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Nucleolus : from structure to dynamics**Danièle Hernandez-Verdun****Nuclei and Cell Cycle****CNRS, Université Paris VI, Université Paris VII****Institut Jacques Monod, 2 place Jussieu, 75251 Paris Cedex 05. France****Corresponding author: Hernandez-Verdun D****Phone: 331 44 27 40 38****Fax : 331 44 27 59 94****E. mail: dhernand@ccr.jussieu.fr**

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 informations. 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 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.

Key words: 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 neighboring. 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. In this review, information coming from 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.

1 - Organization of the nucleolus reflect its activity

1-a) 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).

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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; Hozák et al., 1994; Puvion-Dutilleul et al., 1997 ; Shaw and Jordan, 1995). As far 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, fibrillar and nucleolar proteins 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 intra-nucleolar chromatin is probably low since global DNA staining (by DAPI) excludes the nucleolus. Presently, the identification and the role of the intra-nucleolar 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 and identified in nucleolus by proteomic analysis (Andersen et al., 2005).

1-b) What maintains this organization?

To the question of what normally maintains a nucleolus in its usual shape and structure, it has been proposed that transient association of functionally related components is necessary to generate a morphologically defined nucleolus with its three distinct components (see below, 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)).

a- 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 fibrillar-in-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 relocation 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.

b- 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-ribo-furanosylbenzimidazole (DRB) induces unraveling of nucleoli into necklace and several discrete masses. The necklace structures correspond to the 3-D dispersion of the rDNA (Granick, 1975b ; Haaf et al., 1991; Haaf and Ward, 1996; 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

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3 sufficient to attract the processing proteins. This also proves that the rRNA processing
4 proteins can form nuclear structures independently of the transcripts.
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10 **2 – Dynamics of the nucleolar proteins in interphase**

11 In the past 5 years, the fluorescent fusion proteins observed in living cells have become
12 essential for the analysis of intracellular dynamics. Time-lapse videomicroscopy can track the
13 movement of large fluorescent complexes in the cell volume (3-D + time) and fluorescent
14 recovery after photobleaching (FRAP) can measure the intracellular mobility or the residence
15 time of fluorescent proteins (Lippincott-Schwartz et al., 2001). The inverse FRAP (iFRAP)
16 quantifies the loss of fluorescence of the region of interest (ROI) after complete bleaching
17 outside this region (Dundr et al., 2004). This is a direct evaluation of time residency of the
18 proteins in the ROI. These technologies applied to nuclear dynamics have brought new
19 dimensions and unexpected concepts concerning nuclear functional compartmentation. The
20 mobility of several GFP-tagged nuclear proteins (nucleolar proteins, histone, DNA binding
21 proteins, transcription factors, splicing factors, nuclear receptors) has been estimated by FRAP
22 and the recovery of fluorescence was slower than would be predicted for isolated diffusing
23 proteins of similar size. FRAP recovery rates change with inhibition of transcription, with
24 decreased temperature and with depletion of ATP indicating that recovery is correlated with
25 nuclear activity.
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38 It was demonstrated that nucleolar proteins rapidly associate and dissociate with nucleolar
39 components in a continuous exchange with the nucleoplasm (Phair and Misteli, 2000). The
40 recovery curve of GFP-fibrillarin (DFC marker) in the nucleolus reached a plateau 60 sec after
41 bleaching, and the plateau indicated an immobile fraction of about 15% (Phair and Misteli,
42 2000). The diffusion coefficient of fibrillarin (estimated between 0.02 and 0.046 $\mu\text{m}^2 \text{s}^{-1}$) was
43 10 times lower in the nucleolus than in the nucleoplasm (Chen and Huang, 2001; Phair and
44 Misteli, 2000; Snaar et al., 2000). This value is proposed to reflect the time of residency of
45 fibrillarin engaged in nucleolar activity. The nucleolar proteins engaged in rRNA transcription
46 (UBF) and processing (B23, Nop52, nucleolin, Rpp29) also move rapidly with recovery rates
47 in the nucleolus that are similar to that of fibrillarin (Chen and Huang, 2001 ; Louvet et al.,
48 2005). Conversely the recovery rates of ribosomal proteins is slow (~ 3 times slower than
49 nucleolar proteins) and was proposed to reflect a slower process for assembly of ribosomes
50 compared with transcription and processing (Chen and Huang, 2001). Alternatively this could
51 be due to more stable associations of ribosomal proteins with the pre-rRNAs. In contrast with
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3 the well-defined nucleolar structures visible by electron microscopy, all the nucleolar proteins
4 involved in ribosome biogenesis presently examined cycle between the nucleolus and the
5 nucleoplasm in interphase cells. It has been demonstrated that localization of B23 requires
6 GTP to localize in the nucleolus and ATP for translocation to the nucleoplasm (Finch and
7 Chan, 1996; Finch et al., 1993).

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

32 33 *- Traffic of nucleolar proteins under pol I transcription inhibition*

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35 Pol I transcription inhibition by ActD does not prevent the traffic of nucleolar proteins.
36 However if the diffusion coefficient of nucleolar proteins in the nucleoplasm is similar for
37 active and repressed pol I transcription, the traffic in segregated nucleolus changes
38 differentially for different nucleolar components. The traffic of UBF in the nucleolus is
39 decreased by ActD, whereas it is similar or increased for nucleolar and ribosomal proteins
40 (Chen and Huang, 2001). The question is how nucleolar components continue to be
41 maintained in segregated nucleoli in spite of the absence of transcription or pre-rRNA
42 processing. Nucleolar proteins may still be capable of forming complexes during transcription
43 inhibition, but why these complexes remain juxtaposed is presently unknown. Recently, the
44 relocalization of proteins in specific caps of segregated nucleoli (after pol I and pol II
45 inhibition of transcription) was found to be an energy-dependent repositioning process that
46 requires active metabolism of the cells (Shav-Tal et al., 2005). It was proposed that cell
47 energy in the form of ATP and GTP is most probably required. This is in accordance with the
48 fact that translocation of B23 to the nucleoplasm requires ATP and nucleolar recruitment
49 requires GTP (Finch and Chan, 1996; Finch et al., 1993).
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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 sec 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 versus dephosphorylation of the processing proteins could play a role in rRNA transcript association in active nucleoli versus absence of association with rRNA in the DRB-induced masses. Such a role of phosphorylation versus dephosphorylation was demonstrated for B23 during the cell cycle (Okuwaki et al., 2002).

3 - Nucleolar assembly at the end of mitosis

In higher eukaryotic cells, the nucleolus is assembled at the transition mitose/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

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3 which relocate to the chromosome periphery are components of the DFC and GC of the active
4 nucleolus (Gautier et al., 1992a). This is the case for example of fibrillarin (DFC marker), and
5 B23 and Nop52 proteins (GC markers) (Fomproix et al., 1998; Gautier et al., 1994). These
6 proteins are respectively in the early rRNA processing machinery and in the late rRNA
7 processing machinery. In living cells, the nucleolar proteins tagged with GFP are concentrated
8 around the chromosomes during mitosis and migrate with the chromosomes (Angelier et al.,
9 2005; Savino et al., 2001). The coordinated movements of the nucleolar proteins and
10 chromosomes suggest that these proteins maintain their interactions during this process. Such
11 chromosome association is also supported by DNase solubilization of B23 during mitosis
12 (Zatsepina et al., 1997), by the presence of the proteins at the surface of isolated chromosomes
13 (Gautier et al., 1992c), and by localization of the proteins observed by electron microscopy
14 (Gautier et al., 1992b). However, the mechanism(s) maintaining interactions of nucleolar
15 processing proteins with chromosomes during mitosis are not yet characterized. In anaphase,
16 early and late processing proteins (respectively fibrillarin and Bop1, B23, Nop52) were
17 homogeneously distributed around the chromosomes (Angelier et al., 2005). During telophase
18 and early G1, along the translocation pathway between chromosome periphery and sites of
19 transcription, processing proteins are concentrated in foci designated prenucleolar bodies
20 (PNBs) (Angelier et al., 2005; Azum-Gélade et al., 1994; Dundr et al., 2000; Jiménez-García
21 et al., 1994; Ochs et al., 1985a; Savino et al., 2001). The PNBs are discrete fibrogranular
22 structures visible by electron microscopy in cycling cells of animals and plants (Fig 4B). The
23 PNBs are also formed during *Xenopus* development before complete nucleolar assembly and
24 they can be assembled *in vitro* in *Xenopus* egg extracts (Bell et al., 1992; Verheggen et al.,
25 1998). Thus PNB formation is a general phenomenon occurring during cell cycle and
26 development. The PNBs formed along the recruitment pathway of the nucleolar processing
27 proteins provide a physiological situation to investigate the formation, control and dynamics
28 of body belonging to nucleolar machineries.
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53 4- Dynamics of processing proteins during nucleolar assembly

54 The dynamics of the processing nucleolar proteins was analyzed at the transition
55 mitosis/interphase. Rapid time-lapse videomicroscopy was used to follow GFP-tagged
56 processing proteins in the volume of the cells from metaphase to G1 (Fig 5). High frequency
57 recordings of the GFP-tagged proteins reveal that the first detectable concentration of proteins
58 in foci occurred on the chromosome surface during telophase (Savino et al., 2001). This
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3 conclusion was supported by electron microscopy observations. After this phase of formation,
4 the prediction was that the PNBs would move nuclear bodies involved in the delivery of
5 preassembled processing complexes to the site of rDNA transcription. PNB dynamics in
6 living cells do not reveal such directional movement towards the nucleolus (Fig 5) (Dundr et
7 al., 2000; Savino et al., 2001). Rather, the progressive delivery of proteins of the PNB to the
8 nucleoli is ensured by directional flow between PNBs and between PNBs and the nucleolus
9 (Savino et al., 2001).

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11 Based on observations of fixed cells, it was concluded that early processing proteins
12 (fibrillarin) are recruited first on sites of transcription while the majority of late processing
13 proteins are still in PNBs (Fomproix et al., 1998; Savino et al., 1999). This schedule was
14 confirmed in living HeLa cells. Fibrillarin is in PNBs before recruitment to the nucleolus
15 during a short period (~15 min) while Nop52 is maintained in PNBs for a longer period (~80
16 min) (Savino et al., 2001). These observations were made in different cells, and because the
17 time window is very short, we cannot exclude that early and late processing proteins are in the
18 same PNBs during this short period (~ 15 min) of telophase. Occasionally in fixed cells,
19 fibrillarin, Nop52 and Bop1 can colocalized in some PNBs (Fig. 4C). To answer this question,
20 it was necessary to compare the relative movement of green and red tagged proteins in the
21 same cells. The relative dynamics of early and late rRNA processing proteins at the time of
22 PNB formation was examined using co-expression of GFP-fibrillarin and DsRed-B23. The
23 kinetics of translocation of the two proteins was analyzed in the same living cell by rapid 2
24 color 4D imaging (volume + time for both proteins) (Angelier et al., 2005). In all the cells
25 examined, once near the poles, one or two min after the onset of telophase, numerous bright
26 fluorescent foci containing both GFP-fibrillarin and DsRed-B23 became visible almost
27 simultaneously. In telophase, GFP and Ds-Red were quantified in the same foci and compared
28 to dispersed proteins in the same nucleus. The relative amount of B23 in foci was 5 to 6 times
29 higher that of the dispersed proteins; amount of fibrillarin in the same foci was 3 to 4 times
30 higher that of dispersed proteins for about 10 min. After this time, fibrillarin was released
31 while B23 was still present in foci. This clearly illustrates the presence of the two types of
32 nucleolar processing proteins in the same PNBs and suggests differential sorting of these
33 proteins. Conversely in the same observation conditions, similar dynamics and flow of GFP-
34 Nop52 and DsRed-B23 (late rRNA processing) was found. The relative amounts of Nop52
35 and B23 in PNBs were very similar and the amounts of both proteins varied simultaneously.
36 This could indicate that these late processing proteins are released from PNBs as complexes
37 and could interact along the pathway.

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3 The role of the complex delivery of the nucleolar processing proteins at exit of mitosis
4 remains an open question. When nucleolar function is established during interphase, the
5 recruitment of processing proteins is not associated with PNB formation. PNB formation
6 could be a cell cycle-dependent process. Strikingly, fibrillarin concentrates in PNBs and
7 rDNA clusters when a decrease in Cdk1 activity overcomes the mitotic repression of RNA
8 pol I transcription (Clute and Pines, 1999), while Nop52 and other GC proteins are recruited
9 later on transcripts. This late recruitment is under the control of cyclin-dependent kinases
10 since CDK inhibitors block this process (Sirri et al., 2002). Thus, it seems that recruitment of
11 the processing machinery at the time of nucleolar assembly is a regulated process most
12 probably dependent on cell cycle progression.
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24 **5- Dynamics of processing protein interaction during nucleolar assembly**

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26 The role of the PNBs along the recruitment pathway of the processing nucleolar proteins is
27 presently not clear (Fig 4A). The main question is why these intermediate steps and not a
28 direct recruitment on the newly transcribed rRNAs. The processing proteins should interact
29 with the pre-rRNAs at transcription sites. In telophase/early G1 nuclei, beside the sites of
30 rDNA transcription, pre-rRNAs pass through mitosis and localize in PNBs (Dousset et al.,
31 2000). It has been proposed that the processing complexes forming PNBs can be nucleated by
32 these pre-rRNAs (Dousset et al., 2000; Dunder et al., 2000; Dunder and Olson, 1998; Pinol-
33 Roma, 1999). This hypothesis of PNB formation by association around pre-rRNAs could
34 explain the temporal order of nucleolar delivery of the processing machinery driven by pre-
35 rRNA stability.
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43 To better understand the role of PNBs in nucleolar assembly, we questioned the possibility
44 that processing protein complexes could be formed on these pre-rRNAs. Since the formation
45 of PNBs is very dynamic and occurs along the pathway between chromosomes and nucleoli it
46 is necessary to search for protein interactions in living cells. Time-lapse analysis of
47 fluorescence resonance energy transfer (FRET) was chosen to determine whether nucleolar
48 processing proteins interact along the recruitment pathway (Angelier et al., 2005). The
49 principle is based on the distance of fluorescent tags. If donor and acceptor fluorescent tags
50 are in close proximity (typically less than 7 nm for the GFP/DsRed couple), FRET reduces the
51 fluorescence lifetime of GFP. The apparatus used for FRET determination by the time- and
52 space-correlated single photon counting method was described (Emiliani et al., 2003). This
53 technique directly gives the picosecond time-resolved fluorescence decay for every pixel by
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3 counting and sampling single emitted photons. FRET was measured for several nucleolar
4 protein couples first in nucleoli and then during nucleolar assembly. The positive FRET
5 between GFP-Nop52 and DsRed-B23 in nucleoli indicates that the distance and most
6 probably interactions between proteins can be evaluated by this approach (Angelier et al.,
7 2005). The absence of FRET between GFP-fibrillarin and DsRed-B23 provide a good control
8 of non-colocalized nucleolar proteins since fibrillarin is concentrated in the DFC and B23 in
9 GC. Since it is possible to detect FRET between B23 and Nop52 in nucleoli, we decided to
10 track FRET during the recruitment of these proteins into nucleoli from anaphase to early G1.
11 FRET was never detected during anaphase at the periphery of the chromosomes whereas
12 FRET was registered in 20% of PNBs at the beginning of telophase, in about 40% at the end
13 of telophase, and in 55% in early G1. Thus, interaction between GFP-Nop52 and DsRed-B23
14 was established progressively in PNBs, as the number of PNBs exhibiting FRET increased.
15 Such data indicate that Nop52 and B23 did not interact until they were recruited in PNBs. It is
16 noteworthy that a given PNB can alternatively present FRET or not present FRET. Based on
17 these protein couples, one hypothesis is that late rRNA processing proteins already interact in
18 PNBs. If this is confirmed for other rRNA processing complexes, PNBs could be proposed as
19 assembly platform of processing complexes at this period of the cell cycle. It would be very
20 interesting to know if this role can be extended to the early rRNA processing machinery.

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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.

6 - 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

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3 relationship of CBs with the nucleolus in neurons correlate with nuclear transcription
4 activities (Pena et al., 2001). The CB is highly enriched in small nuclear and nucleolar
5 ribonucleoproteins (snRNPs and snoRNPs) required for the maturation of pre-mRNAs and
6 pre-rRNAs and shares with the nucleolus the proteins fibrillarin, Nopp 140, and NAP57
7 (Matera, 1999) and subunits of the RNase P (Jarrous et al., 1999). It was proposed that Nopp
8 140 functions as a molecular link between the nucleolus and the CB with the possibility to
9 chaperone the transport of other molecules (Isaac et al., 1998). The CB is proposed to be a
10 platform in the biogenesis pathways of snRNPs and snoRNPs (Matera, 1999). This later
11 function could explain the preferential interactions between the nucleolus and the CB.
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14 In telophase, the serine-arginine (SR) rich splicing factors transiently localize around rDNA
15 transcription sites before reaching the nuclear speckles (Bubulya et al., 2004). The authors
16 suggest that this pathway depends of the SR protein kinase localized around new nucleolus at
17 this period. These observations demonstrate the coordination that exists between assembly of
18 several nuclear domains at this period of the cell cycle.
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21 The nucleolus was recently proven to also be involved in other cell functions in addition to
22 ribosome biogenesis (for a review see (Carmo-Fonseca et al., 2000; Pederson, 1998, Olson,
23 2000 #5; Visitin and Amon, 2000)). This is based on the presence of molecules in the
24 nucleolus that are not involved in ribosome biogenesis itself. The presence of the RNA of the
25 signal recognition particle (SRP) and three SRP proteins found in the nucleolus and SRP RNA
26 localization differs from the classical sites of ribosome biogenesis (Politz et al., 2002; Politz
27 et al., 2000). There are also indications that at least some of the tRNAs are processed in the
28 nucleoli (Bertrand et al., 1998; Jarrous et al., 1999), and that the U6 spliceosomal RNA cycles
29 through the nucleolus to undergo methylation and pseudouridylation (Ganot et al., 1999). The
30 nucleolus is also a domain of sequestration or retention for molecules related to cell cycle, life
31 span, and apoptosis (for a review see (Cockell and Gasser, 1999; Guarente, 2000; Visitin and
32 Amon, 2000)).
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53 **Conclusions and remarks**

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

10
11 The second major conclusion concerns the presence in the nucleolus of complexes not
12 involved in ribosome biogenesis. Indeed, the nucleolus appears more at the center of a
13 trafficking pathway for several RNP rather than a domain exclusively dedicated to rRNA
14 synthesis and processing. What is the link between the nucleolar ribosome biogenesis activity
15 per se and the other functions? We propose that ribosome biogenesis creates the
16 compartmentation of a molecular machinery that can be of benefit to other functions. In this
17 case, the prediction is that destruction of ribosome biogenesis activity would also stop the
18 other functions occurring in an active nucleolus. This puts the active nucleolus at the parting
19 of the ways of several nuclear functions.
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Figure Legends

Figure 1. Nucleolar organization viewed by electron microscopy

The three nucleolar components observed in a HeLa cell: the fibrillar centers (asterisks), the dense fibrillar component (arrows) and the granular component (G). Bar: 0.3 μm .

Figure 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 .

Figure 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 .

Figure 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 4 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 (arrows), the same PNBs also contain Bop1-GFP (Bop) and Nop52 (Nop). DNA is revealed by DAPI. Bar: 1 μm .

Figure 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 additionnal data. Bar: 1 μm .

Additionnal data:

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3 movies 1: GFP-Nop52 at early G1 in HeLa cell (courtesy of L. Lo Presti).
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5 movies 2: GFP-Nop52 and DsRed-B23 from metaphase to early G1 in HeLa cell
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7 (courtesy of N. Angelier).
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58
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60

For Peer Review

References

- 1
2
3
4
5
6
7 Andersen, J.S., Y.W. Lam, A.K. Leung, S.E. Ong, C.E. Lyon, A.I. Lamond, and M.
8 Mann. 2005. Nucleolar proteome dynamics. *Nature*. 433:77-83.
- 9
10 Angelier, N., M. Tramier, E. Louvet, M. Coppey-Moisan, T.M. Savino, J.R. De Mey,
11 and D.D. Hernandez-Verdun. 2005. Tracking the Interactions of rRNA
12 Processing Proteins during Nucleolar Assembly in Living Cells. *Mol Biol Cell*.
13 16.
- 14
15 Azum-Gélade, M.-C., J. Noaillac-Depeyre, M. Caizergues-Ferrer, and N. Gas. 1994.
16 Cell cycle redistribution of U3 snRNA and fibrillarin. Presence in the
17 cytoplasmic nucleolus remnant and in the prenucleolar bodies at telophase. *J*.
18 *Cell Sci*. 107:463-475.
- 19
20 Bell, P., M.C. Dabauvalle, and U. Scheer. 1992. In vitro assembly of prenucleolar
21 bodies in *Xenopus* egg extract. *J. Cell Biol*. 118:1297-1304.
- 22
23 Bertrand, E., F. Houser-Scott, A. Kendall, R.H. Singer, and D.R. Engelke. 1998.
24 Nucleolar localization of early tRNA processing. *Genes Dev*. 12:2463-2468.
- 25
26 Biggiogera, M., S. Fakan, S.H. Kaufmann, A. Black, J.H. Shaper, and H. Busch. 1989.
27 Simultaneous immunoelectron microscopic visualization of protein B23 and
28 C23 distribution in the HeLa cell nucleolus. *J. Histochem. Cytochem*. 37:1371-
29 1374.
- 30
31 Boulon, S., C. Verheggen, B.E. Jady, C. Girard, C. Pescia, C. Paul, J.K. Ospina, T. Kiss,
32 A.G. Matera, R. Bordonne, and E. Bertrand. 2004. PHAX and CRM1 are
33 required sequentially to transport U3 snoRNA to nucleoli. *Mol Cell*. 16:777-87.
- 34
35 Bubulya, P.A., K.V. Prasanth, T.J. Deerinck, D. Gerlich, J. Beaudouin, M.H. Ellisman,
36 J. Ellenberg, and D.L. Spector. 2004. Hypophosphorylated SR splicing factors
37 transiently localize around active nucleolar organizing regions in telophase
38 daughter nuclei. *J Cell Biol*. 167:51-63.
- 39
40 Carmo-Fonseca, M., L. Mendes-Soares, and I. Campos. 2000. To be or not to be in the
41 nucleolus. *Nature Cell Biol*. 2:107-112.
- 42
43 Carmo-Fonseca, M., M. Platani, and J.R. Swedlow. 2002. Macromolecular mobility
44 inside the cell nucleus. *Trends Cell Biol*. 12:491-5.
- 45
46 Chan, P.K., Y. Qi, J. Amley, and C.A. Koller. 1996. Quantitation of the
47 nucleophosmin/B23-translocation using imaging analysis. *Cancer Lett*.
48 100:191-7.
- 49
50 Chen, D., and S. Huang. 2001. Nucleolar components involved in ribosome
51 biogenesis cycle between the nucleolus and nucleoplasm in interphase cells. *J*.
52 *Cell Biol*. 153:169-176.
- 53
54 Clute, P., and J. Pines. 1999. Temporal and spatial control of cyclin B1 destruction in
55 metaphase. *Nature cell Biol*. 1:82-87.
- 56
57 Cmarko, D., P.J. Verschure, L.I. Rothblum, D. Hernandez-Verdun, F. Amalric, R. van
58 Driel, and S. Fakan 2000. Ultrastructural analysis of nucleolar transcription in
59 cells microinjected with 5-bromo-UTP. *Histochem. Cell Biol*. 113:181-187.
- 60
Cockell, M.M., and S.M. Gasser. 1999. The nucleolus: nucleolar space for rent. *Current
Biology*. 9:R575-R576.
- David-Pfeuty, T., Y. Nouvian-Dooghe, V. Sirri, P. Roussel, and D. Hernandez-
Verdun. 2001. Common and reversible regulation of wild-type p53 function

- and of ribosomal biogenesis by protein kinases in human cells. *Oncogene*. 20:5951-5963.
- Dousset, T., C. Wang, C. Verheggen, D. Chen, D. Hernandez-Verdun, and S. Huang. 2000. Initiation of nucleolar assembly is independent of RNA polymerase I transcription. *Mol. Biol. Cell*. 11:2705-2717.
- Dundr, M., M.D. Hebert, T.S. Karpova, D. Stanek, H. Xu, K.B. Shpargel, U.T. Meier, K.M. Neugebauer, A.G. Matera, and T. Misteli. 2004. In vivo kinetics of Cajal body components. *J Cell Biol*. 164:831-42.
- Dundr, M., T. Misteli, and M.O.J. Olson. 2000. The dynamics of postmitotic reassembly of the nucleolus. *J. Cell Biol*. 150:433-446.
- Dundr, M., and M.O.J. Olson. 1998. Partially processed pre-rRNA is preserved in association with processing components in nucleolus derived foci during mitosis. *Mol. Biol. Cell*. 9:2407-2422.
- Emiliani, V., D. Sanvitto, M. Tramier, T. Piolot, Z. Petrasek, K. Kemnitz, C. Durieux, and M. Coppey-Moisan. 2003. Low-intensity two-dimensional imaging of fluorescence lifetimes in living cells. *Appl. Phys. Lett*. 83:2471-2473.
- Fatica, A., and D. Tollervy. 2002. Making ribosomes. *Curr. opin. Cell Biol*. 14:313-318.
- Finch, R.A., and P.K. Chan. 1996. ATP depletion affects NPM translocation and exportation of rRNA from nuclei. *Biochem Biophys Res Commun*. 222:553-8.
- Finch, R.A., G.R. Revankar, and P.K. Chan. 1993. Nucleolar localization of nucleophosmin/B23 requires GTP. *J Biol Chem*. 268:5823-7.
- Fomproix, N., J. Gébrane-Younès, and D. Hernandez-Verdun. 1998. Effects of anti-fibrillarin antibodies on building of functional nucleoli at the end of mitosis. *J. Cell Sci*. 111: 359-372.
- Fromont-Racine, M., B. Senger, C. Saveanu, and F. Fasiolo. 2003. Ribosome assembly in eukaryotes. *Gene*. 313:17-42.
- Gall, J.G. 2000. Cajal bodies: the first 100 years. *Annu. Rev. Cell Dev. Biol*. 16:273-300.
- Ganot, P., B.E. Jady, M.-L. Bortolin, X. Darzacq, and T. Kiss. 1999. Nucleolar factors direct the 2'-O-ribose methylation and pseudouridylation of U6 spliceosomal RNA. *Mol. Cell. Biol*. 19:6906-6917.
- Gautier, T., C. Dauphin-Villemant, C. André, C. Masson, J. Arnoult, and D. Hernandez-Verdun. 1992a. Identification and characterization of a new set of nucleolar ribonucleoproteins which line the chromosomes during mitosis. *Exp. Cell Res*. 200:5-15.
- Gautier, T., N. Fomproix, C. Masson, M.C. Azum-Gélade, N. Gas, and D. Hernandez-Verdun. 1994. Fate of specific nucleolar perichromosomal proteins during mitosis : Cellular distribution and association with U3 snoRNA. *Biol. Cell*. 82:81-93.
- Gautier, T., C. Masson, C. Quintana, J. Arnoult, and D. Hernandez-Verdun. 1992b. The ultrastructure of the chromosome periphery in human cells. An *in situ* study using cryomethods in electron microscopy. *Chromosoma*. 101:502-510.
- Gautier, T., M. Robert-Nicoud, M.-N. Guilly, and D. Hernandez-Verdun. 1992c. Relocation of nucleolar proteins around chromosomes at mitosis- A study by confocal laser scanning microscopy. *J. Cell Sci*. 102:729-737.
- Gébrane-Younès, J., V. Sirri, H.R. Junéra, P. Roussel, and D. Hernandez-Verdun. 2005. Nucleolus : An essential nuclear domain. In *Visions of the cell nucleus*. P.H.a.S. Diekmann., editor. ASP, CA. 120-135.

- 1
2
3 Ginisty, H., F. Amalric, and P. Bouvet. 1998. Nucleolin functions in the first step of
4 ribosomal RNA processing. *EMBO J.* 17:1476-1486.
- 5
6 Granick, D. 1975b. Nucleolar necklaces in chick embryo fibroblast cells. II.
7 Microscope observations of the effect of adenosine analogues on nucleolar
8 necklace formation. *J. Cell Biol.* 65:418-427.
- 9
10 Guarente, L. 2000. Sir2 links chromatin silencing, metabolism, and aging. *Genes Dev.*
11 14:1021-1026.
- 12
13 Guillot, P.V., S. Martin, and A. Pombo. 2005. The organization of transcription in the
14 nucleus of mammalian cells. In *Visions of the cell nucleus*. P.H.a.S. Diekmann.,
15 editor. ASP, CA. 95-105.
- 16
17 Haaf, T., D.L. Hayman, and M. Schmid. 1991. Quantitative determination of rDNA
18 transcription units in vertebrate cells. *Exp. Cell Res.* 193:78-86.
- 19
20 Haaf, T., and D.C. Ward. 1996. Inhibition of RNA polymerase II transcription causes
21 chromatin decondensation, loss of nucleolar structure, and dispersion of
22 chromosomal domains. *Exp. Cell Res.* 224:163-173.
- 23
24 Hadjiolov, A.A. 1985. The nucleolus and ribosome biogenesis. Springer-Verlag,
25 Wien, New-York. 1-268 pp.
- 26
27 Hadjiolova, K.V., A. Hadjiolov, and J.-P. Bachelierie. 1995. Actinomycin D stimulates
28 the transcription of rRNA minigenes transfected into mouse cells.
29 Applications for the *in vivo* hypersensitivity of rRNA gene transcription. *Eur.*
30 *J. Biochem.* 228:605-615.
- 31
32 Heix, J., A. Vente, R. Voit, A. Budde, T.M. Michaelidis, and I. Grummt. 1998. Mitotic
33 silencing of human rRNA synthesis: inactivation of the promoter selectivity
34 factor SL1 by cdc2/cyclin B-mediated phosphorylation. *EMBO J.* 17:7373-7381.
- 35
36 Hernandez-Verdun, D. 2004. Behavior of the nucleolus during mitosis. *Eurekah.com*
37 and kluwer Academic/plenum publishing. 41-57 pp.
- 38
39 Hernandez-Verdun, D., and H.R. Junéra. 1995. The nucleolus. In *Principles of*
40 *Medical Biology, Cellular organelles*. Vol. 2. Jai Press Inc. Ed. 73-92.
- 41
42 Hozak, P., P.R. Cook, C. Schöfer, W. Mosgöller, and F. Wachtler. 1994. Site of
43 transcription of ribosomal RNA and intranucleolar structure in HeLa cells. *J.*
44 *Cell Sci.* 107:639-648.
- 45
46 Hozak, P., J.T. Novak, and K. Smetana. 1989. Three-dimensional reconstructions of
47 nucleolus-organizing regions in PHA-stimulated human lymphocytes. *Biol.*
48 *Cell.* 66:225-233.
- 49
50 Isaac, C., Y. Yang, and T. Meier. 1998. Nopp140 functions as a molecular link between
51 the nucleolus and the coiled bodies. *J. Cell Biol.* 142:319-329.
- 52
53 Janicki, S.M., and D.L. Spector. 2003. Nuclear choreography: interpretations from
54 living cells. *Curr Opin Cell Biol.* 15:149-57.
- 55
56 Jarrous, N., D. Wolenski, D. Wesolowski, C. Lee, and S. Altman. 1999. Localization in
57 the nucleolus and coiled bodies of protein subunits of the ribonucleoprotein
58 ribonuclease P. *J. Cell Bio.* 146:559-571.
- 59
60 Jiménez-García, L.F., M.d.L. Segura-Valdez, R.L. Ochs, L.I. Rothblum, R. Hannan,
and D.L. Spector. 1994. Nucleologenesis: U3 snRNA-containing prenucleolar
bodies move to sites of active Pre-rRNA transcription after mitosis. *Mol. Biol.*
Cell. 5:955-966.
- Junéra, H.R., C. Masson, G. Géraud, and D. Hernandez-Verdun. 1995. The three-
dimensional organization of ribosomal genes and the architecture of the
nucleoli vary with G1, S and G2 phases. *J. Cell Sci.* 108:3427-3441.

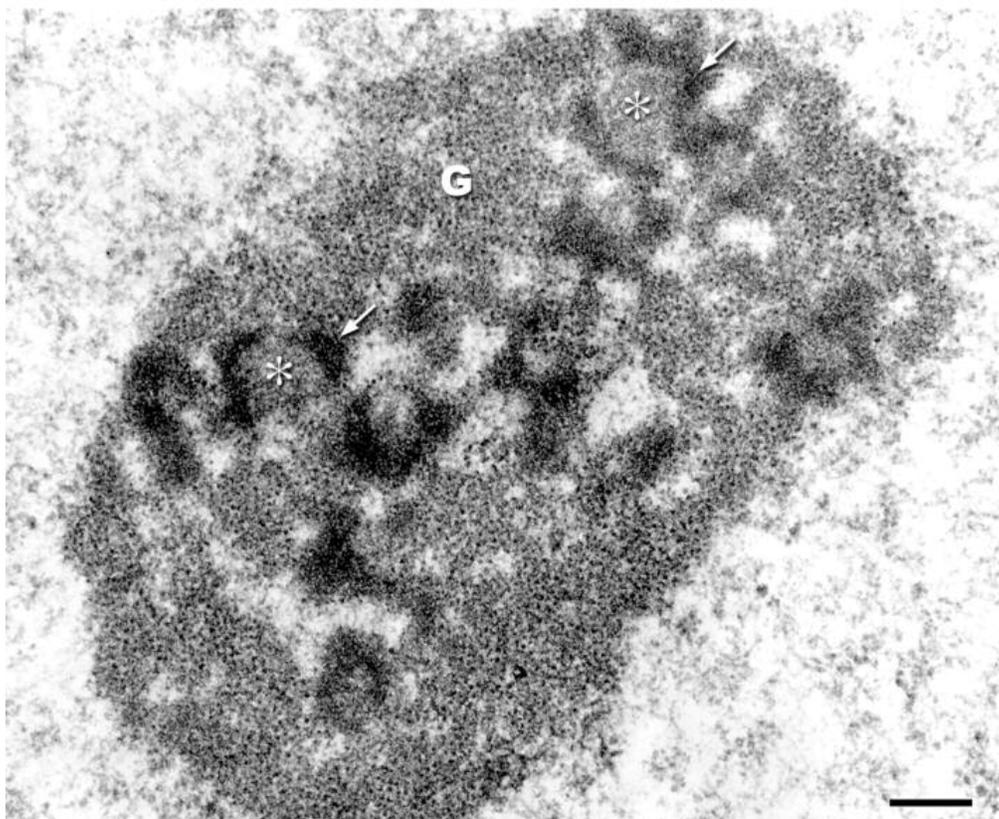
- 1
2
3 Junéra, H.R., C. Masson, G. Géraud, J. Suja, and D. Hernandez-Verdun. 1997.
4 Involvement of in situ conformation of ribosomal genes and selective
5 distribution of UBF in rRNA transcription. *Mol. Biol. Cell.* 8:145-156.
- 6
7 Le Panse, S., C. Masson, L. Héliot, J.-M. Chassery, H.R. Junéra, and D. Hernandez-
8 Verdun. 1999. 3-D Organization of single ribosomal transcription units after
9 DRB inhibition of RNA polymerase II transcription. *J. Cell Sci.* 112: 2145-2154.
- 10
11 Lippincott-Schwartz, J., E. Snapp, and A. Kenworthy. 2001. Studying protein
12 dynamics in living cells. *Nat Rev Mol Cell Biol.* 2:444-56.
- 13
14 Louvet, E., H.R. Junera, S. Le Panse, and D. Hernandez-Verdun. 2005. Dynamics and
15 compartmentation of the nucleolar processing machinery. *Exp. Cell Res.*
16 304:457-470.
- 17
18 Matera, A.G. 1999. Nuclear bodies: multifaceted subdomains of the interchromatin
19 space. *Trends Cell Biol.* 9:302-309.
- 20
21 Mélése, T., and Z. Xue. 1995. The nucleolus : an organelle formed by the act of
22 building a ribosome. *Curr. Opin. Cell Biol.* 7:319-324.
- 23
24 Misteli, T. 2001. Protein dynamics: implications for nuclear architecture and gene
25 expression. *Science.* 291:843-847.
- 26
27 Ochs, R.L., M.A. Lischwe, E. Shen, R.E. Carroll, and H. Busch. 1985a. Nucleogenesis:
28 composition and fate of prenucleolar bodies. *Chromosoma.* 92:330-336.
- 29
30 Ochs, R.L., M.A. Lischwe, W.H. Spohn, and H. Busch. 1985b. Fibrillarin: a new
31 protein of the nucleolus identified by autoimmune sera. *Biol. Cell.* 54:123-134.
- 32
33 Okuwaki, M., M. Tsujimoto, and K. Nagata. 2002. The RNA binding activity of a
34 ribosome biogenesis factor, nucleophosmin/B23, is modulated by
35 phosphorylation with a cell cycle-dependent kinase and by association with its
36 subtype. *Molec. Biol. Cell.* 13:2016-2030.
- 37
38 Pébusque, M.J., and Seite. 1981. Electron microscopic studies of silver-stained
39 proteins in nucleolar organizer regions: location in nucleoli of rat sympathetic
40 neurons during light and dark periods. *J. Cell Sci.* 51:85-94.
- 41
42 Pederson, T. 1998. The plurifunctional nucleolus. *Nucleic Acids Res.* 26:3871-3876.
- 43
44 Pena, E., M.T. Berciano, R. Fernandez, J.L. Ojeda, and M. Lafarga. 2001. Neuronal
45 body size correlates with the number of nucleoli and Cajal bodies, and with
46 the organization of the splicing machinery in rat trigeminal ganglion neurons.
47 *J. Comp. Neurol.* 430:250-263.
- 48
49 Phair, R.D., and T. Misteli. 2000. High mobility of proteins in the mammalian cell
50 nucleus. *Nature.* 404:604-609.
- 51
52 Pinol-Roma, S. 1999. Association of nonribosomal nucleolar proteins in
53 ribonucleoprotein complexes during interphase and mitosis. *Mol. Biol. Cell.*
54 10:77-90.
- 55
56 Platani, M., I. Golberg, J.R. Swedlow, and A.I. Lamond. 2000. In vivo analysis of Cajal
57 body movement, separation, and joining in live human cells. *J. Cell Biol.*
58 151:1561-1574.
- 59
60 Politz, J.C., L.B. Lewandowski, and T. Pederson. 2002. Signal recognition particle
RNA localization within the nucleolus differs from the classical sites of
ribosome synthesis. *J Cell Biol.* 159:411-8.
- Politz, J.C., S. Yarovoi, S.M. Kilroy, K. Gowda, C. Zwieb, and T. Pederson. 2000.
Signal recognition particle components in the nucleolus. *Proc. Natl. Acad. Sci.*
USA. 97:55-60.

- 1
2
3 Puvion-Dutilleul, F., J.-P. Bachellerie, and E. Puvion. 1991. Nucleolar organization of
4 HeLa cells as studied by *in situ* hybridization. *Chromosoma*. 100:395-409.
- 5 Puvion-Dutilleul, F., E. Puvion, and J.-P. Bachellerie. 1997. Early stages of pre-rRNA
6 formation within the nucleolar ultrastructure of mouse cells studied by *in situ*
7 hybridization with 5'ETS leader probe. *Chromosoma*. 105:496-505.
- 8
9 Roussel, P., C. André, L. Comai, and D. Hernandez-Verdun. 1996. The rDNA
10 transcription machinery is assembled during mitosis in active NORs and
11 absent in inactive NORs. *J. Cell Biol.* 133:235-246.
- 12 Rubbi, C.P., and J. Milner. 2003. Disruption of the nucleolus mediates stabilization of
13 p53 in response to DNA damage and other stresses. *Embo J.* 22:6068-77.
- 14 Savino, T.M., R. Bastos, E. Jansen, and D. Hernandez-Verdun. 1999. The nucleolar
15 antigen Nop52, the human homologue of the yeast ribosomal RNA processing
16 RRP1, is recruited at late stages of nucleologenesis. *J. Cell Sci.* 112:1889-1900.
- 17 Savino, T.M., J. Gébrane-Younès, J. De Mey, J.-B. Sibarita, and D. Hernandez-Verdun.
18 2001. Nucleolar assembly of the rRNA processing machinery in living cells. *J.*
19 *Cell Biol.* 153:1097-1110.
- 20
21
22
23 Scheer, U., and R. Benavente. 1990. Functional and dynamic aspects of the
24 mammalian nucleolus. *BioEssays*. 12:14-21.
- 25
26 Scheer, U., and R. Hock. 1999. Structure and function of the nucleolus. *Curr. Opin.*
27 *Cell Biol.* 11:385-390.
- 28
29 Scheer, U., and K.M. Rose. 1984. Localisation of RNA polymerase I in interphase cells
30 and mitotic chromosomes by light and electron microscopic
31 immunocytochemistry. *Proc. Natl. Acad Sci. USA.* 81:1431-1435.
- 32
33 Scheer, U., M. Thiry, and G. Goessens. 1993. Structure, function and assembly of the
34 nucleolus. *Trends Cell Biol.* 3:236-241.
- 35
36 Schul, W., L. de Jong, and R. van Driel. 1998. Nuclear neighbours: the spatial and
37 functional organization of genes and nuclear domains. *J. Cell. Biochem.* 70:159-
38 171.
- 39
40 Shav-Tal, Y., J. Blechman, X. Darzacq, C. Montagna, B.T. Dye, J.G. Patton, R.H.
41 Singer, and D. Zipori. 2005. Dynamic Sorting of Nuclear Components into
42 Distinct Nucleolar Caps during Transcriptional Inhibition. *Mol Biol Cell.*
43 16:2395-413.
- 44
45 Shaw, P.J., and E.G. Jordan. 1995. The nucleolus. *Annu. Rev. Cell Dev. Biol.* 11:93-121.
- 46
47 Sirri, V., D. Hernandez-Verdun, and P. Roussel. 2002. Cyclin-dependent kinases
48 govern formation and maintenance of the nucleolus. *J. Cell Biol.* 156:969-981.
- 49
50 Sirri, V., P. Roussel, and D. Hernandez-Verdun. 2000. *In vivo* release of mitotic
51 silencing of ribosomal gene transcription does not give rise to precursor
52 ribosomal RNA processing. *J. Cell Biol.* 148:259-270.
- 53
54 Sleeman, J.E., and A.I. Lamond. 1999. Newly assembled snRNPs associated with
55 coiled bodies before speckles, suggesting a nuclear snRNP maturation
56 pathway. *Current Biol.* 9:1065-1074.
- 57
58 Snaar, S., K. Wiesmeijer, A.G. Jochemsen, H.J. Tanke, and R.W. Dirks. 2000.
59 Mutational analysis of fibrillarin and its mobility in living human cells. *J. Cell*
60 *Biol.* 151:653-662.
- Sollner-Webb, B., K.T. Tycowski, and J.A. Steitz. 1996. Ribosomal RNA processing in
eukaryotes. *In* Ribosomal RNA: structure, evolution, processing, and function
in protein biosynthesis. CRC Press, New York. 469-490.
- Spector, D.L. 2001. Nuclear domains. *J. Cell Sci.* 114:2891-2893.

- 1
2
3 Strouboulis, J., and A.P. Wolffe. 1996. Functional compartmentalization of the
4 nucleus. *J. Cell Sci.* 109:1991-2000.
- 5
6 Thiry, M., and G. Goessens. 1996. The nucleolus during the cell cycle. Springer-
7 Verlag, Heidelberg. 146 pp.
- 8
9 Tollervey, D. 1996. Trans-acting factors in ribosome synthesis. *Exp. Cell Res.* 229:226-
10 232.
- 11
12 Verheggen, C., S. Le Panse, G. Almouzni, and D. Hernandez-Verdun. 1998. Presence
13 of pre-rRNAs before activation of polymerase I transcription in the building
14 process of nucleoli during early development of *Xenopus laevis*. *J. Cell Biol.*
15 142:1167-1180.
- 16
17 Visitin, R., and A. Amon. 2000. The nucleolus: the magician's hat for cell cycle tricks.
18 *Curr. Opin. Cell Biol.* 12:372-377.
- 19
20 Weisenberger, D., and U. Scheer. 1995. A possible mechanism for the inhibition of
21 ribosomal RNA gene transcription during mitosis. *J. Cell Biol.* 129:561-575.
- 22
23 Zatsepina, O.V., I.T. Todorov, R.N. Philipova, C.P. Krachmarov, M.F. Trendelenburg,
24 and E.G. Jordan. 1997. Cell cycle-dependent translocations of a major
25 nucleolar phosphoprotein, B23, and some characteristics of its variants. *Eur J*
26 *Cell Biol.* 73:58-70.
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Fig 1

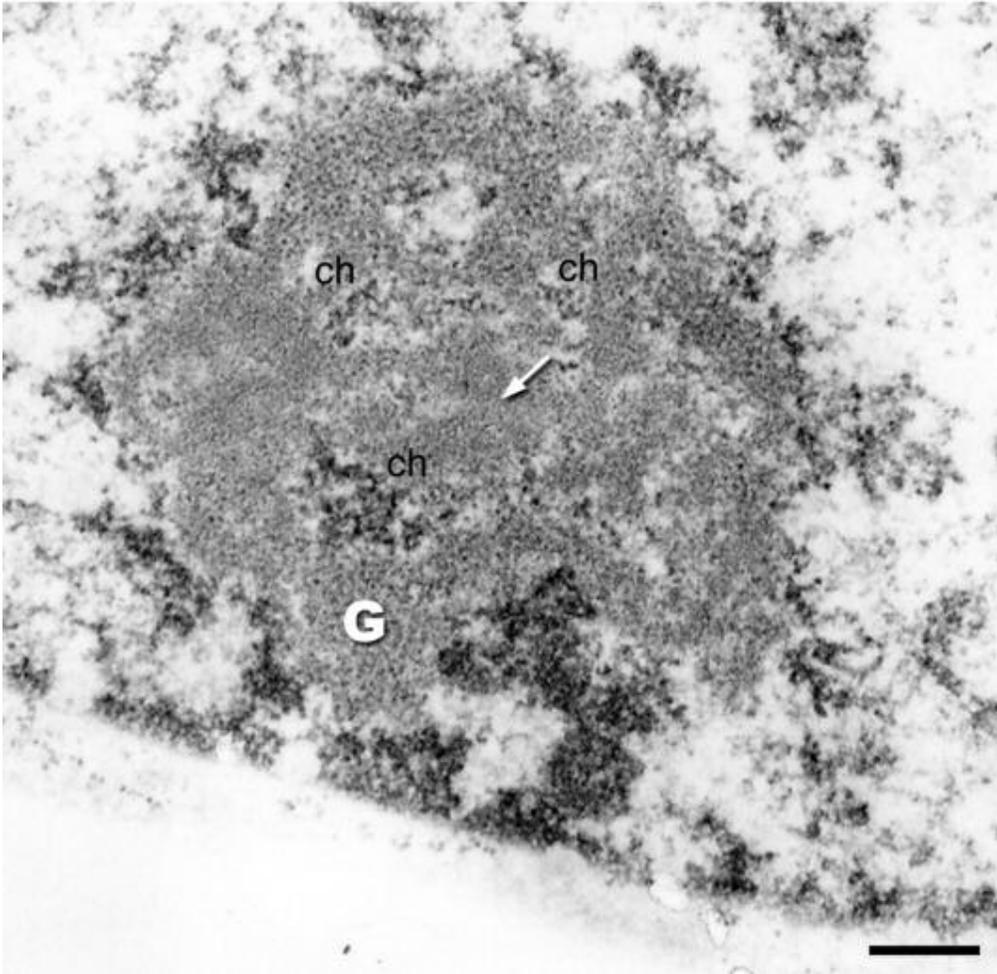


120x110mm (150 x 150 DPI)



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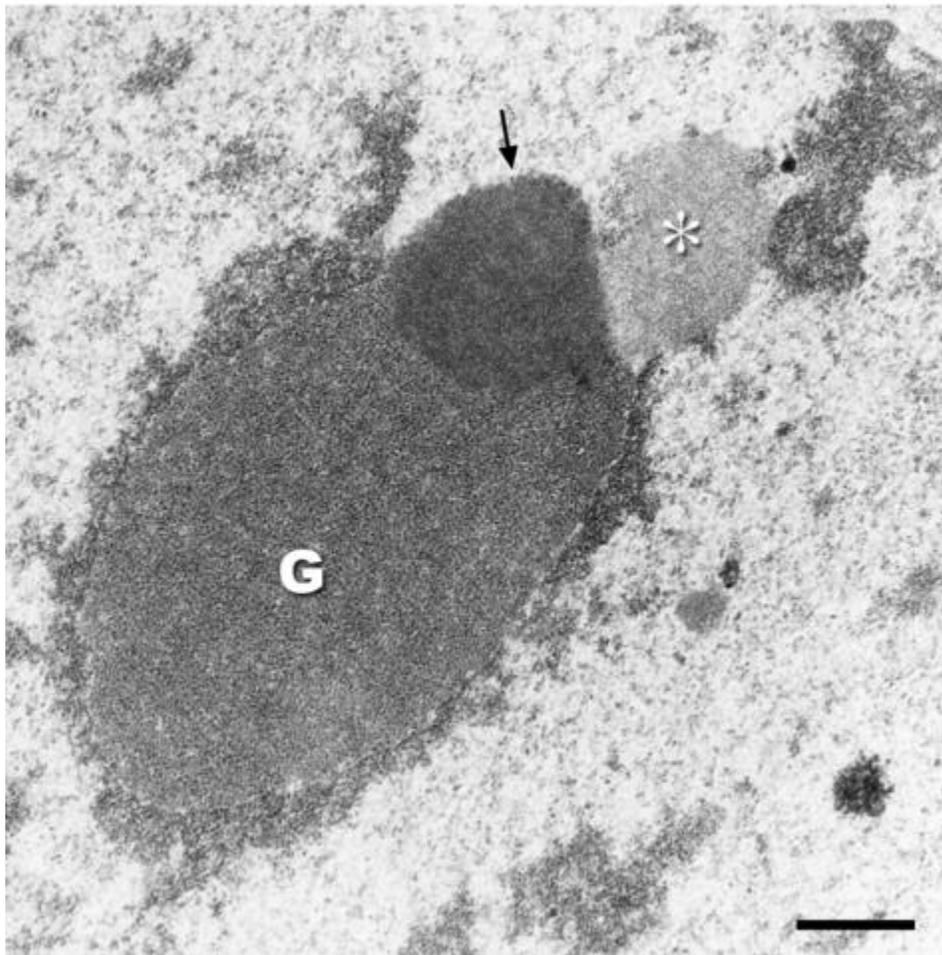
Fig 2



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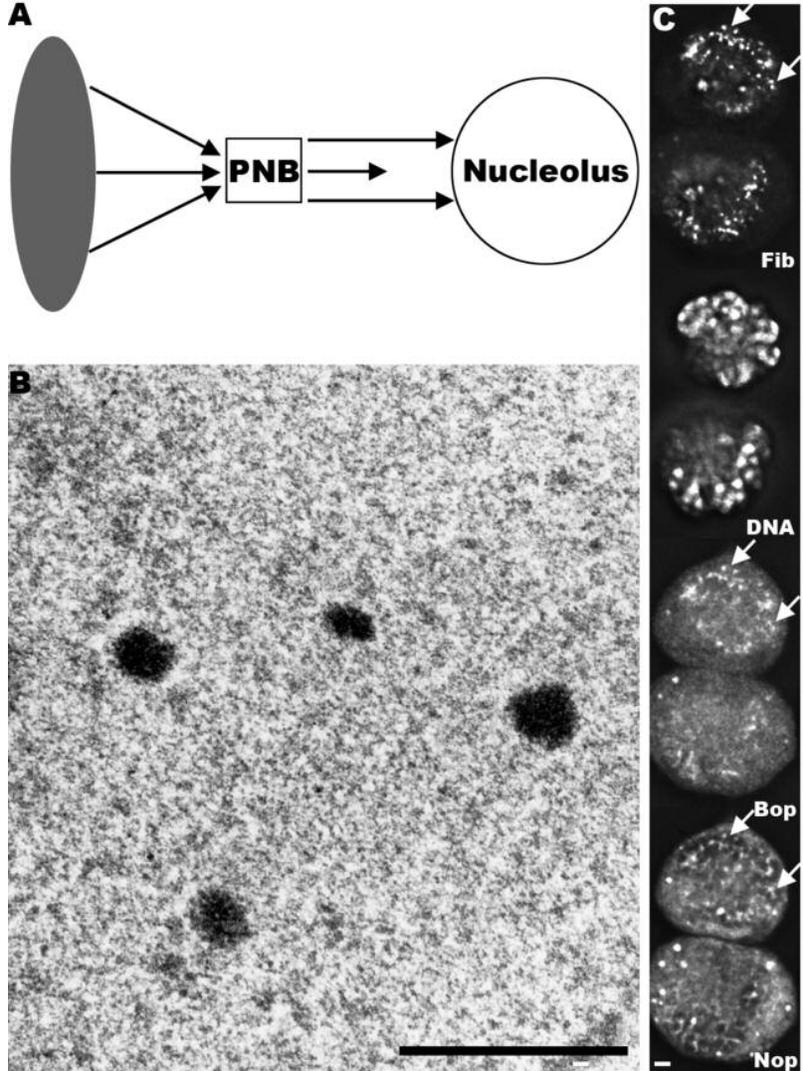
Fig 3



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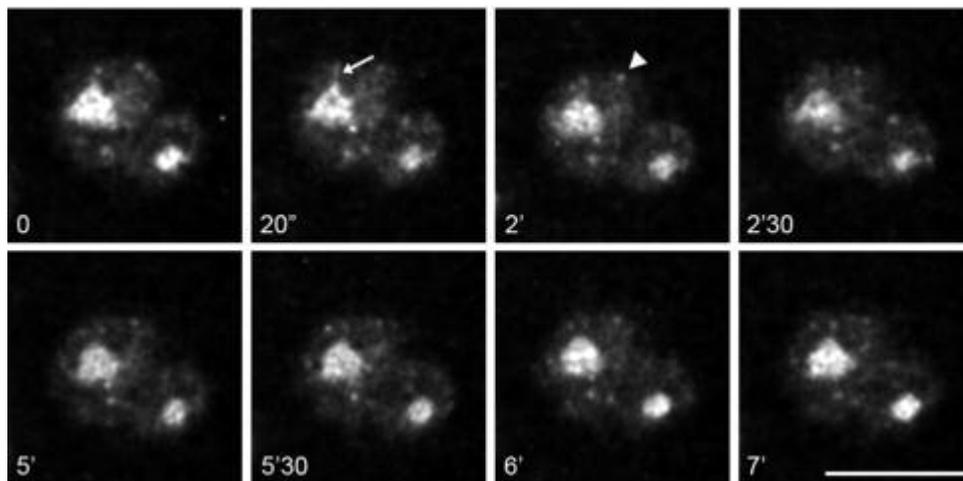
Fig 4



109x163mm (150 x 150 DPI)

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Fig 5



82x47mm (150 x 150 DPI)

Review