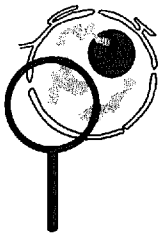
14 LOGOTHETIS, D. E., KURACHI, Y., GALPER, J., NEER, E. J. and CLAPHAM, D. E. (1987) *Nature* 325, 321–326
15 BIRNBAUMER, L. (1987) *Trends Pharmacol. Sci.* 8, 209–211
16 DIETZEL, C. and KURJAN, J. (1987) *Cell* 50, 1001–1010
17 WHITEWAY, M. *et al.* (1989) *Cell* 56, 467–477
18 LEBERER, E., DIGNARD, D., HOUGAN, L., THOMAS, D. Y. and WHITEWAY, M. (1992) *EMBO J.* 11, 4805–4813
19 TANG, W-J. and GILMAN, A. G. (1991) *Science* 254,1500–1503
20 GAO, B. and GILMAN, A. G. (1991) *Proc. Natl Acad. Sci. USA* 88, 10178–10182
21 FEDERMAN, A. D., CONKLIN, B. R., SCHRADER, K. A., REED, R. R. and BOURNE, H. R. (1992) *Nature* 356, 159–161
22 STERNWEIS, P. C. and SMRCKA, A. V. (1992) *Trends Biochem. Sci.* 17, 502–506
23 CAMPS, M., HOU, C. F., SIDIROPOULOS, D., STOCK, J. B., JAKOBS, K. H. and GIERSCHIK, P. (1992) *Eur. J. Biochem.* 206, 821–831
24 SMRCKA, A. V. and STERNWEIS, P. C. *J. Biol. Chem.* (in press)
25 BLANK, J. L., BRATTAIN, K. A. and EXTON, J. H. (1992) *J. Biol. Chem.* 267, 23069–23075
26 HAGA, K. and HAGA, T. (1992) *J. Biol. Chem.* 267, 2222–2227
27 PITCHER, J. A. *et al.* (1992) *Science* 257, 1264–1267
28 LEE, R. H., TING, T. D., LIEBERMAN, B. S., TOBIAS, D. E., LOLLEY, R. N. and HO, Y. K. (1992) *J. Biol. Chem.* 267, 25104–25112
29 BAUER, P. H. *et al.* (1992) *Nature* 358, 73–76
30 LEE, R. H., LIEBERMAN, B. S., YAMANE, H. K., BOK, D. and FUNG, B. K-K. (1992) *J. Biol. Chem.* 267, 24776–24781
31 SCHMIDT, C. J., THOMAS, T. C., LEVINE, M. A. and NEER, E. J. (1992) *J. Biol. Chem.* 267, 13807–13810
32 FAWZI, A. B., FAY, D. S., MURPHY, E. A., TAMIR, H., ERDOS, J. J. and NORTHUP, J. K. (1991) *J. Biol. Chem.* 266, 12194–12200
33 LAW, S. F., MANNING, D. and REISINE, T. (1991) *J. Biol. Chem.* 266, 17885–17897
34 KLEUSS, C., HESCHELER, J., EWEL, C., ROSENTHAL, W., SCHULTZ, G. and WITTIG, B. (1991) *Nature* 353, 43–48
35 KLEUSS, C., SCHERUBL, H., HESCHELER, J., SCHULTZ, G. and WITTIG, B. (1992) *Nature* 358, 424–426
36 KLEUSS, C., SCHERUBL, H., HESCHELER, J., SCHULTZ, G. and WITTIG, B. (1993) *Science* 259, 832–834
37 BERSTEIN, G., BLANK, J. L., JHON, D. Y., EXTON, J. H., RHEE, S. G. and ROSS, E. M. (1992) *Cell* 70, 411–418
38 DETERRE, P. and PFISTER, C. (1992) *J. Biol. Chem.* 267, 22018–22021
39 BREITWIESER, G. E. and SZABO, G. (1988) *J. Gen. Physiol.* 91, 469–493



Structure, function and assembly of the nucleolus

Ulrich Scheer, Marc Thiry and Guy Goessens

Most events of ribosome biogenesis – such as transcription of the ribosomal RNA (rRNA) genes, processing of their primary transcripts into mature rRNAs and assembly with ribosomal and nonribosomal proteins to form the preribosomes – are confined to a special nuclear compartment, the nucleolus. Immunogold labelling and in situ hybridization at the ultrastructural level are providing novel insights into structure–function relationships of the nucleolus, and in vitro systems are beginning to shed light on the molecular mechanisms involved in the reforming of nucleoli after mitosis.

Nucleoli, the sites of ribosome biogenesis, provide the most compelling evidence that there is order in the cell nucleus. The genes coding for ribosomal RNA (rRNA) and the transcription products of these genes are not randomly and diffusely distributed throughout the interphase nucleus, but instead are concentrated in a specific nuclear domain occupied by the nucleolus. Although extranucleolar genes and their RNA transcripts may also be localized to specific nuclear territories, nucleoli are a particularly good model for correlating molecular aspects of gene expression with nuclear structures.

A nucleolus is often regarded as a cluster of transcriptionally active rRNA genes surrounded by a cloud of densely packed transcription products in the form of preribosomal particles at various stages of their maturation pathway. According to this view, nucleolar structures are generated from rRNA-gene transcription units and locally accumulated products of transcription and ribosome assembly. However, this model ignores structural elements within nucleoli such as high-salt- and detergent-resistant filaments that might be involved in the functional compartmentalization of the nucleolus (see Ref. 1 for a review). Furthermore, it is hardly compatible with the well-known fact that inhibition of transcription by drugs such as actinomycin D does not lead to the disappearance of nucleoli. Under such conditions the nucleolar components lose most, if not all, resident pre-rRNAs and yet retain their characteristic ultrastructural features[2]. This observation underlines the importance of a structured framework as a primary determinant of nucleolar architecture and morphology. At first sight, the dynamic changes of the nucleolus accompanying mitosis, i.e. disassembly at prophase and reassembly at telophase, seem to argue against the presence of nucleolar structural elements. However, the fate of the nuclear lamina during mitosis clearly illustrates the cell's ability to induce extensive and rapid structural changes of karyoskeletal elements, probably by protein phosphorylation.

Various aspects of rRNA gene transcription, processing of the primary transcripts into the mature rRNAs, and assembly of preribosomes have been reviewed recently[3–5]. Both pre-mRNA processing events and pre-rRNA maturation appear to be directed by small nuclear ribonucleoproteins (snRNPs), in all the species investigated from yeast to mammals[6–8]. The most abundant nucleolar snRNP contains U3 RNA and several proteins, including the evolutionarily highly conserved protein fibrillarin. How the processes of ribosome biogenesis may be linked to nucleolar structures has been examined in a number of recent articles[9–14]. At present, there is considerable controversy over the location of the rRNA genes within the nucleolar body (see below). In addition to ribosomal proteins, nucleoli contain a large number of nonribosomal proteins involved in various aspects of ribosome biogenesis and maintenance of nucleolar structure. Some of them have been characterized in detail and their biochemical properties and potential functions summarized[15]. The purpose of this review is to provide, against this background, a current view of the functional significance of the various nucleolar components during interphase, and their interactions during postmitotic formation of nucleoli, a process called nucleologenesis.

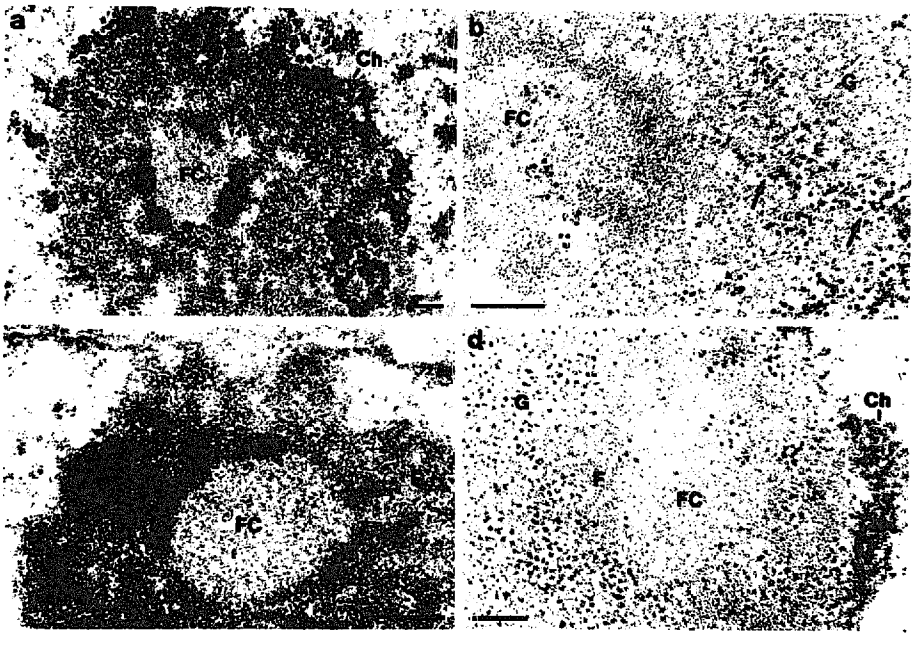


FIGURE 1

(a) The characteristic ultrastructural features of a nucleolus from a cultured human HEp-2 cell. Condensed chromatin (Ch) is visible around and within the nucleolus. The dense fibrillar component (F) is interrupted by an interstice in which chromatin (arrow) approaches the fibrillar centre (FC). G, granular component. (b) Detection of DNA by the terminal transferase assay in a HEp-2 nucleolus[22]. Strands of condensed chromatin, heavily labelled with gold particles (arrows), run through the granular component (G) towards the dense fibrillar component (F). Numerous gold particles are seen over the fibrillar centre (FC). (c) Immunogold–EM localization of the rDNA transcription factor UBF in the nucleolus of a mouse 3T3 cell (Lowicryl section). Antibodies raised in guinea pigs against purified mouse UBF were kindly provided by I. Grummt (Heidelberg). The fibrillar centre (FC) is selectively labelled. (d) Intranucleolar distribution of RNA in an Ehrlich tumour cell as visualized with the polyadenylate nucleotidyl transferase method[31]. Gold particles are present in all regions of the nucleolus, including the fibrillar centre (FC). Bars, 0.2 μm.

Architecture of the interphase nucleolus

When observed with the electron microscope (EM), nucleoli exhibit (with some exceptions) a strikingly similar organization from protists to humans. Generally, three morphologically distinct nucleolar components are recognized (Fig. 1a)[9,16]. The bulk of the nucleolus in metabolically active cells is made of particles resembling ribosomes, and is hence termed the granular component (GC). Within the granular mass are one or several rounded regions of low electron density, the fibrillar centres (FC). They are surrounded by a compact layer of densely staining fibrous material, the dense fibrillar component (DFC), which is especially prominent in plant cells. Nucleoli are generally surrounded by a shell of condensed nucleolus-associated chromatin which, in certain places, penetrates deeply into the nucleolar body through nucleolar interstices and reaches the FCs. Although in sections these nucleolar invaginations often appear as separate islands of intranucleolar chromatin, or interstices containing some chromatin strands, together they establish a structural continuity between the perinucleolar chromatin and the FCs[16]. Chromatin-filled interstices are often in direct contact with the surface of FCs, where they interrupt the layer of the DFC (Fig. 1a). As discussed below, highly decondensed chromatin fibres extend from these interstices into the FCs, where they can be traced only by specific detection methods in the EM.

How are these nucleolar structures linked to transcription of the rRNA genes, processing of the primary transcripts into mature 28S, 18S and 5.8S rRNAs, and assembly with ribosomal and nonribosomal proteins into preribosomal subunits? It is generally thought that ribosome biogenesis is a vectorial process, which may be envisaged as an assembly line, beginning in the fibrillar part and continuing into the surrounding granular portion of the nucleolus.

Tracing the rRNA genes

There is considerable disagreement over the exact intranucleolar location of the transcribing rRNA genes[17,18]. Several experimental strategies have been developed to approach this issue, which is of

Ulrich Scheer is at the Department of Cell and Developmental Biology, Theodor-Boveri-Institute, University of Würzburg, Am Hubland, D-8700 Würzburg, Germany; and Marc Thiry and Guy Goessens are at the Laboratory of Cell and Tissue Biology, University of Liège, rue de Pitteurs 20, B-4020 Liège, Belgium.

paramount importance for understanding nucleolar architecture. EM autoradiographic[16], cytochemical[19,20] and immunocytochemical studies[12,20–22] have clearly established the presence of DNA in the FCs of both plant and animal nucleoli. An extremely useful method for detecting DNA in ultrathin sections of conventionally fixed material, which combines high sensitivity with optimal structural preservation, exploits the ability of terminal deoxynucleotidyl transferase to add nucleotides to the free ends of DNA that are generated at the surface of the sections (Fig. 1b)[22]. To identify the intranucleolar DNA, nonradioactive *in situ* hybridizations for the EM have been performed by several groups. Some authors have concluded that the rRNA genes are located exclusively in the FCs and in some masses of intranucleolar condensed chromatin[2,23–25]; others have located the majority of the rRNA genes in the surrounding DFC[14,26]. As discussed elsewhere[18], the latter view most likely stems from an erroneous identification of the chromatin-containing interstices at the periphery of the FCs as part of the DFC. In fact, those interstices in close contact with the surface of the FCs may contain nontranscribed spacer sequences separating the tandemly repeated rRNA genes[24], or even longer stretches of transcriptionally silent rDNA. Their striking association with the periphery of the FCs suggests that the transition from compacted and hence transcriptionally inactive chromatin into the extended chromatin of the FCs takes place here[12,16].

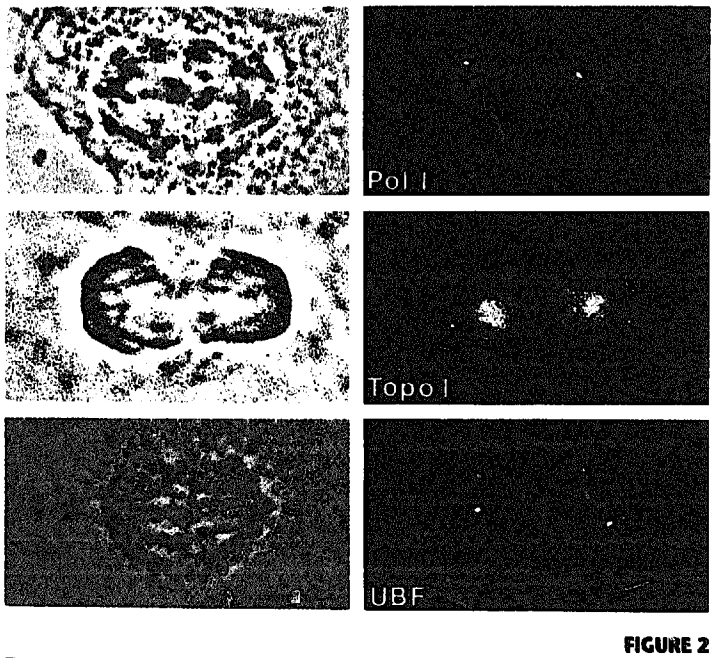


FIGURE 2

The chromosomal nucleolus-organizing regions (NORs) contain pol I, topo I and the transcription factor UBF, as shown by immunofluorescence microscopy of mitotic PtK2 cells (these cells, derived from a male kangaroo rat, have only one NOR located on the X chromosome). Corresponding phase-contrast images are shown in the left panel. Bar, 10 μm.

FCs contain not only rDNA but also enzymes essential for transcription of the rRNA genes, notably RNA polymerase I (pol I)[12,21,27] and DNA topoisomerase I (topo I)[12,21]. It has been proposed that the FCs merely store these enzymes, and that transcription actually takes place in the surrounding DFC[14,17]. However, the reported absence of a nuclear pool of free pol I molecules[28] clearly contradicts this view. Furthermore, the mouse nucleolar upstream binding factor (mUBF), a transcription factor required for the formation of stable initiation complexes by pol I (Ref. 29), is also enriched in the FC and absent in significant amounts from other regions of the nucleolus (Fig. 1c).

Earlier EM autoradiographic studies of cells pulse-labelled with ^{3}H-labelled uridine revealed preferential incorporation of ^{3}H into the DFC. This result is usually taken as evidence for the origin of nascent rRNA in this nucleolar component[9,14,17]. However, after prolonged exposure of autoradiographs the FCs also appear labelled[30]. Hence the autoradiographic data are fully compatible with the view that pre-RNAs are synthesized in the FCs and accumulate transiently in the surrounding DFC. Further support for this notion comes from the demonstration of RNA and, more specifically, rRNA in the FCs by the polyadenylate transferase immunogold technique (Fig. 1d), immunolabelling with anti-RNA antibodies, and *in situ* hybridization[31].

Taken together, all the available evidence points to the nucleolar FCs as the exclusive sites of the transcriptionally active rRNA genes. They must be extremely densely packed since a single rDNA transcription unit, as seen in Miller-type spread preparations, is as long as the entire nucleolus[12]. This high packing density may provide a mechanism for bringing termination and initiation sites of the rRNA genes close together, thus maximizing transcriptional activity[32].

It is tempting to speculate that a major function of the DFC is to establish a boundary around the FC that keeps the active rRNA genes together with the factors necessary for their transcription in this compartment. The DFC is not simply generated by the aggregation of transcription products but rather represents a distinct structure. Thus, when rRNA gene transcription is selectively inhibited by microinjection of antibodies to pol I, the DFC abandons the nucleolus and emerges in the nucleoplasm in the form of numerous dense fibrillar bodies[33]. This behaviour illustrates that ongoing transcription of the rRNA genes is required to maintain the association of the DFC with the surface of the FCs.

Relating ribosome production to nucleolar structures

In situ hybridization studies in the EM, using appropriate hybridization probes, have made it possible to link each step of the pre-rRNA maturation pathway with nucleolar structures. However, results obtained with probes directed against transcribed spacer sequences should be interpreted

with some reservation since it is difficult to assess whether the observed labelling reflects the distribution of pre-rRNAs or the excised fragments that might accumulate to some extent before being degraded. After their release from the template, the pre-rRNA transcripts transiently accumulate in the DFC, where primary processing events are thought to occur, before they pass into the surrounding GC[2,13]. The location of U3 snRNA in the DFC as well as the GC[13,34] is consistent with its proposed role in early and late processing steps[6,7]. Although major proteins of the DFC such as nucleolin, fibrillarin and a phosphoprotein of 140 kDa (nopp140) have been sequenced[35–40], their precise functions remain to be explored. Nucleolin is associated (at least partly) with preribosomes[15]; fibrillarin may play a role in pre-rRNA processing and the structural organization of the nucleolus[38]. A protein of 180 kDa is thought to contribute to the general structure of the DFC[41].

The GC contains preribosomal particles in later stages of the maturation pathway[9]. Although it is generally believed that the granular appearance of this nucleolar component reflects the presence of preribosomal particles, recent results[2] raise some doubts about this correlation. When transcription is blocked by actinomycin D, most of the pre-rRNA molecules leave the nucleolus but neither the granular component nor the DFC is lost. Hence it is conceivable that the granules form part of a structural framework of the nucleolus, providing support to preribosomes. Most of the ribosomal proteins are associated with maturing preribosomal subunits as well as some nonribosomal proteins, notably B23/NO38, whose primary structure has been determined[15,42,43]. It is striking that not only B23/NO38 but also nucleolin and nopp140 shuttle between the nucleus and cytoplasm, and hence may play a role in nucleocytoplasmic transport processes[40,43]. Now that antibodies against other ribosomal and nonribosomal proteins are available, a clearer picture of the order of protein addition during ribosome assembly should emerge in the near future.

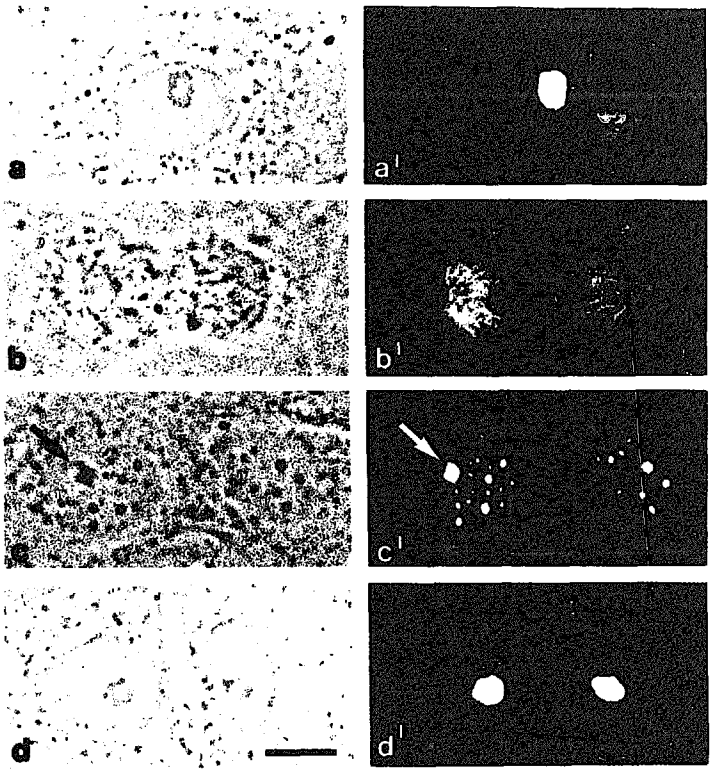


FIGURE 3

Distribution of fibrillarin during mitosis of cultured PtK2 cells as revealed by immunofluorescence microscopy (a'–d'). Corresponding phase-contrast images are also shown (a–d). In interphase cells (a,d), fibrillarin is localized to the nucleoli. In metaphase and anaphase (b), fibrillarin uniformly coats the chromosome surfaces. In late telophase (c), the reforming nucleoli are stained (arrows) as well as numerous prenucleolar bodies. Bar, 10 μm.

The chromosomal nucleolus organizer

When cells of higher eukaryotes enter mitosis, the nucleoli disintegrate in various stages. The first component to disappear is the DFC, and this is followed by disappearance of the GC. Throughout mitosis the chromosomal nucleolus-organizing region (NOR), which harbours the tandemly repeated rRNA genes and reveals a striking morphological similarity to the FCs, persists[16]. The reformation of nucleoli at telophase follows the reverse order, i.e. the DFC forms around the NORs before the appearance of the GC[16]. The persistent association of pol I, topo I and the transcription factor UBF with the chromosomal NORs during mitosis (Fig. 2) indicates that NORs and nucleolar FCs are equivalent structures, thus confirming earlier conclusions based on morphological similarities and the presence of silver-binding proteins[16]. There have been reports that the DFC proteins nucleolin, fibrillarin and nopp140 are present in chromosomal NORs[15,44]. However, recent studies have failed to reproduce these observations (P. Bell and U. Scheer, unpublished).

The absence of rRNA synthesis during mitosis, despite the continued presence of the transcriptional machinery, represents an extremely interesting case of gene regulation. Since the NOR-bound pol I can be reactivated by heparin, the enzyme apparently exists as an initiated complex with rDNA during mitosis[45]. At present the mechanisms that suppress or retard transcription of the rRNA genes during the mitotic block, and whether the nascent transcripts are actually shed from the template, are not known. The mitotic inhibition of rRNA synthesis may involve the recently identified pol I inhibitory activity in growth-arrested mouse cells; this factor does not affect the preinitiation complex, but inhibits later stages of rDNA transcription[46].

Postmitotic nucleolar assembly

Nucleolar reformation begins at telophase with the formation of numerous prenucleolar bodies (PNBs) that contain several proteins characteristic of the DFC in functional nucleoli (see Ref. 47 for a

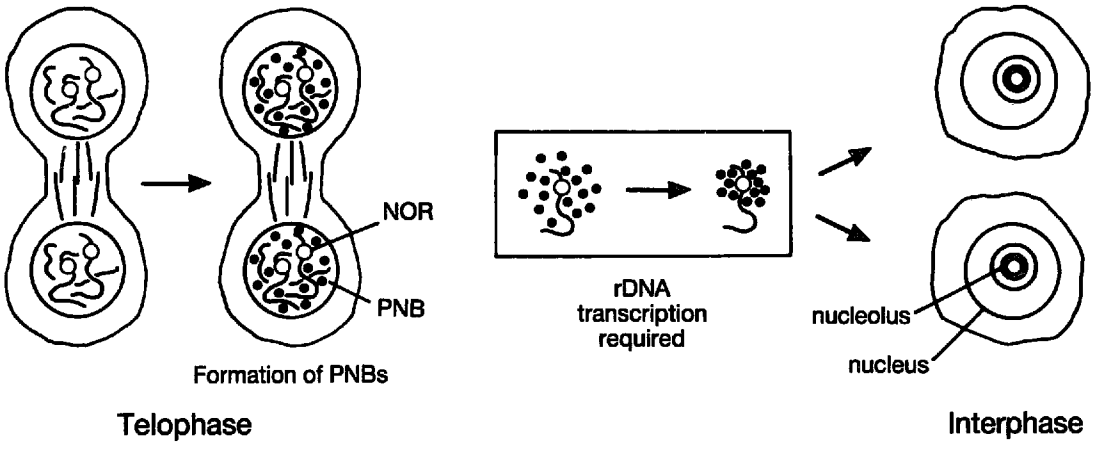


FIGURE 4

Schematic view of postmitotic nucleologenesis. Nucleolar reformation begins at telophase with the aggregation of nucleolar material, derived from the mother cell, into numerous prenucleolar bodies (PNBs). Subsequently the PNBs migrate towards the nucleolus-organizing region (NOR) where they eventually fuse to become the dense fibrillar component of the developing nucleolus. The site-specific fusion of the preformed nucleolar entities around the NOR requires transcriptional activity of the rRNA genes (boxed).

compilation of data). Figure 3 illustrates the distribution of one of these proteins, fibrillarin, during mitosis as seen by immunofluorescence microscopy. At metaphase and anaphase fibrillarin is uniformly associated with the surface of all chromosomes. At telophase, however, it appears in numerous PNBs. In a subsequent step these PNBs then coalesce around the chromosomal NOR(s) into the developing nucleolar body as illustrated schematically in Fig. 4. This site-specific fusion of the PNBs requires transcriptional activity of the rRNA genes. Exposure of cells to actinomycin D[48] or the topo-I-specific inhibitor campthotecin[49], or microinjection of antibodies to pol I into mitotic cells[50], prevents the fusion of PNBs, which then remain dispersed throughout the nucleus (for similar results with plant cells see Ref. 51).

How are PNBs targeted to the NOR and induced to fuse and to become the DFC? Is the ability of the NOR to serve as a nucleation site in this assembly process an intrinsic property of the rDNA-containing chromatin or a property of the nascent transcripts? In a revealing experiment Karpen *et al.*[52] demonstrated that a single rRNA gene inserted randomly into the genome of *Drosophila* is sufficient to organize a functional nucleolus. Based on the association of fibrillarin with the free 5'-ends of nascent transcripts of the rRNA genes it was proposed that they may directly interact with the PNBs during nucleologenesis[12]. However, a truncated rRNA gene consisting of the promotor region, the 5'-external transcribed spacer and the adjacent 18S sequences failed to organize a nucleolus[53], indicating that nucleolar formation also requires the 28S sequences.

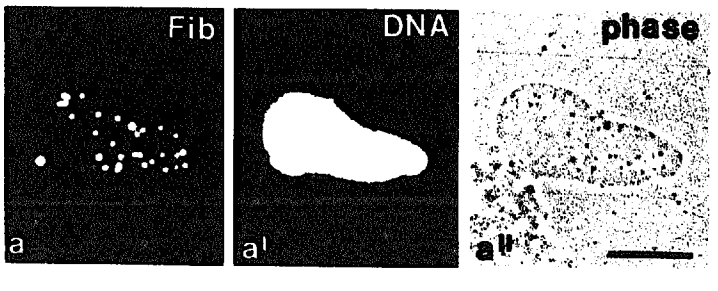


FIGURE 5

Nuclei reconstituted from lambda DNA in a *Xenopus* egg extract contain PNB-like bodies as revealed by immunofluorescence using antibodies against fibrillarin (a) and by phase-contrast microscopy (a''). The corresponding DNA-specific Hoechst fluorescence is also shown (a'). For details see Ref. 47. Bar, 20 μm.

Early steps of nucleolar assembly *in vitro*

Elucidation of the molecular mechanisms involved in nucleologenesis would be greatly facilitated by the availability of a cell-free system capable of reproducing this process. A first step in this direction was taken recently with the observation that nuclei assembled in *Xenopus* egg extract from purified DNA contain numerous distinct aggregates that are indistinguishable from authentic PNBs by ultrastructural and compositional criteria[47] (an example is shown in Fig. 5). Removal of specific nucleolar proteins from the egg extract by immunodepletion showed that assembly of the PNB-like structures requires nucleolin and the 180 kDa protein[41] present in the DFC of *Xenopus* nucleoli, but is independent of fibrillarin (P. Bell, 1993, PhD thesis, University of Würzburg). So far, this experimental system allows the reproduction of the early steps of nucleologenesis up to the PNB stage. By reconstitution of nuclei around rDNA it should be feasible to establish conditions promoting the fusion of the pre-assembled PNBs into a coherent structure and to decipher the molecular inter-

actions that take place between PNBs and transcribing rRNA genes during postmitotic nucleolar reformation.

References

1 BENAVENTE, R., KROHNE, G., SCHMIDT-ZACHMANN, M., HÜGLE, B. and FRANKE, W. W. (1984) *J. Cell Sci. Suppl.* 1, 161–186
2 PUVION-DUTILLEUL, F., MAZAN, S., NICOLOSO, M., PICHARD, E., BACHELLERIE, J-P. and PICHARD, E. (1992) *Eur. J. Cell Biol.* 58, 149–162
3 GERBI, S., SAVINO, R., STEBBINS-BOAZ, B., JEPPESEN, C. and RIVERA-LEO N, R. (1990) in *The Ribosome – Structure, Function and Evolution* (Hill, W. E. *et al.*, eds), pp. 452–469, American Society for Microbiology
4 REEDER, R. H. (1990) *Trends Genet.* 6, 390–395
5 SOLLNER-WEBB, B. and MOUGEY, E. B. (1991) *Trends Biochem. Sci.* 16, 58–62
6 KASS, S., KAZIMIERZ, T., STEITZ, J. A. and SOLLNER-WEBB, B. (1990) *Cell* 60, 897–908
7 SAVINO, R. and GERBI, S. A. (1990) *EMBO J.* 9, 2299–2308
8 FOURNIER, M. J. and MAXWELL, E. S. (1993) *Trends Biochem. Sci.* 18, 131–135
9 HADJIOLOV, A. A. (1985) *The Nucleolus and Ribosome Biogenesis*, Springer Verlag
10 SOMMERVILLE, J. (1986) *Trends Biochem. Sci.* 11, 438–442
11 WARNER, J. R. (1990) *Curr. Opin. Cell Biol.* 2, 521–527
12 SCHEER, U. and BENAVENTE, R. (1990) *BioEssays* 12, 14–21
13 FISCHER, D., WEISENBERGER, D. and SCHEER, U. (1991) *Chromosoma* 101, 133–140
14 SCHWARZACHER, H. G. and WACHTLER, F. (1991) *Ann. Génét.* 34, 151–160
15 OLSON, M. O. J. (1990) in *The Eukaryotic Nucleus, Vol. 2* (Strauss, P. R. and Wilson, S. H., eds), pp. 519–559, The Telford Press
16 GOESSENS, G. (1984) *Int. Rev. Cytol.* 87, 107–158
17 JORDAN, E. G. (1991) *J. Cell Sci.* 98, 437–442
18 THIRY, M. and GOESSENS, M. (1992) *Exp. Cell Res.* 200, 1–4
19 DERENZINI, M., THIRY, M. and GOESSENS, M. (1990) *J. Histochem. Cytochem.* 38, 1237–1256
20 MOTTE, P. M., LOPPES, R., MENAGER, M. and DELTOUR, R. (1991) *J. Histochem. Cytochem.* 39, 1495–1506
21 THIRY, M., SCHEER, U. and GOESSENS, G. (1991) *Electron Microsc. Rev.* 4, 85–110
22 THIRY, M., PLOTON, D., MENAGER, M. and GOESSENS, G. (1993) *Cell Tissue Res.* 271, 33–45
23 THIRY, M. and THIRY-BLAISE, L. (1989) *Eur. J. Cell Biol.* 50, 235–243
24 THIRY, M. and THIRY-BLAISE, L. (1991) *Nucleic Acids Res.* 19, 11–15
25 PUVION-DUTILLEUL, F., BACHELLERIE, J-P. and PUVION, E. (1991) *Chromosoma* 100, 395–409
26 WACHTLER, F. *et al.* (1992) *Exp. Cell Res.* 198, 135–143
27 SCHEER, U. and ROSE, K. M. (1984) *Proc. Natl Acad. Sci. USA* 81, 1431–1435
28 HALLECK, M. S., SCHLEGEL, R. A. and ROSE, K. M. (1989) *J. Cell Sci.* 92, 101–109
29 VOIT, R. *et al.* (1992) *EMBO J.* 11, 2211–2218
30 THIRY, M. and GOESSENS, G. (1991) *J. Cell Sci.* 99, 759–767
31 THIRY, M. (1992) *Nucleic Acids Res.* 20, 6195–6200
32 JOHNSON, S. P. and WARNER, J. R. (1989) *Mol. Cell. Biol.* 9, 4986–4993
33 BENAVENTE, R., REIMER, G., ROSE, K. M., HÜGLE-DÖRR, B. and SCHEER, U. (1988) *Chromosoma* 97, 115–123
34 PUVION-DUTILLEUL, F., MAZAN, S., NICOLOSO, M., CHRISTENSEN, M. E. and BACHELLERIE, J-P. (1991) *Eur. J. Cell Biol.* 56, 178–186
35 LAPEYRE, B., BOURBON, H. and AMALRIC, F. (1987) *Proc. Natl Acad. Sci. USA* 84, 1472–1476
36 LAPEYRE, B. *et al.* (1990) *Mol. Cell. Biol.* 10, 430–434
37 CAIZERGUES-FERRER, M. *et al.* (1989) *Genes Dev.* 3, 324–333
38 JANSEN, R. P. *et al.* (1991) *J. Cell Biol.* 113, 715–729
39 ARIS, J. P. and BLOBEL, G. (1991) *Proc. Natl Acad. Sci. USA* 88, 931–935
40 MEIER, U. T. and BLOBEL, G. (1992) *Cell* 70, 127–138
41 SCHMIDT-ZACHMANN, M., HÜGLE, B., SCHEER, U. and FRANKE, W. W. (1984) *Exp. Cell Res.* 153, 327–346
42 SCHMIDT-ZACHMANN, M., HÜGLE-DÖRR, B., SCHEER, U. and FRANKE, W. W. (1987) *EMBO J.* 6, 1881–1890
43 BORER, R. A., LEHNER, C. F., EPPENBERGER, H. M. and NIGG, E. A. (1989) *Cell* 56, 379–390
44 MEIER, U. T. and BLOBEL, G. (1990) *J. Cell Biol.* 111, 2235–2245
45 MATSUI, S. and SANDBERG, A. A. (1985) *Chromosoma* 92, 1–6
46 KERMEKCHIEV, M. and MURAMATSU, M. (1993) *Nucleic Acids Res.* 21, 447–453
47 BELL, P., DABAUVALLE, M-C. and SCHEER, U. (1992) *J. Cell Biol.* 118, 1297–1304
48 OCHS, R. L., LISCHWE, M. A., SHEN, E., CARROLL, R. E. and BUSCH, H. (1985) *Chromosoma* 92, 330–336
49 WEISENBERGER, D., SCHEER, U. and BENAVENTE, R. *Eur. J. Cell Biol.* (in press)
50 BENAVENTE, R., ROSE, K. M., REIMER, H., HÜGLE-DÖRR, B. and SCHEER, U. (1987) *J. Cell Biol.* 105, 1483–1491
51 DE LA TORRE, C. and GIMENEZ-MARTIN, G. (1982) in *The Nucleolus* (Jordan, E. G. and Cullis, C. A., eds), pp. 153–177, Cambridge University Press
52 KARPEN, G. H., SCHAEFER, J. E. and LAIRD, C. D. (1988) *Genes Dev.* 2, 1745–1763
53 MCKEE, B. D., HABERA, L. and VRANA, J. A. (1992) *Genetics* 132, 529–544

Acknowledgements

We thank I. Grummt, U. T. Meyer and G. Reimer for antibodies; H. Merkert and F. Skivée for skilful technical assistance; and R. Benavente and P. Bell for helpful discussions. Work in the authors' laboratories was supported by grants from the Deutsche Forschungsgemeinschaft (Sche 157/8-1), the Fonds de la Recherche Scientifique Médicale (3.4512.90) and the Actions de Recherche Concertées (91/95-152).

The next article in the series on nuclear structures involved in RNA biogenesis is scheduled to appear in the September issue of *TCB*.

REVIEWS

Reviews in *Trends in Cell Biology* aim to provide objective, authoritative and concise overviews of recent progress in a research area of broad interest. Review articles are usually commissioned by the Editor. The submission of completed reviews without prior consultation is discouraged. All reviews are sent to recognized specialists for refereeing and are subject to stringent editing before being accepted for publication: commissioning does not guarantee publication.