Only the intense labeling of these loose networks, which corresponded to the clusters of interchromatin granules, allowed their clear delineation from the surrounding innumerable chromatin fibers. Labeled clusters of interchromatin granules were observed both adjacent to (Fig. 4A) and at a considerable distance from (Fig. 4B) the interchromatin granule-associated zones. Coiled bodies were recognizable only following the use of the U2 DNA probe, which intensely labeled small, clearly delineated tangles of coiled filaments (Fig. 4C). Gold particles were directly associated with the latter filaments and never with their interstices. After the use of the U1 DNA probe, the coiled bodies were difficult to distinguish among the loosened chromatin fibers because of their minimal level of labeling.

In nuclear matrices, the clusters of interchromatin granules remained labeled with the U1 (Fig. 5A) and U2 DNA probes (Fig. 5B). Once again, their associated zones were labeled with the U1 DNA probe (Fig. 5A) and never with the U2 DNA probe (Fig. 5B). Conversely, coiled bodies were intensely labeled with the U2 DNA probe and more slightly with the U1 DNA probe (Fig. 5B,C).

**Immunofluorescence labeling of U1 and U2 snRNAs by optical in situ hybridization**

Following in situ hybridization of U1 DNA probe (Fig. 3E) on formaldehyde/Triton-treated cells, the extranucleolar region of the nuclei showed a diffuse staining in which more bright spots were observed. Following the use of the U2 DNA probe (Fig. 3F), the general nucleoplasmic labeling is less intense, rendering bright patches more visible.

**DISCUSSION**

In previous publications (Visa et al., 1993a; Puvion-Dutilleul et al., 1994) we have described the results of studies identifying those intranuclear structures of HeLa cells which are accumulation sites for spliceosome components. In these reports, we presented evidence that, in addition to the well-known coiled bodies and clusters of interchromatin granules, a third structure concomitantly accumulates snRNP. This new structure was designated the interchromatin granule-associated zone because of its usual contiguity with the clusters of interchromatin granules (Visa et al., 1993a). In addition, we observed that the distribution of spliceosome components is dynamic and depends upon the physiological state of the cell, since only one type of spliceosome-component accumulation site persists in cells infected with either adenovirus (Puvion-Dutilleul et al., 1994) or herpes simplex virus (Besse et al., 1994) when the replicative activity of viral genomes becomes intense. In the current investigation, we extend our earlier observations with evidence that all three spliceosome-component accumulation sites are morphologically stable structures which persist without changes in their snRNA contents in nuclear matrices, i.e. following a high salt treatment of cells, as well as following experimentally induced mild loosening of nucleoproteins by a low salt treatment which spreads the nucleoproteins enough to allow a clear in situ visualization of actively transcribed genes (Puvion-Dutilleul and Puvion, 1980; Puvion-Dutilleul, 1993). In order to facilitate the observation of coiled bodies among the residual ribonucleoprotein network and the partially spread nucleoproteins, we used the anti-p80-coilin antibody as a marker (Andrade et al., 1993; Carmo-Fonseca et al., 1993; Lamond and Carmo-Fonseca, 1993b) and discovered that p80-coilin was present in the interchromatin granule-associated zones, not only in untreated cells but also after high or low salt treatment, as well as in the coiled bodies.

The most intriguing finding of this study is the binding of anti-p80-coilin antibody to the interchromatin granule-associated zones as well as the coiled bodies. Indeed, the anti p80-coilin antibody decorated the interchromatin granule-associated zones intensely. Thus electron microscopy reveals that the location of p80-coilin is not restricted to the coiled bodies, since anti-p80-coilin antibody binds to another structure which is different in shape (round, ovoid or cylindrical), appearance (compact and fibrillar) and snRNA composition (devoid of U2 snRNA). These electron microscope data are reinforced by immunofluorescence data which demonstrate the binding of anti-p80-coilin antibody over two morphologically distinct structures, i.e. spherical and cylindrical structures. In addition, fluorescence microscopy revealed that only 10% of the nuclei contain labeled elongated structures in addition to bright spots, while labeled interchromatin granule-associated zones observed with the electron microscope were present in about 50% of the sectioned nuclei. Taken together, these observa-
tions confirm that the interchromatin granule-associated zones are pleomorphic structures and reveal that the rounded ones are part of the spotted fluorescence. This is interesting in the light of previous study by Carmo-Fonseca et al. (1993) showing that coiled bodies are kinetic structures and that their steady-state size changes depending upon the metabolic activity of the cell.
whereas the intracellular level of p80-coilin remains stable, which implies that p80-coilin must exist outside the coiled bodies.

Variations in the ionic strength affected the aspect of coiled bodies only. After a high salt treatment of cells in order to obtain nuclear matrices, coiled bodies appear to consist of thin

Fig. 4. Localization of U1 (A) and U2 (B,C) snRNAs by in situ hybridization of U1 and U2 DNA probes, respectively. Lowicryl sections of mildly loosened nucleoproteins of HeLa cells. Uranyl acetate staining. (A) Localization of U1 snRNA. Gold particles labeled the interchromatin granule-associated zone (star) and the cluster of interchromatin granules (ig). A few gold particles are scattered over the surrounding loosened nucleoproteins. (B) Localization of U2 snRNA. Gold particles are mainly present over the round cluster of interchromatin granules (ig). (C) Localization of U2 snRNA. Gold particles are intensely accumulated over the filaments of the coiled body (cb). Bars, 0.5 µm.
filaments enclosed in a round, well-delineated, moderately electron-opaque matrix, as seen in Fig. 2C. The loosening procedure markedly modified the appearance of the coiled bodies by also reducing the diameter of their filaments; however, without concomitant formation of a matrix. As seen in Fig. 2B, following a low salt treatment of cells, the coiled bodies are indistinguishable morphologically from the surrounding loosened nucleoproteins. Only the binding of anti-p80-coilin antibody and U2 DNA sequences to round networks of filaments allowed us to identify the coiled bodies, and they clearly revealed that the low salt treatment did not induce swelling of the coiled bodies and did not disperse their filaments but simply made them thinner. On the other hand, the shape and fibrillar organization of the interchromatin granule-associated zones were preserved after combined hypotonic shock and detergent treatment, as well as after combined DNase digestion and high salt treatment, which renders these structures identifiable even in the absence of labeling. The association of the interchromatin granule-associated zones with the clusters of interchromatin granules was preserved.

Fig. 5. Localization of U1 (A,C) and U2 (B,D) snRNAs by in situ hybridization of U1 and U2 DNA probes, respectively. Lowicryl sections of nuclear matrices. Uranyl acetate staining. (A,C) Following the use of the U1 DNA probe, gold particles are numerous over the cluster of interchromatin granules (ig) and its associated zones (stars) and rarer over the coiled body (cb). (B,D) Following the use of the U2 DNA probe, gold particles are numerous over the cluster of interchromatin granule (ig) and the coiled body (cb). The interchromatin granule-associated zones (stars) are entirely devoid of labeling. Bars, 0.5 µm.
whatever the salt treatment used, which reinforces our assumption that the two structures are morphologically and functionally linked. Taken together, the data demonstrate that the interchromatin granule-associated zones, like coiled bodies and interchromatin granules, are elements of the nuclear matrix. In addition, the level of p80-coilin and snRNAs in both coiled bodies and interchromatin granule-associated zones was not sensitive to the low and high salt treatment of the cell, which suggests that p80-coilin and snRNAs are integral components of these nuclear matrix structures.

The structure of the clusters of interchromatin granules was not markedly altered after the loosening method as well as after the high salt treatment of cells. The clusters still appeared to be networks of interconnected particles, which, however, were more difficult to distinguish among the deoxyribonucleoprotein fibers released by the loosening of chromatin. Following loosening treatment of cells, therefore, only labeling with p80-coilin and snRNAs was detected in both unloosened HeLa cells and nuclear matrices. This suggests that p80-coilin and snRNAs are integral components of the clusters of interchromatin granules and revealed their presence, often in close proximity to the interchromatin granule-associated zones. It appears, therefore, that the respective topological localizations of the clusters of interchromatin granules and their associated zones are not sensitive to the high and low salt treatment. This suggests that both structures are tightly bound to or are constituents of the intranuclear matrix. This agrees, therefore, with previous studies which clearly demonstrated such an association of the clusters of interchromatin granules with the nuclear matrix (Berezney, 1979; Rzeszowska-Wolny et al., 1988) and the presence of snRNA within the nuclear matrix (Miller et al., 1978; Gallinaro et al., 1983; Long and Schrier, 1983; Raska et al., 1991).

In conclusion, the results of electron microscopic in situ hybridization of U DNA probes performed on loosened and unloosened HeLa cells and nuclear matrices demonstrate preservation of the topological distribution of the U1 and U2 snRNAs following low and high salt treatment of cells, and reinforce the concept of the crucial role of the intranuclear matrix in spliceosome formation and/or storage. The so-called interchromatin granule-associated zones, like the coiled bodies and the clusters of interchromatin granules, are elements of the intranuclear matrix. In addition, the unexpected immunogold detection of p80-coilin in the interchromatin-associated zone, which differs from the coiled bodies by its morphology and U snRNA content, raises the question of its belonging to the class of structures called ‘nuclear bodies’, and accentuates the complexity of the nuclear domains involved in gene expression.

The authors thank Drs J. P. Bachellerie and Mrs N. Nicoloso (Centre de Recherche de Biochimie et Génétique du CNRS, Toulouse, France) for the preparation of bionylated U1 DNA and U2 DNA probes. They thank Drs J. E. Dahlberg (University of Wisconsin, Madison, WI, USA) and T. Pederson (Worcester Foundation For Experimental Biology, Shrewsbury, MS, USA) for the gift of the plasmids used for preparing the probes. They thank Mrs E. Pichard for her expert technical assistance, and Mr F. Harper and Mrs M. Harper for the gift of HeLa nuclear matrices and cell cultures, respectively. This work was funded by general grants from the Centre National de la Recherche Scientifique. Françoise Puvion-Dutilleul is a member of the Institut National de la Santé et de la Recherche Médicale. Sylvie Besse was the recipient of a fellowship from the Comité Départemental du Val-de-Marne de la Ligue Nationale contre le Cancer.

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(Received 29 July 1994 - Accepted 23 November 1994)