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REVIEWS

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Nucleolus: from structure to dynamics

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Abstract The nucleolus, a large nuclear domain, is the ribosome factory of the cells. Ribosomal RNAs are synthesized, processed and assembled with ribosomal proteins in the nucleolus, and the ribosome subunits are then transported to the cytoplasm. In this review, the structural organization of the nucleolus and the dynamics of the nucleolar proteins are discussed in an attempt to link both information. By electron microscopy, three main nucleolar components corresponding to different steps of ribosome biogenesis are identified and the nucleolar organization reflects its activity. Time-lapse videomicroscopy and fluorescent recovery after photobleaching (FRAP) demonstrate that mobility of GFP-tagged nucleolar proteins is slower in the nucleolus than in the nucleoplasm. Fluorescent recovery rates change with inhibition of transcription, decreased temperature and depletion of ATP, indicating that recovery is correlated with cell activity. At the exit of mitosis, the nucleolar processing machinery is first concentrated in prenucleolar bodies (PNBs). The dynamics of the PNBs suggests a steady state favoring residence of processing factors that are then released in a control- and time-dependent manner. Time-lapse analysis of fluorescence resonance energy transfer demonstrates that processing complexes are formed in PNBs. Finally, the nucleolus appears at the center of several trafficking pathways in the nucleus.

Keywords Nucleolus · Organization · Dynamics · Assembly

Introduction

The nucleolus, the ribosome factory, is a large structure visible in the cell nucleus that can be isolated from the neighborhood. The molecular machines necessary for ribosome biogenesis are targeted in the nucleolus, and consequently the nucleolus was proposed to be the paradigm of nuclear functional compartmentalization (Strouboulis and Wolffe 1996). Ribosome biogenesis implies the transcription of ribosomal genes (rDNA), processing and cleavages of the 47S ribosomal RNA (rRNA), and assembly with ribosomal proteins and the 5S RNA to form the small and large pre-ribosome subunits (selected reviews: Gébrane-Younès et al. 2005; Hadjiolov 1985; Hernandez-Verdun and Junéra 1995; Scheer and Hock 1999; Scheer et al. 1993; Shaw and Jordan 1995; Thiry and Goessens 1996). Specific complexes successively participate in accomplishing the different steps, interacting either with rDNA or with rRNA directly or indirectly but they are not associated with mature ribosomes. Indeed, the confinement of these machineries to the nucleolus provides an interesting situation to analyze the functional compartmentalization of transcription and processing in the nucleus. Recently an interesting review concentrated on the moving parts of the nucleolus (Olson and Dundr 2005). The authors concluded that the nucleolus is a dynamic structure. They proposed that this dynamics is necessary not only for nucleolar functions such as assembly of ribosomes but also for communication with the other parts of the cell.

In this review, information on the structural organization of the nucleolus and the dynamics of the nucleolar proteins are reported and discussed in an attempt to link both aspects of nucleolar function.

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Organization of the nucleolus reflect its activity

The three canonical nucleolar components visible by electron microscopy

The nucleolus is "formed by the act of building a ribosome" (Mélèse and Xue 1995). Consequently the size of the nucleolus varies greatly in appearance depending on cellular activity, proliferation or differentiation. Nevertheless, three main specific components can be identified in most eukaryotic nucleoli (Fig. 1). Clear areas, the fibrillar centers (FCs), are partly or entirely surrounded by a highly contrasted region, the dense fibrillar component (DFC). The FCs and DFC are embedded in the granular component (GC), mainly composed of granules of 15–20 nm in diameter. The FCs form discrete structures (their number doubles in G2 compared to G1) located in different levels throughout the nucleoli and connected by a network of DFC (Junéra et al. 1995). It has become apparent that nucleoli of different cell types exhibit a variable number of FCs of variable sizes, with an inverse proportion between size and number (Hozak et al. 1989; Pébusque and Seïte 1981). It was recently proposed that the presence of three distinct nucleolar components emerges during evolution from a two component—fibrillar and granular—nucleolus (Thiry and Lafontaine 2005). This interesting idea is based on the size of the intergenic spacers of the rDNA. However, the limit of this classification resides in the difficulty encountered in identifying the presence or the absence of the FC in certain samples.

General mapping of ribosome biogenesis in these morphologically distinct nucleolar components has been achieved. The nascent transcripts appear in the junction region between the FCs and DFC and accumulate in the DFC (Cmarko et al. 2000; Guillot et al. 2005; Hozàk et al. 1994; Puvion-Dutilleul et al. 1997; Shaw and Jordan 1995). As for processing of the 47S rRNA, it starts at the site of transcription in the DFC (Cmarko et al. 2000) and continues during the intranucleolar migration of the rRNA towards the GC. This complex series of maturation and processing events, presently better characterized in yeast than in higher eukaryotes has been extensively reviewed (Fatica and Tollervey 2002; Fromont-Racine et al. 2003; Sollner-Webb et al. 1996; Tollervey 1996). In the nucleoli, the vectorial distribution of the machineries successively involved correlates with the different processing steps. For example, fibrillarin and nucleolin that participate in the early stages of rRNA processing, localize in the DFC along with the U3 snoRNA (Biggiogera et al. 1989; Ginisty et al. 1998; Ochs et al. 1985b; Puvion-Dutilleul et al. 1991), whereas proteins B23 and Nop52 that are involved in intermediate or later stages of ribosome biogenesis have been localized to the GC (Biggiogera et al. 1989; Gautier et al. 1994). These findings have led to assigning specific functions to specific compartments of the nucleolus.

Of these three main nucleolar components, condensed chromatin is visible by electron microscopy (Fig. 2). The global amount of intranucleolar chromatin is probably low since global DNA staining (by DAPI) excludes the nucleolus. Presently, the identification and the role of the intranucleolar chromatin that does not correspond to rDNA are unknown. It will be important to characterize this DNA with respect to the numerous proteins not directly involved in ribosome biogenesis

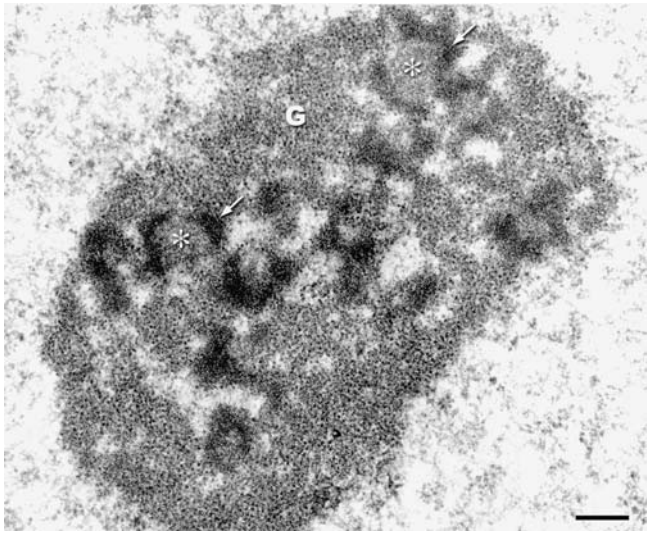

Fig. 1 Nucleolar organization viewed by electron microscopy. The three nucleolar components observed in a HeLa cell: the fibrillar centers (*asterisks*), the dense fibrillar component (*arrow*) and the granular component (*G*). *Bar:* 0.3 μm

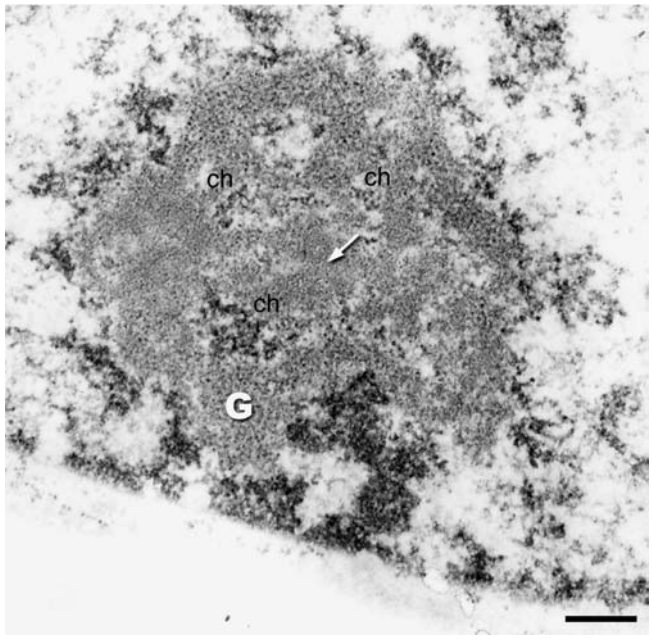

Fig. 2 DNA and RNA in the nucleolus. Nucleic acids were contrasted by the methylation–acetylation procedure (Junéra et al. 1995). Distribution of DNA around the nucleolus as well as among the nucleolar component is visible (*ch*). The contrast in the dense fibrillar component (*arrow*) and in the granular component (*G*) is due to RNAs. *Bar:* 0.3 μm

and identified in the nucleolus by proteomic analysis (Andersen et al. 2005).

What maintains this organization?

Regarding the question of what normally maintains a nucleolus in its usual shape and structure, it has been proposed that a transient association of functionally related components is necessary to generate a morphologically defined nucleolus with its three distinct components (see below for protein dynamics). Hence this organization is the consequence of the activity of ribosome biogenesis. This hypothesis is supported by the spectacular reorganization of the nucleolus induced by blockage of ribosome biogenesis. The two most typical nucleolar reorganizations are presented here but this is not an exhaustive overview of nucleolar reorganization (reviewed in: Gébrane-Younès et al. 2005; Hadjiolov 1985).

Inactivation of rDNA transcription: nucleolar segregation

Nucleolar segregation is observed in conditions of rDNA transcriptional arrest either in physiological conditions or induced by low doses (0.01–0.04 μg/ml) of actinomycin D (ActD). The segregation of nucleoli is characterized by the separation of the nucleolar components, which remain superimposed but no longer intermingle (Fig. 3) (for reviews see: Hadjiolov 1985; Hernandez-Verdun and Junéra 1995; Puvion-Dutilleul et al. 1997; Scheer and Benavente 1990). The effect of ActD on nucleolar organization follows sequential changes: first the fibrillar components condense and migrate towards the nucleolar periphery, then the nucleolar components segregate to finally form a central body associated with caps (Dousset et al. 2000; Hadjiolova et al. 1995). In the caps, several proteins related to the RNA pol I transcription machine such as UBF are present close to fibrillarin-containing caps. In the central body are proteins derived from the GC with the progressive release of some proteins as for example B23. It was recently demonstrated that some nucleolar caps of segregated nucleoli could recruit factors involved in mRNA splicing. In this case relocalization is induced by inhibition of both pol I (rRNA transcription arrest) and pol II (mRNA transcription arrest) (Shav-Tal et al. 2005). This is not observed when only pol I is inhibited, indicating that the composition of a segregated nucleolus can be more complex when induced by general transcription inhibition.

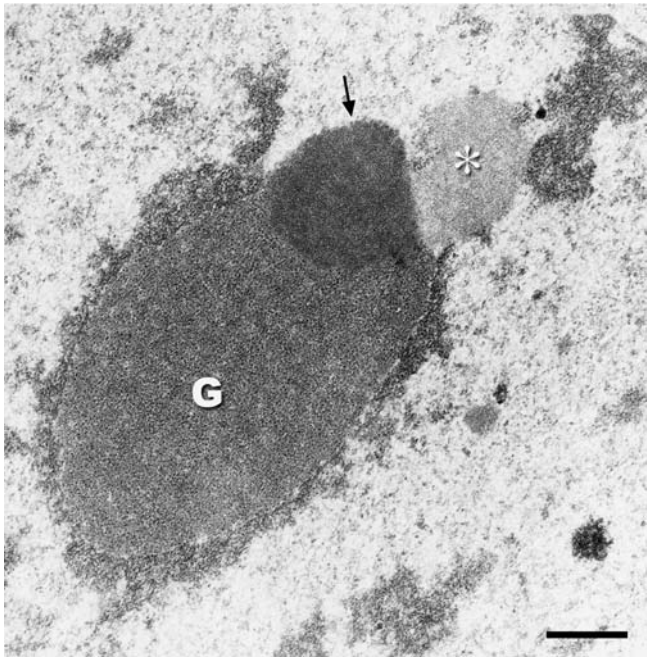

Fig. 3 Disorganization of nucleolar organization of HeLa cells. The three main nucleolar components are segregated by actD inhibition of pol I transcription: the fibrillar centers (*asterisks*), the dense fibrillar component (*arrow*) and the granular component (*G*). *Bar:* 0.5 μm

Separation of transcription sites from processing proteins

Disconnection between rDNA transcription sites and the late rRNA processing proteins can be induced (Chan et al. 1996; Hadjiolov 1985; Rubbi and Milner 2003). The casein kinase (CK2) inhibitor 5,6-dichloro-1-ribofuranosylbenzimidazole (DRB) induces unraveling of nucleoli into necklace and several discrete masses. The necklace structures correspond to the 3D dispersion of the rDNA (Granick 1975; Haaf and Ward 1996; Haaf et al. 1991; Junéra et al. 1997) while several late processing proteins are located in the masses (Louvet et al. 2005). Each bead of the necklace contains rDNA, rRNAs, pol I, DNA topoisomerase I, UBF and fibrillarin (Haaf et al. 1991; Junéra et al. 1997; Le Panse et al. 1999; Scheer and Benavente 1990; Scheer and Rose 1984; Weisenberger and Scheer 1995). EM studies revealed that the nucleolar necklace is composed of small FCs linked to each other by DFC (Junéra et al. 1997; Le Panse et al. 1999) and of masses containing late rRNA processing proteins derived from the GC (Louvet et al. 2005). The rRNA processing proteins can be disconnected from the rRNA transcription sites indicating that rRNA transcripts are not sufficient to attract the processing proteins. This also proves that the rRNA processing proteins can form nuclear structures independently of the transcripts.

The use of drugs is not the only way to induce the separation of the nucleolar machineries with independent distributions of the fibrillar and GCs. Recently it was demonstrated that deletion of the nucleolar trimethyl guanosine synthase I (Tgs1p) can induce such a separation in yeast (Colau et al. 2004). The authors proposed that the interactions of Tgs1p with the snoRNP proteins contribute to the coalescence of nucleolar components. This could indicate that the

connection between the fibrillar and granular nucleolar components is achieved through the interactions of the U3 small ribosomal subunit processome and 90S preribosomes.

Dynamics of the nucleolar proteins in interphase

In the past five years, the fluorescent fusion proteins observed in living cells have become essential for the analysis of intracellular dynamics. Time-lapse videomicroscopy can track the movement of large fluorescent complexes in the cell volume (3D + time) and fluorescent recovery after photobleaching (FRAP) can measure the intracellular mobility or the residence time of fluorescent proteins (Lippincott-Schwartz et al. 2001). The inverse FRAP (iFRAP) quantifies the loss of fluorescence of the region of interest (ROI) after complete bleaching outside this region (Dundr et al. 2004). This is a direct evaluation of time residency of the proteins in the ROI. These technologies applied to nuclear dynamics have brought new dimensions and unexpected concepts concerning nuclear functional compartmentation. The mobility of several GFP-tagged nuclear proteins (nucleolar proteins, histone, DNA binding proteins, transcription factors, splicing factors and nuclear receptors) has been estimated by FRAP and the recovery of fluorescence was slower than would be predicted for isolated diffusing proteins of similar size. FRAP recovery rates change with inhibition of transcription, with decreased temperature and with depletion of ATP indicating that recovery is correlated with nuclear activity.

It was demonstrated that nucleolar proteins rapidly associate and dissociate with nucleolar components in a continuous exchange with the nucleoplasm (Phair and Misteli 2000). The recovery curve of GFP-fibrillarin (DFC marker) in the nucleolus reached a plateau 60 s after bleaching, and the plateau indicated an immobile fraction of about 15% (Phair and Misteli 2000). The diffusion coefficient of fibrillarin (estimated between 0.02 and 0.046 $\mu m^2\ s^{-1}$) was ten times lower in the nucleolus than in the nucleoplasm (Chen and Huang 2001; Phair and Misteli 2000; Snaar et al. 2000). This value is proposed to reflect the time of residency of fibrillarin engaged in nucleolar activity. The nucleolar proteins engaged in rRNA transcription (pol I subunits and transcription factors) (Dundr et al. 2002) and processing (B23, Nop52, nucleolin and Rpp29) also move rapidly with recovery rates in the nucleolus that are similar to that of fibrillarin (Chen and Huang 2001; Louvet et al. 2005). Conversely the recovery rates of ribosomal proteins is slow (approximately three times slower than nucleolar proteins) and was proposed to reflect a slower process for an assembly of ribosomes compared with transcription and processing (Chen and Huang 2001). Alternatively this could be due to more stable associations of ribosomal proteins with the pre-rRNAs. In contrast with the well-defined nucleolar structures visible by electron microscopy, all the nucleolar proteins involved in ribosome biogenesis presently examined cycle between the nucleolus and the nucleoplasm in interphase cells. It has been demonstrated that localization of B23 requires GTP to localize in the nucleolus and ATP for translocation to the nucleoplasm (Finch and Chan 1996; Finch et al. 1993).

Certain nucleolar proteins are shared with other nuclear domains in particular the Cajal body (CB). In the CB, fibrillarin has a significantly larger mobile fraction than in the nucleoli (Snaar et al. 2000). Using iFRAP, it was demonstrated that the nucleolar proteins, fibrillarin, Nopp140 and B23, exhibit similar dissociation kinetics from CB but the dissociation kinetics of proteins from the nucleoli were significantly slower (Dundr et al. 2004). The authors suggest the existence of compartment-specific retention mechanisms for proteins in the CB and nucleoli. In active nucleoli, the fluorescence recovery rate or the loss of fluorescent signal (FRAP or iFRAP) of fluorescent nucleolar proteins indicates rapid diffusion in the nucleoplasm and permanent recruitment in the nucleolus (Chen and Huang 2001; Dundr et al. 2004; Phair and Misteli 2000; Snaar et al. 2000). This also indicates that the residence time depends on specific interactions (Misteli 2001).

Traffic of nucleolar proteins under pol I transcription inhibition

Pol I transcription inhibition by ActD does not prevent the traffic of nucleolar proteins. However, if the diffusion coefficient of nucleolar proteins in the nucleoplasm is similar for active and repressed pol I transcription, the traffic in segregated nucleolus changes differentially for different nucleolar components. The traffic of UBF in the nucleolus is decreased by ActD, whereas it is similar or increased for nucleolar and ribosomal proteins (Chen and Huang 2001). The question is how nucleolar components continue to be maintained in segregated nucleoli in spite of the absence of transcription or pre-rRNA processing. Nucleolar proteins may still be capable of forming complexes during transcription inhibition, but why these complexes remain juxtaposed is presently unknown. Recently, the relocalization of proteins in specific caps of segregated nucleoli (after pol I and pol II inhibition of transcription) was found to be an energy-dependent repositioning process that requires active metabolism of the cells (Shav-Tal et al. 2005). It was proposed that cell energy in the form of ATP and GTP is most probably required. This is in accordance with the fact that translocation of B23 to the nucleoplasm requires ATP and nucleolar recruitment requires GTP (Finch and Chan 1996; Finch et al. 1993). GTP binding was also shown to function as a driving mechanism of translocation for nucleostemin from nucleoplasm to the nucleolus (Tsai and McKay 2005). This dynamic system generates a bidirectional, fast and sensitive measurement

for the control of the amount of nucleostemin engaged in the nucleolus.

Traffic of nucleolar proteins in masses disconnected from transcription sites

It is possible to trigger redistribution of late rRNA processing proteins in masses at a distance from the transcription sites (David-Pfeuty et al. 2001; Rubbi and Milner 2003; Sirri et al. 2002). In these masses no rRNAs can be detected (Louvet et al. 2005), and no rRNA processing activity can occur. We were interested in characterizing the dynamics of processing proteins in these conditions. Surprisingly, it was found that the traffic of processing proteins is similar in the DRB-induced masses and in the GC of active nucleoli (Louvet et al. 2005). Fluorescence recovery was observed in 60 s and immobile fractions of B23 and Nop52 were found for both active and inactive processing proteins. This steady state is compatible with formation of the masses but the mechanism maintaining these immobile fractions is presently unknown. It was proposed that protein–protein interactions could be at the origin of these interactions since there are no rRNAs in the masses (Louvet et al. 2005). In this case, phosphorylation vs dephosphorylation of the processing proteins could play a role in rRNA transcript association in active nucleoli vs absence of association with rRNA in the DRB-induced masses. Such a role of phosphorylation vs dephosphorylation was demonstrated for B23 during the cell cycle (Okuwaki et al. 2002).

Nucleolar assembly at the end of mitosis

In higher eukaryotic cells, the nucleolus is assembled at the transition mitosis/interphase (Hernandez-Verdun 2004). Nucleolar assembly takes place when cells exit from mitosis around the rDNA clusters and coincides with the reactivation of rDNA transcription. During mitosis, the rDNA transcription machinery remains associated with or close to the rDNA throughout the cell cycle (Roussel et al. 1996). rDNA transcription is repressed at the entrance to mitosis (Heix et al. 1998) and maintained repressed during mitosis (Sirri et al. 2000) by phosphorylation of components of the rDNA transcription machinery directed by the cyclin-dependent kinase (CDK) 1-cyclin B. Consequently, inactivation of the CDK1-cyclin B occurring normally in telophase is sufficient to release mitotic repression of rDNA transcription.

In addition to the reactivation of the rDNA transcription machinery, the assembly of the nucleolus requires relocalization and reactivation of the pre-rRNA processing machinery. Proteins involved in pre-rRNA processing leave the nucleolus in prophase and localize mainly at the periphery of chromosomes during mitosis (Gautier et al. 1994). The nucleolar proteins which relocate to the chromosome periphery are components of the DFC and GC of the active nucleolus (Gautier et al. 1992a). This is the case for example of fibrillarin (DFC marker), and B23 and Nop52 proteins (GC markers) (Fomproix et al. 1998; Gautier et al. 1994). These proteins are, respectively, in the early rRNA processing machinery and in the late rRNA processing machinery. In living cells, the nucleolar proteins tagged with GFP are concentrated around the chromosomes during mitosis and migrate with the chromosomes (Angelier et al. 2005; Savino et al. 2001). The coordinated movements of the nucleolar proteins and chromosomes suggest that these proteins maintain their interactions during this process. Such chromosome association is also supported by DNAse solubilization of B23 during mitosis (Zatsepina et al. 1997), by the presence of the proteins at the surface of isolated chromosomes (Gautier et al. 1992c), and by localization of the proteins observed by electron microscopy (Gautier et al. 1992b). However, the mechanism(s) maintaining interactions of nucleolar processing proteins with chromosomes during mitosis are not yet characterized. In anaphase, early and late processing proteins (respectively, fibrillarin and Bop1, B23, Nop52) were homogeneously distributed around the chromosomes (Angelier et al. 2005). During telophase and early G1, along the translocation pathway between chromosome periphery and sites of transcription, processing proteins are concentrated in foci designated prenucleolar bodies (PNBs) (Angelier et al. 2005; Azum-Gélade et al. 1994; Dundr et al. 2000; Jiménez-Garcia et al. 1994; Ochs et al. 1985a; Savino et al. 2001). The PNBs are discrete fibrogranular structures visible by electron microscopy in cycling cells of animals and plants (Fig. 4b). The PNBs are also formed during *Xenopus* development before complete nucleolar assembly and they can be assembled in vitro in *Xenopus* egg extracts (Bell et al. 1992; Verheggen et al. 1998). Thus PNB formation is a general phenomenon occurring during cell cycle and development. The PNBs formed along the recruitment pathway of the nucleolar processing proteins provide a physiological situation to investigate the formation, control and dynamics of body belonging to nucleolar machineries.

Dynamics of processing proteins during nucleolar assembly

The dynamics of the processing nucleolar proteins was analyzed at the transition mitosis/interphase. Rapid time-lapse videomicroscopy was used to follow GFP-tagged processing proteins in the volume of the cells from metaphase to G1 (Fig. 5). High frequency recordings of the GFP-tagged proteins reveal that the first detectable concentration of proteins in foci occurred on the chromosome surface during telophase (Savino et al. 2001). This conclusion was supported by electron microscopy observations. After this phase of

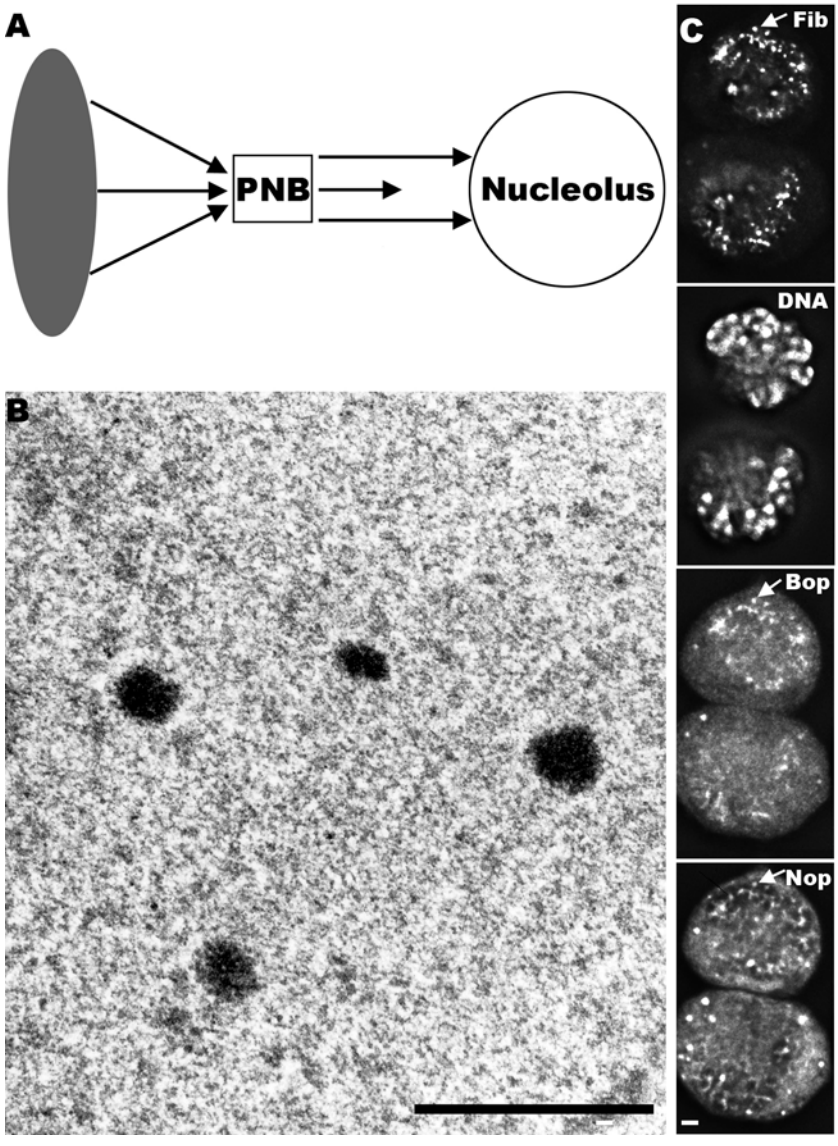

Fig. 4 The prenucleolar bodies (PNBs). (**a**) Schematic representation of PNB formation at the end of mitosis. The nucleolar processing proteins which localized around the chromosomes (*ellipse*) during mitosis, assemble in PNBs at telophase. During early G1, the PNB proteins are progressively recruited in the nucleolus. (**b**) Standard electron microscopy of PNBs in HeLa cells. Note the fibrillo-granular structure of the four PNBs. *Bar:* 0.1 μm (courtesy of J. Gébranne-Younes). (**c**) Localization of early and late nucleolar processing proteins in the same PNBs in telophase. A pair of daughter cells are visible. The presence of fibrillarin (Fib) is detected in PNBs (*arrow*), the same PNBs also contain Bop1-GFP (Bop) and Nop52 (Nop). DNA is revealed by DAPI. *Bar:* 1 μm

formation, the prediction was that the PNBs would move nuclear bodies involved in the delivery of preassembled processing complexes to the site of rDNA transcription. PNB dynamics in living cells do not reveal such directional movement towards the nucleolus (Fig. 5) (Dundr et al. 2000; Savino et al. 2001). Rather,

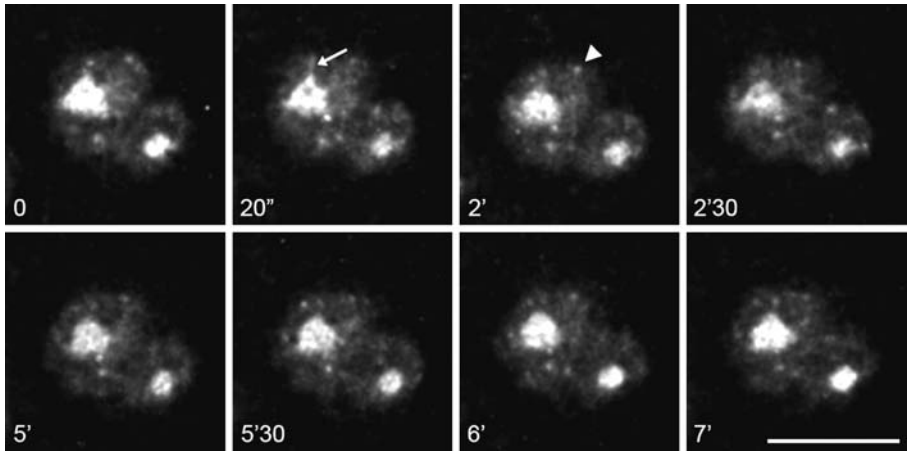

Fig. 5 Dynamics of prenucleolar bodies (PNBs) in a living cell. The dynamics of PNBs was recorded in living cells, in an early G1 HeLa cell expressing GFP-Nop52. The amount of GFP-Nop52 varies in the same PNBs (*arrowhead*) and the nucleolar delivery is visible as a flux (*arrow*). See *movies 1* as additional data. *Bar:* 1 μm. Additional data: *movies 1*—GFP-Nop52 at early G1 in HeLa cell (courtesy of L. Lo Presti); *movies 2*—GFP-Nop52 and DsRed-B23 from metaphase to early G1 in HeLa cell (courtesy of N. Angelier)

the progressive delivery of proteins of the PNB to the nucleoli is ensured by directional flow among PNBs and between PNBs and the nucleolus (Savino et al. 2001).

Based on observations of fixed cells, it was concluded that early processing proteins (fibrillarin) are recruited first on sites of transcription while the majority of late processing proteins are still in PNBs (Fomproix et al. 1998; Savino et al. 1999). This schedule was confirmed in living HeLa cells. Fibrillarin is in PNBs before recruitment to the nucleolus during a short period (~15 min) while Nop52 is maintained in PNBs for a longer period (~80 min) (Savino et al. 2001). These observations were made in different cells, and because the time window is very short, we cannot exclude that early and late processing proteins are in the same PNBs during this short period (~15 min) of telophase. Occasionally in fixed cells, fibrillarin, Nop52 and Bop1 can be colocalized in some PNBs (Fig. 4c). To answer this question, it was necessary to compare the relative movement of green and red tagged proteins in the same cells. The relative dynamics of early and late rRNA processing proteins at the time of PNB formation was examined using co-expression of GFP-fibrillarin and DsRed-B23. The kinetics of translocation of the two proteins was analyzed in the same living cell by rapid two color 4D imaging (volume + time for both proteins) (Angelier et al. 2005). In all the cells examined, once near the poles, 1 or 2 min after the onset of telophase, numerous bright fluorescent foci containing both GFP-fibrillarin and DsRed-B23 became visible almost simultaneously. In telophase, GFP and Ds-Red were quantified in the same foci and compared to dispersed proteins in the same nucleus. The relative amount of B23 in foci was five to six times higher than that of the dispersed proteins; amount of fibrillarin in the same foci was three to four times higher than that of the dispersed proteins for about 10 min. After this time, fibrillarin was released while B23 was still present in foci. This clearly illustrates the presence of the two types of nucleolar processing proteins in the same PNBs and suggests differential sorting of these proteins. Conversely in the same observation conditions, similar dynamics and flow of GFP-Nop52 and DsRed-B23 (late rRNA processing) was found. The relative amounts of Nop52 and B23 in PNBs were very similar and the amounts of both proteins varied simultaneously. This could indicate that these late processing proteins are released from PNBs as complexes and could interact along the pathway.

The role of the complex delivery of the nucleolar processing proteins at exit of mitosis remains an open question. When nucleolar function is established during interphase, the recruitment of processing proteins is not associated with PNB formation. PNB formation could be a cell cycle-dependent process. Strikingly, fibrillarin concentrates in PNBs and rDNA clusters when a decrease in CdK1 activity overcomes the mitotic repression of RNA pol I transcription (Clute and Pines 1999), while Nop52 and other GC proteins are recruited later on transcripts. This late recruitment is under the control of cyclin-dependent kinases since CDK inhibitors block this process (Sirri et al. 2002). Thus, it seems that recruitment of the processing machinery at the time of nucleolar assembly is a regulated process most probably dependent on cell-cycle progression.

Dynamics of processing protein interaction during nucleolar assembly

The role of the PNBs along the recruitment pathway of the processing nucleolar proteins is presently not clear (Fig. 4a).Why these intermediate steps and not a direct recruitment on the newly transcribed rRNAs is the main question. The processing proteins should interact with the pre-rRNAs at transcription sites. In telophase/early G1 nuclei, beside the sites of rDNA transcription, pre-rRNAs pass through mitosis and localize in PNBs (Dousset et al. 2000). It has been proposed that the processing complexes forming PNBs can be nucleated by these pre-rRNAs (Dousset et al. 2000; Dundr and Olson 1998; Dundr et al. 2000; Pinol-Roma 1999). This hypothesis of PNB formation by association around pre-rRNAs could explain the temporal order of nucleolar delivery of the processing machinery driven by pre-rRNA stability.

To understand the role of PNBs in nucleolar assembly better, we questioned the possibility that processing protein complexes could be formed on these pre-rRNAs. Since the formation of PNBs is very dynamic and occurs along the pathway between chromosomes and nucleoli it is necessary to search for protein interactions in living cells. Time-lapse analysis of fluorescence resonance energy transfer (FRET) was chosen to determine whether nucleolar processing proteins interact along the recruitment pathway (Angelier et al. 2005). The principle is based on the distance of fluorescent tags. If donor and acceptor fluorescent tags are in close proximity (typically less than 7 nm for the GFP/DsRed couple), FRET reduces the fluorescence lifetime of GFP. The apparatus used for FRET determination by the time- and space-correlated single photon counting method was described (Emiliani et al. 2003). This technique directly gives the picosecond time-resolved fluorescence decay for every pixel by counting and sampling single emitted photons. FRET was measured for several nucleolar protein couples first in the nucleoli and then during nucleolar assembly. The positive FRET between GFP-Nop52 and DsRed-B23 in the nucleoli indicates that the distance and most probably interactions between proteins can be evaluated by this approach (Angelier et al. 2005). The absence of FRET between GFP-fibrillarin and DsRed-B23 provide a good control of non-colocalized nucleolar proteins since fibrillarin is concentrated in the DFC and B23 in GC. Since it is possible to detect FRET between B23 and Nop52 in the nucleoli, we decided to track FRET during the recruitment of these proteins into

nucleoli from anaphase to early G1. FRET was never detected during anaphase at the periphery of the chromosomes whereas FRET was registered in 20% of PNBs at the beginning of telophase, in about 40% at the end of telophase, and in 55% in early G1. Thus, interaction between GFP-Nop52 and DsRed-B23 was established progressively in PNBs, as the number of PNBs exhibiting FRET increased. Such data indicate that Nop52 and B23 did not interact until they were recruited in PNBs. It is noteworthy that a given PNB can alternatively present FRET or not present FRET. Based on these protein couples, one hypothesis is that late rRNA processing proteins already interact in PNBs. If this is confirmed for other rRNA processing complexes, PNBs could be proposed as assembly platform of processing complexes at this period of the cell cycle. It would be very interesting to know if this role can be extended to the early rRNA processing machinery.

In conclusion, the dynamics of PNBs at the mitosis/interphase transition suggests a steady state favoring at this period of the cell-cycle residence of processing factors close to the condensed chromatin either by self-assembly of processing factors or by specific interaction with pre-rRNAs. The processing machinery is first concentrated in PNBs; some complexes are formed and then released in a control- and time-dependent manner.

The nucleolus in the nuclear environment

Several nuclear domains (for a review see: Matera 1999; Schul et al. 1998; Spector 2001), interact physically with the nucleoli. The question is whether these nuclear domains and nucleoli are functionally linked and communicate with each other. The best example is provided by the CB, which was discovered in the neuronal nucleus as "accessory body" of the nucleolus (for a review see: Gall 2000; Matera 1999). The interaction between the CB and the nucleolus has been well documented by electron microscopy (for a recent reference see: Pena et al. 2001) and its dynamics analyzed in living cells (Dundr et al. 2004; Platani et al. 2000; Sleeman and Lamond 1999). It was demonstrated that the number of CBs and the relationship of CBs with the nucleolus in neurons correlate with nuclear transcription activities (Pena et al. 2001). The CB is highly enriched in small nuclear and nucleolar ribonucleoproteins (snRNPs and snoRNPs) required for the maturation of pre-mRNAs and pre-rRNAs and shares with the nucleolus the proteins fibrillarin, Nopp 140, and NAP57 (Matera 1999) and subunits of the RNase P (Jarrous et al. 1999). It was proposed that Nopp 140 functions as a molecular link between the nucleolus and the CB with the possibility to chaperone the transport of other molecules (Isaac et al. 1998). The CB is proposed to be a platform in the biogenesis pathways of snRNPs and snoRNPs (Matera 1999). This later function could explain the preferential interactions between the nucleolus and the CB.

In telophase, the serine–arginine (SR) rich splicing factors transiently localize around rDNA transcription sites before reaching the nuclear speckles (Bubulya et al. 2004). The authors suggest that this pathway depends of the SR protein kinase localized around new nucleolus at this period. These observations demonstrate the coordination that exists between an assembly of several nuclear domains at this period of the cell cycle.

The nucleolus was recently proven to also be involved in other cell functions in addition to ribosome biogenesis (for a review see: Carmo-Fonseca et al. 2000; Olson 2000; Olson and Dundr 2005; Pederson 1998; Visitin and Amon 2000). This is based on the presence of molecules in the nucleolus that are not involved in ribosome biogenesis itself. The presence of the RNA of the signal recognition particle (SRP) and three SRP proteins found in the nucleolus and SRP RNA localization differs from the classical sites of ribosome biogenesis (Politz et al. 2000, 2002). There are also indications that at least some of the tRNAs are processed in the nucleoli (Bertrand et al. 1998; Jarrous et al. 1999), and that the U6 spliceosomal RNA cycles through the nucleolus to undergo methylation and pseudouridylation (Ganot et al. 1999). The nucleolus is also a domain of sequestration or retention for molecules related to cell cycle, life span and apoptosis (for a review see: Cockell and Gasser 1999; Guarente 2000; Visitin and Amon 2000).

Conclusions and remarks

The organization and structure of the nucleolus have been well characterized by electron microscopy since the 1970 s. To form this domain, the targeting of large multiprotein complexes should be achieved. The dynamics of the machines or complexes necessary to build a ribosome would support the nucleolar organization described in electron microscopy. How these molecular machines reach the correct place at the right time and participate in the assembly of active functional nucleoli is under investigation by the analysis of the dynamics of proteins and complexes in living cells. The first conclusion is that the diffusion of nucleolar proteins appears most likely to be the major mode of movement in the nucleoplasm similar to other nuclear complexes (for a general review see: Carmo-Fonseca et al. 2002; Janicki and Spector 2003; Misteli 2001; Roix and Misteli 2002). However, the existence of transport factors cannot be excluded, as recently demonstrated for the routing of U3 precursors between the CB and nucleolus that requires CRM1 (Boulon et al. 2004).

The second major conclusion concerns the presence in the nucleolus of complexes not involved in ribosome biogenesis. Indeed, the nucleolus appears more at the center of a trafficking pathway for several RNP rather than a domain exclusively dedicated to rRNA synthesis and processing. What is the link between the nucleolar ribosome biogenesis activity per se and the other func-

tions? We propose that ribosome biogenesis creates the compartmentation of a molecular machinery that can be of benefit to other functions. In this case, the prediction is that destruction of ribosome biogenesis activity would also stop the other functions occurring in an active nucleolus. This puts the active nucleolus at the parting of the ways of several nuclear functions.

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