Structure and function of the nucleolus in the spotlight
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The nucleolus is the most obvious and clearly differentiated nuclear sub-compart-ment. It is where ribosome biogenesis takes place, but it is becoming clear that the nucleolus also has non-ribosomal functions. In this review we discuss recent progress in our understanding of how both ribosome biosynthesis and some non-ribosomal functions relate to observable nucleolar structure. We still do not have detailed enough information about the in situ organization of the various processes taking place in the nucleolus. However, the present power of light and electron microscopy techniques means that a description of the organization of nucleolar processes at the molecular level is now achievable, and the time is ripe for such an effort.

Introduction

Ribosome biogenesis requires RNA polymerase I (pol I)-driven synthesis, cleavage and modification of precursor rRNAs (pre-rRNAs), assembly with r-proteins and transient interactions with numerous small nucleolar ribonucleoproteins (snoRNPs) and non-ribosomal nucleolar proteins, culminating in the export of almost mature r-subunits. Although this would appear to be a simple scenario, in fact the biogenesis of ribosomes is a very much more complex process involving many more steps and factors than was foreseen [2,11,12**].

In the past few years, our understanding of ribosome biosynthesis has been revolutionized by proteomic research backed by genetic and biochemical analyses [2], particularly in yeast. Many of the individual steps in ribosome biogenesis have now been described at the molecular level, and most of the macromolecular components involved have been identified. However, the synthesis of these data to understand the various interactions of the different nucleolar factors and r-proteins in vivo remains at an early stage, even in yeast [2,11,12**,13,14*,15*,16,17**].

Proteomic research [12**,18,19,20*,21*] is also rapidly increasing our understanding of ribosome biosynthesis in higher eukaryotes, although at a slower pace than in yeast. It is important to emphasize that, although RNA and r-proteins have been highly conserved among eukaryotes, it is not always possible to extrapolate yeast data to metazoa and other eukaryotes. At the molecular level, for example, the factors involved in the earliest steps of the pol I activation, the pre-initiation complex (PIC), differ between yeast and mammals [4,5]. At the cellular level, the nucleolus in the yeast Saccharomyces cerevisiae does not disassemble at mitosis, in contrast to what occurs in mammals and plants. Furthermore, important findings with relevance to human medicine are very specific to mammalian or even to human cells [22*,23*,24].

Ribosomal genes comprise a transcribed sequence and an intergenic spacer, and are located on one or (usually) several chromosomes in arrays of head-to-tail tandem repeats called nucleolus organizer regions (NORs). For instance, human diploid cells contain ~400 r-genes on five pairs of NOR-bearing chromosomes, each NOR containing several tens of repeats. During interphase, r-repeats from more than one NOR-bearing chromosome often cluster together and participate in the formation of a given nucleolus. In mitosis, rRNA synthesis ceases as a result of phosphorylation of the relevant nucleolar factors and the nucleoli disassemble; at the end of mitosis RNA synthesis resumes and nucleoli reform [6*,7*,8*,9*,10].

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proteins or macromolecular complexes are often encountered in nucleoli [3,25]. Different location of a macromolecule suggests a different function, and indeed there is much emerging evidence that nucleoli have a number of ‘non-canonical’ functions in addition to their roles as ribosome factories, such as virus infection control, maturation of non-nucleolar RNAs or RNP, senescence and regulation of telomerase function, regulation of cell cycle, tumor suppressor and oncogene activities, cell stress sensing and signaling [3,7,25,26,27,28]. The best-characterised of these other activities is in the assembly of the signal recognition particle, a complex of an RNA with several proteins that targets translation of certain proteins to the endoplasmic reticulum [25,29–32]. This activity is...
relatively easily rationalized as being at least connected with ribosome biogenesis and activity.

In this review, we discuss recent progress in deciphering how the processes of the ribosome biosynthetic pathway are integrated into the nucleolar structure, with the emphasis on the important conceptual changes since the last structurally oriented nucleolar review published in this journal in 1999 [33]. It is impossible to survey such a vast literature adequately in a short review, and we have had to be very selective. For the most part we have concentrated where possible on human nucleoli, but have included data from other species, particularly yeast, where relevant, or where human data is still unavailable.

**Nucleolar constituents are enormously dynamic**

Breakthrough photobleaching experiments have led to a complete reassessment of the extent of nucleolar and nuclear dynamics [7,9,34,35,36,37]; similar results demonstrate the rapid diffusion of RNA [38]. Current models show that proteins freely diffuse through the nuclear space, including the nucleolus [39], and that the mean residence time of most nucleolar proteins in nucleoli can be calculated to be only a few tens of seconds. The nucleolus exists as a discrete structure because certain proteins, some of which remain to be characterized, bind to the rDNA, forming a more or less stable core on which the complex set of nucleolar interactions and dynamic processes is built. The surrounding nucleoplasmic components continually exchange with this nucleolar ‘super-complex’, and the steady-state composition of the nucleolus is to a great extent the result of the fact that the nucleolar residence time of non-nucleolar proteins, which do not find interacting partners in nucleoli, is one or more orders of magnitude shorter than that of nucleolar proteins. Thus, the nucleolus represents a steady-state structure with its components in dynamic equilibrium with the surrounding nucleoplasm [7,9,34,35,36,37]. It should be noted that nucleolar proteins are not recruited to nucleoli through a common nucleolar targeting signal, but through functional interactions with other macromolecules already present in nucleoli; for both nucleoli and other nuclear sub-compartments, the term ‘retention signal’ is more accurate than ‘targeting signal’ [40]. A GTP-driven cycle has now been identified as a first mechanism for protein retention in the nucleolus [41].

**Pol I activity alone is not sufficient for the maintenance of nucleolar morphology**

What is responsible for the maintenance of the typical steady-state nucleolar structure (Figures 1 and 2)? The classical explanation [33] is that pol I-driven transcription,
with r-genes serving as nucleation sites, organizes and maintains nucleoli. Importantly, Gonda et al. [42] have shown in *Xenopus* that the maintenance of nucleolar morphology can be uncoupled from pol I-driven transcription by the action of specific intrinsic proteins. These disassembly mediator proteins, under conditions of ongoing pol I transcription, reversibly disassemble the nucleolus *in vitro* and *in vivo*. The abundant nucleolar phosphoprotein B23 is an interacting partner of the proteins [43*]. Therefore, although transcriptional activity of pol I is necessary, it is not sufficient for the maintenance of nucleolar structure, which must depend on the complex intermolecular interaction of nucleolar components [44].

Thus, our current understanding of the functional organization of the nucleolus is that it is a self-organizing, dynamic system [37]. Nucleolar components are continuously exchanged with the nucleoplasm, their detailed dynamics generating the observed stable overall nucleolar configuration. In turn, the fine control and tuning of gene expression is ultimately responsible for the generation and maintenance of this highly dynamic, but permanent, sub-compartment. Conversely, the nucleolus is an open system, and even though it is a steady-state structure, it is far from equilibrium (Figure 3). Its morphology is directly linked to its functional status and can quickly and even dramatically react to external stimuli [36*,44,45*].

**Electron microscopy illuminates the molecular organization of active ribosomal genes**

The molecular organization of active r-genes in the form of Christmas trees (CTs; Figure 1) is seen in electron micrographs of isolated and highly loosened chromatin spreads [46,47]. We emphasize that these standard views of CTs are only obtained for certain cell types, typically yeast cells but also maturing amphibian (or insect) oocytes possessing a high number of extra-chromosomal nucleoli [48,49]. Because of the presence of interfering chromatin structures in mammalian somatic cells, a complete mammalian r-gene tandem repeat has never been visualized; the few pictures of mammalian r-genes that have been obtained show either CTs with maximal pol I loading, or inactive r-genes covered by nucleosomes. These results were interpreted to support the binary model of r-genes in which each gene is a ‘binary unit’ that is either on or off; when the gene is on, it is heavily loaded with transcribing pol I. Recent findings by French et al. [50] in yeast challenged the binary model (Figure 1b). They demonstrated the presence of intermingled active and inactive genes randomly distributed within spreads of a tandem repeat of r-genes. In addition, they showed that the individual active genes had different levels of pol I loading.

The terminal CT knobs have been shown in *Xenopus* cells to represent processing complexes containing U3 snoRNP [51]. In *Dictyostelium*, electron microscopy (EM) of CTs has revealed that early rRNA processing occurs co-transcriptionally [52]. Until recently, apart from the latter study, rDNA transcription and processing have been considered to be separable steps in gene expression. In yeast, this idea has recently been refuted (Figure 1c). By means of genetic, biochemical and EM studies it has been shown that the large terminal knob is indeed the processome, a huge processing complex containing U3 snoRNA, but that >50% of nascent transcripts are cleaved during transcription [53**–55**]. The cleavage is accompanied by the liberation of the small pre-ribosomal subunit and loss of the large terminal knob in CTs (Figure 1c). In addition, the factor responsible for small-subunit processome assembly and compaction has been identified [56].

**Where are the Christmas trees in the nucleolar forest?**

EM thin-section images of eukaryotic nucleoli can be classified into three components (Figures 1 and 2): the fibrillar center (FC), the dense fibrillar component (DFC) and the granular component (GC). This statement, which regularly appears in publications on eukaryotic nucleoli, is extremely strong and needs to be examined critically from various structural and functional points of view.

Depending on the species, cell type and physiological state of the cell, there is considerable diversity in both the prevalence and the arrangement of the three nucleolar components [1,57,58]. However, the tripartite nucleolar organization has been recently challenged with a hypothesis that only the amniotes, including mammals, possess the three structural components, while the anamniote nucleoli have no FCs [59*]. The hypothesis is based on the idea that amniotes have much longer intergenic rDNA sequences than anamniotes. This hypothesis cannot be completely true, since, for example, anamniotic amphibian nucleoli do possess FCs ([60]; Figure 1). Also, FCs were originally defined by their morphology and cytochemistry [61]. Later it was shown by immunocytochemistry that FCs contained pol I [62]. Interestingly, in native cryosectioned mammalian nucleoli, one cannot distinguish between FC and DFC ([63] and the references therein). But this is rather due to the lack of amplitude contrast, since in freeze-substituted nucleoli, the structure of which should best mimic the native cryopreparations, the tripartite organization of nucleoli is seen.

Recent studies from McStay and colleagues have shed new light on the molecular arrangement of rDNA *in situ* [64*,65*]. Efficient transcription by pol I requires the formation of a PIC on an r-gene promoter. The PIC comprises upstream binding factor (UBF) and selectivity factor complex SL1, which has a high affinity for the promoter sequence. PICs recruit an initiation-competent
subfraction of pol I, defined by the presence of transcription initiation factor IA (TIF-IA/Rrn3). UBF has been shown by both biochemical and microscopic approaches to be present not only at the promoter, but also extensively over the r-gene cluster, and is responsible not only for the specialized decondensed state of r-chromatin in interphase, but also for the secondary constrictions seen in mitosis. Since SL1 and TIF-IA/Rrn3 also show similarly extensive binding, it is possible that pol I subunits bind to PICs outside the promoter. <10% of nucleolar pol I is engaged in transcription [66], and this non-promoter binding may explain why such a great excess of non-transcribing pol I is sequestered in nucleoli [65*]. This naturally raises the question of whether the high concentration of pol I seen in FCs corresponds to transcriptionally active or inactive enzyme. In our opinion, current evidence indicates that it is non-transcribing pol I that accumulates in FCs, and that transcribing pol I is in the DFC; when a non-transcribed r-gene within the FC becomes activated, it automatically becomes a part of the DFC [67].

Nucleolar morphology has been correlated with various steps in ribosome biogenesis through mapping of various ribosomal and nucleolar components. So far, CTs have not been unequivocally identified in situ within nucleoli [68] and an intense debate about their location has raged since pol I was first seen in FCs by a pre-embedding EM immunocytochemical approach in 1984 [44,57,58,62,67,69]. This challenged numerous previously published autoradiographic studies situating nucleolar transcription in the DFC [70], and produced an avalanche of rectified autoradiographic studies. Later on, it became apparent that the pre-embedding result [62] could be biased since the approach revealed pol I in FCs, but would miss it in the DFC [58,67].

The best current approach for identifying CTs is directly to map incorporated modified nucleotides such as bromouridine (BrU) into nascent rRNA transcripts, since this method allows sensitive detection with a resolution far greater than is achieved with autoradiographic methods. Current data using mapping of incorporated BrU into nascent transcripts shows that CTs, at least in mammalian cells as well as in onion and pea root cells, are found in the DFC, including the border region between the DFC and separated, and “remote” regulation is therefore possible in this scheme. Such remote regulation may be missing in in vitro systems. This underlines the importance of in situ studies of the cell. Importantly, the dynamics of transient interactions, producing shorter or longer residence times of components in a given (nucleolar) compartment, cannot be shown in this simplified drawing. The scheme illustrated in this figure is applicable to both ribosomal and ‘non-canonical’ functions of nucleoli even though this simple scheme does not explicitly encompass the complex intermolecular interaction of nucleolar components as shown for instance in [42,43] (from the work of Ivo Melčák and Ivan Raška; copyright permission of Vesmír).
FC [44,57,58,71*]. Thus, there is unequivocal evidence for C Ts within the DFC, which necessarily must include the DFC/FC borders. There is no convincing evidence that C Ts are found exclusively within FCs. Furthermore, since there is a consensus that pre-rRNA processing and pre-ribosomal assembly are organized vectorially away from the r-genes, location of C Ts purely at the DFC/FC border would seem to be sterically impossible. Ultimately this question will be subsumed by high-resolution structural data showing the molecular organization of the different complexes and their higher order topology; already the argument about DFC versus FC seems somewhat archaic.

Once transcription has started, the biogenesis of ribosomes is a vectorial process. FCs correspond to interphase counterparts of mitotic NORs and comprise inactive r-genes (Figure 2). However, in growing cells, clustered r-genes from one NOR must unravel and give rise to several FCs because FCs increase in number in rapidly growing cells, although the total number of r-genes remains constant [67]. Pre-rRNA initially accumulates in DFC and its first processing steps take place in DFC. Later steps of ribosomal biogenesis take place in GC and nearly mature r-subunits move out of the nucleolus and reach the cytoplasm [7,70]. Nucleolar export is apparently a simple diffusional process, as has been shown in the case of 60S r-subunits [38]. Actin and myosin I have been detected within nucleoli [72], raising the question of whether these motor proteins are involved in the movement of pre-rRNP complexes through the nucleolus. Although it has been shown that myosin I is important for the initiation of transcription and that actin is involved in both initiation and elongation, there is no current evidence that these proteins are involved in facilitating the movement of nucleolar pre-rRNP complexes [72,73].

In an important study, Dundr et al. [66] tagged subunits of pol I and PIC with green fluorescent protein (GFP) and determined the kinetics of assembly and elongation of the pol I transcription complexes by FRAP (fluorescence recovery after photobleaching) and related techniques. The authors concluded that the pol I complex is disassembled after the termination of transcription and that a new complex is reassembled from subunits on r-genes for a new round of transcription. However, this ‘hit-and-run’ mechanism for pol I has been recently questioned in yeast. Biochemical studies show that the pol I complex remains stable after its disengagement from the DNA matrix, the two largest subunits within the pol I complex remaining associated through many rounds of transcription [74]. These results support the idea that preassembled, ready-to-use multiprotein pol I transcription factories are present in nucleoli. In addition, it appears that in human cells, separate subunits of pol I move coordinately, either as part of a stable complex or through the actions of common receptors [12**]. Since pol I subunits and the mechanism of transcription are conserved between yeast and mammals, it would be worthwhile confirming the dynamics of pol I subunit exchange in mammalian cells by more direct means like those used by Schneider and Nomura [74].

The cell needs the coordinated activities of all three polymerases to make ribosomes: pol I for rRNA, pol II for r-protein genes and pol III for 5S rRNA, as well as precisely equimolar amounts of rRNA and r-proteins [4]. Recent work suggests that this balance may be achieved post-transcriptionally or post-translationally rather than at the level of transcriptional regulation ([12**]; Al Lamond, personal communication). Mass spectrometric proteomic analysis showed that, after proteasome-mediated protein degradation is blocked, there is a large nuclear accumulation of r-proteins, indicating that normally there is a large turnover of r-proteins by the proteasome, and that r-proteins are synthesized more rapidly than any other nucleolar proteins. The rapid synthesis and turnover of r-proteins has been confirmed by analysis of individual GFP-tagged r-proteins in stable cell lines, using photobleaching and pulse-labeling methods. A proposed working model is that r-proteins are made in excess and rapidly imported into the nucleus and nucleolus. Then either they associate with rRNA, assemble into an r-subunit and are exported to the cytoplasm, or else they get ubiquitinated and degraded in the nucleus ([12**]; Al Lamond, personal communication). This model received recent support from Stavreva et al. [75], who showed that ubiquitin and the proteasome play a role in ribosomal biogenesis. Interestingly in this respect, three new studies show that the oncoprotein Myc, which is known to drive cell division, also enhances rRNA synthesis by pol I in addition to controlling pol II- and pol III-regulated gene transcription. This suggests that Myc promotes the generation of crucial components of a functional ribosome ([76] and references therein).

Conclusions
The nucleolus harbors hundreds of different biochemical processes taking place at any moment in a growing cell. This activity produces many protein and nucleoprotein complexes of different sizes, including pre-ribosomal particles. This simple fact necessarily makes any simple structural model of the nucleolus, such as the tripartite model, an oversimplification. Of course, leaving aside perinucleolar condensed chromatin masses, other nucleolar components, such as small clumps of condensed intranucleolar chromatin, nucleolar interstices and nucleolar vacuoles or cavities, are regularly observed within nucleoli. But even allowing for these exceptions, a simple model is clearly insufficient. For example, GC granules have been considered to represent pre-ribosomes at various stages of maturation [1], and are thus necessarily heterogeneous, but many granules do not contain RNA [77*] and presumably cannot therefore
correspond to pre-ribosomal particles. Similarly, fibrillar-larin, a specific marker of the DFC, is located close to r-genes during transcription, probably associated with nascent transcripts, but moves away from r-genes during their replication [78]. This suggests that the DFC is functionally heterogeneous. The sub-nuclear Cajal bodies are known to share a number of common protein and RNP factors with nucleoli [26] and are often associated with, or even engulfed in, the nucleolus [79]. Moreover, a number of recent studies describe non-standard functions for the nucleolus and show that ‘non-nucleolar’ proteins can frequently be sequestered in nucleolar subdomains [27,41**,77].

Our knowledge of nucleolar ribosome biogenesis as well as many new nucleolar non-ribosomal processes has expanded dramatically in the past few years. However, we are still lacking detailed in situ structural information about the cascade of steps in ribosome biogenesis, as well as about the non-canonical nucleolar functions. Even a question as elementary as the location of the active r-genes has been debated for >20 years [44,80]. However, rather than trying to force observations into a particular simplistic preconceived model, we should concentrate on obtaining an ultrastructural description of the nucleolus at the molecular level.

Are current methods and technology good enough to describe the functional organization of nucleoli in molecular detail? We are confident that in many cases the answer is yes, through either straightforward high-resolution microscopy or immunolabeling techniques or both in combination [81]. Huge advances in the resolution of light microscopy are now being made and these will help to bridge the gap between light microscopy (LM) and EM [82,83]. Another powerful approach is provided by correlative LM and EM, including tomographic EM and ultrastructural identification of the nucleolar distribution of proteins tagged with GFP or other markers [67,84*]. A first step is to identify CTS clearly in situ; a start has been made in this [67,85], and modern developments of LM and EM make this goal eminently achievable. We are confident that this will open the way to describe the nucleolus at the same level of molecular detail as has already been achieved with the Golgi and endocytic pathways.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest


This exhaustive review also describes basic facts about rRNA and ribosome biogenesis (see also [2,11]). Pol I synthesizes a pre-rRNA in which the successively transcribed 18S, 5.8S and 28S rRNA sequences are separated by internal transcribed spacer sequences and flanked by external transcribed sequences. SS rRNA is synthesized by pol III. The small r-subunit (SSU) contains 18S rRNA, while the large r-subunit contains 28S, 5.8 S and 5S rRNAs. During nucleolar biogenesis of ribosomes, the non-coding transcribed spacer sequences are removed through the action of endonucleases and exonucleases before or during the assembly of the small and large r-subunits; pseudouridine synthases and methyltransferases modify ~200 nucleotides of the rRNA, guided by cognate snoRNAs; RNA helicases and RNA chaperones mediate RNP folding and remodelling; GTPases and AAA-ATPases (ATPase associated with various cellular activities) facilitate protein associations and disassociations. SS rRNA, synthesized (in mammalian and plant cells) in the nucleolus, and r-proteins, synthesized in the cytoplasm, are recruited into the nucleolus and become integrated into pre-ribosomal particles. Nearly completed r-subunits then move to the cytoplasm with the help of special adaptor molecules.


This study makes use of an isotopic labeling method in mass spectrometry termed SILAC (stable isotope labeling by amino acids in cell culture) to analyze the dynamics of the nucleolar proteins, adding valuable quantitative data to the mass spectrometric analysis. By growing cells in isotopically substituted amino acids, cultures are differentially labeled, and each culture subjected to a different treatment by a metabolic inhibitor. The cultures are then pooled and nucleoli are purified and analyzed by mass spectrometry. In this way the same peptides are produced from each set of nucleoli in the same way, but their source can be identified by their differing masses, and the ratios of their abundance in the nucleoli of the differently treated cell cultures can be calculated. This and similar approaches provide very powerful methods
for examining the behavior and dynamics of defined sets of proteins by proteomic approaches. This paper, for example, provides convincing evidence for the rapid turnover of r-proteins.


Little is known about the function of r-proteins in vivo and the systematic analysis of yeast r-proteins of the small subunit has revealed that most eukaryotic r-proteins fulfill different roles in ribosome biogenesis, making them indispensable for growth. Different r-proteins control distinct steps of nuclear and cytoplasmic pre-18S rRNA processing and thus ensure that only properly assembled ribosomes become engaged in translation. Comparative analysis of dynamic and steady state maturation assays reveals that several r-proteins are required for efficient nuclear export of pre-18S rRNA, suggesting that they form an interaction platform with the export machinery. In contrast, the presence of other r-proteins is mainly required before nuclear export is initiated. The study draws a correlation between the in vitro assembly, structural localization, and in vivo function of r-proteins.


Although the name suggests its involvement in nonsense-mediated decay of mRNAs, the Nmd3p protein rather plays a role as an adapter molecule in the export of both small and large subunits and provides the nuclear export signal [13,16]. In yeast, Nmd3p apparently binds to the large subunit in the nucleus. The export of the large subunit is receptor-mediated; the relevant chromosome region maintenance 1 (CRM1) receptor recognizes Nmd3p, allowing the formation of an export complex with RanGTP and unidirectional trafficking of nascent 60S subunits to the cytoplasm. It is proposed that Rpl10p protein binds to the 60S subunit only in the cytoplasm, and Nmd3p is then released through the action of two other cytoplasmic factors, the Rpl10p chaperone and a GTPase.


17. Leger-Silvestre I, Gas N: The nucleolar ultrastructure in yeast.


- The authors discuss the functional organization of the yeast nucleolus. In their previous studies [86,87], they used modern EM approaches in order to preserve nucleolar structure optimally. They fixed the cells by rapid freezing so as to freeze the water as amorphous ice. While maintaining the frozen cells at very low temperatures, they gradually increased the temperature of the specimen and substituted water molecules for monoatomic resin (epoxy or methacrylate), then polymerized the resin. The embedded cells were thin-sectioned and immunogold labeling was performed. In the yeast nucleolus, FCs are seen by EM only after the use of rapid freezing and freeze-substitution; using classical processing of cells for EM, yeast FCs are not observed.


This is the first systematic proteomic analysis of nucleolar proteins in a plant. ~200 proteins are identified in purified nucleolus, and are compared with the human nucleolar proteome. In addition to the expected nucleolar proteins, this study found a large number of unexpected proteins, including proteins involved in small nuclear and nucleolar RNA maturation, spliceosomal proteins, and many unknown proteins. Approximately half of the identified proteins are expressed as GFP fusion proteins, which confirms the nucleolar location of the vast majority. The presence of many of the proteins of the mRNA-associated post-splicing exon junction complex is particularly surprising, suggesting a role for the plant nucleolus in mRNA surveillance. ~70% of the identified nucleolar proteins have homologues in the human proteome; others were plant-specific, with no close mammalian homologue.


This extensive review provides a comprehensive guide to the current state of nucleolar proteome research.


The perinucleolar compartment (PNC) is a dynamic nuclear sub-structure in which newly synthesized RNAs (primarily pol III transcripts) and RNA-binding proteins are highly concentrated. The formation of the PNC is closely linked to malignant transformation as demonstrated both in vitro and in vivo. Although the function of the PNC remains unclear, findings from various groups suggest that the PNC is involved in trafficking of a subset of newly synthesized pol III RNA.


This study links the prevalence of the PNC with the progression of breast cancer in vivo and suggests that PNC-containing cells have metastatic advantages. The results also show the potential of PNC prevalence as a prognostic marker for breast cancer.


The authors show that nucleolar sequestration is a general phenomenon and that the nucleolus can capture and release proteins in response to different cellular signals and thus regulate the output of dynamic molecular networks by switching proteins between static (sequenced) and mobile states.


Nucleolar segregation is controlled under some physiological conditions of transcriptional arrest. This process can be mimicked, for example by...
transcriptional arrest after actinomycin D treatment, leading to the segregation of nucleolar components and the formation of unique structures termed nucleolar caps surrounding the central nucleolar body. Surprisingly, nucleolar caps contain nucleoplasmic components as well as components of Cajal and PML bodies, in addition to nucleolar proteins and fluorescent dextran probes. Many nucleolar components disperse under these conditions. Nucleolar caps are dynamic structures, as shown by photobleaching experiments, and require energy for their formation. The results emphasize the dynamic characteristics of nuclear domain formation in response to cellular stress.


There is a lack of information about the basic physical parameters of the nucleoplasm and nuclear sub-compartments. Because of its size, the Xenopus germinal vesicle is a convenient system for such measurements. The authors measure the refractive index, and consequently the density, of nucleoli and nucleolar components as well as that of nuclear speckles and Cajal bodies. They obtain evidence that the density of sub-compartment is higher than the density of the nucleoplasm, but the density difference is not as high as anticipated. In nucleoli, the DFC has a higher density than the GC. The authors also test permeability by means of fluorescent dextrans and show that nucleoli have a sponge-like structure. In other words, nucleoli are highly permeable and contain a lot of nucleoplasm. The DFC is less permeable than the GC.


Nucleolar proteins, including nucleostemin, are increasingly recognized as potential regulators of cell growth and proliferation. Nucleostemin has GTP binding sites and is reported to be preferentially present in stem cells and to be downregulated during differentiation. The GTP-bound form of nucleostemin is localized to the nucleolus. Exchange of GTP in the nucleolus reverses the nucleolar binding and nucleostemin is released to the nucleoplasm. The involvement of a GTP switch indicates that nucleolar localization can be regulated and may be responsive to extracellular stimuli through signaling pathways. Interestingly enough, nucleolar proteins may mutually affect their localization since the nucleolar reorganization/disassembly in vitro as well as in vivo and that RNA non-sequence-specific binding motifs are involved in this process. Importantly, nucleolar disassembly, which takes place in the presence of continuing transcription, is reversible. In the present study, the authors propose that FRG2a and its human related protein YB1 disassemble nucleoli by sequestering B23, a major nucleolar phosphoprotein. It is possible that RNA itself plays a structural role in nucleolar maintenance, as the role of these proteins in nucleolar disassembly may be through their non-sequence-specific RNA binding, which may thus prevent the interaction of rRNA with other RNAs or proteins.


The same group has shown in [42] that two RNA binding proteins, FRG2Ya and FRG2Yb, involved not in the maintenance but in the disassembly of nucleoli, are identified in extracts of X. laevis oocytes. [42] provides the first evidence that the maintenance of nucleolar morphology can be uncoupled from pol I driven transcription by specific intrinsic proteins. The recombinant FRG2a and FRG2b proteins induce nucleolar reorganization/disassembly in vitro as well as in vivo and that RNA non-sequence-specific binding motifs are involved in this process. Importantly, nucleolar disassembly, which takes place in the presence of continuing transcription, is reversible. In the present study, the authors propose that FRG2a and its human related protein YB1 disassemble nucleoli by sequestering B23, a major nucleolar phosphoprotein. It is possible that RNA itself plays a structural role in nucleolar maintenance, as the role of these proteins in nucleolar disassembly may be through their non-sequence-specific RNA binding, which may thus prevent the interaction of rRNA with other RNAs or proteins.


Drug-induced inhibition of rRNA processing events causes dispersion of rDNA clusters and disassociation of the late rRNA processing proteins, which accumulate in distinct masses away from the transcription sites. Removal of the drug leads to nucleolar reformation, reversing this disassociation and restarting rRNA processing. The functional implications with regard to the generation and maintenance of nucleoli are discussed.


This is an important study, which showed for the first time that the small r-subunit is produced and processed co-transcriptionally. See also [54**, 55**].


See annotation to [65].

65. Prieto JL, McStay B: Nuclear biogenesis: the first small steps.

This review describes initial steps in rRNA synthesis (see also [64**]). In addition, it elucidates the structure of NORs, as it has been generally agreed, but never proved, that NORs containing active r-genes during DNA replication and transcription in vertebrates, in yeast. As far as we know, this is the first non-gold signals due to incorporated fluoro-uridine are observed in the DFC, but not in the FCs, in neuronal cells. In this study, living rats are injected with fluoro-uridine. Later on, the authors show that ubiquitin is present in nucleoli and that complexes of proteins or protein complexes that serve other functions.


71. Casafont I, Navascues J, Pera E, Lafarga M, Berciano MT: Nuclear organization and dynamics of transcription sites in rat sensory ganglia neurons detected by incorporation of 5'-fluorouridine into nascent RNA. Neuroscience 2006, in press.

In this study, living rats are injected with fluoroo-uridine. Later on, the animals are sacrificed and, after processing of tissues for EM, immunogold signals due to incorporated fluoroo-uridine are observed in the DFC, but not in the FCs, in neuronal cells. As far as we know, this is the first non-isotopic study employing living animals to show sites of nucleolar RNA transcription in situ. This result provides a very strong argument for the DFC encompassing CTs.


By means of immunofluorescence microscopy and immunoprecipitation, the authors show that ubiquitin is present in nucleoli and that complexes associated with pre-rRNA processing factors are ubiquitinated. Inhibition of proteasome degradation affects some, but not all, factors involved in ribosome biogenesis, induces the accumulation of 90S pre-ribosomes, slows release of nature rRNA from the nucleolus and leads to a depletion of 18S and 28S rRNAs. These data suggest that the ubiquitin-proteasome system is involved in many steps in ribosome biogenesis.


Nucleostemin is a p53-interacting cell cycle progression factor that shuffles between the nucleus and nucleolus. Within nucleoli, it accumulates in rRNA-deficient domains within the GC. This finding is supported by electron spectroscopic imaging studies of the nitrogen and phosphorus distribution in the GC, which reveal GC regions that are very rich in protein and yet devoid of nucleic acid. These results suggest that the ultrastructural texture of the nucleolar granular component represents not only ribosomal particles but also RNA-free zones populated by proteins or protein complexes that serve other functions.


A novel correlitive LM and EM approach that allows the ultrastructural localization of proteins tagged with GFP is described.


