Inhibition of Nucleolar Reformation after Microinjection of Antibodies to RNA Polymerase I into Mitotic Cells

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Abstract. The formation of daughter nuclei and the reformation of nucleolar structures was studied after microinjection of antibodies to RNA polymerase I into dividing cultured cells (PtK_2). The fate of several nucleolar proteins representing the three main structural subcomponents of the nucleolus was examined by immunofluorescence and electron microscopy. The results show that the RNA polymerase I antibodies do not interfere with normal mitotic progression or the early steps of nucleologenesis, i.e., the aggregation of nucleolar material into prenucleolar bodies. However, they inhibit the telophasic coalescence of the prenucleolar bodies into the chromosomal nucleolar organizer regions, thus preventing the formation of new nucleoli. These prenucleolar bodies show a fibrillar organization that also compositionally resembles the dense fibrillar component of interphase nucleoli. We conclude that during normal nucleologenesis the dense fibrillar component forms from preformed entities around nucleolar organizer regions, and that this association seems to be dependent on the presence of an active form of RNA polymerase I.

NUCLEOLI are sites of assembly, processing, and maturation of eukaryotic ribosomal particles (for review see Busch and Smetana, 1970; Hadjiolov, 1985). Although considerable progress has been made in assigning functional roles to distinct substructures of the fully formed nucleolus (Hadjiolov, 1985), the assembly and maintenance of this structure are still poorly understood.

There is growing experimental evidence that nucleoli contain, in addition to pre-rRNA transcription units and preribosomal particles, abundant proteins that are thought to provide a structural support for the spatial arrangement of the rRNA genes and to play a role in the ordered translocation of preribosomal particles from their primary site of assembly to the nucleolar periphery (e.g., Franke et al., 1981; Moreno Diaz de la Espina et al., 1982; Krohne et al., 1982; Schmidt-Zachmann et al., 1984; Benavente et al., 1984*a*; for review see Benavente et al., 1984*b*).

Although nucleoli are stably organized and contain a structural framework, they are also highly dynamic. During mitotic prophase they disintegrate and their various constituents are released and become differentially distributed within the dividing cell. For example, at least some of the RNA polymerase I and the nucleolar protein C23 (nucleolin) remain associated with the rRNA genes of the nucleolar organizer region (NOR;[1] Scheer and Rose, 1984; Ochs et al., 1983; Gas et al., 1985). In contrast, preribosomal components, including ribosomal proteins and several nucleolus-restricted proteins such as the basic protein fibrillarin, nucleolar protein B23, and an acidic protein termed NO38 disperse, during metaphase, over the surfaces of all chromosomes and seem to remain in the perichromosomal cytoplasm (Fan and Penman, 1971; Ochs et al., 1983; 1985*a*, *b*; Hügle et al., 1985*a*, *b*; Schmidt-Zachmann et al., 1987). Other structural proteins are dispersed throughout the entire cytoplasm during mitosis (Benavente et al., 1984*a*; Schmidt-Zachmann et al., 1984). During telophase these disparate nucleolar constituents rapidly reassemble in an apparently coordinate fashion and accumulate in the NORs, contributing to the reformation of functioning nucleoli in the daughter cells. A first step in this telophasic reconstitution of nucleoli appears to be the aggregation of nucleolar material into larger entities, termed prenucleolar bodies (PNBs), which then seem to associate with the NORs (for reviews see Busch and Smetana, 1970; De la Torre and Gimenez-Martin, 1982).

From such observations it has been suggested that the NORs play a crucial role in the topologically specific assembly of nucleolar material during telophase. The question of whether the reformation of nucleoli is dependent on the onset of pre-rRNA synthesis has been addressed by several inves-

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1. *Abbreviations used in this paper*: AMD, actinomycin D; NOR, nucleolar organizer region; PNB, prenucleolar body.

tigators (e.g., Phillips, 1972; Phillips and Phillips, 1973; Gimenez-Martin et al., 1974; Semeshin et al., 1975; Morcillo and De la Torre, 1979) but the results are quite contradictory. A major problem with all these studies is that they are based on the use of drugs, such as actinomycin D (AMD), which are known to interfere with rRNA synthesis, but at the concentrations used might also have had effects on the synthesis of other RNAs, including pre-mRNAs. Furthermore, AMD has also been reported to interfere with several posttranscriptional events such as processing and nucleocytoplasmic transport of RNA (e.g., Eckert et al., 1975) and to affect other metabolic processes (for references see Scheer et al., 1975; Kostura and Craig, 1986).

In this study we have chosen to selectively impair transcription of the rRNA genes directly by microinjection of antibodies to RNA polymerase I into dividing cells. It has been recently shown that these antibodies inhibit transcription of rRNA genes both in vitro (Rose et al., 1981) and in vivo when microinjected into the cell nucleus or into the cytoplasm of mitotic cells (Mercer et al., 1984; Schlegel et al., 1985; Reimer et al., 1987*a*). We have examined the distribution of several nucleolar proteins serving as markers for the different nucleolar components by immunofluorescence microscopy. In addition, we have reinvestigated the effect of AMD on nucleolar reformation.

Materials and Methods

Cells and Microinjection

Rat kangaroo kidney epithelial cells (PtK_2) were grown as reported (Franke et al., 1978). Microinjection of cells was performed as previously described (Ansorge, 1982; Kreis and Birchmeier, 1982) with a microinjector (model 5242; Eppendorf Gerätebau, Hamburg, Federal Republic of Germany) equipped with glass capillaries (GC 150; Clark Electromedical Instrumental, Reading, United Kingdom). Cells undergoing mitosis (prometaphase and metaphase stages) were injected (see also Benavente and Krohne, 1986).

Antibodies

Human autoimmune serum S18 (from a patient suffering from scleroderma) containing high titers of antibodies to RNA polymerase I was recently described (Reimer et al., 1987*a*). Immunoglobulins were purified from this serum by incubation of the serum with protein A-sepharose CL-4B (Pharmacia, Uppsala, Sweden). Bound immunoglobulins were eluted with PBS (137 mM NaCl, 2.7 mM KCl, 7 mM Na_2HPO_4, 1.5 mM KH_2PO_4) containing an additional 3 M KSCN and dialyzed against PBS. IgG fraction of RNA polymerase I antibodies raised in rabbits was described and characterized in detail (Rose et al., 1981). IgG of monoclonal antibody $L_0$46F7, specific for *Xenopus* lamins L_{III} and L_{IV} (Benavente et al., 1985; Benavente and Krohne, 1985), was purified from ascites fluid by ammonium sulfate precipitation, followed by DE52 chromatography (Johnstone and Thorpe, 1982). Goat anti-rabbit IgG conjugated to Texas red was purchased from Dianova (Hamburg, FRG). In all cases antibodies were dissolved in PBS and microinjected in various concentrations, ranging from 1 to 20 mg/ml. Monoclonal murine antibodies (72B9) against a protein of the dense fibrillar component related to U3 containing RNP particle (Reimer et al., 1987*b*) and RS1-105 against ribosomal protein S1 (Hügle et al., 1985*a*) were also used. A human autoimmune serum (Scl C) from a patient suffering from scleroderma was used for comparison. This reacted with a protein of the dense fibrillar component, putatively identified as fibrillarin, and probably related to the antigen of monoclonal antibody 72B9 (Reimer et al., 1987*b*).

Immunofluorescence Microscopy

At various culturing times after antibody microinjection, the cell monolayers were fixed in methanol (−20°C) for 10 min, dipped for a few seconds into acetone (−20°C), and then air-dried. Microinjected antibodies were visualized by incubating coverslips with secondary antibodies conjugated with Texas red for 10 min. The fate of specific nucleolar proteins was monitored by double-labeling immunofluorescence, using secondary antibodies coupled to FITC (Dianova). Alternatively, AMD (Serva, Heidelberg, FRG) was added to the culture at concentrations ranging from 0.08 to 0.2 μg/ml, 10–40 min before microinjection. Individual mitotic cells were labeled by microinjection with control antibody $L_0$46F7 or with goat anti-rabbit IgG. 3 h later the cells were fixed and processed as described above.

Electron Microscopy

Cells microinjected with RNA polymerase I antibodies were fixed with 2.5% glutaraldehyde for 15 min and then postfixed with 2% osmium tetrox-

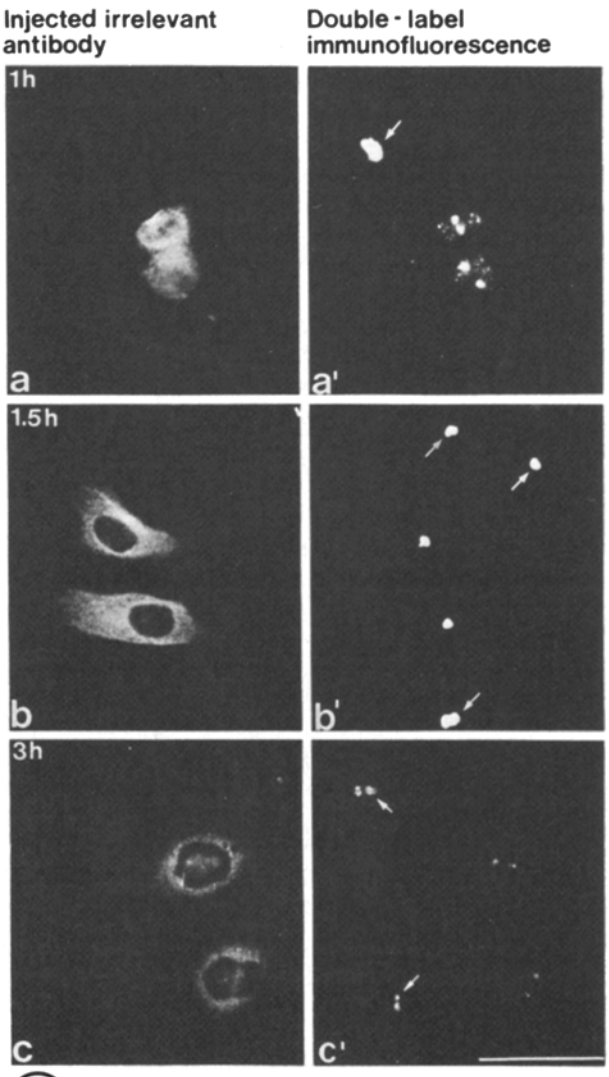


Figure 1. Fluorescence micrographs showing the time course of mitosis in PtK_2 cells microinjected with a nonspecific antibody at metaphase. (*a–c*) Distribution of the microinjected antibody $L_0$46F7 (reacting only with amphibian but not with PtK_2 lamins) after 60 (*a*), 90 (*b*), and 180 min (*c*). Double-label immunofluorescence of the same cells with the nucleolus-specific autoimmune serum Scl C (*a'* and *b'*) illustrates the progression of nucleologenesis. 90 min after microinjection nucleoli are reformed and cannot be distinguished from those of noninjected cells (*b'*). RNA polymerase I antibodies (S18) also reveal an identical staining pattern in microinjected and noninjected cells (*c'*). The noninjected cells are indicated by arrows in *a'–c'*. Note the exclusion of microinjected antibodies from the daughter nuclei (*b* and *c*). Bar, 50 μm.

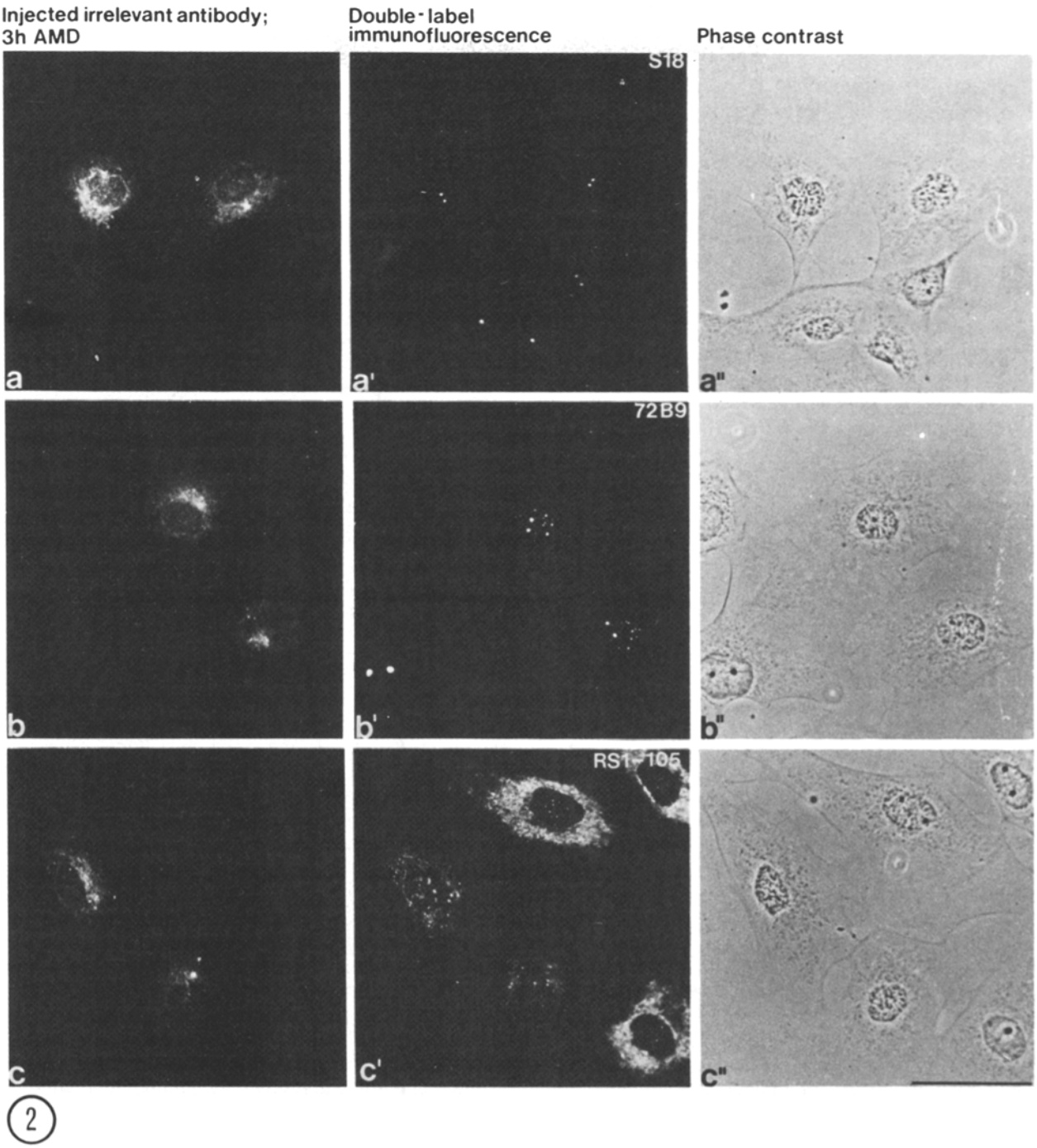


Figure 2. Inhibition of nucleolar reformation by AMD as visualized by immunofluorescence microscopy. To identify postmitotic cells, dividing PtK_2 cells were microinjected with goat anti-rabbit IgG conjugated with Texas red and cultured for 3 h in the presence of AMD (*a–c*). Double-label immunofluorescence was performed with antibodies to RNA polymerase I (S18, *a'*), monoclonal antibody 72B9 reacting with the fibrillar component of the nucleolus (*b'*), and monoclonal antibody RS1-105 against ribosomal protein S1 (*c'*). (*a"–c"*) Phase-contrast photographs of the same cells. Bar, 50 μm.

ide (15 min) at 4°C, stained overnight with 0.5% uranyl acetate, dehydrated in ethanol series, and embedded in Epon. Ultrathin sections were double-stained with uranyl acetate and lead citrate according to standard procedures (see Benavente and Krohne, 1986).

Results

In a previous study we have demonstrated that nonspecific Igs, i.e., Igs not reactive with antigens present in PtK_2 cells, had no noticeable effect on the progression of cell division and formation of daughter cells after microinjection into dividing PtK_2 cells (Benavente and Krohne, 1986). Here we show (Fig. 1) that nucleolar reformation is also unaffected in cells microinjected in this way. Cells injected during mitosis (prometaphase–metaphase) with monoclonal antibody $L_0$46F7, which is specific for amphibian lamins but does not react with lamins of higher vertebrates (Benavente and

Krohne, 1985; Benavente et al., 1985), showed, by 60 min after microinjection, that their nucleoli had partially reformed. At this point of time the autoimmune antibody Scl C, which recognizes a constituent of the dense fibrillar component, stained the reforming nucleoli as well as a number of PNBs still scattered in the nucleus (Fig. 1*a'*; see also Reimer et al., 1987*b*). By 90 min after microinjection, PNBs could no longer be recognized and nucleoli appeared to be completely reformed, as they were indistinguishable from those of nonmicroinjected adjacent interphase cells (Fig. 1*b'*). Furthermore, the distribution of RNA polymerase I was identical in injected and noninjected cells, as monitored by immunofluorescence microscopy (Fig. 1, *c* and *c'*; see also Scheer and Rose, 1984). From these controls it is also evident that, with the onset of telophase, antibody $L_0$46F7 was excluded from the reforming daughter nuclei (Fig. 1, *a–c*) in agreement with the notion that immunoglobulin molecules generally do not migrate across an intact nuclear envelope (Einck and Bustin, 1984). This is, however, not the case when IgGs are bound to a karyophilic protein or oligopeptide (Lanford et al., 1986; Madsen et al., 1986).

Inhibition of Nucleolar Reformation by AMD

We first analyzed the distribution of the various nucleolar components after exposure of PtK_2 cells to AMD, i.e., under conditions that are known to severely affect nucleologenesis (Fig. 2; for references see introduction). Concurrent with AMD administration, mitotic cells were labeled by microinjection of goat anti-rabbit IgG coupled to Texas red. 3 h later the cells were fixed and processed for immunofluorescence microscopy. By this procedure we marked a cell population that progressed synchronously into the next interphase. Under these experimental conditions cells were able to complete cell division. However, typical interphase nucleolar structures could not be detected by phase-contrast microscopy (Fig. 2, *a''–c''*), indicating that nucleolar reformation had been inhibited.

The fate of specific nucleolar components in postmitotic AMD-treated cells was analyzed by immunofluorescence microscopy. RNA polymerase I appeared to remain in association with small, distinct spheres (Fig. 2 *a'*), which sometimes could be identified in phase contrast (Fig. 2 *a''*). The distribution of other nucleolar components differed considerably from the normal interphase situation. Monoclonal antibody 72B9, which reacts with a component of the dense fibrillar region of nucleoli, stained numerous bodies dispersed throughout the nuclei of postmitotic daughter cells (Fig. 2 *b'*). In addition, the somewhat larger structures (usually two) visible in phase contrast and positive for RNA polymerase I were also stained by this antibody (Fig. 2 *b'*). In contrast, antibodies to ribosomal protein S1, a marker for the granular component of interphase nucleoli (Hügle et al., 1985*a*) gave an almost uniform or irregularly punctate nucleoplasmic fluorescence (Fig. 2 *c'*) different from that seen with antibodies to RNA polymerase I and 72B9. This fluorescence pattern suggested that precursor particles to the small ribosomal subunit were rather evenly distributed in the nuclear interior or occurred in the form of small aggregates which, however, were clearly different from PNBs (Fig. 2 *c'*). The cytoplasmic fluorescence seen in control and injected cells reflected the presence of ribosomal protein S1 in the cytoplasmic ribosomes (cf. Hügle et al., 1985*a*).

Inhibition of Nucleolar Reformation by Microinjected Antibodies to RNA Polymerase I

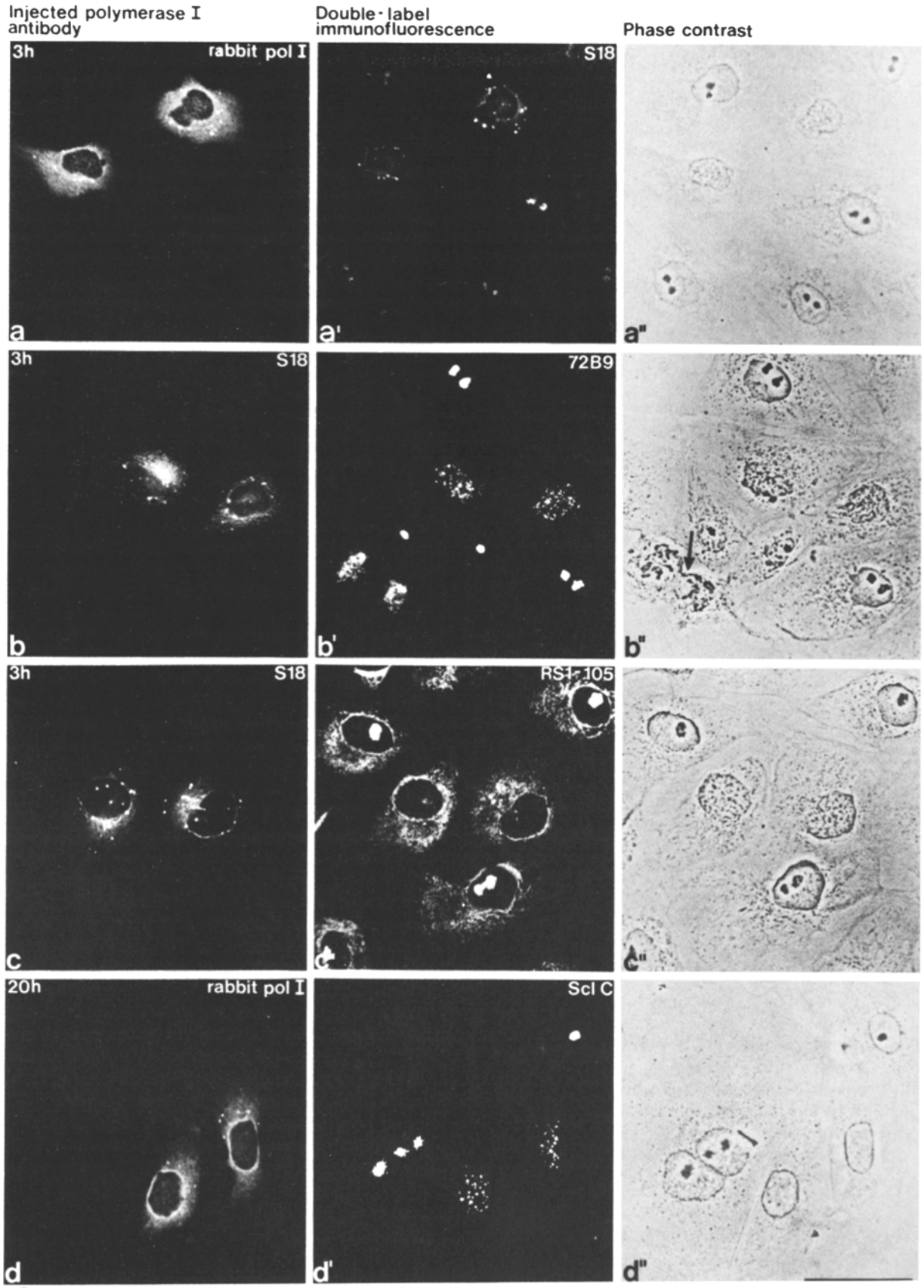
Microinjection of RNA polymerase I antibodies into mitotic cells also had dramatic effects on nucleolar reorganization at telophase. After microinjection, the majority of the antibody-associated fluorescence occurred in several clusters in the perinuclear cytoplasm of the postmitotic daughter cells, resulting in a punctate pattern (Fig. 3, *a–d*; the cytoplasmic localization of the fluorescing dots was established by examining the cells at different focal levels). These clusters most probably represent aggregates of RNA polymerase I molecules complexed with microinjected antibodies, since they were stained, in double-label immunofluorescence, both with antibodies to RNA polymerase I and antibodies recognizing the injected Igs (Fig. 3, *a–a''*). In contrast to the results obtained with AMD, virtually no RNA polymerase I staining was found within the postmitotic daughter nuclei, suggesting that the injected RNA polymerase I antibodies may have caused the release of the polymerase molecules from the NORs of metaphase chromosomes (Fig. 3 *a'*). In addition, these cytoplasmic aggregates may also contain the soluble form of RNA polymerase I, i.e., those molecules not bound to the NORs during mitosis. A similar perinuclear accumulation of immunocomplexes has recently been reported after microinjection of lamin antibodies into dividing PtK_2 cells (cf. Benavente and Krohne, 1986).

The distribution of antigens specific for the fibrillar and granular regions of nucleoli also differed markedly from that of noninjected cells. The dense fibrillar zone nucleolar protein recognized by antibody 72B9 was found in association with the numerous bodies randomly dispersed throughout the nuclear interior (Fig. 3 *b'*). Antibodies to ribosomal protein S1, a marker for the granular region of nucleoli, did not stain the same nuclear structures but showed a relatively weak nucleoplasmic fluorescence. In addition, this antibody reacted, as expected, with the cytoplasmic ribosomes (Fig. 3 *c'*; cf. Hügle et al., 1985*a*). Inspection of the microinjected cells at different postinjection times (up to 20 h) revealed the same pattern of fluorescence with no evidence for the formation of nucleolus-like structures (Fig. 3, *d–d''*).

Ultrastructural Observations

We also studied nucleologenesis at the ultrastructural level

Figure 3. Effect of RNA polymerase I antibodies on nucleolar reformation. Dividing PtK_2 cells were injected with rabbit (*a* and *d*) or human (*b* and *c*) antibodies to RNA polymerase I and incubated for 3 h (*a–c*) and 20 h (*d*). The distribution of the injected antibodies is shown in *a–d*. Double-label immunofluorescence of the fixed cells was performed with anti-polymerase I serum S18 (*a'*), monoclonal antibody 72B9 reacting with the fibrillar nucleolar component (*b'*), monoclonal antibody RS1-105 against ribosomal protein S1 (*c'*), and the nucleolus-specific serum Scl C (*d'*). (*a''–d''*) Phase-contrast photographs of the same cells. Noninjected cells in early telophase are indicated by the arrow in *b''*. Bar, 50 μm.


Injected polymerase I antibody
Double-label immunofluorescence
Phase contrast
3h
rabbit pol I
S18
a
a'
a"
3h
S18
72B9
b
b'
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S18
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20h
rabbit pol I
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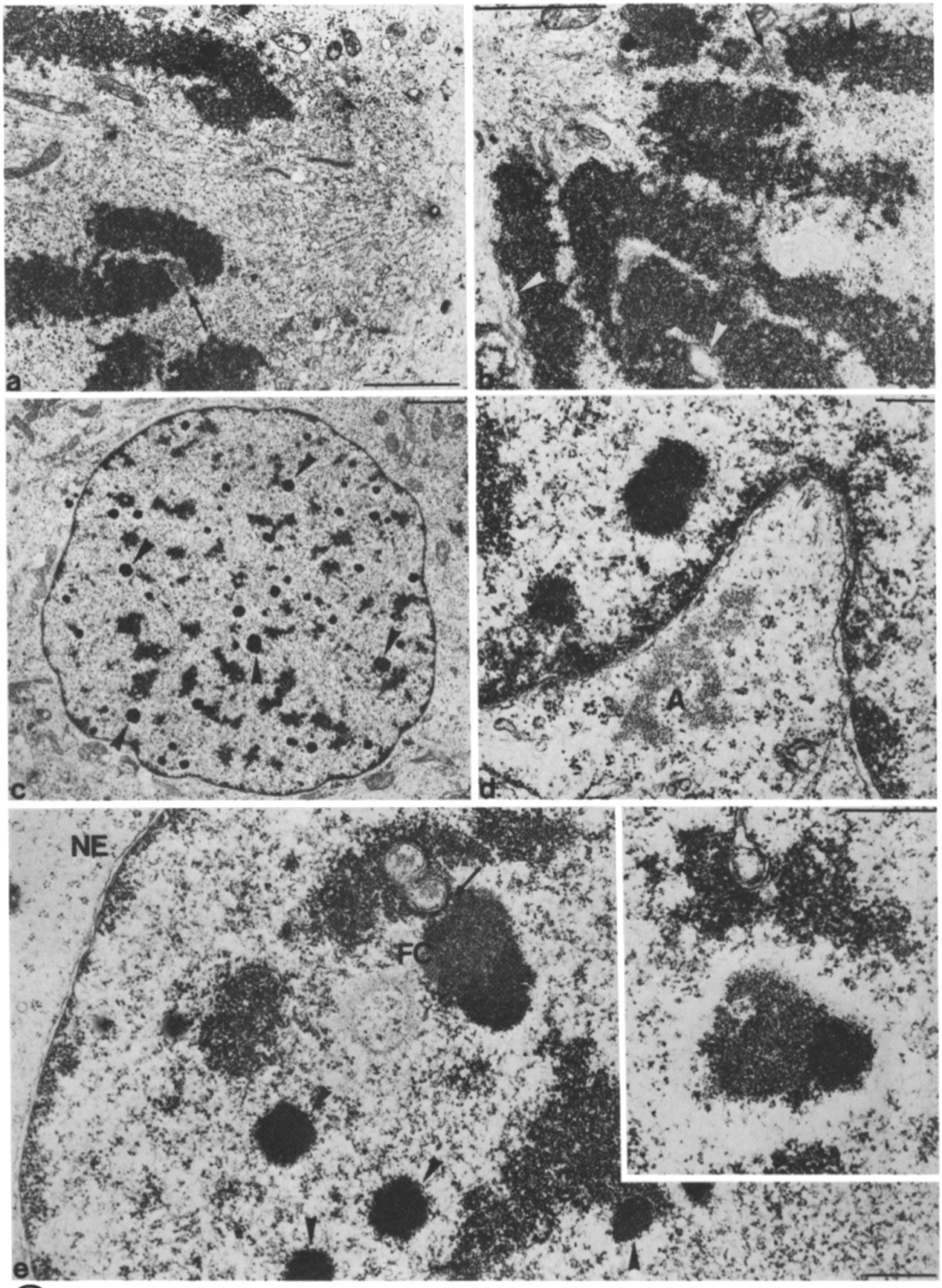
3



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by comparing control cells with postmitotic cells that had been injected with antibodies to RNA polymerase I in the preceding metaphase (Fig. 4). In noninjected cells, NORs could be identified during different stages of mitosis as chromosomal regions of conspicuously low staining density surrounded by the highly condensed chromatin of the mitotic chromosomes (Fig. 4, *a* and *b*; for review see Goessens, 1984). At the time of nuclear envelope reformation (Fig. 4 *b*) evidence for the presence of additional nucleolar components could not be found at the level of the NORs (see also Hernandez-Verdun et al., 1979). Recruitment of nucleolar material around the NORs was only visualized later during telophase (not shown; for review see Goessens, 1984), in agreement with the results obtained by immunofluorescence microscopy (Fig. 1 *a'*).

The distribution of nucleolar material in postmitotic cells containing antibodies to RNA polymerase I was clearly different. Injected cells could be identified unequivocally in the electron microscope since they occurred in pairs (daughter cells) and contained characteristic cytoplasmic aggregates of granular material that were absent in control cells (Fig. 4 *d*). These aggregates are likely to represent immunocomplexes containing RNA polymerase I molecules, i.e., the ultrastructural equivalent to the cytoplasmic fluorescent spots seen in Fig. 3, *a–d*. In support of this interpretation, cells microinjected with lamin antibodies also contained similar cytoplasmic aggregates of immunocomplexes of lamin antibodies (Benavente and Krohne, 1986). However, the cytoplasmic aggregates of the present study differed from those containing lamins by the fact that they were not associated with membrane vesicles and cisternae, probably reflecting the affinity of lamins for nuclear membrane structures.

A careful examination of numerous serial sections of postmitotic daughter cells that had originated from mitotic cells microinjected with RNA polymerase I antibodies revealed a complete absence of typical nucleolar structures. Instead, the daughter nuclei contained, 3 h after mitosis, numerous roundish aggregates scattered throughout the nuclear interior, composed primarily of densely packed fibrillar material (Fig. 4 *e*). In addition, discrete spherical structures were also recognized, which, according to their morphology and heavy metal staining property, were tentatively identified as the interphase counterpart of the NORs (fibrillar centers; see Lepoint and Goessens, 1978). They were usually found in association with peripheral condensed chromatin close to the nuclear envelope, i.e., sites at which the NOR pedicles are known to occur in many species (cf. Busch and Smetana, 1970; Rae and Franke, 1972; for review see Bouteille et al., 1982). Occasionally, these fibrillar centers were seen in association with intensely stained structures resembling the free aggregates of densely packed fibrillar material (Fig. 4 *e* and inset).

Discussion

Synthesis of rRNA and Nucleolar Reformation at Telophase

The postmitotic reformation of a functional nucleolus is a complex process involving the precise targeting of old nucleolar material (derived from the mother cell) to the NORs of the telophase chromosomes of the daughter nuclei, the rapid assembly into the nucleolar body, and the resumption of pre-rRNA synthesis (e.g., Fan and Penman, 1971; Lepoint and Goessens, 1978; Morcillo and De la Torre, 1979; Hernandez-Verdun et al., 1980; Hügle et al., 1985*a*, *b*; Ochs et al., 1985*b*; for review see De la Torre and Gimenez-Martin, 1982). To find out whether this fast and precise reassembly event is dependent on the onset of pre-rRNA synthesis we have experimentally inhibited the transcriptional status of the rRNA genes by microinjecting antibodies to RNA polymerase I into dividing cells (see Schlegel et al., 1985).

The present study indicates that the transcriptional status of the rRNA genes plays a crucial role in the reformation of nucleoli. Inhibition of the transcriptional activation of the rRNA genes during and after mitosis leads to a block in nucleolar reconstitution. Interestingly, two steps of postmitotic nucleologenesis can be distinguished in these experiments. While the assembly of nucleolar material around the NORs is severely affected, the first step, i.e., the fusion of some nucleolar components into PNBs is not impaired to any noticeable extent. In control cells, this first step of nucleologenesis is very short (see Fig. 1 *a'*). Inhibition of transcriptional activity of the rRNA genes by microinjection of antibodies to RNA polymerase I apparently freezes this stage. This suggests that during late telophase an active form of RNA polymerase I is required for the specific assembly of the PNBs around the NORs.

At first sight, this conclusion may seem to be in conflict with the situation found in the embryos of the anucleolate *O-nu* mutant of *Xenopus laevis* (Elsdale et al., 1958), which forms structures resembling nucleoli (pseudonucleoli; Hay and Gurdon, 1967) despite the apparent absence of rRNA genes and rRNA synthesis (Brown and Gurdon, 1964; Wallace and Birnstiel, 1966). Recent studies have further shown that these pseudonucleoli contain certain nucleolar antigens (Steele et al., 1984). In addition, DNA sequences related to the rRNA genes were identified in *O-nu* mutants (Steele et al., 1984), which contain at least the enhancer elements, the promoter region, and a portion of the 18S rRNA gene, although in a considerably deleted and modified form (Tashiro et al., 1986). Therefore, it is possible that the pseudonucleoli of the *O-nu* mutants are able to bind RNA polymerase I and perhaps also to transcribe some rDNA-like sequences and hence represent the expression of these residual, albeit mu-

Figure 4. (*a–b*) Electron micrographs showing two different noninjected PtK_2 cells undergoing anaphase. NORs are indicated by arrows. Mitosis is more advanced in *b*. The nuclear envelope is already partly reassembled on the surface of the chromosomes (*arrowheads*). (*c–e*) Electron micrographs of the daughter nuclei 3 h after microinjection with polymerase I antibodies (S18). As shown in the survey micrograph nucleoli are absent but numerous PNBs are recognized (some of them are denoted by arrowheads in *c*). Often aggregates of a granular substance occur in the perinuclear cytoplasm (*A* in *d*). Most prenucleolar bodies are composed of densely packed fibrils (*arrowheads* in *e*). Occasionally, individual PNBs are associated with the fibrillar centers (*FC*). The fibrillar centers are usually in close contact with invaginations of the nuclear envelope (*arrow* in *e*) and condensed chromatin in the nuclear periphery (*e* and *inset*). *NE*, nuclear envelope; *Ch*, condensed chromatin. Bars: (*a–c*) 2 μm; (*d–e*) 0.5 μm.

tated, genes. Clearly, clarification of the situation in the *Xenopus O-nu* mutants through the combination of nucleic acid techniques and immunolocalization of nucleolar proteins will aid our understanding of nucleologenesis.

PNBs, Dense Fibrillar Component, and Nucleolar Organization

As shown by immunofluorescence microscopy, PNBs contain a nucleolar protein that is a component of the dense fibrillar region of interphase nucleoli and that probably corresponds to fibrillarin (Reimer et al., 1987*b*; see also Fig. 3, *d–d″* of the present study) as well as protein C23 (Ochs et al., 1985*b*). In the present study, however, constituents of the granular component such as ribosomal protein S1, which is associated with precursor particles to the small ribosomal subunit, have not been detected in PNBs. This finding is in agreement with the purely fibrillar nature of PNBs as seen in electron micrographs but does not exclude the fact that normal PNBs contain, in addition to the fibrillar components, a coat of granular material representing preribosomal particles (see Hügle et al., 1985*a*, *b*). It is conceivable that during the prolonged PNB stage, under the experimental conditions of this study, this granular component dissociates from the PNBs and becomes redistributed in the nucleus.

Our results show that the association of the preformed fibrillar PNBs with the NORs is an important step in the reconstitution of nucleoli. Whether this site-specific assembly is mediated directly by the nascent transcripts cannot be answered at present. Reports that PNBs can coalesce to some extent at the NORs during telophase in the presence of AMD and other inhibitory drugs (for references see also Morcillo and De la Torre, 1979) might be due to the fact that AMD at the low concentrations usually used does not completely abolish pre-rRNA synthesis (Mercer et al., 1984). This interpretation is in agreement with our results (Fig. 2, *b′* and *b″*) and additional experiments, which showed that after injection of limiting amounts of RNA polymerase I antibodies an extension of the PNB stage of nucleologenesis was noted but, finally, some small nucleolar structures appeared (data not shown).

It is remarkable that during an early stage of nucleolar reorganization two nucleolar components, the NOR and the dense fibrillar material, occur as separate structural entities. The NOR contains the tandemly repeated rRNA genes and tightly bound RNA polymerase I molecules (Scheer and Rose, 1984) as well as topoisomerase I (Guldner et al., 1986) and, at least some of protein C23 (nucleolin; Ochs et al., 1983; Gas et al., 1985). The PNBs, on the other hand, are related to the fibrillar component of interphase nucleoli, as both structures are positive with antibodies 72B9 and Scl C. The widely held view that the dense fibrillar component of interphase nucleoli is a transient structure formed by the transcriptionally active rRNA genes and their primary transcription products (for review see Bouteille et al., 1982; Goessens, 1984) is not compatible with our observation that in telophase this component exists as preformed spheroidal entities physically separated from the rRNA genes. On the contrary, the results of the present study suggest that the dense fibrillar component of nucleoli is a structure *sui generis*, independent of the transcriptional apparatus. This view is strongly supported by the observation that functionally inactive nucleoli lacking RNA polymerase I, which occur naturally in nucleated erythrocytes of amphibians and birds, resemble the dense fibrillar component since they are positive with antibody 72B9 (unpublished observations) and contain an M_r 180,000 protein that is also a marker of the dense fibrillar component (Schmidt-Zachmann et al., 1984). Therefore, the function and mode of assembly of the dense fibrillar nucleolar units require further investigation.

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