

Ultrastructure of Free Ribonucleoprotein Complexes in Spread Mammalian Nuclei

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ABSTRACT Mouse erythroleukemia cell nuclei obtained by three different methods were spread for electron microscopy under low ionic conditions. It was found that this procedure allows the observation of free large ribonucleoprotein (RNP) complexes released from the nuclei during the centrifugation. The morphology of these complexes was readily affected by the conditions of cell treatment and spreading. Two extreme forms of free nuclear RNP structures were obtained, both consisting of spherical particles with diameters of ~17–20 nm. The first type was of loosened complexes of irregularly assembled particles interconnected with RNA fibrils. The second represented tightly packed particles forming mostly branched structures. The latter structures appeared to be closer to the native form of the nuclear RNP particles, differing from polyribosomes by their characteristic branching and stability in EDTA solutions.

Since the discovery that 30-40S nuclear ribonucleoprotein (RNP) particles are monomers of a more complex polysomelike structure (29), the electron microscopic morphology of these complexes has been studied mostly on isolated material. Such preparations consist almost exclusively of single, or a few irregularly assembled particles (5, 9, 14, 16, 18, 28), due to the rapid degradation of the large complexes during their isolation. These data do not help us to understand the structure of the whole complex. Moreover, some rearrangement of proteins has been shown to take place during the degradation process (35). Electron microscopical studies on ultrathin sections of fixed material have revealed the presence of RNA-protein complexes in the nucleus (2, 21), but they do not reveal their detailed ultrastructural morphology. Only in the case of amphibian oocytes have free RNP complexes been described as large irregular aggregates that can be transformed into linear arrangements of particles upon treatment with formamide (17, 22).

On the other hand, electron microscope observations on nonribosomal RNA transcripts in spread nuclear preparations show that they appear as DNA-associated lateral fibers representing complexes of particles (1, 15, 26) which contain newly synthesized RNA (38). The morphology of these complexes, however, showed great variability: irregularly branched, or bushlike particulate structures, thick fibers, linear arrays of particles more or less regularly distributed, or covering only some regions of RNA (1, 3, 8, 11, 23, 25–27). It is not clear whether this is due to a real heterogeneity in the ultrastructural organization of different RNA transcripts or to their high sensitivity to environmental conditions. Thus, it is difficult to

deduce the exact morphology of the nuclear RNP complexes, although different models have been proposed (18, 29, 31, 34, 37). The resolution of this problem may be facilitated by methods that allow ultrastructural observations of free nuclear RNP particles without their preliminary isolation and under minimal treatment of the nuclei.

Here we show that the spreading of nuclei for electron microscopy makes possible the observation of free RNP complexes released from the nucleus. Their morphology is readily affected by the experimental procedures. The structures which seem to be the closest to the native state are tightly packed polyparticles showing a characteristic branching.

MATERIALS AND METHODS

Friend erythroleukemia cells, clone F4N, grown in Eagle's minimum essential medium (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) supplemented with 10% calf serum, were used in all experiments. The cells were maintained in exponential growth by daily subculturing in fresh medium at an initial density of 2.5×10^5 cells/ml. Nuclei were prepared by three different procedures and spread for electron microscopy.

In the first procedure the cells were washed with 0.25 M sucrose containing 0.025 M CaCl_2 , 0.05 M Tris-HCl, pH 7.4, and resuspended in 0.25 M sucrose, 0.001 M CaCl_2 , 0.01 M Tris-HCl, pH 8.0 ($\sim 3 \times 10^6$ cells/ml). They were treated with 0.2% Nonidet P-40 (NP-40) for 5 min in an ice bath and the nuclei were pelleted at 600 g. These preparations are referred to as Ca^{2+} -nuclei. In some experiments the nuclei were suspended in distilled water adjusted to pH 9.0 with borate buffer (19) and were incubated with 1 $\mu\text{g}/\text{ml}$ trypsin (Worthington Biochemical Corp., Freehold, NJ) for 15–30 min in an ice bath.

In the second procedure, the cells were washed with phosphate-buffered saline (PBS) buffer (0.14 M NaCl, 2.7 mM KCl, 1.47 mM KH_2PO_4 , 8.1 mM Na_2HPO_4 , pH 7.8), resuspended in the same buffer, treated with NP-40 as above and the nuclei pelleted at 600 g. These preparations are referred to as PBS-nuclei.

In the third procedure the cells were lysed in water of pH 9.0 containing 0.01%

of the household detergent Joy (19) for 2 min in an ice bath and used for spreading without a preliminary isolation of the nuclei.

As a source of polysomes, reticulocytes were isolated from the blood of phenylhydrazine-treated mice (6) and treated with Joy as above. To discriminate between polysomes and nuclear RNP polyparticles, samples of the material suspended in pH-9 water (19) were treated with 0.2 mM EDTA (5) for 30 min in the cold before centrifugation on the grid.

To check for the presence of RNA in different particulate structures, the material was treated with pancreatic RNase (Worthington Biochemical Corp.) in two different ways—either in solution before spreading or on the grids after spreading. In the first case treatment was for 10–15 min at 37°C in pH-9 water containing 10 µg/ml RNase. In the second case, the grids with the spread material were floated on a drop of RNase, 10 µg/ml in 0.05 M Tris-HCl, pH 7.

For comparison, free RNP particles were also isolated by a slightly modified classical procedure involving 0.14 M NaCl extraction of isolated nuclei and fractionation of the extracted material in a sucrose density gradient (12).

For electron microscopy, the material (isolated nuclei, Joy-treated Friend cells, or reticulocytes) was suspended in pH-9 water and layered on 0.1 M sucrose with or without 10% formaldehyde, pH 8.6, in a microcentrifugation chamber (19). The samples were centrifuged for 8 min at 4,000 rev/min and 4°C on carbon-Formvar-coated grids that had been glow-discharged in the air just before use. After dehydration in 70%, 96%, and absolute ethanol, the material was stained with uranyl acetate in ethanol and rotary shadowed with tungsten dioxide.

In the case of free RNP particles extracted from nuclei, a drop of the gradient was placed directly on the electron microscope grid, the solution was withdrawn with filter paper, and the sample dehydrated and stained as above.

Micrographs were taken at 80 kV in a JEM B electron microscope. The exact magnification was determined by means of a Polaron grating replica with 2,160 lines/mm.

RESULTS AND DISCUSSION

When isolated PBS-nuclei were dispersed on the grid they were surrounded by complexes of irregularly arranged particles ~20 nm in diameter, interconnected with RNase sensitive filaments (Fig. 1A). Different complexes contained different number of particles but no consistent structural arrangement was apparent.

A different morphology of the free particulate structures was seen when Ca²⁺-nuclei were spread under the same conditions. These appeared as branched structures composed of tightly packed particles, sometimes arranged in zig-zag and all without visible interconnecting filaments (Fig. 1B). Treatment with EDTA did not destroy these structures. Treatment with RNase on the grid did not affect visibly the tightly packed complexes of the type shown in Fig. 1B, but digestion in solution led to their decomposition into single particles or small groups of two to four particles (Fig. 2). The tightly packed RNP complexes were relatively more resistant to RNase than the loose complexes obtained from PBS nuclei (data not shown).

The most rapid way of obtaining RNA-containing particulate structures was centrifugation onto the grid of whole Joy-treated cells. With this procedure two types of structures were obtained: branched complexes similar to those released from Ca²⁺-nuclei (Fig. 3) and linear arrays of particles showing as a rule interconnecting filaments (Fig. 4A). The same linear beads-on-a-string arrangements of particles were obtained also from mouse reticulocytes. The only difference was that there were five to six particles in a linear complex of the reticulocytes (Fig. 4A, bottom row), while in the Friend cells much larger complexes were observed. All these linear complexes disappeared completely after treatment with 0.2 mM EDTA (data not shown). They were very sensitive to RNase both in solution and when treated on the grid (Fig. 4B). Thus, we identify the linear complexes in the spread Joy-treated cells (Fig. 4A) as polyribosomes, having the same morphology as described for polyribosomes isolated from rabbit reticulocytes (33).

Unlike the linear and loosely arranged polyribosomes (Fig. 4A), the branched and tightly packed complexes of the Joy-treated cells (Fig. 3) were not destroyed by EDTA and were

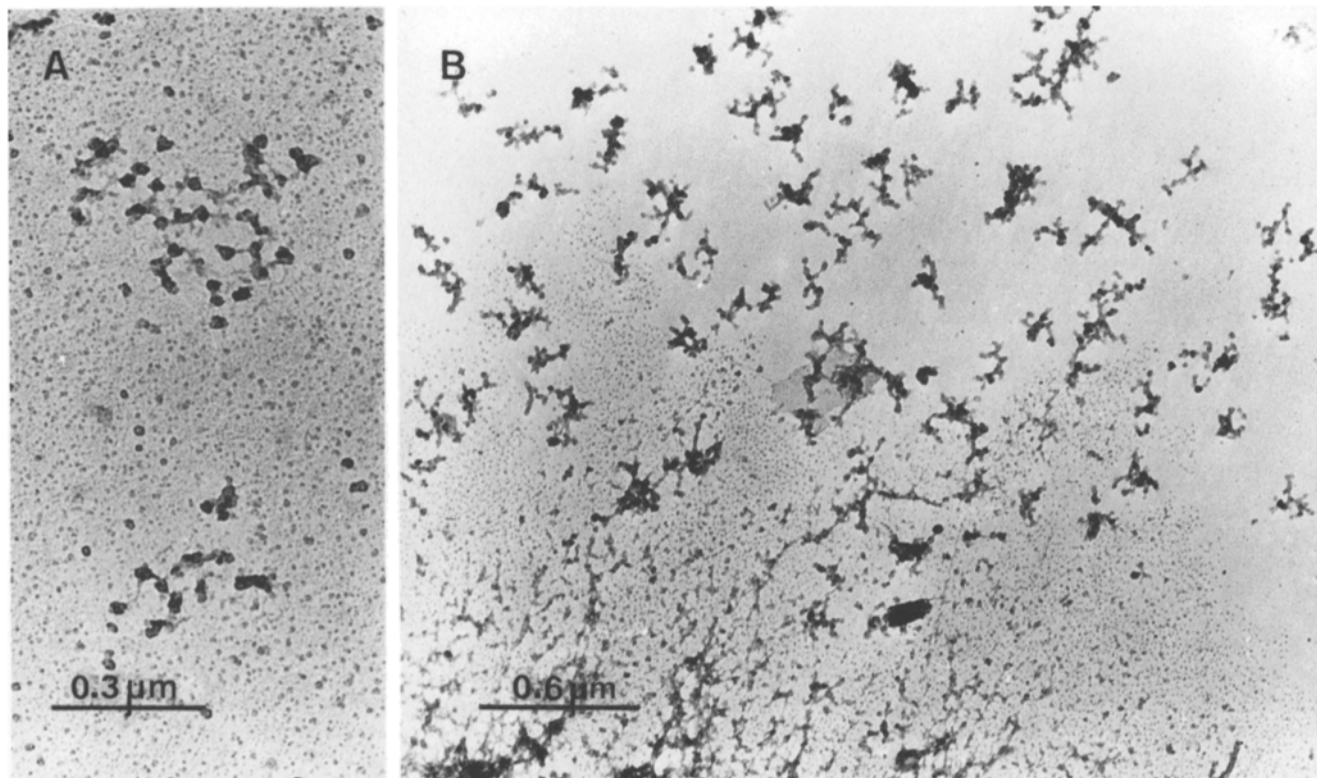


FIGURE 1 (A) RNP complexes released from spread PBS nuclei. (B) RNP complexes released around the periphery of a spread Ca²⁺-nucleus mildly treated with trypsin (see Materials and Methods). Stained with alcoholic uranylacetate and rotary shadowed with WO₂. × 20,000.

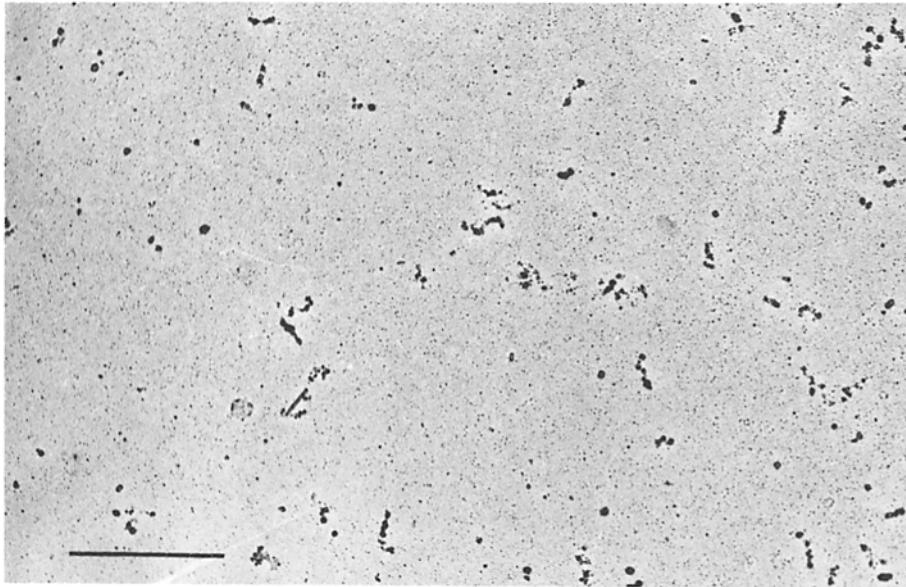


FIGURE 2 RNP particles released from spread Ca^{2+} -nuclei treated with RNase in solution (see Materials and Methods). Staining as in Fig. 1. Bar, $1 \mu\text{m}$. $\times 10,000$.

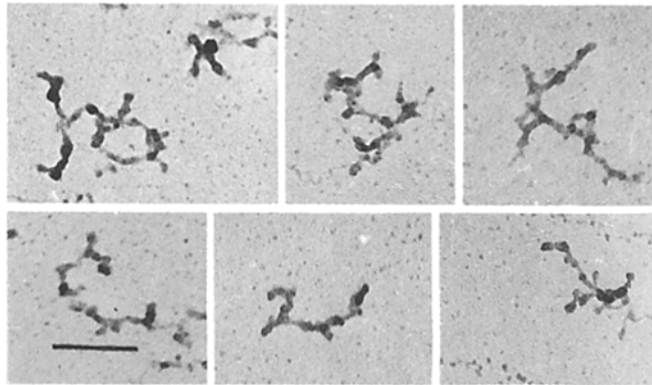


FIGURE 3 RNP complexes released from Joy-treated Friend cells. Staining as in Fig. 1. Bar, $0.2 \mu\text{m}$. $\times 20,000$.

affected by RNase when treated in solution only (data not shown). Thus, in all respects these structures resembled those obtained from Ca^{2+} -nuclei (Fig. 2). Therefore, we conclude that they also represent nuclear RNP particles. The differences between polyribosomes and free nuclear RNP particles can be summarized as follows: (a) polyribosomes are linear arrangements of particles usually with visible interconnecting filaments; nuclear RNP particles are branched structures with closely packed particles. (b) Polyribosomes are dissociated by EDTA; nuclear RNP particles are resistant to this treatment. (c) Polyribosomes are sensitive to RNase when treated on the grid; nuclear RNP particles are more resistant and are affected by the enzyme only in solution.

In all preparations of spread nuclei single particles were relatively rare, while the procedure of obtaining RNP complexes by salt extraction of isolated nuclei (12) yielded predominantly monparticles (Fig. 5 B). Their morphology was identical to that of the particles in the large complexes (Fig. 5 A), except that they appeared somewhat larger. In the large complexes (Fig. 5 A), the size of the monomers varied between 17 and 22 nm, whereas the size of the isolated monparticles (Fig. 5 B) was 20–30 nm. This difference was probably due to the different ionic conditions used in the two preparations.

Our data show that the morphology of the nuclear RNP complexes is readily affected by the ionic conditions. Although

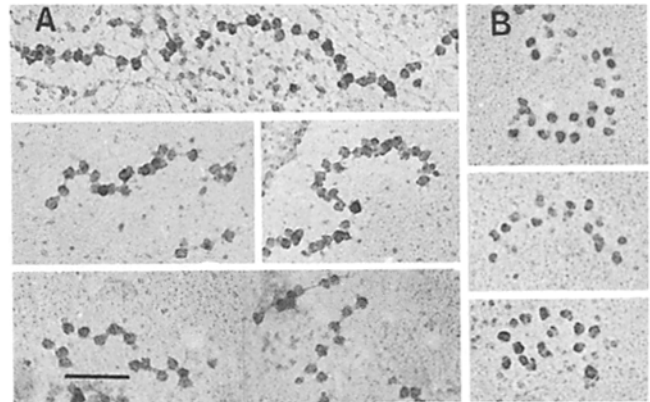


FIGURE 4 (A) Polysomes released from Joy-treated Friend cells and Joy-treated mouse reticulocytes (bottom row). (B) Polysomes treated with RNase on the grid after spreading. Staining as in Fig. 1. Bar, $0.15 \mu\text{m}$. $\times 20,000$.

it is difficult to prove which of the observed morphologies corresponds most closely to the native form of these structures, we favor the tightly packed particles of the kind shown in Figs. 1 B, 3, and 5 A. This is suggested by the fact that the RNP complexes observed as nascent nonribosomal RNA transcripts consist of tightly packed particles with all three methods of nuclear spreading (Fig. 6). Moreover, such compact complexes are obtained when whole cells are lysed on the grid by low concentrations of Joy under low ionic strength, conditions which could hardly be expected to artifactually induce compact structures.

The tight arrangement of the particles in the large, compact complexes obscures their size heterogeneity. This becomes evident, however, after RNase treatment (Fig. 2) or when the complexes are deliberately allowed to form monparticles during their isolation (Fig. 5 B). This is in agreement with other observations on isolated monparticles (9).

A careful examination of the particles reveals very often an electron-dense center both in the poly- and in the monparticles (Fig. 5, arrows). This dark spot might be interpreted as a central protein core as suggested by the first model proposed (29) and also by a similar observation on nucleosomes (36). However, under our experimental conditions such a central

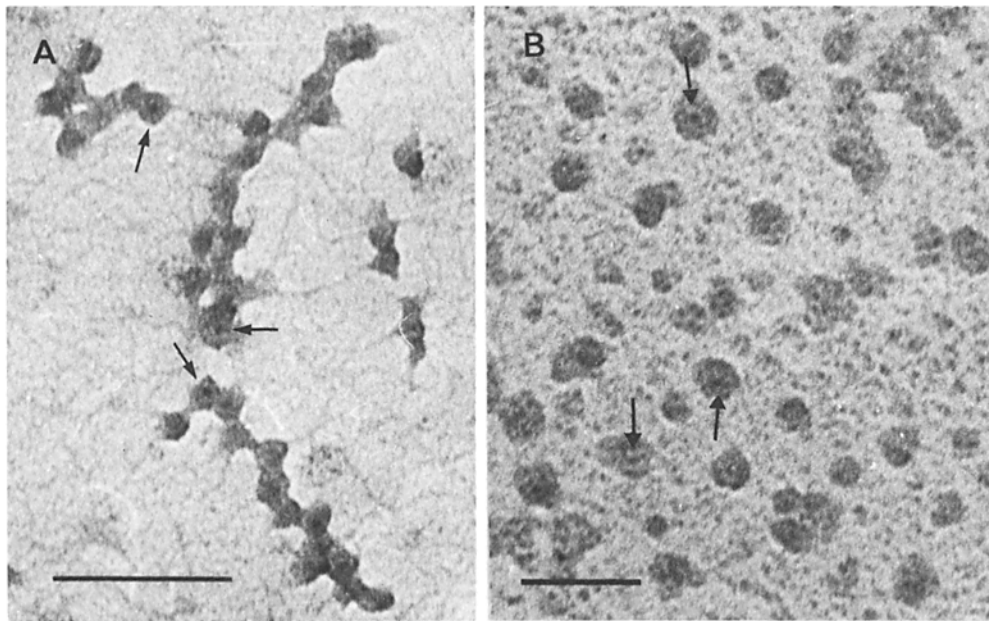


FIGURE 5 (A) RNP complexes in a Joy-treated Friend cell showing electron-dense centers (arrows). Treated with RNase on the grid. Bar, 0.1 μm . $\times 50,000$. (B) RNP monoparticles with similar morphology as in A obtained from salt extracts of isolated nuclei (12). Staining as in Fig. 1. Bar, 0.1 μm . $\times 20,000$.

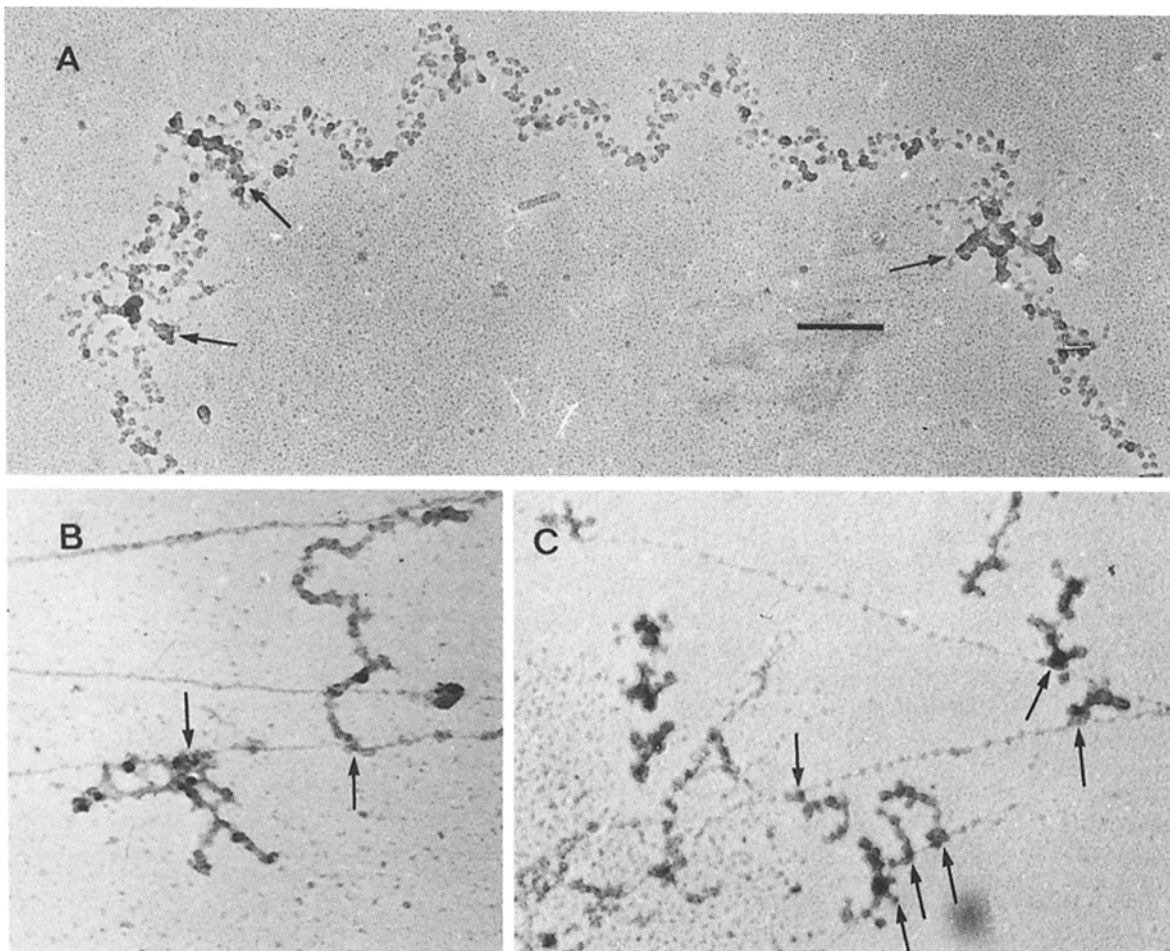


FIGURE 6 Nonribosomal RNA transcripts associated with DNA fibers in spread PBS nuclei (A), Joy-treated cells (B), and Ca^{2+} -nuclei (C). Staining as in Fig. 1. Bar, 0.15 μm . $\times 20,000$.

dark spot was observed also in the polysomal particles, making more difficult the interpretation as discussed earlier by others (33).

The general structure of the free RNP polyparticles appears to be one of closely opposed particles forming variable

branched structures without visible interconnecting RNA filaments. When these complexes are still associated with DNA fibers, both branched and linear structures can be seen (Fig. 6). It is possible that branching is a second step in the formation of the RNP complexes that are typical of the free polyparticles

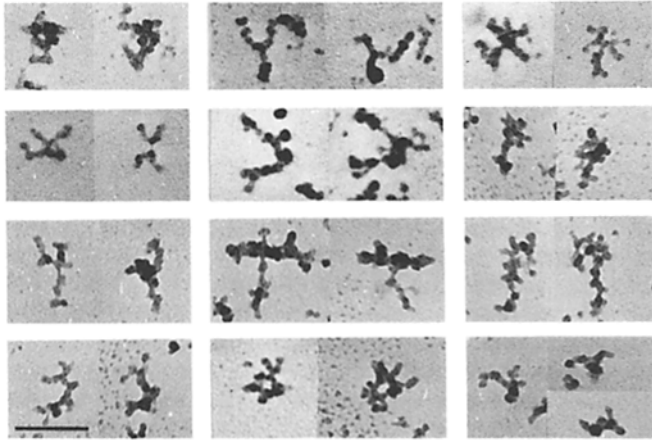


FIGURE 7 Pairs of RNP complexes released from spread Ca^{2+} -nuclei and showing similar morphological patterns. Staining as in Fig. 1. Bar, 0.2 μm . $\times 20,000$.

of the so-called RNP network (7, 10, 20, 32), while during transcription the branching pattern is still unstable and can be easily linearized (3, 15, 19).

Beyond the similar overall organization of the nuclear RNP complexes, it is interesting to note that among the heterogeneous population of variously branched structures it is not difficult to discover similar patterns. Fig. 7 shows selected pairs of polyparticles having similar configurations. This suggests the possibility that the various branching forms observed might not be the result of a purely random twisting of linear arrays of polyparticles but might instead represent regular patterns determined by some structural features of the primary RNA transcripts. One such factor could be the spacing of alternating single- and double-stranded RNA regions that have been shown to be present in native nuclear RNP structures (4). It is tempting to suggest that different branching patterns may be characteristic of different RNA transcripts. Such a sequence-related arrangement of the RNP particles could be important for the correct splicing of the premessenger RNA (10, 13), a process which takes place while RNA is still organized in RNP complexes (24, 30).

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