The nucleolus

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The transcriptionally active rRNA genes have the remarkable ability to organize and integrate the biochemical pathway of ribosome production into a structural framework, the nucleolus. The past year has seen numerous advances in our understanding of the relationships between nucleolar substructures, the site of ribosomal RNA (rRNA) gene transcription and the pathway of ribosome maturation. Progress has also been made both in the molecular identification of nucleolar constituents and in our understanding of the interactions between these components and their assembly into higher order structures.

Current Opinion in Cell Biology 1994, **6**:354–359

Introduction

Nucleoli are readily visualized by light and electron microscopy as solitary or multiple non-membranous nuclear structures that occupy a substantial portion of the total nuclear interior (Fig. 1). Within nucleoli takes place the production of pre-ribosomal components, including the transcription of the rRNA genes, the processing of the primary transcripts into mature 18S, 5.8S and 28S rRNAs, the addition of proteins to the nascent pre-ribosomes, and the incorporation of the 5S RNA, which is synthesized outside of the nucleolus [1]. In addition to the rRNA gene clusters and flanking sequences, the nucleolus harbors a large number of protein and RNA components which are not part of mature cytoplasmic ribosomes, but rather are involved in the transcriptional [2,3] and post-transcriptional [4,5] regulation of ribosome synthesis. Among these are small nucleolar RNAs (snoRNAs; [6,7]), non-ribosomal proteins that may be components of nucleolar structural elements, proteins that are transiently associated with pre-ribosomes, and nucleocytoplasmic shuttle proteins.

Nucleoli undergo extensive structural changes during the cell cycle of higher eukaryotes; they disappear at prophase and reappear at telophase. This dynamic behavior is useful in the analysis of how the various nucleolar components interact with one another to build the intricate structure of the nucleolus. Recent reviews have summarized the organization of the various nucleolar structures and their possible functional links to ribosome biosynthesis (e.g. [1,4,8–14]). In this review we discuss some recent progress in our understanding of nucleolar architecture, its assembly and its relationship to nucleolar function as reported in the preceding year.

Molecular architecture of the nucleolus

Most nucleoli display a concentric arrangement of three structural components (Fig. 1). The central element is a pale-staining region, the fibrillar center, which contains RNA polymerase I, DNA topoisomerase I, and the transcription factor UBF [13,15,16]. Fibrillar centers are surrounded, either wholly or in part, by a compact layer termed the dense fibrillar component. A major constituent of this region is fibrillarin, a protein associated with several snoRNAs, and involved in the post-transcriptional assembly of pre-ribosomes [17••]. The outermost layer has a grainy appearance and is hence termed the granular component. Pulse-chase labeling and *in situ* hybridization experiments have clearly demonstrated that pre-ribosome biogenesis is a vectorial process. Nascent pre-ribosomes move from the fibrillar area to the granular component of the nucleolus, and are then released into the nucleoplasm as nearly mature ribosomal subunits (e.g. [1,11,18]). It is still not clear, however, whether the various events in ribosome maturation can be strictly assigned to particular nucleolar structures. The nucleolus may contain either a number of spatially fixed 'stations' where specific events of pre-rRNA processing and/or assembly take place as the pre-ribosomes pass by, or alternatively, each transcript may carry its own processing machinery that functions independently of its specific position within the nucleolus.

The intranucleolar site of ribosomal DNA (rDNA) transcription is still under serious debate (for possible models, see [10]). By using a variety of cytochemical and immunocytochemical approaches, rDNA has been localized either to the fibrillar centers exclusively [19••,20,21], to the fibrillar center and the proximal regions of the surrounding dense fibrillar component

Abbreviations

NLS—nuclear localization signal; **NOR**—nucleolus organizer region; **PNB**—pre-nucleolar body; **rDNA**—ribosomal DNA; **rRNA**—ribosomal RNA; **snoRNA**—small nucleolar RNA;

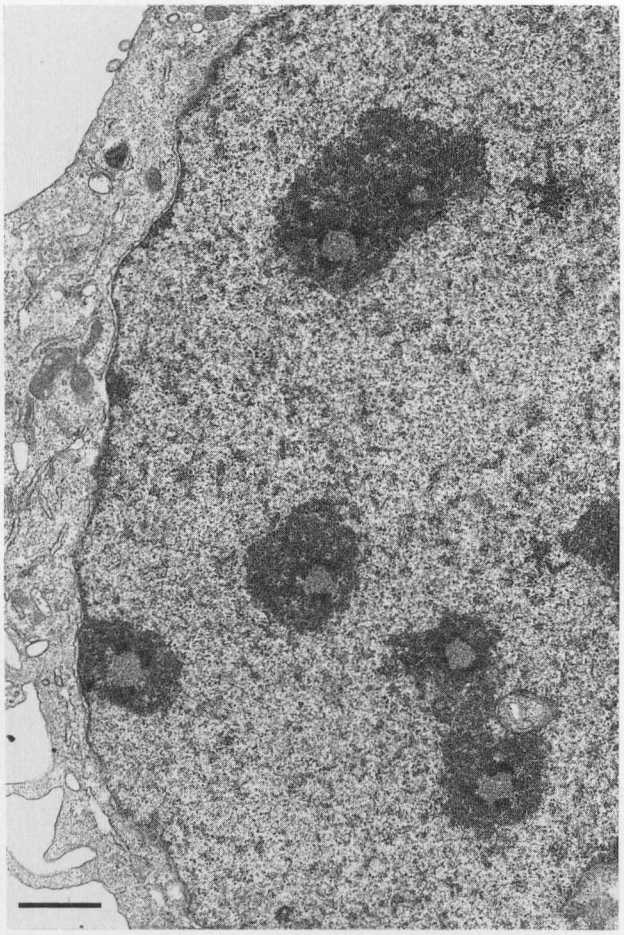

Fig. 1. Electron micrograph of a human bladder carcinoma cell. Each of the multiple nucleoli is subdivided into three components described in the text. Embedded in the main body of the nucleolus (the granular component) are rounded structures of low contrast, the fibrillar centers. These are surrounded by a compact layer of the dense fibrillar component. Bar indicates 1 μm.

[22–24], or predominantly to the dense fibrillar component [25]. To settle the present controversy about where the rDNA is located, improved DNA localization methods are required which combine high sensitivity with optimal preservation of biological structures (see [19••]).

Using an alternative approach, results from the incorporation of UTP analogs followed by immunogold electron microscopy have suggested that rRNA gene transcription takes place at the boundary between the fibrillar center and the dense fibrillar component [26•,27•]. According to the model proposed by Hozák *et al.* [27•], the rDNA transcription units are attached through active RNA polymerases to the surface of the fibrillar centers, which serve both as the central skeletal element of the nucleolus and as a storage site for disengaged components of the transcription machinery, such as RNA polymerase I and DNA topoisomerase I. This view emphasizes the importance of the boundary region between the fibrillar center and the dense fibrillar component, and importantly, is reconcilable with most previous localization studies.

'Christmas trees' and nucleolar structure

Nascent transcripts are anchored by closely spaced 10–14 nm particles containing RNA polymerase I to the chromatin axes of rRNA genes, and their free 5′-ends carry an approximately 25 nm thick granule, the 'terminal ball'. These morphological features are readily seen in Miller-type spread preparations, even when the transcribing rRNA genes (in the form of the characteristic 'Christmas trees') are closely juxtaposed (Fig. 2). The terminal balls are the ultrastructural counterparts of pre-rRNA processing complexes, consisting of U3 snoRNA and a number of proteins which co-assemble close to the 5′-end of the nascent pre-rRNA [28••]. These complexes mediate the U3-dependent first cleavage within the 5′-external transcribed spacer in species as distant as mouse and *Xenopus* [29••].

In Miller spreads, the rDNA of transcribed nucleolar genes is in an extended B-form, in which a single mammalian rRNA transcription unit, with a length of ≈4 μm, could easily span the entire nucleolus *in situ* [8]. Thus, the rRNA genes must be packed extremely tightly within the nucleolus of a living cell. It is remarkable that transcribing rRNA genes have not yet been directly visualized in ultrathin sections of cells fixed *in situ*. Although we do not know the specific conformation of the nascent transcripts with their terminal processing complexes *in vivo*, it should be feasible in the future to identify active rRNA genes in ultrathin sections of nucleoli, using refined methods such as electron microscope tomography.

Nucleolar targeting

Once transported into the nucleus, how are nucleolar proteins recruited to the nucleolus? Evidence is accumulating that recruitment is not due to a common nucleolar targeting signal, but rather to multiple functional interactions with other macromolecules already present in the nucleolus. A quantitatively major nucleolar protein, nucleolin, is known to bind to pre-rRNA and is thought to be involved in its processing, or in the early stages of ribosome assembly [4]. As shown by deletional analyses, nucleolin is, like most karyophilic proteins, transported into the nucleus by a specific nuclear localization signal (NLS). Nucleolar targeting, however, requires two additional domains which are capable of interacting with RNA [30•–32•]. The combination of an RNA recognition motif (RRM), the glycine/arginine rich (GAR) domain, and the NLS were sufficient to direct a hybrid protein to the nucleolus ([32•]; see, however, [30•,31•]). The nucleolar localization of the yeast NSR1 protein, which is related to mammalian nucleolin, also appears to be mediated by NSR1 protein domains interacting with other nucleolar components [33•].

Molecular interactions between the nucleolar targeting domain of Rex, a regulatory protein of the human retrovirus HTLV-I, and nucleolar constitutents have been studied by affinity chromatography [34•]. The nucleolar protein B23, a putative ribosome-assembly factor, is the

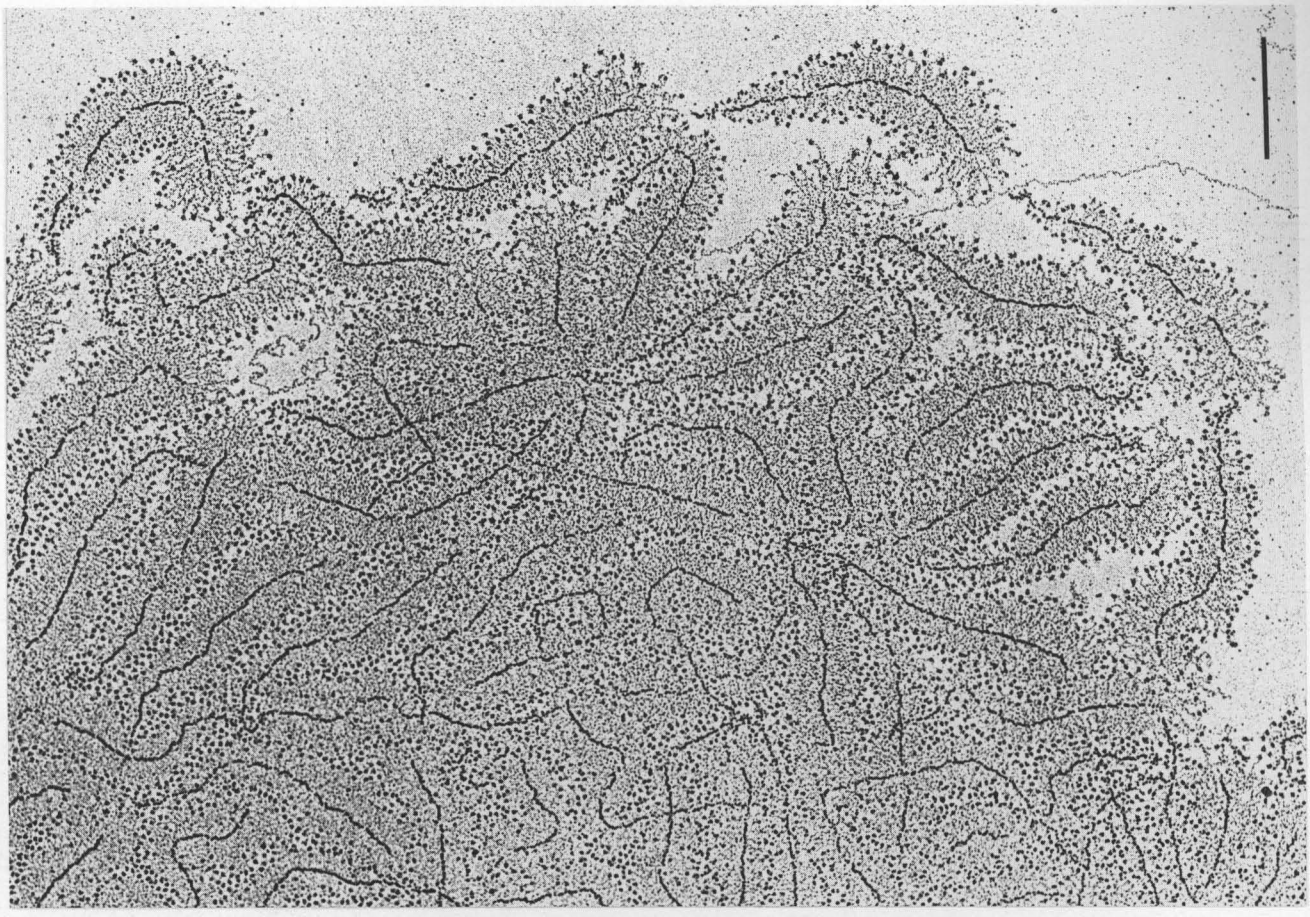

Fig. 2. Ribosomal RNA genes in full transcriptional activity, as seen in a spread preparation of an oocyte of the salamander *Pleurodeles waltl*. The high packing density of the genes probably reflects their *in vivo* situation. Each transcription unit is characterized by closely spaced transcription complexes and the terminal balls of the nascent ribonucleoprotein fibrils. Bar indicates 1 μm.

major binding host for Rex. Interestingly, protein B23 (and its amphibian counterpart NO38) belongs to the group of nucleolar proteins that shuttle between the nucleolus and the cytoplasm. Hence, it is conceivable that the Rex protein can 'piggyback' into the nucleolus simply by interacting with B23 [34•]. In support of this idea, other shuttling nucleolar proteins such as rat liver Nopp140 [35], mammalian nucleolin and its yeast counterpart, NSR1 [36•], recognize the NLS of karyophilic proteins and may thus provide a shuttle service for nucleolar accumulation (as well as a mechanism for the export of ribosomal subunits).

Taken together, these and earlier nucleolar targeting experiments with the protein NO38/B23 ([37]; see also [38]) and the rDNA transcription factor UBF [39] have indicated that different nucleolar proteins are sequestered in the nucleolus by multiple pathways, which depend on the particular binding host within the nucleolus. This may also be true for the nucleolar accumulation of RNAs or ribonucleoproteins such as 5S rRNA and various snoRNPs.

Maintenance of nucleolar structure

The nucleolar targeting experiments have indicated that a cascade of protein–protein and protein–nucleic acid interactions are involved in establishing and maintaining the intricate nucleolar architecture. This concept has received further support from the observation that solubilized components of amoebae nucleoli can reassociate *in vitro* into nucleolus-like structures with granular and dense fibrillar components [40••]. But what is the primary determinant of nucleolar organization? It is now well established that a single rRNA gene is sufficient to organize a nucleolus [41], and that the structural integrity of a nucleolus is dependent upon the transcriptional activity of the rRNA genes [8]. Thus, a single rDNA transcription unit has the potential to organize and maintain nucleolar structure. In principal, rDNA-associated proteins, such as transcription factors or transcribing polymerases, nascent pre-rRNAs, or proteins and snoRNAs that are bound to them could be involved in this activity. It has been proposed that the 5′-ends of the nascent pre-rRNA transcripts may be involved in the formation and maintenance of the dense fibrillar component, which could, in turn, provide the necessary binding sites for elements of the granular component [8,13,42]. However, recent results obtained from a mutant yeast strain which synthesizes rRNA exclusively by RNA polymerase II have indicated that RNA polymerase I itself, rather than the growing transcripts, may play a role as a structural element for

nucleolar architecture [43••]. These polymerase I deletion yeast mutants contain fibrillarin-positive dense bodies (termed mini-nucleolar bodies) which resemble the dense fibrillar component fragments induced in mammalian cells after inhibition of rDNA transcription [8,44]. Oakes *et al.* [43••] have thus come to the provocative conclusion that an intact nucleolar structure is not absolutely required for ribosome biosynthesis, but may simply enhance the efficiency of this process. Perhaps the yeast polymerase I mutant system may provide clues as to why eukaryotes have developed a nucleolus despite the fact that their prokaryotic ancestors were perfectly capable of synthesizing ribosomes in the absence of such a structure.

The nucleolus organizer and pre-nucleolar bodies

During mitosis, rRNA synthesis is downregulated; yet proteins that play a central role in rDNA transcription, such as RNA polymerase I, DNA topoisomerase I, and UBF remain associated with the chromosomal nucleolus organizer regions (NORs), which also harbor the rRNA genes [13]. The mechanisms that modulate the activity of rRNA genes during mitosis may provide an interesting case by which to analyze the control of gene activity and structure. Postmitotic reformation of nucleoli is dependent on the interaction of two separate entities, the NOR and the pre-nucleolar bodies (PNBs). PNBs appear at telophase, and are initially scattered throughout the reforming daughter nuclei as they progress into the G_1 phase (Fig. 3). The PNBs contain a number of proteins characteristic for the dense fibrillar component of interphase nuclei [45], as well as U3 snoRNA [42]. PNB-like structures can also form *in vitro* during the assembly of nuclei in *Xenopus* egg extract independent of the presence of rDNA sequences [45]. Interestingly, the 'synthetic' PNBs share several components with coiled bodies such as spliceosomal small nuclear (sn) RNAs and the protein coilin [46•], indicating that there is a possible relationship between these nuclear structures. Coiled bodies, which, like the PNBs, contain fibrillarin and disassemble during mitosis, often reveal a close physical association with nucleoli [47,48]. When the rRNA genes resume their transcriptional activity, PNBs fuse around the NOR of the developing nucleolus [13]. In HeLa cells, however, the postmitotic reformation of PNBs and coiled bodies follows different kinetics, and there is currently no evidence that they are assembled from a common precursor structure [49]. The identification of the components involved in this selective targeting process of the PNBs, and their specific mode of interaction, will be important for understanding the dynamic rearrangements of nucleoli during mitosis.

Conclusion

The transcriptionally active rRNA genes have the remarkable ability to organize and integrate the biochemical pathway of ribosome production into a structural framework, the nucleolus. We presently believe that the transcribing rRNA genes serve as a nucleation site where the primary binding and assembly of nucleolar components occurs. Do these, in turn, provide the next level of binding sites for other nucleolar proteins or RNAs necessary for higher order organization? Are the active RNA polymerase I molecules, the nascent transcripts or the rDNA-containing chromatin the determinants of nucleolar higher order structure? Answers should come in the near future from the use of genetic approaches in yeasts [43••] and from refinements in cell-free systems presently available [40••,45,46•].

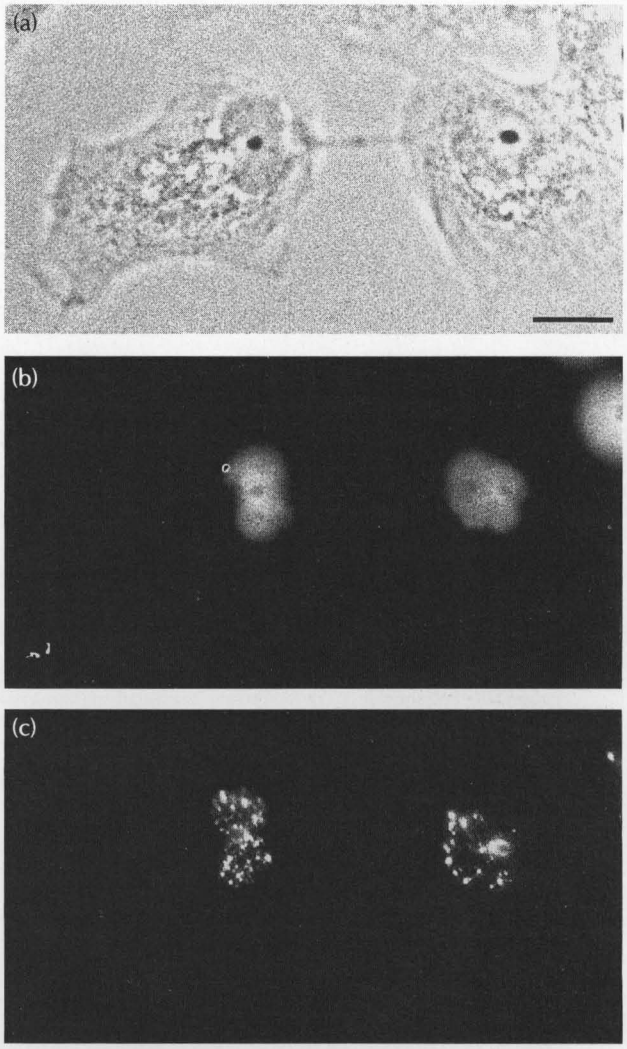

Fig. 3. Nucleoli reform after mitosis by the coalescence of preformed entities, the PNBs. **(a)** A phase contrast image (note the residual cytoplasmic bridge between the daughter cells, arrow) and **(b)** Hoechst staining are shown. **(c)** PNBs are visualized by immunofluorescence microscopy of *Xenopus* A6 cells with mAb No-114, which recognizes a protein of the dense fibrillar component of interphase nucleoli (for references see [45]). Bar indicates 10 μm.

What is a nucleolus? Is it just a cloud of transcription products with associated proteins and factors that gather around the transcribing rRNA genes? Or are structural elements involved in nucleolar function, such as the skeletal network extending throughout the am-

plified nucleoli of *Xenopus* [50]? Future progress in understanding nucleolar function and ribosome biosynthesis will depend on finding answers to these questions.

Acknowledgements

We thank Hilde Merkert for providing Fig. 1, Drs D Lourim and R Benavente for critical comments on this manuscript and our colleagues for communicating results before publication. The authors' research is supported by a grant from the Deutsche Forschungsgemeinschaft (Sche 157/8-2).

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