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Nucleolar changes after microinjection of antibodies to RNA polymerase I into the nucleus of mammalian cells

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Abstract. After microinjection of antibodies against RNA polymerase I into the nuclei of cultured rat kangaroo (PtK_2) and rat (RVF-SMC) cells alterations in nucleolar structure and composition were observed. These were detected by electron microscopy and double-label immunofluorescence microscopy using antibodies to proteins representative of the three major components of the nucleolus. The microinjected antibodies produced a progressive loss of the material of the dense fibrillar component (DFC) from the nucleoli which, at 4 h after injection, were transformed into bodies with purely granular component (GC) structure with attached fibrillar centers (FCs). Concomitantly, numerous extranucleolar aggregates appeared in the nucleoplasm which morphologically resembled fragments of the DFC and contained a protein (fibrillarin) diagnostic for this nucleolar structure. These observations indicate that the topological distribution of the material constituting the DFC can be experimentally influenced in interphase cells, apparently by modulating the transcriptional activity of the rRNA genes. These effects are different from nucleolar lesions induced by inhibitory drugs such as actinomycin D-dependent "nucleolar segregation". The structural alterations induced by antibodies to RNA polymerase I resemble, however, the initial events of nucleolar disintegration during mitotic prophase.

Introduction

The initial processes of ribosome biogenesis, i.e. synthesis and processing of precursors to cytoplasmic rRNA molecules (pre-rRNAs) and their assembly with specific proteins, take place in the nucleolus (for reviews see Smetana and Busch 1974; Goessens 1984; Hadjiolov 1985). Various stages of the complex biochemical pathway have been localized to specific subnucleolar compartments. By electron microscopy, three nucleolar components can be generally distinguished: the fibrillar center(s) (FC), the dense fibrillar component (DFC) which surrounds the FCs, and the granular component (GC) which usually constitutes the main body of the nucleolus (Goessens 1984). Based on autoradiographic and biochemical studies primary steps of pre-ribosome formation have been assigned to the DFC whereas subsequent maturation stages occur in the GC (Hadjiolov 1985). The intranucleolar location of the transcriptionally active rRNA genes is still a matter of debate. An immunocytochemical approach has located RNA polymerase I in the FC, indicating that transcription of rDNA occurs within this nucleolar component (Scheer and Rose 1984; Reimer et al. 1987a, b; Scheer and Raska 1987). In contrast, it has been suggested from earlier studies based on the distribution of short-term labeled RNA that transcription takes place in – or at the border of – the DFC (for references see Goessens 1984; Hadjiolov 1985).

One possible approach to the study of the functional organization of the nucleolus and the roles of the various nucleolar components in ribosome formation is the use of drugs that interfere with certain steps of ribosome biogenesis. Most of these inhibitors cause extensive alterations of the nucleolar architecture (for reviews see Busch and Smetana 1970; Bernhard 1971; Simard et al. 1974; Hadjiolov 1985). However, the interpretation of such structural rearrangements in relation to the molecular mechanism of drug action is often hindered due to the fact that inhibitors are not specific for the ribosome pathway but may also interfere with the synthesis and processing of other RNA classes. In addition, side effects of a given drug on other cellular metabolic pathways might occur which makes a correlation of the observed structural changes with certain molecular processes even more difficult.

The group of inhibitors which interfere with the synthesis of RNA molecules, including pre-rRNA, by DNA binding induce a characteristic redistribution of the nucleolar components, called "nucleolar segregation" (Busch and Smetana 1970; Bernhard 1971; Simard et al. 1974; Daskal 1979; Goessens 1984). Actinomycin D (AMD), a DNA-intercalating drug, is the most widely used member of this group. However, in interpretations of the changes of nucleolar structure induced by treatment with AMD it has to be kept in mind that AMD inhibits preferentially but not exclusively the transcription of the rRNA genes (Perry 1966). Hence, effects on other genes may also contribute to any phenomena observed. Furthermore, numerous reports have shown that AMD might also affect the nucleocytoplasmic translocation of ribosomes, the stability of mRNA in the cytoplasm, and the translational machinery (for references see Eckert et al. 1975; Kostura and Craig 1986). In this context it is also noteworthy that drugs known to inhibit pre-rRNA synthesis by different molecular mech-

Abbreviations: FC Fibrillar center; *DFC* dense fibrillar component; *GC* granular component; *AMD* actinomycin D

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anisms may also have different effects on nucleolar structure (see Simard et al. 1974; Hadjiolov 1985). For example, while AMD induces extensive segregation of the nucleolar components, galactosamine D, which depletes the intracellular UTP pool, leads to a loss of the GC of nucleoli in rat hepatocytes (e.g. Hadjiolov 1985; Hadjiolova et al. 1986).

Recent experiments have shown that pre-rRNA synthesis can be selectively affected by introducing antibodies to RNA polymerase I, i.e. the enzyme responsible for rDNA transcription, into living cells (Mercer et al. 1984; Schlegel et al. 1985; Reimer et al. 1987a). These findings prompted us to study the effects of microinjected antibodies to RNA polymerase I on the organization and assembly of nucleolar components. Recently we have shown that cells microinjected with antibodies to RNA polymerase I during mitosis proceed through division and form daughter cells but are unable to reconstitute functional nucleoli (Benavente et al. 1987). Using a series of antibodies reacting with other nucleolar proteins we were able to follow, by immunofluorescence and electron microscopy, the behavior of the three major nucleolar components during these stages of experimentally repressed nucleologenesis. In the present study we report that antibodies to RNA polymerase I and the reduction of rDNA transcription cause extensive structural changes and rearrangements of the nucleolar constituents which are, however, different from the segregation phenomenon induced by AMD treatment.

Materials and methods

Cells and microinjection. PtK_2 (rat kangaroo kidney epithelial) and RVF-SMC cells (derived from vascular smooth muscle of rat vein) were grown on coverslips (for details see Franke et al. 1978, 1980). Microinjection into nuclei of interphase cells was performed as described (Benavente and Krohne 1986) with instrumentation as designed by Ansorge (1982).

Antibodies. The following antibodies were used for microinjection: (a) human S18 autoantibodies including antibodies directed against RNA polymerase I (Reimer et al. 1987a). IgGs were purified by chromatography as described (Benavente et al. 1987). (b) The IgG fraction of a rabbit serum containing antibodies to RNA polymerase I (Rose et al. 1981). (c) Monoclonal antibody L_O46F7 against *Xenopus* lamins L_{III} and L_{IV} (Benavente et al. 1985; Benavente and Krohne 1985) used as IgG from ascites fluid (Benavente and Krohne 1986). All antibodies were dissolved in PBS (137 mM NaCl, 2.8 mM KCl, 7 mM Na_2HPO_2, 1.5 mM KH_2PO_4, pH 7.4) and used for microinjection at concentrations ranging from 3 to 20 mg/ml.

For immunofluorescence microscopy, the following antibodies were used in addition: (a) monoclonal antibody 72B9 against a protein of the DFC, related to the U3 ribonucleoprotein (RNP) complex (Reimer et al. 1987c). (b) Human Scl C autoantibodies recognizing a protein of the DFC identified as fibrillarin, that is probably related to the antigen recognized by the monoclonal antibody 72B9 (Reimer et al. 1987c). (c) Monoclonal antibody RS1-105 against ribosomal protein S1 (Hügle et al. 1985).

Immunofluorescence microscopy. Double-label immunofluorescence microscopy of microinjected cells was performed essentially as described (Benavente and Krohne 1986; Benavente et al. 1987). Briefly, after methanol/acetone fixation coverslips were incubated with fluorescein isothiocyanate (FITC)-conjugated antibodies (Dianova, Hamburg, FRG) directed against the microinjected antibody. Then the cells were incubated with antibody 72B9, Scl C or RS1-105 followed by the appropriate secondary antibodies conjugated to Texas Red (Dianova, Hamburg, FRG). Cells grown in the presence of 0.08 to 0.2 μg/ml AMD (Serva, Heidelberg, FRG) were processed for double-label immunofluorescence with RNA polymerase I antibodies and monoclonal antibodies 72B9 or RS1-105.

Electron microscopy. Coverslips with the microinjected cells were fixed with 2.5% glutaraldehyde followed by 1% osmium tetroxide and processed for electron microscopy according to standard procedures (see Benavente and Krohne 1986; Benavente et al. 1987). For immunogold electron microscopy, cells were fixed in methanol (−20° C) for 10 min, dipped for a few seconds into cold acetone at −20° C and then air-dried. Coverslips were then incubated with monoclonal antibody 72B9 for 15 min followed by incubation with secondary antibodies coupled to 5 nm gold particles (Janssen Life Sciences, Beerse, Belgium) diluted 1:5 in PBS containing 0.2% bovine serum albumin for 1 h. After washing in PBS, the cells were fixed with 2.5% glutaraldehyde in 50 mM sodium cacodylate buffer (pH 7.2) for 15 min (4° C), postfixed with 1% osmium tetroxide (15 min) at 4° C, dehydrated in an ethanol series and embedded in Epon (for details see Benavente and Krohne 1986; Benavente et al. 1987). Ultrathin sections were stained with uranyl acetate and lead citrate.

Results

Following a 4 h exposure of PtK_2 cells to low concentrations of AMD (0.2 μg/ml), electron microscopy revealed the characteristic segregation of the three main nucleolar components (data not shown; cf. Ochs et al. 1985). In ultrathin sections, AMD-segregated nucleoli usually exhibited a stratified organization, i.e. the FC was located as a cap-like structure at the nucleolar periphery, separated from the GC by an intervening layer of DFC material (cf. Simard et al. 1974). These ultrastructural observations were confirmed by immunofluorescence microscopy of AMD-treated PtK_2 cells. Antibodies to RNA polymerase I, which in untreated cells decorate specifically the FCs of nucleoli (Scheer and Rose 1984; Reimer et al. 1987b), stained small, cap-like structures in the outer nucleolar zone (Fig. 1a; for the immunofluorescence pattern of untreated PtK_2 cells see Benavente et al. 1987). In contrast, monoclonal antibody 72B9 reacting specifically with the DFC (Reimer et al. 1987b) stained one or two roundish and much larger nucleolar structures which could often be correlated with a region of the segregated nucleolus appearing light by phase contrast (Fig. 1a'; see also Schmidt-Zachmann et al. 1984; Hügle et al. 1985; Ochs et al. 1985). In the course of such AMD treatments, ribosomal protein S1, a marker for the GC (Hügle et al. 1985), progressively disappeared from the PtK_2 cell nucleoli (Fig. 2a', b'), indicative of an AMD-induced clearance of precursor particles to the small ribosomal subunit from the nucleoli which in PtK_2 cells occurred much faster than in *Xenopus* cells of line A6 (Hügle et al. 1985). It should be emphasized that the AMD-induced

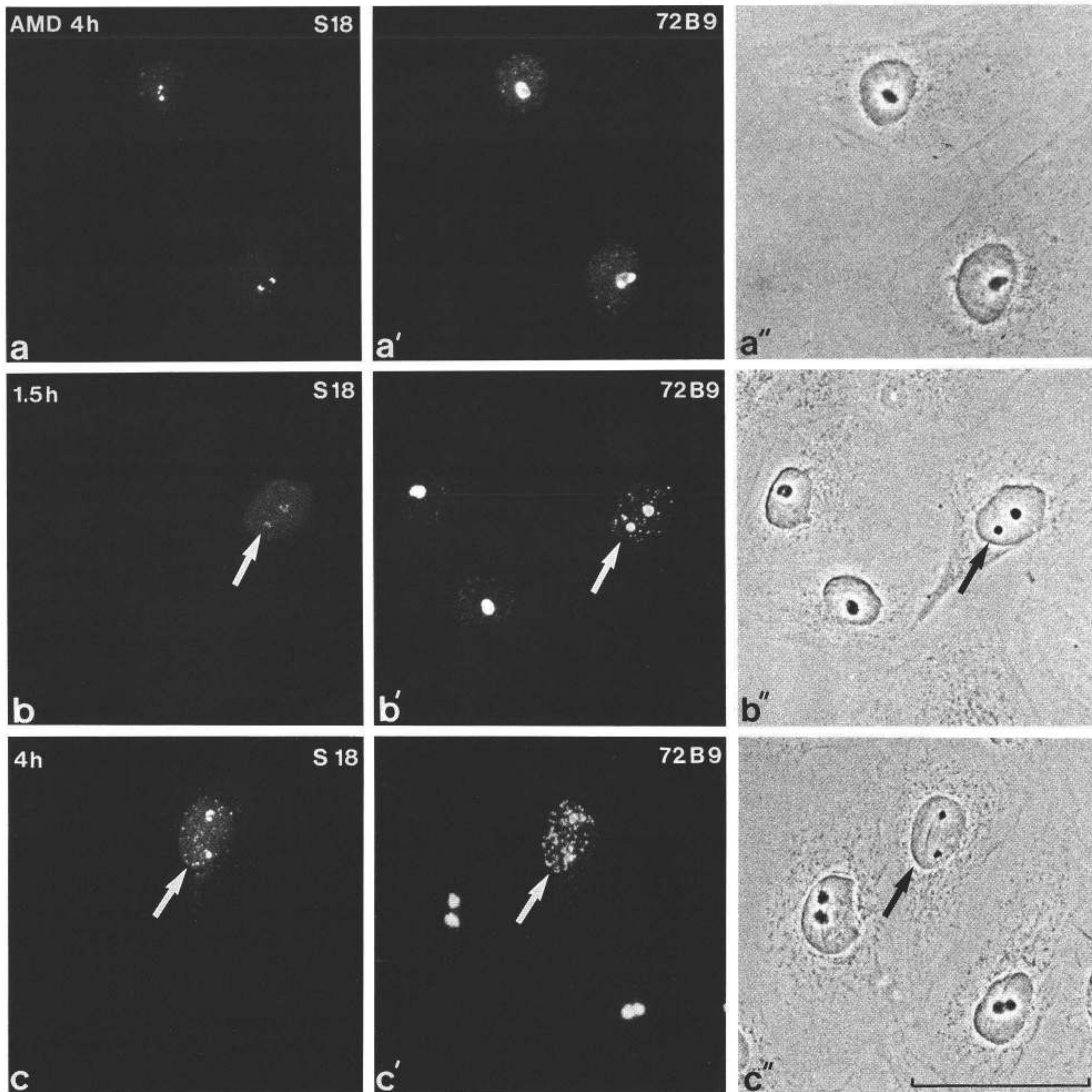

Fig. 1a–c. PtK_2 cells after exposure to actinomycin D (AMD) (**a–a''**) and microinjection of RNA polymerase I (S18) antibodies into their nuclei (**b–b''**, **c–c''**) as seen by immunofluorescence (**a–c**, **a'–c'**) and phase contrast (**a''–c''**) microscopy. **a–a''** Cells cultivated for 4 h in the presence of 0.2 μg/ml AMD. Regions containing RNA polymerase I are reduced to small caps at the nucleolar periphery as revealed by immunofluorescence microscopy with S18 antibodies (**a**). Antibody 72B9 stains adjacent, much larger structures (**a'**). **b–b''** Injection of antibodies to RNA polymerase I into a cell (*arrows*) 1.5 h prior to fixation. The injected antibodies are concentrated in the nucleoli (**b**). Antibody 72B9 stains, in addition to the nucleoli, numerous nucleoplasmic entities in the injected cell (*arrow*) but not in adjacent control cells (**b'**). **c–c''** 4 h after microinjection of S18 antibodies. The distribution of the injected antibodies is shown in **c** (*arrow*). The nucleolar material recognized by antibody 72B9 is dispersed in numerous small entities throughout the nucleoplasm of the injected cell (*arrow* in **c'**). Note the different fluorescence pattern of the adjacent nontreated cells. Bar represents 50 μm

redistribution of the nucleolar components did not result in the eventual disintegration of the nucleolar body (Figs. 1a'', 2b''). Even after prolonged exposure of cells to AMD, segregated nucleoli remained as compact entities which, however, appeared gradually to lose the RNA polymerase I-dependent fluorescence, probably as a consequence of the progressive release of the enzyme molecules from the rDNA template (see Scheer and Rose 1984).

When antibodies to RNA polymerase I were injected into nuclei of interphase PtK_2 cells, a considerable proportion accumulated in the nucleoli, as judged by immunofluorescence microscopy with FITC-labeled secondary antibodies directed against the injected immunoglobulins (Figs. 1b, c, 2c). This observation also demonstrated the accessibility of the nucleolar polymerases – which most likely represent the template-engaged form – to microinjected antibodies. At about 90 min after antibody injection, the first signs of nucleolar fragmentation became visible: antibody 72B9, which normally reacts with the DFC, stained an increasing number of small nucleoplasmic bodies, in ad-

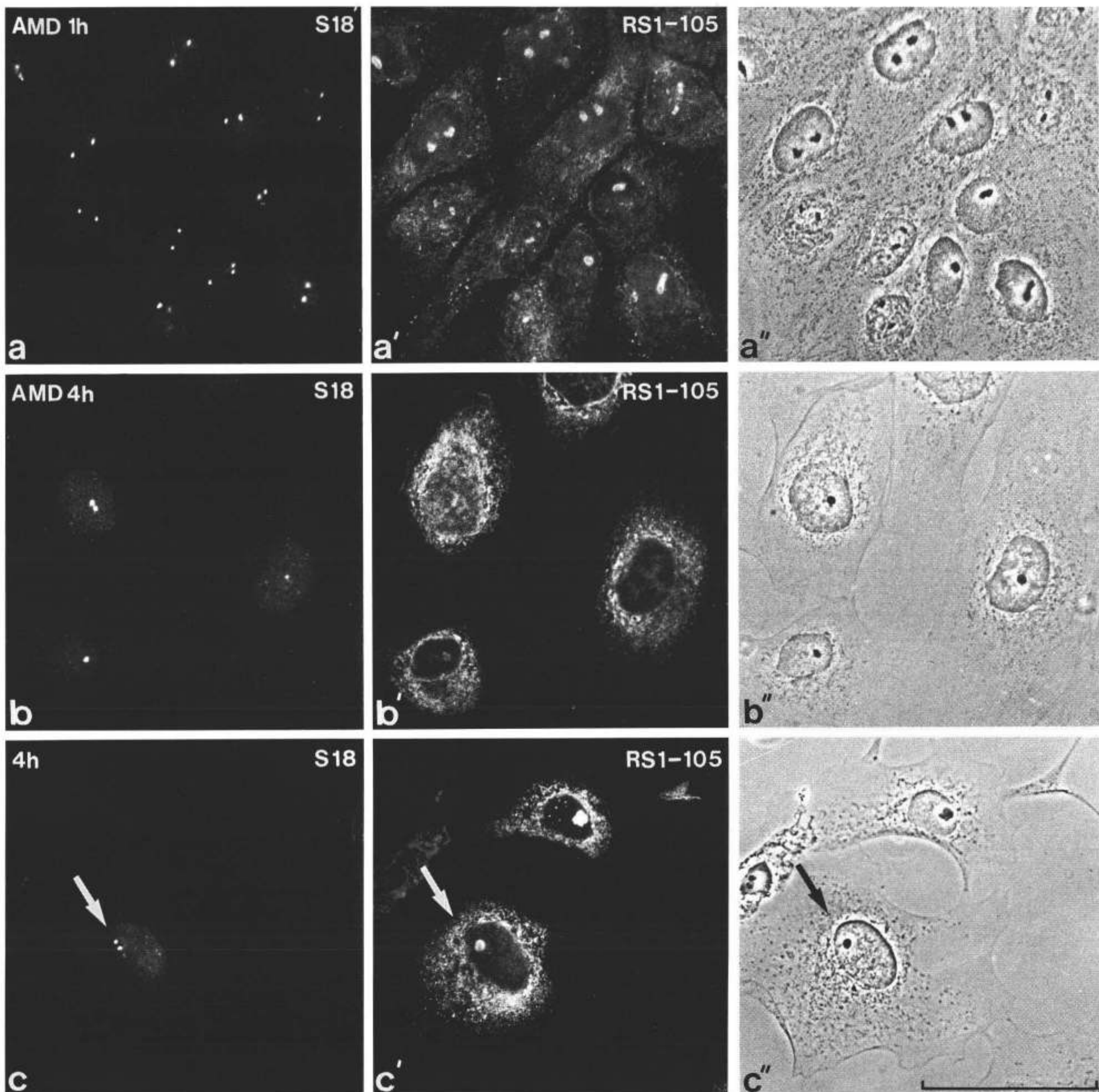

Fig. 2a–c. Comparison of the effects of actinomycin D (AMD) (**a–a"**, **b–b"**) and RNA polymerase I (S18) antibodies microinjected into nuclei of PtK_2 cells (**c–c"**) as demonstrated by immunofluorescence (**a–c**, **a'–c'**) and phase contrast (**a"–c"**) microscopy. In cells cultivated for 1 h (**a–a"**) or 4 h (**b–b"**) in the presence of AMD, RNA polymerase I-containing regions are reduced to small caps at the nucleolar periphery as revealed by immunofluorescence microscopy with S18 antibodies (**a, b**). Antibody RS1-105 stains, besides the cytoplasmic ribosomes, nucleoli of cells exposed for 1 h to AMD (**a'**). However, after 4 h of AMD treatment nucleoli are almost negative (**b'**). **c–c"** 4 h after microinjection of RNA polymerase I antibodies. The distribution of the injected antibodies is shown in **c** (the *arrow* denotes the injected cell). Antibody RS1-105 stains the nucleolus of both the injected (**c'**, *arrow*) and the control cell (upper cell in **c'**). The cytoplasmic fluorescence is caused by the binding of the antibodies to ribosomes. Bar represents 50 μm

dition to the residual nucleoli (Fig. 1b'). The autoantibody Scl C produced the same pattern of immunofluorescence (not shown). At 4 h, the DFC fragmentation into small individual entities dispersed over the nucleoplasm had progressed (Fig. 1c'). Such extranucleolar bodies reacting with antibody 72B9 were never observed in interphase control cells (Fig. 1b', c'). Nucleolar remnant structures, which were still visible by phase contrast optics (Fig. 1c"), were only weakly stained with antibody 72B9, suggestive of a considerable loss of the DFC (Fig. 1c'). However, the same nucleolar remnant structures retained a significant portion of ribosomal protein S1, as judged from the fluorescence intensity observed with monoclonal antibody RS1-105 (Fig. 2c'). In contrast, the numerous nucleoplasmic bodies were not stained by the S1 antibodies (Fig. 2c').

Microinjection of RNA polymerase I antibodies into the nucleus of rat cells (RVF-SMC) gave essentially identical results (not shown). However, the smaller size and higher number of nucleoli in RVF-SMC cells made the interpretation of the results more complicated. In addition, the nucleoli of the rat cells did not show the same clear pattern of segregation after AMD treatment as the PtK_2 cells.

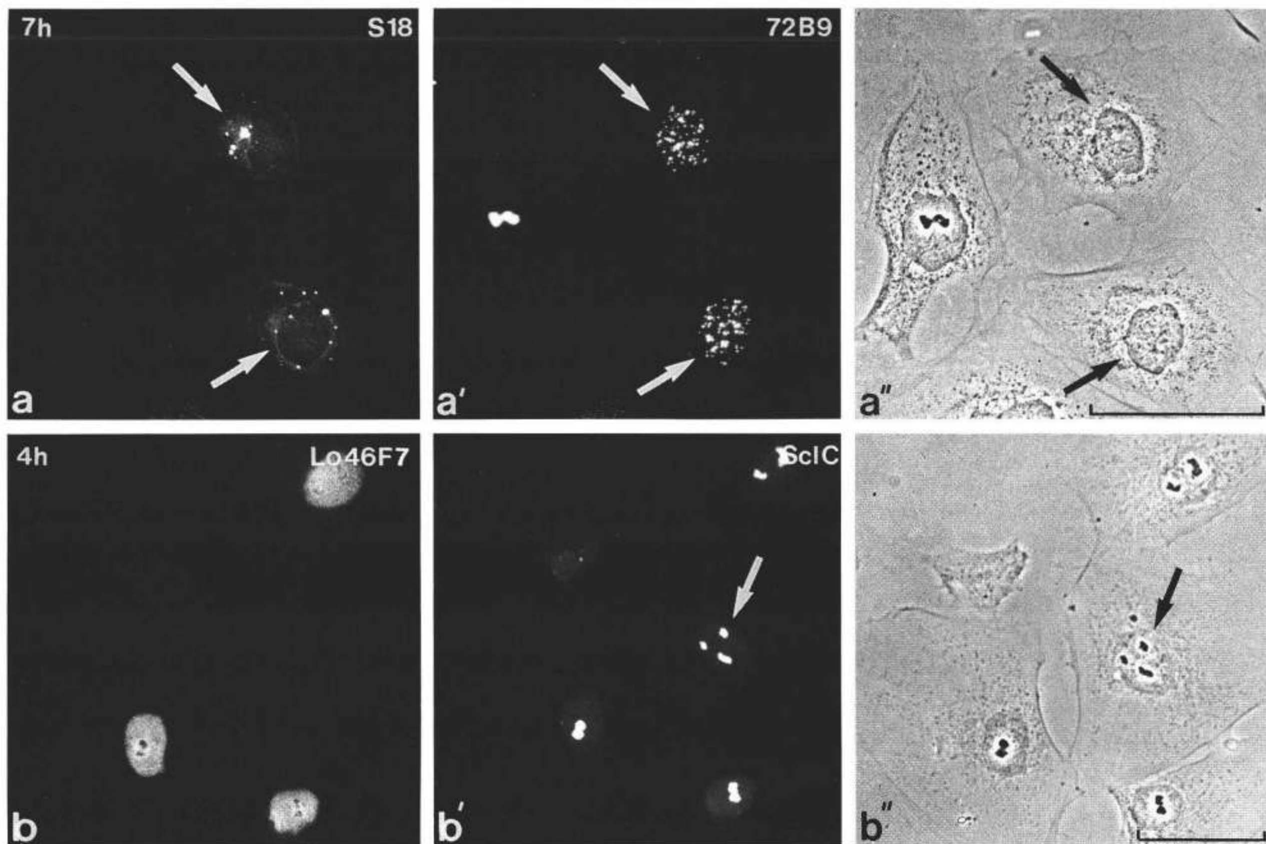

Fig. 3. a–a" Daughter cells derived from a PtK_2 mother cell which was injected 7 h before with RNA polymerase I (S18) antibodies (*arrows*). The injected antibodies are concentrated in perinuclear aggregates in the two daughter cells (**a**). Antibody 72B9 stains numerous nucleoplasmic entities in the daughter cells whereas in the adjacent non-treated cell the fluorescence is confined to the nucleoli (**a'**). The corresponding phase contrast image is shown in **a"**. Note the absence of nucleoli in the daughter cells in contrast to the adjacent control cell. **b–b"** Microinjection of control antibodies (L_O46F7) into the nuclei of PtK_2 cells. After 4 h incubation the cells were fixed and processed for immunofluorescence microscopy (**b, b'**). The distribution of the injected antibodies is shown in **b**. Double-label immunofluorescence microscopy with the nucleolus-specific antibody Scl C (**b'**). The corresponding phase contrast image is shown in **b"**. A non-injected cell is denoted by *arrows* (**b', b"**). Bar represents 50 μm

Two types of control experiments are shown in Figure 3. When cells were cultured for prolonged periods of time after microinjection of RNA polymerase I antibodies, they proceeded through normal mitosis, indicating that the microinjection procedure did not interfere with this basic cellular process (Fig. 3a-a"). In the resulting daughter cells, the RNA polymerase I antibodies appeared to be largely excluded from the nuclei and were mostly recovered in certain cytoplasmic, perinuclear aggregates, perhaps IgG aggregates (Fig. 3a). Reformation of nucleoli, however, was apparently prevented in these cells (Fig. 3a"). These effects were indistinguishable from those observed after microinjection of the polymerase antibodies into mitotic PtK_2 cells (Benavente et al. 1987). When probed with the monoclonal antibody 72B9, the daughter nuclei formed from the injected mother cell were seen to contain numerous fluorescent dots, reminiscent of the prenucleolar bodies that normally occur exclusively during early telophase (Fig. 3a'; see also Benavente et al. 1987; cf. De la Torre and Gimenez-Martin 1982). None of the nucleolar effects described was seen after injection of other, i.e. "irrelevant" antibodies (Fig. 3b-b"). For example, PtK_2 cells injected with the monoclonal antibody L_O46F7 which reacts with *Xenopus laevis* lamins L_{III} and L_{IV} but not with mammalian lamins (Krohne and Benavente 1986) were indistinguishable from control cells: DFC antibodies (Scl C) exclusively stained the nucleoli in both microinjected and control cells (Fig. 3b').

The electron microscopic appearance of nucleoli from PtK_2 and RVF-SMC cells, before and after microinjection of antibodies to RNA polymerase I, is presented in Figures 4 and 5. Nucleoli of PtK_2 cells are of the compact type (Fig. 4a) whereas the rat cell nucleoli reveal a nucleolonema organization (Fig. 4b; the three main nucleolar components are indicated). By 4 h after microinjection of RNA polymerase I antibodies, nucleoli from both cell types appeared as relatively small and highly contrasted structures (Fig. 5a; for corresponding light microscopical appearance see also Fig. 1c"). Higher magnification revealed that the main body of these residual nucleoli consisted essentially of granular material, with one or two FCs usually at the periphery (Fig. 5d, e), corresponding to the fluorescent cap structures containing the microinjected antibodies to RNA polymerase I (compare Figs. 1c and 2c with Fig. 5d, e). The third nucleolar structure, the DFC, was either absent or considerably reduced in such residual nucleoli (Fig. 5d, e). On the other hand, numerous roundish aggregates composed of tightly packed fibrillar material could be recognized, which were scattered throughout the nuclear interior and did not show any structural connections to

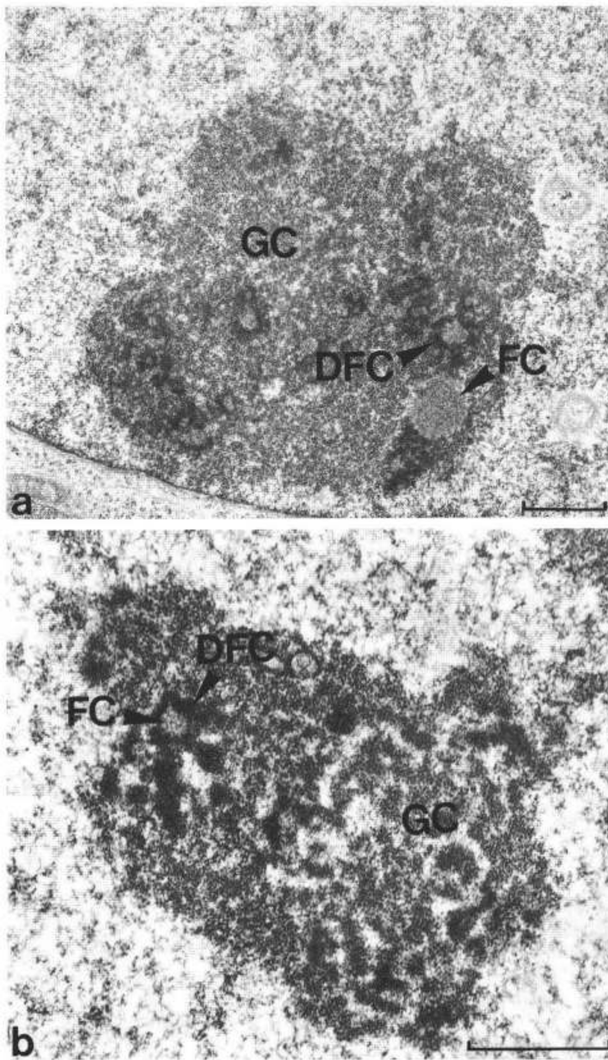

Fig. 4a, b. Electron micrographs of nucleoli of untreated rat kangaroo (PtK_2, **a**) and rat (RVF-SMC, **b**) cells. The three main nucleolar components are clearly visible, i.e. *FC* fibrillar center, *DFC* dense fibrillar component and *GC* granular component. Bars represent 1 μm

the nucleolar remnants (Fig. 5a). These spheroidal bodies resembled in their texture the DFC of normal nucleoli (Fig. 5b). In fact, antibody 72B9, which reacts specifically with DFC, decorated these nucleoplasmic bodies as demonstrated by immunogold electron microscopy (Fig. 5c; for immunofluorescence see Fig. 1c').

Discussion

In the present study we have examined the ability of antibodies directed against RNA polymerase I to induce structural changes in nucleoli when directly microinjected into the nuclei of interphase cells. The two kinds of antibodies used, i.e. experimentally raised antibodies to rat RNA polymerase I and human RNA polymerase I autoantibodies have previously been shown to neutralize RNA polymerase I activity in vitro and to reduce or even suppress rRNA synthesis upon microinjection into cultured mammalian cells and *Xenopus* oocytes (Rose et al. 1981; Mercer et al. 1984; Schlegel et al. 1985; Reimer et al. 1987a). In addition, recent microinjection experiments have demonstrated that these antibodies also inhibit the postmitotic reformation of nucleoli when microinjected into mitotic PtK_2 cells (Benavente et al. 1987). In view of the general experience that almost any agent interfering with the complex process of ribosome biogenesis may cause one or another structural change in interphase nucleoli (for references see Bernhard 1971; Simard et al. 1974; Smetana and Busch 1974; Daskal 1979; Bouteille et al. 1982; Hadjiolov 1985) it is not surprising that nuclear microinjection of RNA polymerase I antibodies into living interphase cells alters the nucleolar architecture. However, our results with RNA polymerase I antibodies are particularly novel and significant as this experimental approach allowed the selective inhibition of transcription of the rRNA genes with a minimum of side effects on other genes and other proteins. Remarkably, the pattern of structural alterations of the nucleolus induced by the microinjected antibodies is different from that produced by most inhibitory drugs.

The most striking nucleolar change observed after inhibition of rDNA transcription by microinjected antibodies to RNA polymerase I was a progressive loss of DFC material from the nucleoli, with the concomitant occurrence of small, purely fibrillar extranucleolar bodies scattered throughout the nucleoplasm. These morphological changes were first detectable at about 1–2 h after antibody injection. Based on ^{3}H-uridine incorporation studies, it has been shown that inhibition of nucleolar RNA synthesis by microinjection of RNA polymerase I antibodies reaches a maximum at this time point and is subsequently maintained for several hours (Mercer et al. 1984). By morphological and immunological criteria the experimentally induced extranucleolar bodies resembled fragments of the DFC of normal nucleoli as they contained the protein fibrillarin which is a specific marker of this nucleolar structure (Ochs et al. 1985; Reimer et al. 1987b). The nucleoplasmic bodies present in microinjected *Xenopus* A6 cells contained, in addition, another specific DFC protein with an M_r of 180,000 (data not shown; cf. also Schmidt-Zachmann et al. 1984). In contrast, precursor particles to the small ribosomal subunit as well as RNA polymerase I molecules could not be detected in these bodies. They apparently remained bound to the nucleolar remnant structures which consisted almost purely of GC with FCs still attached.

At first sight it seems puzzling that the morphological changes in the nucleoli induced by nuclear injection of RNA polymerase I antibodies are clearly different from the distinctive segregation phenomenon caused by a variety of DNA-binding drugs such as AMD (e.g. Bernhard 1971; Simard et al. 1974) although both treatments interfere with pre-rRNA synthesis. However, it has to be emphasized that reduction of pre-rRNA synthesis alone does not necessarily lead to nucleolar segregation, as illustrated by a variety of drugs and treatments which efficiently block nucleolar RNA synthesis but do not induce nucleolar segregation (for refs. see Bernhard 1971; Simard et al. 1974; Hadjiolov 1985). In addition, nucleolar segregation by AMD can be prevented by combination with other drugs (see Monneron 1971), again indicating that the segregation phenomenon is not a simple response to inhibition of rDNA transcription but is the result of more complex processes (for discussion see Hadjiolov 1985). On the other hand, the morphological changes described in the present study are also different from other typical drug-induced nucleolar lesions such as

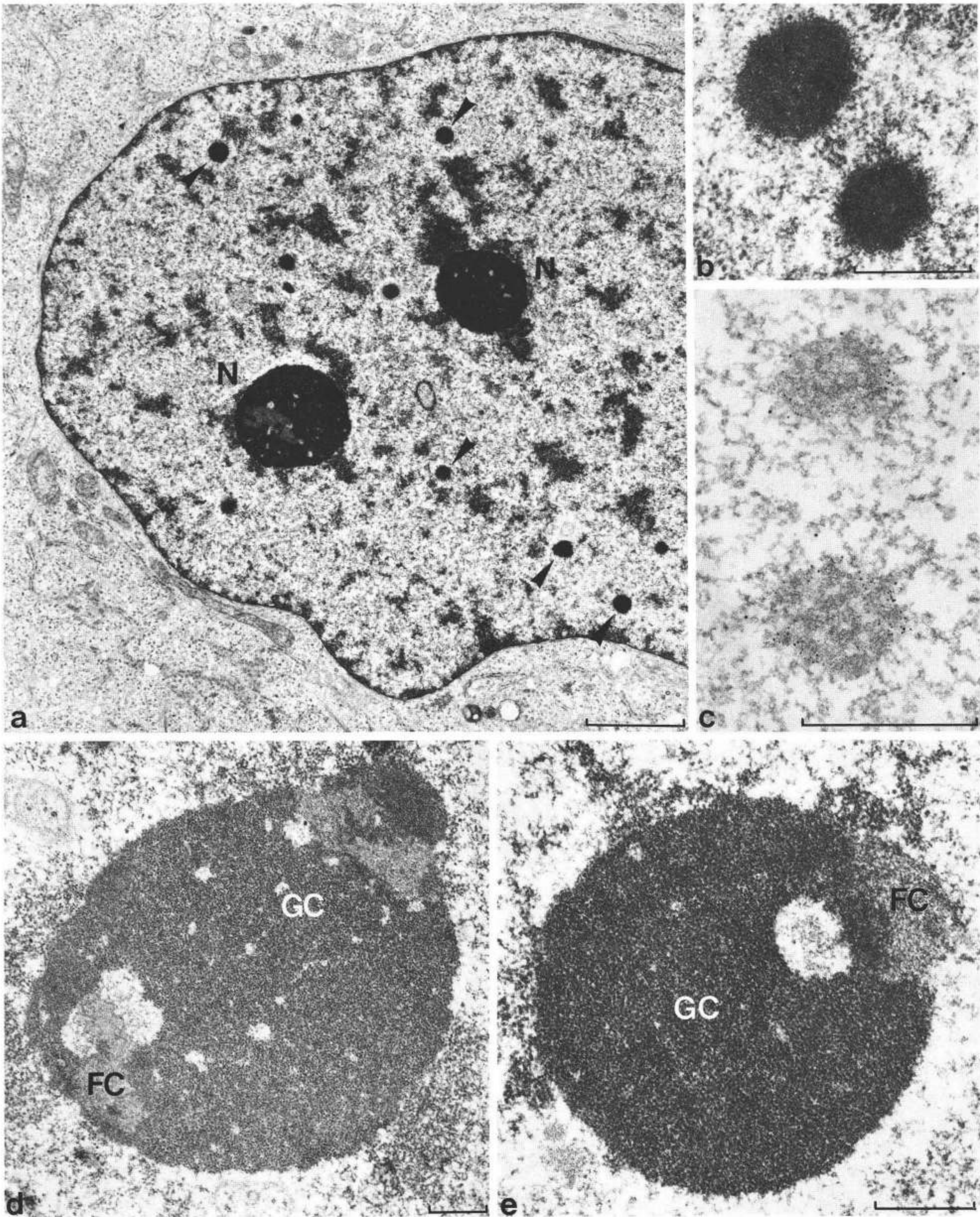

Fig. 5a–e. Effects of human (**a, b, d**) and rabbit (**c, e**) RNA polymerase I antibodies 4 h after intranuclear injection into PtK_2 (**a, b, d**) and RVF-SMC (**c, e**) cells as shown by electron microscopy. Residual nucleoli appear as compact structures (*N* in **a**). In addition, numerous smaller spherical bodies of high contrast are present in the nucleoplasm (some of them are denoted by *arrowheads* in **a**). They consist primarily of fibrillar material (**b**) and contain the antigen recognized by monoclonal antibody 72B9 as shown by the distribution of the 5 nm gold particles (**c**). **d, e** The residual nucleolar structures are composed primarily of the granular component (*GC*) with fibrillar centers (*FC*) at their periphery. The dense fibrillar component is almost absent from these structures. Bars represent 2 μm (**a**) and 0.5 μm (**b–e**)

nucleolar degranulation and fragmentation (for review see Simard et al. 1974) and resemble only the "100% granular nucleoli" described in rat hepatocytes after treatment with both AMD and thioacetamide (Narayan et al. 1966).

Most strikingly, the gradual disappearance of the DFC from the nucleoli observed after RNA polymerase I antibody injection resembles early stages in their natural nucleolar breakdown during mitotic prophase. As shown by Goessens and Lepoint (1974; reviewed by Goessens 1984) the DFC disappears first in prophase, leaving behind a nucleolus of pure GC with attached FC(s). It is, therefore, tempting to speculate that the experimentally induced disappearance of the DFC by the microinjected antibodies mimics to some extent the naturally occurring primary steps of nucleolar disintegration at the beginning of mitosis in that both events are initiated by reduced or blocked activity of RNA polymerase I. In addition, the nucleoplasmic bodies described in the present study resemble by morphological and immunological criteria the prenucleolar bodies which appear toward the end of mitosis during the early stages of nucleologenesis (see Fig. 3a-a"; for the behavior of the DFC during mitosis see Schmidt-Zachmann et al. 1984; Reimer et al. 1987c). These naturally occurring prenucleolar bodies arise in the absence of pre-rRNA synthesis but their assembly into the nucleolar body requires the activity of the rRNA genes (for a detailed discussion see Benavente et al. 1987).

Taken together, our findings lend further support to our previous suggestion that the DFC represents a genuine component of the nucleolus and is not a transient structure formed by the superposition of rDNA transcription units and primary transcriptional products (Schmidt-Zachmann et al. 1984; Benavente et al. 1987). Furthermore our data show that the nucleolar location and the integration of the DFC into nucleoli is critically dependent on ongoing transcription of the rRNA genes. We therefore propose, in contrast to the currently prevailing concept of DFC structure and function, that the nascent transcripts of the rRNA genes serve as specific nucleation sites for DFC assembly.

Based on the present data as well as our previous study (Benavente et al. 1987) we also conclude that microinjection of antibodies directed against nucleolar constituents into living cells combined with double-label immunocytochemistry is a valuable experimental approach in studies of the relationship between nucleolar structure and function. We hope that the use of antibodies as highly specific tools will lead to a better understanding of the functional organization of the nucleolus and the interplay between specific structures and biochemical reactions.

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