DISTRIBUTION OF THE 70K U1 RNA-ASSOCIATED PROTEIN DURING INTERPHASE AND MITOSIS
CORRELATION WITH OTHER U RNP PARTICLES AND PROTEINS OF THE NUCLEAR MATRIX

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SUMMARY
Earlier studies suggested that the 70K (70×10^3 M_2) polypeptide is a nuclear matrix (associated) protein since it is the only U1 RNP-associated antigen that is not released from the nucleus after treatment of the cell with, successively, detergents, DNase I and/or RNase A and high salt. The possibility that the 70K protein functions in the binding of U1 RNP to the nuclear matrix is now further substantiated by the finding that U1 RNP particles that did or did not contain the 70K protein could be isolated, depending on the method of isolation. When U1 RNP particles were obtained by means of sonic disruption of the nucleus they contained the 70K polypeptide, whereas particles that were isolated by extraction at room temperature and a slightly alkaline pH lacked the 70K protein but contained the intact U1 RNA and the other U1 RNP-associated proteins.

During interphase the localization of the 70K protein is restricted to the nucleus, giving a dot-like distribution pattern with exclusion of the nucleoli. During prophase to late anaphase the protein is dispersed throughout the entire cytoplasm with the exception of the chromatin regions. Immunofluorescence studies, using a monoclonal anti-70K antibody in combination with human autoimmune sera that react with U1 RNA-associated proteins, demonstrate that the 70K protein is localized in those areas of the cell where other U RNP proteins occur, also during mitosis. Topoisomerase I and nuclear laminas, typical nuclear matrix proteins, show completely different distribution patterns in all phases of the cell cycle.

Assembly of the nuclear envelope is attended by the re-formation of the clustered appearance of the 70K antigen. These results suggest that, although associated with the nuclear matrix fraction in interphase cells, the 70K protein remains associated with the U1 RNP particles during cell division.

INTRODUCTION
The small nuclear ribonucleoprotein (snRNP) particles U1 to U6 are present in nuclei of all eukaryotic cells (reviewed by Reddy & Busch, 1983; Brunel et al. 1985). In interphase cells the U1, U2, U4, U5 and U6 (U1–U6) RNP particles are mainly located in the nucleoplasm, whereas U3 RNP can only be detected in nucleoli.

Evidence has been provided that U1, U2 and probably other snRNPs as well are essential components in the processing of mRNA (Krämer et al. 1984; Black et al. 1985; Chabot et al. 1985; Krainer & Maniatis, 1985). During this process the U1

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RNP particle is involved in the recognition of the 5' splice sites of pre-mRNA (Krämer et al. 1984).

To date, U1 RNP particles are known to contain at least nine different protein constituents, namely the 70K protein ($70 \times 10^3 M_r$) and proteins A ($32 \times 10^3 M_r$), B' ($28 \times 10^3 M_r$), C ($21 \times 10^3 M_r$), D ($16 \times 10^3 M_r$), E ($13 \times 10^3 M_r$), F ($12 \times 10^3 M_r$) and G ($11 \times 10^3 M_r$) (Billings & Hoch, 1984; Pettersson et al. 1984; Brunel et al. 1985). Polypeptides 70K, A and C are unique to the U1 RNP particle (Billings & Hoch, 1984; Pettersson et al. 1984; Habets et al. 1985), while proteins B', B, D, E, F and G are also associated with the other U snRNAs. Several of these proteins are recognized by autoantibodies from patients with connective tissue diseases (Lerner & Steitz, 1981; Tan, 1982).

It has been suggested that the 70K protein might be involved in binding of U1 RNP to the nuclear matrix, since a substantial part of it is not released from this nuclear substructure by incubation of nuclei with RNase or DNase. Other U1 RNA-associated proteins, especially A and C, are readily extracted under these circumstances (Mariman & van Venrooij, 1985; Habets et al. 1985; Verheijen et al. 1986b). This indicates that the 70K protein interacts directly with components of the nuclear matrix or is an integral part of it.

In order to understand better the function of the various U1 RNP constituents and their possible interaction with the nuclear matrix we have examined the subcellular localization of these components in both interphase and mitotic cells. For this purpose we have used human autoimmune sera, specifically recognizing U snRNA-associated proteins and topoisomerase I, respectively, in combination with monoclonal antibodies to lamins (Burke et al. 1983) and the 70K protein (Billings et al. 1982).

Furthermore, we have isolated U1 RNP particles from HeLa cells and found that such particles may or may not contain the 70K polypeptide depending on the isolation conditions used.

MATERIALS AND METHODS

Antibodies

The hybrid cell lines 2.73 (anti-70K) and 7.13 (anti-D) were provided by Dr S. Hoch (La Jolla, Ca) and have been described (Billings et al. 1982, 1985). The hybrid cell line 41CC4 (anti-lamins) was a kind gift from Dr G. Warren (Heidelberg) and has also been described (Burke et al. 1983). The human sera G15 (anti-U1 RNP), H11 (anti-Sm) and Z3 (van Venrooij et al. 1985; antitopoisomerase I, Shero et al. 1986) were obtained from patients with systemic autoimmune diseases.

Cell culture

Tissue culture media and calf sera were purchased from Flow Laboratories Ltd, Irvine, Scotland.

The cells used in this study were: HeLa S3 (human cervix carcinoma), MR65 (human pulmonary squamous cell carcinoma), T24 (human bladder transitional cell carcinoma), BHK (baby hamster kidney), a bovine lens cell culture and cultured human fibroblasts. All cells were grown on glass slides or coverslips in Eagle's modified Minimum Essential Medium supplemented with 10% newborn calf serum.
Distribution of 70K U1 RNA-associated protein

HeLa S3 cells were also grown in suspension at densities ranging from 0.5 \times 10^6 to 10^6 cells ml\(^{-1}\) on Suspension Minimal Essential Medium supplemented with 10% newborn calf serum and 1.5 g l\(^{-1}\) lactalbumin hydrolysate.

**Immunofluorescence**

Cells grown in monolayer on glass slides or coverslips were rinsed with phosphate-buffered saline (PBS) and fixed by dipping in cold methanol (-20°C) and thereafter in acetone at room temperature (3 times 5 s). The cells could then be stored at -20°C for several days.

The single-antibody indirect immunofluorescence technique was performed essentially as described by Ramaekers et al. (1983).

For triple-labeling the procedure was as follows: the fixed cells were air dried and incubated for 30 min at room temperature successively with: (1) human autoantibody (dilution 1:50); (2) undiluted culture supernatant of the mouse monoclonal antibody; (3) Texas Red-conjugated sheep anti-mouse (Fab\(_2\)) Ig (immunoglobulin; NEN, Dreieich, FRG; dilution 1:50); and (4) FITC-conjugated goat anti-human Ig (heavy and light chains; Nordic, Tilburg, The Netherlands; dilution 1:50). Each step was followed by a washing procedure in PBS containing 0.5% Triton X-100 (3 times 15 s). All serum dilutions were made in PBS. DNA was then stained by incubating the cells for 15 min with Hoechst 33258 (0.1 μg ml\(^{-1}\) in 22 mM-citric acid, 55 mM-disodium hydrogen phosphate). After washing with PBS the cells were mounted in Gelvatol (Monsanto, St Louis, Missouri, USA) containing 100 mg ml\(^{-1}\) 1,4-diazobicyclo[2,2,2]-octane (DABCO; Janssen Pharmaceutica, Beersel, Belgium) and viewed with a Leitz Dialux 20 EB microscope equipped with epi-fluorescent illumination. Pictures were taken on Tri-X film (Kodak) with an automatic camera using an ASA setting of 400.

Controls were processed as described above and included: (1) conjugated second antibody used without first antibodies; (2) mouse monoclonal antibodies were used with FITC-conjugated goat anti-human Ig as second antibody; and (3) human autoantibodies with Texas Red-conjugated sheep anti-mouse (Fab\(_2\)) Ig as second antibody. These control experiments showed that no interspecies cross-reactivity occurred with the second antibodies.

**Isolation of U1 RNP particles**

All chemicals were of analytical grade. Buffers were boiled in the presence of 0.02% diethylpyrocarbonate and then autoclaved. Each solution was supplemented with 0.5 mM-phenylmethylsulphonyl chloride (PMSF) to reduce proteolytic degradation. This agent was added from a freshly prepared stock. Centrifugation steps were carried out for 5 min at 800 g and 2°C. If not indicated otherwise the procedures used for the isolation of U1 particles were carried out at 0-4°C.

HeLa S3 cells in suspension were harvested on frozen NKM buffer (130 mM-NaCl, 5 mM-KCl, 1.5 mM-MgCl\(_2\)), pelleted by centrifugation, washed twice with isotonic NKM solution and pelleted again. Subsequently, the cell pellet was resuspended in reticulocyte suspension buffer (RSB 7-4) (10 mM-Tris-acetate, pH 7.4, 10 mM-NaCl, 1.5 mM-MgCl\(_2\)) and after adjustment to 0.5% (v/v) Nonidet P40 (Fluka, Buchs, Switzerland) the suspension (3\times10^7 cells ml\(^{-1}\)) was placed on ice for 10 min, with occasional stirring.

From this step on we used two different methods to obtain the U1 RNP particles. First, the 'incubation procedure' as described by Wilk et al. (1985). This procedure is based on the endonucleolytic degradation of large hnRNP structures at 22°C and pH 8.0. Second, the 'sonication procedure' in which RNP complexes were released by sonic disruption of the nuclei on ice at pH 7.4.

**Incubation procedure.** The nuclei were washed twice with RSB 7-0 (RSB adjusted to pH 7.0), once with RSB 8-0 (RSB adjusted to pH 8.0) and finally extracted twice in 1.5 ml RSB 8-0 (2\times10^9 nuclei ml\(^{-1}\)) for 30 min at 22°C, with constant stirring.

**Sonication procedure.** The nuclei were washed twice with RSB 7-4 and resuspended in 3 ml RSB 7-4 (1\times10^9 nuclei ml\(^{-1}\)) in the presence of 1 μl silicon antifoam emulsion (J. T. Baker Chemicals). The nuclei were sonicated three times (1 min) on ice with a Branson sonifier, model 8-30 (Branson Instr. Inc., Connecticut, USA) at level 5.
The suspension of sonicated nuclei as well as the combined extracts obtained from the incubation procedure was centrifuged at 16,000 g for 15 min. The supernatants were layered on 15% to 33.5% (w/v) glycerol gradients in RSB, 7.4, and centrifuged in a Beckman SW 40.1 rotor at 25,000 rev. min⁻¹ for 16 h. Absorption was continuously monitored using a Gilford spectrophotometer at 260 nm. The gradients were collected in 0.7-ml fractions, which were used for the determination of protein and RNA content.

RNA analysis

Immediately after fractionation of the gradients, RNA was isolated from the various fractions (van Eckelen & van Venrooij, 1981), precipitated overnight at -20°C by adding 2 vol. of ethanol, 0.1 vol. 3 M-sodium acetate, pH 5-0, and 10 µg yeast tRNA carrier and collected by centrifugation at 16,000 g for 20 min at -20°C. The pellets were dissolved in 5 × SSC (standard saline citrate: 150 mM-NaCl, 15 mM-trisodium citrate) containing 7.5% (v/v) formaldehyde, heated for 10 min at 65°C, chilled in ice and placed on nitrocellulose filters (Schleicher & Schuell membrane filters, BA 85, Dassel, FRG). The blots were rinsed with 5 × SSC, dried, and heated at 80°C for 2 h under vacuum.

Prehybridization was performed for 2 h at 42°C in 50% (v/v) formamide, 5 × SSC, 5 × Denhardt's solution (0.1% (w/v) Ficoll-400, 0.1% (w/v) bovine serum albumin, 0.1% (w/v) polyvinylpyrrolidone-400), 5 mM-EDTA, 50 mM-sodium phosphate, pH 6.8, and complemented with 250 µg ml⁻¹ denatured herring sperm DNA.

Hybridization was carried out for 16 h at 42°C in 50% (v/v) formamide, 5 × SSC, 1 × Denhardt's solution, 5 mM-EDTA, 20 mM-sodium phosphate, pH 6.8, 100 µg ml⁻¹ denatured herring sperm DNA and 2 × 10⁶ cts min⁻¹ of the nick-translated plasmid probe pU1.2 (αβ³²P]-ATP: Amersham, UK, ±3000 Ci mmol⁻¹). This probe contains a SacI fragment from the λ clone pU1-6 (Monstein et al., 1983) and carries the complete U1 RNA complementary sequence.

After hybridization the dot-bLOTS were washed once with the hybridization buffer at 42°C for 1 h and twice with 2 × SSC, 0.1% sodium dodecyl sulphate (SDS) for 15 min at 42°C. Then the blots were dried and used for autoradiography with Kodak XAR-5 film, using intensifying screens.

RNAs isolated from the gradient fractions were also analysed on 10% polyacrylamide gels containing 8.3 M-urea, 9 mM-Tris-borate, pH 8.0, and 2 mM-EDTA. Blotting onto GeneScreen (New England Nuclear, Boston, USA) and hybridization of U1 RNA with the nick-translated probe pU1.2 was performed according to the GeneScreen instruction manual; hybridization of RNA, method II.

Protein analysis

For the characterization of the antibodies used in this study by immunoblotting assays total nuclear protein fractions (Habets et al., 1983) and purified nuclear matrices (Verheijen et al., 1986a) from HeLa cells were used. The nuclei or nuclear matrices were dissolved in SDS sample buffer (62.5 mM-Tris·HCl, pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol) and boiled for 5 min.

Proteins in gradient fractions were precipitated by adding trichloroacetic acid to a final concentration of 10% (w/v). The precipitates were pelleted, washed twice with cold acetone, dried and dissolved in SDS sample buffer.

SDS-polyacrylamide gel electrophoresis (SDS–PAGE) was performed using the Laemmli (1970) buffer system.

After SDS–PAGE, proteins were transferred overnight onto nitrocellulose (Habets et al., 1983), dried, and stored at room temperature. Detection of the antigens on the blots was performed essentially as described by Habets et al. (1983). As primary antibodies mouse monoclonal antisera were used next to human autoimmune sera. The human sera were diluted 1:50 in buffer B (0.3% (w/v) bovine serum albumin, 350 mM-NaCl, 10 mM-Tris·HCl, pH 7.6, 1% (v/v) Triton X-100, 0.5% (w/v) sodium deoxycholate, 0.1% (w/v) SDS), whereas the monoclonal antisera were used as culture supernatant diluted with 1/6 vol. of 6 × concentrated buffer B. The antibody–antigen
Distribution of 70K U1 RNA-associated protein

RESULTS AND DISCUSSION

Characterization of antibodies

Immunooblots containing total nuclear proteins or purified nuclear matrix proteins from HeLa S3 cells were used for the identification of antigens recognized by the various antibodies used in this study (Fig. 1).

In the total nuclear protein fraction the monoclonal antibodies 2.73 and 7.13 recognize the 70K and D snRNP polypeptides, respectively. When tested on nuclear matrix blots it is evident that a substantial part of the 70K protein is retained in the nuclear matrix fraction, while the D protein can only be detected in low amounts in this fraction. Similar observations were made earlier by Habets et al. (1985).

In total nuclei the human anti-Sm serum H11 reacts with the A, B', B and D proteins, whereas the human anti-U1 RNP serum G15 reacts predominantly with the proteins A and C. In the nuclear matrix fraction the B' and B proteins are partly retained while the A and C proteins are completely lost.

The finding that the 70K protein apparently is the only U1 RNP-specific antigen in the nuclear matrix fraction suggests, in line with earlier findings (Habets et al. 1985; Verheijen et al. 1986), that the 70K protein might mediate the binding of U1 RNP to the nuclear matrix.

The human scleroderma serum Z3 recognizes an antigen with an apparent molecular weight of $100 \times 10^3 \text{M}_r$. Previously the molecular weight of this antigen was estimated to be about $86 \times 10^3$ (van Venrooij et al. 1985). Shero et al. (1986), in accordance with Maul et al. (1986), recently identified the protein recognized by this antibody as topoisomerase I having a molecular weight of $100 \times 10^3$. Van Venrooij et al. (1985) had already demonstrated this antigen to be a nuclear matrix component, since it was not released from HeLa nuclei by nuclease and high-salt treatment.

The monoclonal antibody 41CC4 recognizes lamins A and C, both in total nuclear protein fractions and in nuclear matrix preparations (Burke et al. 1983; Verheijen et al. 1986a).

Is the 70K protein involved in the binding of U1 RNP to the nuclear matrix?

To obtain further experimental evidence for a possible role of the 70K protein in linking U1 RNP to the nuclear matrix, HeLa cell nuclei were subjected to two procedures that disrupt the nuclear integrity in completely different ways.

The isolated nuclei were either incubated at a slightly alkaline pH at room temperature (Wilk et al. 1985) or sonicated. In the first method, based on the original Samarina procedure (Samarina et al. 1968) the large hnRNP polyparticle structures are cleaved into 40S hnRNP monoparticles by endogenous ribonuclease activity. The sonication procedure releases RNP particles by nuclear disruption. The released RNP's were fractionated on glycerol gradients. The sedimentation profiles of both
methods showed a major peak with a sedimentation coefficient of approximately 40S, indicating that the large hnRNP complexes had been disrupted.

Screening of gradient fractions for the presence of U1 RNA by the dot-blot technique demonstrates that the highest amounts of U1 RNA were detected on top of the gradients in fractions 3 to 7 (Fig. 2A,B). This holds true for U1 RNP-containing gradient fractions isolated by the incubation procedure as well as by sonication. Both U1 RNP preparations (sedimenting around 17S) contained intact U1 RNA (Fig. 2C) as found by acrylamide gel electrophoresis followed by hybridization with the U1-RNA-specific probe pU1.2 (Monstein et al. 1983).
Protein blots of the U1 RNA-containing fractions were screened with antibody 2.73 (anti-70K) and the human anti-Sm serum H11. Fig. 3A shows that the 70K protein can only be detected in the U1 RNP-containing fractions that were obtained by the sonication procedure (lane 1), while after the incubation procedure (lane 2) no 70K protein is seen in the immunoblotting assay. This difference is not due to a concentration effect since, when an additional incubation of these blots with serum H11 was performed, about the same staining intensities of the A, B', B and D proteins in both lanes were seen (Fig. 3B). This indicates that U1 RNPs isolated after endonuclease activity contain none, or very low amounts, of the 70K protein. The possibility that selective degradation of the 70K protein occurred during the incubation procedure was also considered. Two findings argue against this possibility. First, in the U1 RNP-containing fraction no degradation products with antigenic determinants could be detected, neither with the monoclonal anti-70K (Fig. 3) nor with autoimmune anti-70K antibodies (data not shown). Second, in the pellet remaining after the extraction the triplet of 70K polypeptides was present in an

![Dinot-blot autoradiographs containing U1 RNA from gradient fractions obtained after: A, sonic disruption of the nuclei, and B, incubation of nuclei at 22°C and pH 8.0. Isolated RNA was placed on nitrocellulose paper, and hybridised with the nick-translated probe pU1.2 containing the complete U1 RNA complementary sequence (Monstein et al. 1983). C, Northern blot autoradiographs containing U1 RNA from the pooled gradient fractions nos 1-5 obtained after: sonic disruption of the nuclei (lane 1); and incubation of nuclei at 22°C and pH 8.0 (lane 2). Isolated RNAs were separated on a 10% polyacrylamide gel, blotted onto GeneScreen and hybridized with the nick-translated probe pU1.2. Note that both preparations contain intact U1 RNA.](image-url)
apparently undegraded form, and no degradation products could be observed (unpublished data).

These results demonstrate that the 70K protein can be obtained in association with the ‘free’ U1 RNP particle when the nuclear matrix structure is disrupted. Endonucleolytic degradation of nuclei, however, shows that intact U1 RNA that is not complexed with this 70K protein can be isolated. One of the many possible explanations for this phenomenon could be that (hn)RNA is involved in the binding of the 70K protein to the U1 RNP particle.
Distribution of 70K U1 RNA-associated protein

It should be noted here that the high resolution of this gel permits the recognition of the 70K protein as a triplet (previously described by Habets et al. 1985 and Verheijen et al. 1986a). It is not known if this triplet is a result of distinct proteolytic degradation or of post-translational modifications.

A further analysis of the RNA and protein composition of the gradient fractions will be published elsewhere.

Distribution of the 70K protein during interphase and in mitotic cells

Immunofluorescence studies with the monoclonal antibody 2.73 in various cell cultures (Fig. 4) show similar distribution patterns of the 70K protein in human cells as well as in non-human cells. Also when comparing cells of epithelial origin and cells of non-epithelial origin similar distribution patterns are seen. In all interphase cells the 70K antigen is almost exclusively located in the nucleus, exhibiting a speckled pattern with exclusion of the nucleoli. As discussed by Ringertz et al. (1986) the nuclear speckles seen with Sm and RNP antibodies probably represent transcriptionally active nuclear domains in which there is an accumulation of snRNP complexes because of active processing of RNA polymerase II transcripts.

Fig. 4. Interphase nuclei of various cell types stained with monoclonal antibody 2.73, recognizing the U1 RNA-associated 70K protein. A, MR65 (human pulmonary squamous cell carcinoma); B, HeLa S3 (human cervix carcinoma); C, human fibroblast culture; D, T24 (human bladder transitional cell carcinoma); E, bovine lens cell culture; F, BHK cells. ×800.
Fig. 5. Distribution of the 70K protein, detected by antibody 2.73 (right panels) during the various phases of mitosis in MR65 cells. DNA staining with Hoechst 33258 (left panels). A. Prophase; B, metaphase; C, anaphase; D, telophase; E, cyrokinesis. ×800.

Note that the dot-like distribution of the 70K protein in interphase cells changes into a diffuse cytoplasmic staining during prophase to anaphase. The 70K protein appears not to be associated with the chromatin.
For the immunofluorescent visualization and localization of the 70K polypeptide during mitosis we have used cell line MR65, human pulmonary squamous cell carcinoma cells in culture that remain relatively flat during the mitotic cell cycle and have large nuclei. The localization of the 70K protein in these cells during different phases of mitosis is shown in Fig. 5. During prophase to late anaphase the dot-like staining pattern of the 70K protein is almost completely lost. The protein seems to be dispersed throughout the entire cytoplasm of the cells, but in all mitotic stages the polypeptide appears not to be associated with the condensed chromosomes. In late anaphase/telophase the speckled 70K pattern is regenerated. These fluorescence patterns suggest that no direct interaction of the 70K protein and the DNA exists, at least not in mitotic cells.

Using the triple-labelling immunofluorescence technique we were able to monitor simultaneously the expression and distribution of two different antigens in these cells, in particular mitotic phases. In doing so we have studied the relation of the 70K protein with: (1) other U1 RNP-specific proteins; (2) other (U1–U6)RNP-

Fig. 6. Comparison of the staining patterns of the anti-U1 RNP serum G15 (middle panels) and antibody 2.73 (right panels) in MR65 cells, also stained with Hoechst 33258 (left panels), demonstrating that these two sera recognizing U1 RNP proteins give similar staining patterns in both interphase and mitotic cells. ×800.
associated proteins; and (3) the nuclear matrix protein topoisomerase I. We have also investigated the distribution of the U snRNPs in relation to the nuclear lamins.

70K versus other U1 RNP proteins

As stated before, the anti-U1 RNP serum G15 reacts with the A and C proteins, whereas the monoclonal antibody 2.73 recognizes only the 70K antigen. Since these antigens are all U1 RNP-specific proteins one can anticipate that triple-labelling with these antibodies should result in similar staining patterns, at least in interphase cells. Fig. 6 demonstrates these staining reactions. As expected, the 70K, A and C proteins appear to colocalize in dot-like structures within the nucleus. Localization of the 70K protein in the same areas as the other U1 RNP-specific proteins was also found in
mitotic cells. This result suggests that also during mitosis the 70K protein is complexed with the U1 RNP particle as found earlier by Reuter et al. (1985).

70K versus (U1–U6) RNP proteins

Serum H11 was used to study the correlation between the 70K protein and other (U1–U6) RNP constituents. The anti-Sm serum H11 and the monoclonal antibody 7.13 both react with (U1–U6) RNP particles, as they both recognize the D protein. Fig. 7A shows similar staining patterns of H11 and 7.13. Also G15 and 2.73 (Fig. 6) and G15 and 7.13 (Fig. 7B) recognize antigens that colocalize. Consequently, H11 and 2.73 should show a comparable fluorescence distribution in both mitotic and interphase cells (Fig. 7C). Although the similar fluorescence patterns of these different antibodies might suggest that various U snRNP particles are complexed with each other (see also Reuter et al. 1985; Spector & Smith, 1986), the data should be interpreted with great care. Since U1 and U2 RNP are the most abundant amongst the U snRNPs, the methods used will most probably not permit a separate detection of U4–U6 snRNP as a result of their low concentration. However, as far as

Fig. 8. Triple-labelling in MR65 cells with Hoechst 33258 (left panels), the antitopoisoerase I serum Z3 (middle panels) and antibody 2.73 (right panels), ×800. Note that the staining patterns of topoisoerase I and 70K are almost complementary.
the resolution of the fluorescence method allows it appears that at least part of the U1 RNP, U2 RNP and the 70K protein is colocalized during interphase and mitosis. Such a localization of U1 and U2 RNP during interphase is not surprising as they both participate in the processing of mRNA (Krämer et al. 1984; Black et al. 1985; Krainer & Maniatis, 1985), most likely as components of the spliceosome. It is

Fig. 9. Triple-labelling in MR65 cells at the various stages of mitosis with Hoechst 33258 (left panels), anti-U1 RNP serum G15 (middle panels, A and B), and the anti-Sm serum H11 (middle panels, C-H) and anti-lamin antibody 41CC4 (right panels). ×800.
interesting, however, that also during mitosis the association of U1 RNP, U2 RNP and the 70K seems to be maintained.

70K versus topoisomerase I

During interphase, topoisomerase I and the 70K protein are both located in the nucleus, but their fluorescence patterns are different. The scleroderma serum Z3, recognizing topoisomerase I, stains the whole nucleus diffusely with a very strong reaction in the nucleoli (Fig. 8). From biochemical studies it has been concluded that topoisomerase I is a constituent of the nuclear matrix (van Venrooij et al. 1983).

During the various stages of mitosis antisera Z3 and 2.73 show diffuse but almost complementary staining patterns. Topoisomerase I appears to be associated with the condensed chromosomes, while the 70K protein is not observed in this region. This demonstrates that the 70K protein and topoisomerase I do not exhibit the same structural organization during mitosis. These data fully support those described
above in showing that in mitotic cells the 70K–U1 RNP complex is excluded from the chromatin-containing structure.

U snRNPs versus nuclear lamins

The formation and degradation of the nuclear membrane during mitosis can be monitored with the monoclonal antibody 41CC4, directed against the nuclear lamin polypeptides A and C. Fig. 9A shows the staining patterns of the U snRNP complexes and the lamina in interphase cells as detected by triple-labeling experiments with the sera G15 or H11 and 41CC4, respectively. When the cells enter prophase (Fig. 9B) the chromosomes become condensed and the nuclear lamina disappears. In this phase of the cell cycle the laminae stain diffusely while the speckled U snRNP distribution has become less dense. During metaphase (Fig. 9C) and anaphase (Fig. 9D) the U snRNPs as well as the laminae are found throughout the entire cytoplasm with exclusion of the chromatin.

During progression of telophase (Fig. 9E,F,G) the nuclear lamina is restored. Reformation of the discrete speckled snRNP pattern in the newly formed nucleus of each daughter cell seems strongly correlated with the assembly of the nuclear lamina. At cytokinesis (Fig. 9H) the initial situation is reached again. The pictures shown in Fig. 9E–H suggest a certain sequence of events with respect to re-formation of the nucleus during telophase and cytokinesis. It appears that 70K–U1 RNP aggregation into discrete nuclear protein clusters has already been completed before each group of daughter chromatids is surrounded by a new nuclear envelope (compare Figs 5 and 9).

Conclusions

In summary our studies suggest that: (1) the 70K protein may function in binding U1 RNP particles to the nuclear matrix. (2) During mitosis the 70K protein is dispersed throughout the cytoplasm still associated with other U1 RNP polypeptides, probably as an intact RNP particle. (3) Re-aggregation of the 70K–U1 RNP complex at telophase into discrete nuclear protein clusters appears to be accomplished before the formation of a new nuclear envelope is completed.

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