

In Vitro Assembly of Prenucleolar Bodies in *Xenopus* Egg Extract

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Abstract. Nuclei assembled in *Xenopus* egg extract from purified DNA or chromatin resemble their natural counterparts in a number of structural and functional features. However, the most obvious structural element of normal interphase nuclei, the nucleolus, is absent from the in vitro reconstituted nuclei. By EM, cytological silver staining, and immunofluorescence microscopy employing antibodies directed against various nucleolar components we show that nuclei assembled in vitro contain numerous distinct aggregates that resemble prenucleolar bodies (PNBs) by several criteria. Formation of these PNB-like structures re-

quires pore complex-mediated nuclear transport of proteins but is independent of the genetic content of the in vitro nuclei as well as transcriptional and translational events. Our data indicate that nuclei assembled in vitro are capable of initiating early steps of nucleogenesis but that the resulting PNBs are unable to fuse with each other, probably due to the absence of a functional nucleolus organizer. With appropriate modifications, this experimental system should be useful to define and analyze conditions promoting the site-specific assembly of PNBs into a coherent nucleolar body.

EXTRACT prepared from activated amphibian eggs is capable of assembling purified DNA or chromatin into nuclear-like structures which share several structural and functional features with authentic interphase nuclei (Lohka and Masui, 1983; Newmeyer et al., 1986a; Blow and Laskey, 1986; Lohka and Maller, 1987; Newport, 1987; Lohka, 1988; Laskey and Leno, 1990). These artificial or pseudonuclei are enclosed by a nuclear lamina and a double-layered nuclear membrane with numerous pore complexes (Newmeyer et al., 1986a; Newport, 1987; Lohka, 1988; Sheehan et al., 1988; Laskey and Leno, 1990; Dabauvalle et al., 1990) and are capable of accumulating soluble karyophilic proteins from the surrounding medium by signal sequence and energy-dependent transport processes (Newmeyer et al., 1986a,b; Newmeyer and Forbes, 1988). In addition, they replicate their DNA semiconservatively at discrete intranuclear sites, similar to what has been described for authentic nuclei (Cox and Laskey, 1991). Since cell-free systems are amenable to experimental manipulations and molecular analyses they have provided important insights into the pathways and mechanisms by which cell nuclei are assembled from depolymerized constituents. However, the synthetic nuclei differ from normal interphase nuclei in one important aspect in that they lack the most obvious structural element, the nucleolus.

During the cell division cycle in higher organisms nucleoli emerge at telophase, concomitantly with the reconstitution of the daughter nuclei, by a sequential assembly process and persist throughout interphase until they disintegrate at the onset of the next mitosis. Postmitotic nucleolar assembly in whole cells is initiated with the formation of numerous intranuclear discrete structures termed "prenucleolar bodies"

(PNBs)¹ which subsequently coalesce around the chromosomal nucleolus organizer regions (NORs) into a coherent nucleolar body (De La Torre and Gimenez-Martin, 1982; Ochs et al., 1985a; Benavente et al., 1987; Scheer and Benavente, 1990). In the present study we show that in vitro assembled nuclei, independent of their genetic content, are capable of reproducing early steps of nucleolus formation up to the PNB stage.

Materials and Methods

Animals

Xenopus laevis were purchased from the South African Snake Farm (Fish Hoek, Cape Province, South Africa).

Antibodies

Among the antibodies employed in the present study one was *Xenopus*-specific (No-114); all the others reacted with the indicated antigens from a wide variety of species including *Xenopus laevis*. Antibodies with the following specificities were used. Human autoimmune sera against fibrillarin (S4; Reimer et al., 1987a; for reaction with *Xenopus* fibrillarin see Lapeyre et al., 1990; Caizergues-Ferrer et al., 1991), RNA polymerase I (S18; Reimer et al., 1987b), 7-2-RNP (serum To; Reimer et al., 1988), and U1 small nuclear RNP (snRNP, purified Ig fraction; Reuter et al., 1985); affinity-purified rabbit antibodies against nucleolin (anti-pp105; Pfeiffe and Anderer, 1983; Pfeiffe et al., 1986), DNA topoisomerase I (Rose et al., 1988) and the trimethylguanosine cap of small nuclear RNAs (snRNAs; Lüthmann et al., 1982); mAb (IgG1) against the *Xenopus* nucleolar protein N038, a homologue to the rat nucleolar protein B23 (No-185; Schmidt-

1. *Abbreviations used in this paper:* NOR, nucleolus organizer region; PNB, prenucleolar body; snRNA, small nuclear RNA; snRNP, small nuclear RNP; WGA, wheat germ agglutinin.

Zachmann et al., 1987), mAb (IgG1) against the *Xenopus* 180-kD nucleolar protein (No-114; Schmidt-Zachmann et al., 1984), mAb (IgM) against double- and single-stranded DNA (AK30-10; Scheer et al., 1987; purchased from Boehringer Mannheim, Germany), mAb (IgG1) against lamina proteins including *Xenopus* lamin L_{III} (S49H2; see Dabauvalle et al., 1990), a mAb (IgG2b) reacting with mammalian and *Xenopus* core histones as well as histone H1 (GA 199; Platz, 1991). For immunofluorescence, sera were used at a 1:100 dilution, purified Igs at concentrations ranging from 10 to 50 µg/ml and, in case of mAbs, cell culture supernatants without dilution.

Nuclear Reconstitution

Extract was prepared from dejellied *Xenopus* eggs 20 min after their activation by electric shock (Karsenti et al., 1984) essentially as described by Newport (1987). Aliquots were frozen in liquid nitrogen and could be stored at -70°C for up to 30 d. Purified DNA was added at a concentration of 15 µg/ml to the extract or, alternatively, demembrated *Xenopus* sperm nuclei (600 nuclei/µl extract) together with an ATP-regenerating system to initiate nuclear reconstitution (Newport, 1987). After an incubation time of 90 min at 22°C, the mixture was processed for immunofluorescence microscopy, silver staining, in situ hybridization or EM. Some extracts were manipulated by adding actinomycin D (Serva, Heidelberg, Germany) to a final concentration of 5 µg/ml or wheat germ agglutinin (WGA; Calbiochem, Frankfurt/Main, Germany) to 200 µg/ml before incubation.

DNA templates used for nuclear assembly were bacteriophage lambda DNA (Gibco-BRL, Eggenstein, Germany), total genomic DNA isolated from *Xenopus* liver by the method of Blin and Stafford (1976), and plasmid DNA prepared according to Birnboim and Doly (1979). The plasmid was constructed by cloning an 11.4-kb HindIII fragment containing a full-length *Xenopus* rDNA repeat unit (see Rungger et al., 1979) into pBluescript KS(+) (Stratagene, Heidelberg, Germany). Demembrated sperm nuclei were prepared as described by Blow and Laskey (1986).

Immunofluorescence, Silver Staining, In Situ Hybridization and EM

After incubation with purified DNA or sperm chromatin the extract preparations were fixed by dilution with 4 vol PBS containing 1% formaldehyde (freshly prepared from paraformaldehyde). Nuclei were adsorbed to a coverslip by centrifugation for 10 min at 1,000 rpm in a cyto centrifuge (Cytospin 2, Shandon, Frankfurt/Main, Germany). Specimens were then fixed in acetone at -20°C for 10 min and air dried.

For immunofluorescence microscopy, specimens were incubated for 20 min each with the primary antibodies followed by appropriate secondary antibodies conjugated to Texas red or FITC (Dianova, Hamburg, Germany; diluted 1:50). In case of double-label immunofluorescence the preparations were subsequently incubated with another set of primary and secondary antibodies. DNA was visualized by staining with Hoechst 33258 (5 µg/ml PBS).

To localize silver binding proteins the air dried specimens were fixed with Carnoy's fixative (ethanol/chloroform/glacial acetic acid in a ratio of 6:3:1) for 10 min and stained with silver nitrate as described (Ochs et al., 1985a).

In situ hybridizations were performed with digoxigenin-labeled antisense RNA probes prepared with an RNA labeling kit (Boehringer Mannheim) according to the manufacturer's instructions. Templates were either plasmid pBSK U3, a Bluescript vector containing a *Xenopus* U3 cDNA insert (Jepsen et al., 1988) or the rDNA containing plasmid described above. RNA probes were hybridized at concentrations of 10 µg/ml in 5× SSC containing 100 µg/ml yeast tRNA for 3 h at 65°C. Specifically bound probes were visualized by immunofluorescence using antidigoxigenin antibodies (Boehringer Mannheim).

Photographs were taken with a Zeiss Axiophot (Carl Zeiss, Oberkochen, Germany) equipped with epifluorescence optics and the appropriate filter sets.

Specimens were prepared for EM essentially as described (Dabauvalle et al., 1990).

Results

Ultrastructural Features of In Vitro Assembled Nuclei

A typical pseudonucleus assembled around bacteriophage lambda DNA after a 90-min incubation in *Xenopus* egg ex-

tract is shown in Fig. 1 a. Apart from chromatin and some membrane material which presumably became trapped during the formation of the nuclear envelope, conspicuous electron-dense spheroidal aggregates with diameters of ~0.2 µm are recognized within the newly assembled nucleus. The number of these dense bodies in each section varied from zero to three or more. Comparable structures were also found in pseudonuclei assembled from other DNA templates such as plasmids containing a *Xenopus* rDNA repeat unit or total genomic DNA isolated from *Xenopus* liver.

Upon incubation in egg extract, demembrated sperm nuclei acquire a nuclear envelope with pore complexes and their chromatin decondenses (Lohka and Masui, 1983; Lohka and Maller, 1987). After a 90-min incubation the resulting nuclei also contained electron-dense spheroidal aggregates with diameters ranging from 0.15 to 0.4 µm scattered throughout the nuclear interior (up to eight spheres per section; Fig. 1 b).

Composition of the Dense Bodies

To gain insight into the biochemical composition of the dense bodies we have employed immunofluorescence microscopy in conjunction with antibodies specific for various nucleolar components, the cytological silver staining method, and in situ hybridization. All antibodies used (except the non-nucleolar antibodies directed against DNA, histones, lamins, and U1 snRNP which served as controls) stained the amplified nucleoli of *Xenopus* oocytes as well as nucleoli of somatic *Xenopus* cells. Two antibodies immunostained, in addition to the nucleoli, also nucleoplasmic structures, i.e., the antibodies directed against DNA topoisomerase I and the 5'-trimethylguanosine cap of snRNAs. In fact, DNA topoisomerase I is known to be present both in the nucleolus and the nucleoplasm (Muller et al., 1985; Rose et al., 1988) and the majority of the snRNAs occurs in the extranucleolar space except for U3 and a few other minor snRNA species (for review see Reddy and Busch, 1988).

When nuclei assembled from pure DNA (bacteriophage DNA or the eukaryotic DNA templates mentioned above) or sperm chromatin were examined, no differences were noted. The dense bodies stained heavily with silver as indicated by the dot-like pattern of the in vitro assembled nuclei (Figs. 2 a and 3 a). Antibodies directed against fibrillarin, a constitutive protein of the dense fibrillar component of nucleoli, produced a similar dot-like intranuclear fluorescence pattern (Figs. 2 b and 3 b). Fibrillarin is an evolutionarily highly conserved protein (e.g., Ochs et al., 1985b; Christensen et al., 1986; Reimer et al., 1987a,c; for *Xenopus* see Lapeyre et al., 1990; Caizergues-Ferrer et al., 1991) which in yeast has been shown to function both in pre-rRNA processing and in organizing the nucleolar structure (Jansen et al., 1991). Antibodies against another protein of the dense fibrillar component, the 180-kD protein described in *Xenopus* nucleoli (Schmidt-Zachmann et al., 1984), immunostained the dots in the same fashion as antibodies to fibrillarin (Fig. 2 f" and 3 d). Nucleolin (or C23), an abundant protein occurring in the dense fibrillar component and to a lesser extent also in the granular component of nucleoli from a wide variety of species (Pfeifle and Anderer, 1983; Pfeifle et al., 1986; Biggiogera et al., 1989; for *Xenopus* see Caizergues-Ferrer et al., 1989), has been implicated in early processes of ribosome biogenesis (Olson, 1990). Antibodies against nucleol-

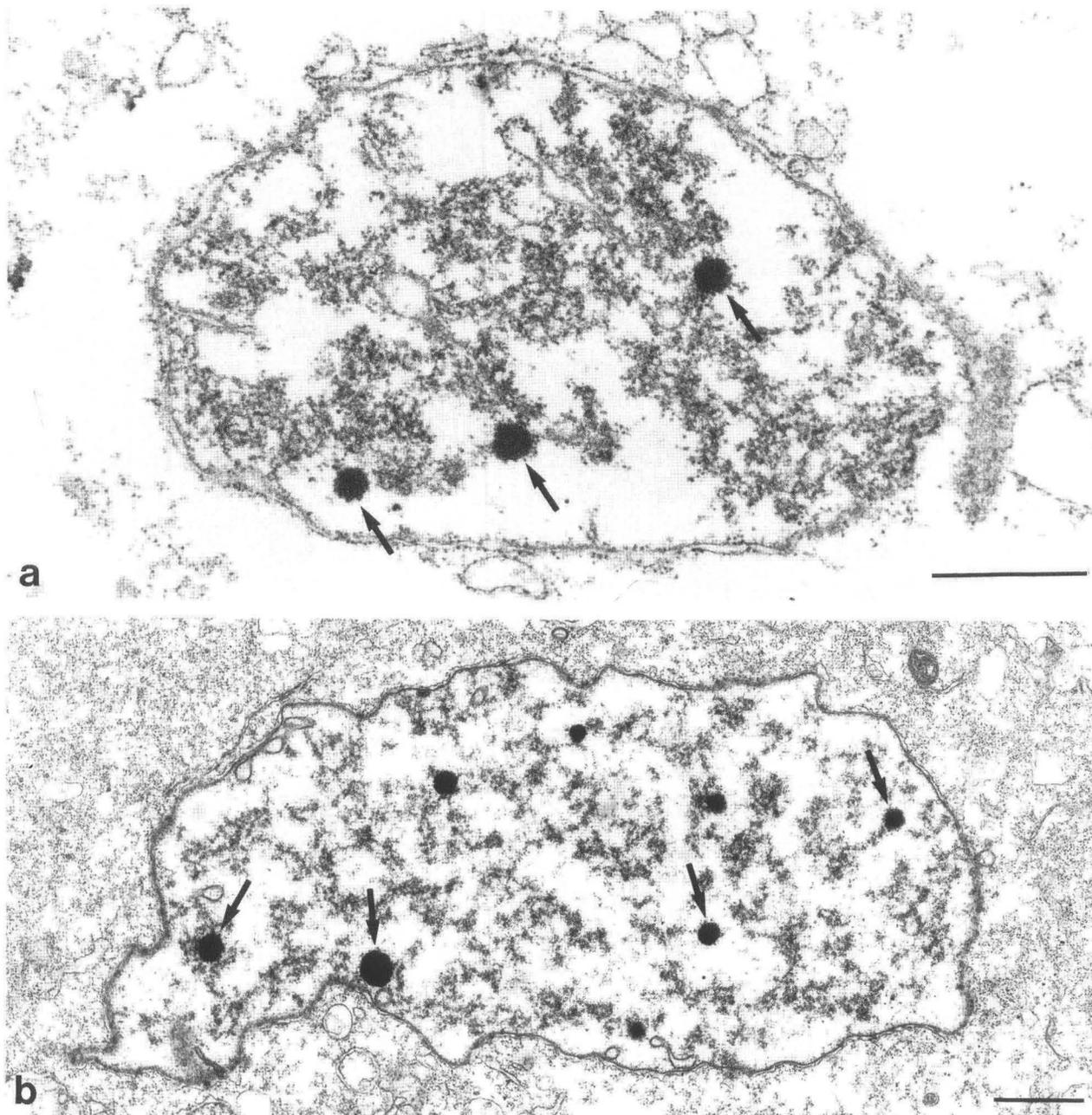


Figure 1. Electron micrographs showing nuclei assembled in *Xenopus* egg extract from bacteriophage lambda DNA (a) and demembrated *Xenopus* sperm chromatin (b). Both nuclei contain apart from chromatin several round, electron dense structures (some are denoted by arrows). Bars, 1 μ m.

lin also resulted in a brightly punctate fluorescence pattern of the pseudonuclei (Figs. 2 c and 3 c). In contrast, antibodies to B23/N038, a nucleolar protein located predominantly, though not exclusively, in the granular component (Biggio-gera et al., 1989; Olson, 1990; for *Xenopus* see Schmidt-Zachmann et al., 1987), stained the nuclear dense bodies only weakly (Fig. 2 d). Finally, double-label immunofluorescence in various combinations produced superimposable patterns, indicating that each nuclear dot contained the whole spectrum of proteins studied (Figs. 2 e'-e'' and f'-f'' and 3 e'-e''). In this context it is worth mentioning that the larger fluorescing dots could be clearly identified by phase-contrast microscopy (Figs. 2, e and f and 3 e).

To clarify whether nuclear assembly and appearance of the dense bodies required transcriptional events, in some experiments we have added actinomycin D to the cell extract. Dense body formation was not affected to any noticeable extent under such conditions, both in nuclei assembled from pure DNA and sperm chromatin.

When probed with antibodies against RNA polymerase I, DNA topoisomerase I, 7-2-RNP and, as negative control, U1 snRNP, the nuclear dense bodies did not fluoresce (Table I). This finding indicates that proteins confined to the fibrillar centers of nucleoli such as RNA polymerase I and DNA topoisomerase I (Scheer and Rose, 1984; Thiry et al., 1991) as well as nucleolar constituents exclusively located in the

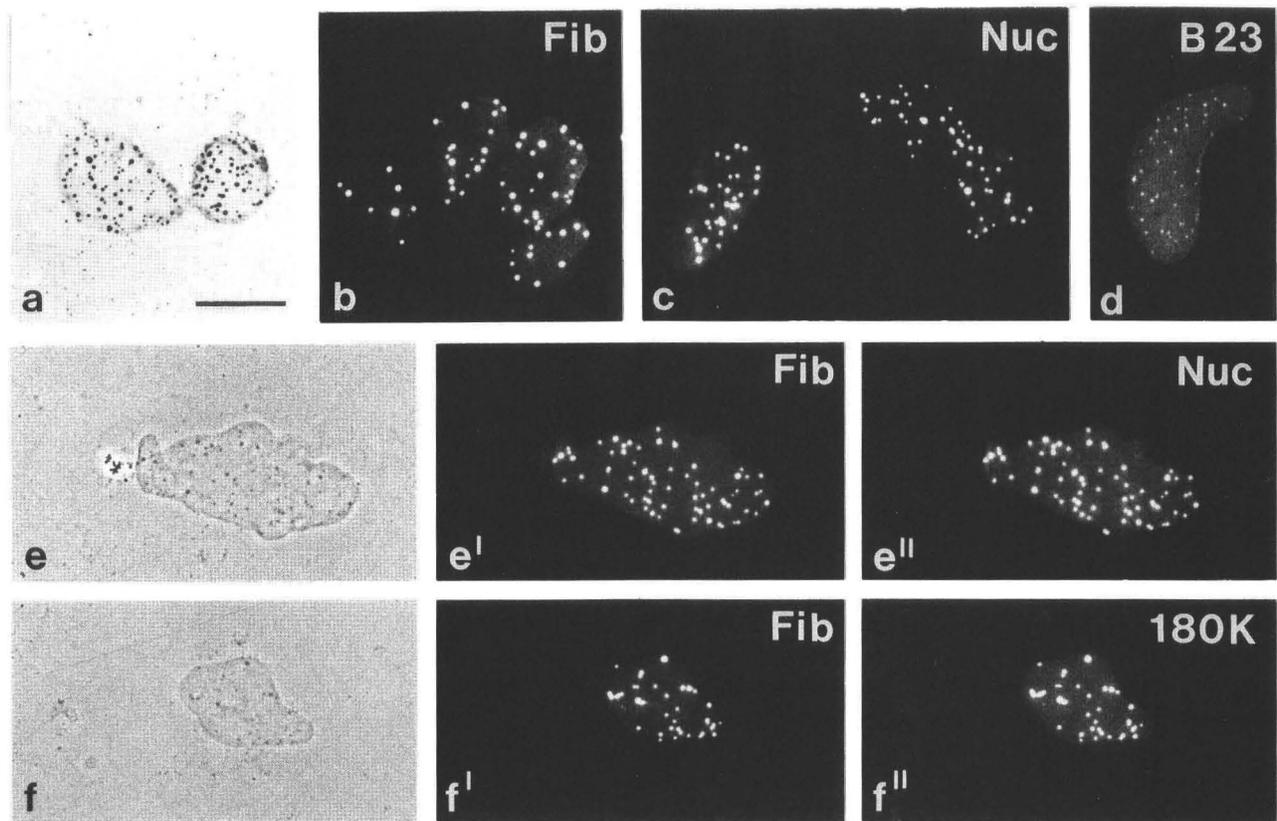


Figure 2. Intranuclear dot-like structures in pseudonuclei assembled from lambda DNA as revealed by silver staining (a) and immunofluorescence microscopy using antibodies against fibrillar (Fib; b, e', f'), nucleolin (Nuc; c, e''), B23/N038 (B23; d) and a 180-kD nucleolar protein (180K; f''). Double-labeling experiments demonstrate colocalization of fibrillar with, respectively, nucleolin (e', e'') and the 180-kD protein (f', f''). Note that the larger nuclear dots can be clearly seen as dense structures by phase-contrast microscopy (e, f). Bar, 20 μ m.

granular component (7-2-RNP; Reimer et al., 1988) are absent from the dense bodies. Antibodies specific for the 5'-trimethylguanosine cap structure of snRNAs (Lührmann et al., 1982; Reuter et al., 1985) labeled the dense bodies slightly above background. However, in situ hybridization with U3-specific probes failed to produce a signal. Thus, the dense bodies do not contain significant quantities of U3 snRNA molecules. Likewise, they also contain no detectable amounts of rRNA as judged by in situ hybridization with appropriate digoxigenin-labeled probes.

We have performed additional control experiments with nonnucleolar antibodies in order to rule out nonspecific binding to the dense bodies. In fact it could be argued that these structures label so strongly because they are the only particulate material apart from chromatin present in the nuclei. However, this is not the case. For instance, with antibodies directed against histones (Fig. 4, a and b) and DNA (Fig. 4 c), nuclei assembled from DNA or sperm chromatin fluoresced almost uniformly with the notable exception of the dense bodies which stood out as dark "holes" (e.g., Fig. 4 b). Yet another fluorescence pattern was obtained with antibodies directed against lamin L_{III} which decorated the periphery of the in vitro nuclei but left the dense bodies unstained (Fig. 4 d). We conclude that the punctate fluorescence pattern obtained with some nucleolus-specific anti-

bodies represents genuine binding to their antigens concentrated in the dense bodies.

Formation of the Dense Bodies Requires Nuclear Transport

The large number and intense fluorescence of the dense bodies present in virtually any pseudonucleus indicates that the nucleolar proteins fibrillar, nucleolin, B23/NO38, and the 180-kD protein are stored in excess in a depolymerized form in the *Xenopus* egg, similar to what has been found for other nuclear components (for refs. see Laskey and Leno, 1990). Is the formation of the dense bodies dependent on nuclear transport processes? To approach this question we have added the lectin WGA to the egg extract before incubation with DNA or chromatin. Previously we have shown that under such conditions nuclei are assembled which are surrounded by a nuclear envelope but lack pore complexes and hence are unable to import karyophilic proteins (Dabauvalle et al., 1990). Nuclei reconstituted around added DNA in the presence of WGA did not contain dense bodies as monitored by phase-contrast and immunofluorescence microscopy with antibodies against fibrillar (Fig. 5). Likewise, nuclei assembled from sperm chromatin in the presence of WGA were also devoid of dense bodies (Fig. 6). Under such conditions

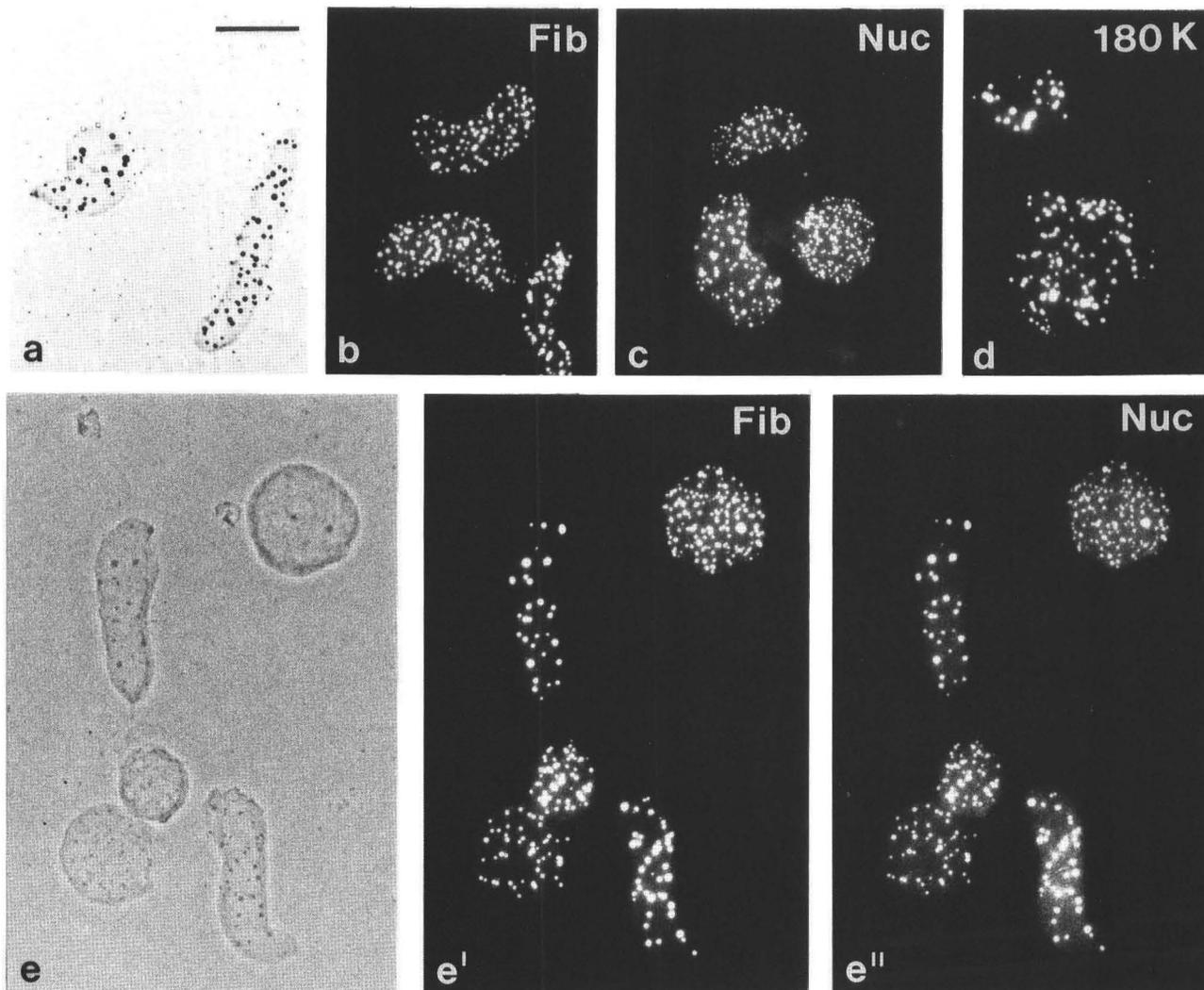


Figure 3. Demembrated sperm nuclei incubated for 90 min in the egg extract and visualized by silver staining (*a*) and immunofluorescence microscopy with antibodies to fibrillar (Fib; *b*, *e'*), nucleolin (*Nuc*; *c*, *e''*) and the 180-kD nucleolar protein (*180K*; *d*). Colocalization of the fibrillar-containing spots with the antinucleolin staining (*e'*, *e''*) is demonstrated by a double-labeling experiment. The larger spots correspond to bodies visible in phase contrast (*e*). Bar, 20 μm .

the sperm nuclei were unable to decondense from elongated into rounded structures (compare Figs. 6, *a* and *b*), most likely due to their inability to import nuclear proteins. We conclude that nuclear uptake and accumulation of maternally stored nucleolar proteins is essential for the formation of larger supramolecular entities.

Discussion

The present study reveals a remarkable feature of *in vitro* assembled nuclei. Irrespective of whether purified DNA or sperm chromatin was added to *Xenopus* egg extract to promote nuclear assembly, the resulting pseudonuclei developed a large number of electron-dense spheroidal structures with diameters ranging from ~ 0.15 to $0.4 \mu\text{m}$ which filled the whole nuclear interior. Clearly, these dense bodies originated *de novo* since they were first detectable, at an incubation time of ~ 45 min, as tiny fluorescent dots which subsequently grew in size. In addition, when WGA was added to

the egg extract in order to reconstitute nuclei lacking pore complexes and hence defective for transport processes (Dabauvalle et al., 1990), dense bodies did not occur. Considered together, these results demonstrate that the dense bodies do not preexist as maternally derived structures and simply become integrated into the nascent nuclei. Rather, their formation requires pore complex-mediated import of the constituent proteins which apparently are stored in the egg in a soluble, depolymerized form (cycloheximide was added to all extract preparations in order to arrest them in interphase; hence new synthesis of proteins during the incubation period is ruled out). Addition of actinomycin D did not inhibit formation of the dense bodies. This result in conjunction with the observation that they occurred in all pseudonuclei irrespective of their genetic content clearly establishes the independence of their formation from specific DNA sequences or transcriptional events.

Our present findings enable us to relate the *in vitro* assembled dense bodies to the PNBs occurring during telophase

Table I. Comparison of Dense Bodies of In Vitro Assembled Nuclei and PNBs of Telophasic Cells as Revealed by Immunofluorescence Microscopy, In Situ Hybridization, and Silver Staining

| Macromolecular component | Nucleolar location | PNBs | Dense bodies* |
|--------------------------------|---|--|---------------|
| Fibrillarin | DFC (Reimer et al., 1987c) | + (Jimenez-Garcia et al., 1989) | + |
| 180-kD protein | DFC (Schmidt-Zachmann et al., 1984) | + | + |
| Nucleolin | DFC+GC (Pfeifle et al., 1986; Biggiogera et al., 1989) | + (Pfeifle et al., 1986; Ochs et al., 1985a) | + |
| B23/NO38 | GC+DFC (Biggiogera et al., 1989) | + (Ochs et al., 1985a; Schmidt-Zachmann et al., 1987) | + |
| RNA polymerase I | FC (Scheer and Rose, 1984) | - (Jimenez-Garcia 1989) | - |
| DNA topoisomerase I | FC (Thiry et al., 1991) | - | - |
| 7-2-RNP | GC (Reimer et al., 1988) | - | - |
| m ³ G-capped snRNAs | DFC+GC+FC (Fischer et al., 1991) | - | ± |
| U3 snRNA | DFC+GC+FC (Fischer et al., 1991) | ND | - |
| rRNA (18+28S sequences) | DFC+GC+FC (Fischer et al., 1991; Puvion-Dutilleul et al., 1991) | ND | - |
| Argyrophilic proteins | DFC+FC (Ploton et al., 1987) | + (Jimenez-Garcia et al., 1989; Ochs et al., 1985a; Ploton et al., 1987) | + |
| U1 snRNP (control) | - | - | - |

* The data refer to dense bodies of pseudonuclei assembled in vitro from bacteriophage lambda DNA or sperm chromatin. DFC, dense fibrillar component; GC, granular component; FC, fibrillar center.

of normal mitotic cells. Both types of nuclear structures are virtually indistinguishable as judged from ultrastructural (for morphological aspects of PNBs see Risueno et al., 1987; Ochs et al., 1985a; Ploton et al., 1987; Benavente et al., 1987) and compositional criteria (Table I). It is notable that, in agreement with their purely fibrillar aspect as seen in electron micrographs, the dense bodies as well as the PNBs contain primarily those proteins that are associated with the dense fibrillar component of functional nucleoli. At first sight, the presence of B23/NO38, a presumed marker protein of the granular component, appears to contradict this notion.

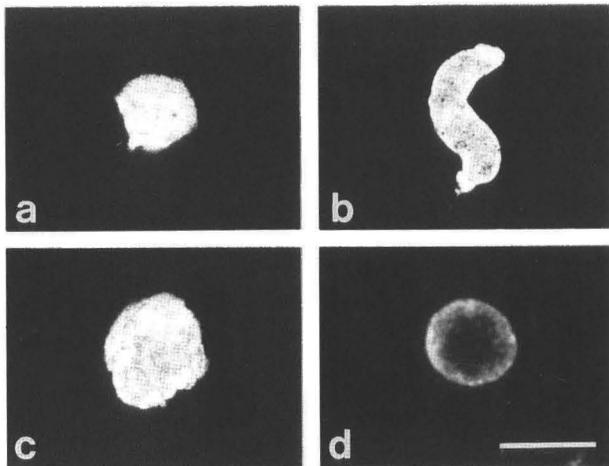


Figure 4. Controls with nonnucleolar antibodies. Nuclei reconstituted from lambda DNA (*a*, *c*, *d*) or sperm chromatin (*b*) were incubated with antibodies against histones (*a*, *b*), DNA (*c*), and lamina proteins (*d*) and visualized by immunofluorescence microscopy. In some preparations the dense bodies are clearly visualized as negative dot-like structures within the fluorescent nucleoplasm (e.g., *b*). Bar, 20 μ m.

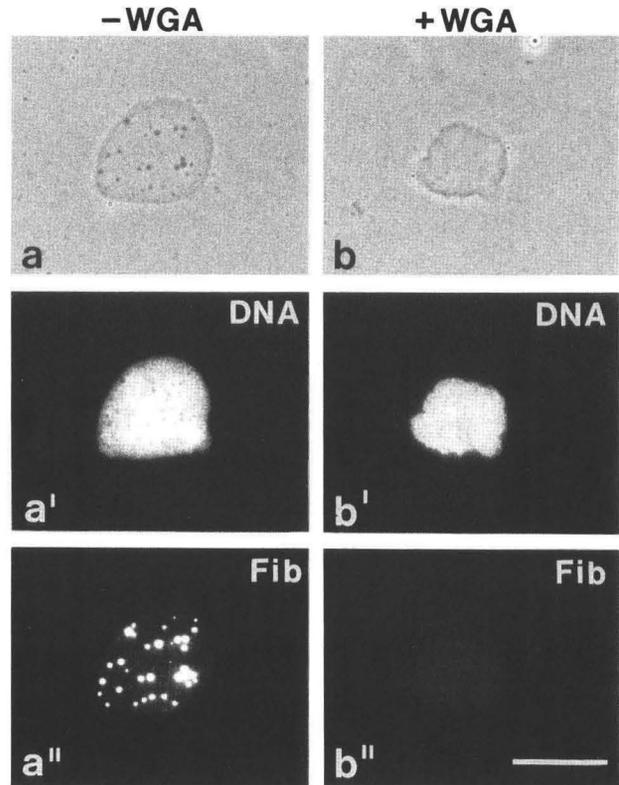


Figure 5. Inhibition of dense body formation in nuclei reconstituted from lambda DNA in the presence of WGA. In controls, i.e., in the absence of WGA (-WGA), numerous dense bodies are present in the newly assembled nuclei as revealed by phase-contrast (*a*) and immunofluorescence microscopy with antibodies against fibrillarin (*Fib*; *a''*). In contrast, in nuclei reconstituted in the presence of WGA (+WGA) dense bodies are not detectable both by phase-contrast (*b*) and immunolabeling with anti-fibrillarin antibodies (*b''*). The corresponding Hoechst fluorescence is shown in the middle panel (*a'*, *b'*). Bar, 20 μ m.

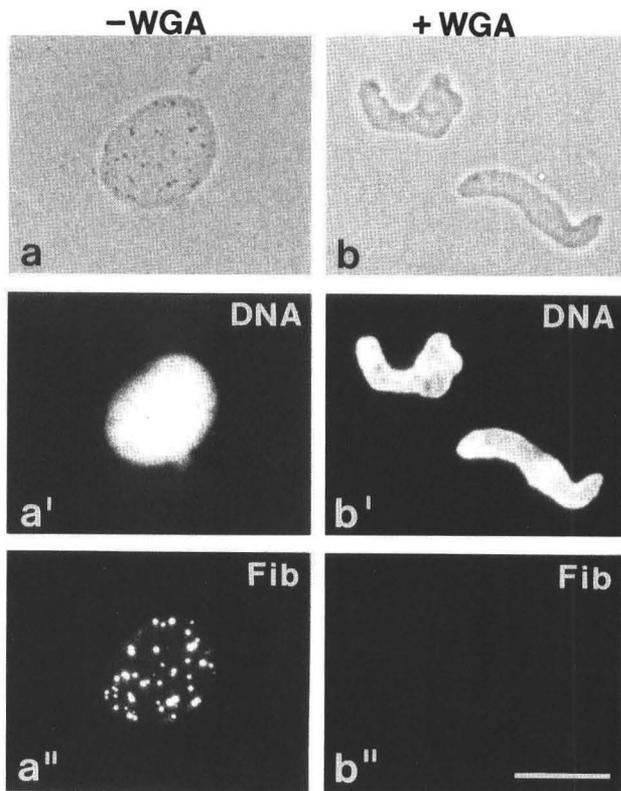


Figure 6. Dense bodies do not form in nuclei reconstituted from sperm chromatin in the presence of WGA. Control nuclei ($-WGA$) contain multiple dense bodies which are clearly visible by phase contrast microscopy (*a*) and immunolabeling with antibodies to fibrillarlin (*Fib*; *a''*). In the presence of WGA ($+WGA$) sperm nuclei are unable to decondense into rounded structures and are devoid of dense bodies as revealed by phase-contrast (*b*) and immunofluorescence microscopy with antifibrillarlin (*b''*). The corresponding Hoechst fluorescence is shown in *a'* and *b'*. Bar, 20 μ m.

However, the relatively weak fluorescence signal of the dense bodies after incubation with antibodies against B23/N038 is explained by the recent finding that this nucleolar protein is not exclusively located in the granular component but to some extent also in the dense fibrillar component (Biggiogera et al., 1989).

In whole cells, PNBs are transitory structures which appear at early telophase and rapidly coalesce around the chromosomal NOR into the developing nucleolar body. While the formation of the PNBs is independent of ongoing protein and RNA synthesis, their subsequent site-specific fusion requires transcriptional activity of the rRNA genes (Benavente et al., 1987; see also De La Torre and Gimenez-Martin, 1982; Ochs et al., 1985a). Thus, the dense bodies dispersed throughout the pseudonuclei might be considered as PNBs unable to fuse into the developing nucleolus due to the absence of a functional organizer, i.e., transcriptionally active rRNA genes. Similar situations are also found in embryos of the anucleolate (0-nu) *Xenopus* mutant which has lost most, if not all, functional rRNA genes (Steele et al., 1984) and in nuclei lacking NORs in so-called micronucleated cells (e.g., Hernandez-Verdun et al., 1979, 1991; Sato, 1988; Benavente et al., 1988). The formation of multiple micronuclei in a single cell can be experimentally induced by prolonged exposure of cell lines to the antimetabolic drug colchi-

cine. Each resulting micronucleus contains a subfraction of the genome, often corresponding to an individual chromosome (Labidi et al., 1987). Only those micronuclei that contain a NOR-bearing chromosome are able to form a normal nucleolus, whereas micronuclei lacking rRNA genes develop multiple fibrillar aggregates with morphological and compositional features similar to those of the dense bodies described in the present study. Thus, fibrillarlin and the 180-kD nucleolar protein have been identified as constituents of the fibrillar aggregates of micronucleated cells but not RNA polymerase I, ribosomal protein S1, and B23/N038 (Benavente et al., 1988; compare Table I). It will be interesting to examine whether other nucleolar proteins described in the fibrillar aggregates (Hernandez-Verdun et al., 1991) are also constituents of the dense bodies of in vitro assembled nuclei.

Based on the association of fibrillarlin with nascent transcripts of the rRNA genes we have recently proposed that the 5'-regions of the growing pre-rRNA molecules are specifically involved in establishing a structural link between the chromosomal NOR and the surrounding dense fibrillar component in intact nucleoli (Scheer and Benavente, 1990). It is tempting to speculate that the site-specific fusion of the PNBs at the NOR is also mediated by the nascent pre-rRNA transcripts which might serve as specific nucleation sites for the coalescence of the PNBs into a continuous layer. The experimental system described here should provide the means to elucidate the molecular mechanisms involved. As a first step it will be necessary to manipulate the egg extract in such a way that rDNA transcription can occur. Experiments in this direction are underway with nuclei assembled from non-nucleolar DNA containing some inserted *Xenopus* rDNA repeats. In this context it is interesting to note that a single rDNA repeat is sufficient to organize a nucleolus (Karpen et al., 1988). In addition, immunodepletion of the extract may lead to the identification of those nucleolar proteins that are essential for PNB formation and interaction with the transcribing rRNA genes.

We are grateful to the following individuals for their kind gifts of antibodies and DNA clones: S. A. Gerbi (U3 clone); G. Krohne (antilamins); R. Lührmann (anti-U1 snRNP and anti-m₃G); J. Pfeife and F. A. Anderer (antinucleolin); G. Reimer (antifibrillarlin, anti-RNA polymerase I, and anti-7-2-RNP); A. Richter (anti-DNA topoisomerase I); D. Rungger (rDNA clones); and M. Schmidt-Zachmann and W. W. Franke (anti-B23/N038 and anti-180-kD protein). We also thank Hilde Merkert for expert help with EM and preparation of the figures and Dagmar Fischer for helpful comments and a critical reading of the manuscript.

This work received financial support from the Deutsche Forschungsgemeinschaft (Sche 157/8-1).

Received for publication 20 March 1992 and in revised form 10 June 1992.

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