Histol Histopathol (2008) 23: 1291-1298

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Nucleolus: The ribosome factory

Dusan Cmarko, Jana Smigova, Lucia Minichova and Alexey Popov

Institute of Cellular Biology and Pathology, First Faculty of Medicine, Charles University in Prague, and Department of Cell Biology, Institute of Physiology, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic

Summary. The nucleolus is a nuclear compartment and represents the most obvious and clearly differentiated nuclear structure seen in the microscope. Within nucleoli most events of ribosome biogenesis, such as ribosomal RNA synthesis, processing, and ribosome subunit assembly, take place. Several lines of evidence now show that the nucleolus has also numerous non-ribosomal functions. This review is focused on the recent progress in our knowledge of how to correlate the known biochemical processes taking place in the nucleolus with nucleolar structures observed in the microscope. We still lack detailed enough information to understand fully the organization and regulation of the processes taking place in the nucleolar sub-structures. However, the present power of microscopy techniques should allow for an *in situ* description of the organization of nucleolar processes at the molecular level in the years to come.

Key words: Nucleolus, Nucleolar architecture, Nucleolar transcription, Ribosome biogenesis

Introduction

The most prominent structure of a eukaryotic nucleus is the nucleolus. Its straightforward role consists of rRNA synthesis and biogenesis of ribosomal subunits (Busch and Smetana, 1970). This nuclear organelle contains relatively little chromosomal DNA but is rich in protein and ribonucleoprotein (RNP) complexes, including the large and small pre-ribosome subunits as they undergo assembly.

The nucleolus is formed around discrete chromosomal loci known as nucleolus organizer regions, or NORs, which consist of tandemly repeated genes for rRNA (Hernandez-Verdun, 2006; Raska et al., 2006a,b). Several NORs can exist within investigated species, but each organizer resides on a separate chromosome. In human cells, the NORs appear on the short arms of the five acrocentric chromosomes (chromosomes 13, 14, 15, 21, and 22) which contain in total about 400 ribosomal genes (Hadjiolov, 1985). During interphase, ribosomal repeats from more than one NOR-bearing chromosome often cluster together and participate in the formation of a given nucleolus. In mitosis, rRNA synthesis ceases as a result of phosphorylation of the relevant nuclear/nucleolar factors and the nucleoli disassemble; at the end of mitosis, phosphorylation of the relevant factors is reversed, RNA synthesis resumes and nucleoli reform. In other words, nucleologenesis is concurrent with the (re)initiation of NOR transcription (DiMario, 2004; Chen et al., 2005; Hernandez-Verdun, 2006).

Using the established evidence from the yeast Saccharomyces cerevisiae, mostly because of its powerful genetic and biochemical suitability that is not readily applied in metazoan systems (Granneman and Baserga, 2003; Huh et al., 2003; Tschochner and Hurt, 2003; Conconi, 2005; Chedin et al., 2007), that can be frequently, but not always, transposed to higher eukaryotes, dramatic progress has been made in the elucidation of nucleolar function through extensive proteomic analysis (Andersen et al., 2002, 2005; Scherl et al., 2002; Pendle et al., 2005). The ability to isolate large amounts of purified nucleoli, and ongoing improvements in protein separation methods prior to high-throughput mass spectrometry allowed scientists to identify over 700 nucleolar proteins in human (Lam et al., 2005; Boisvert et al., 2007). A comparison of such data from human and budding yeast showed that ~ 90 % of human nucleolar proteins have yeast homologues (Andersen et al., 2005). This demonstrates that the nucleolar proteome is largely conserved through evolution (Boisvert et al., 2007). All these studies, together with the application of bibliographic and bioinformatic analyses (Coute et al., 2006; Hinsby et al., 2006; Vollmer et al., 2006; Schuemie et al., 2007), have contributed to the emerging concept of the nucleolus as a compartment that is also involved in the control of numerous (non-ribosomal) cellular functions (Boisvert et al., 2007). Furthermore, time-lapse video microscopy,

Offprint requests to: Dr. Dusan Cmarko, Institute of Cellular Biology and Pathology, First Faculty of Medicine, Charles University in Prague, Albertov 4, 128 01 Prague 2, Czech Republic. e-mail: dusan.cmarko@lf1.cuni.cz

together with fluorescence recovery after photobleaching, fluorescence loss in photo-bleaching assays and fluorescent RNA cytochemistry have shown that most nuclear protein components, but also several types of RNA, are constantly being exchanged with the surrounding nucleoplasm (Pederson, 2001; Hernandez-Verdun, 2006; Lam et al., 2007).

Although a great portion of the mammalian nucleolar proteome (approximately 30%) is associated with rRNA processing and ribosome biogenesis, many other categories of nucleolar proteins and RNAs have discrete, apparently non-ribosomal functions, consistent with additional processes that occur within the nucleolus. Moreover, approximately 30% of the identified proteins lack functional annotation (Andersen et al., 2005; Boisvert et al., 2007). At the same time, many nucleolar proteins are also present at other cellular locations, and some only accumulate transiently, depending on various cell stimuli, in nucleoli (Andersen et al., 2005). Importantly, the interaction between individual multifunctional nucleolar factors is essential for the coordination of the functions of this nuclear compartment and illustrates principles of nucleolar architecture, i. e. a combination of many protein–protein and protein–nucleic acid interactions (Gorski et al., 2006; Hernandez-Verdun, 2006; Raska et al., 2006a; Sirri et al., 2008).

In this review, we focus on recent findings and novel concepts of the nucleolus as a highly dynamic subnuclear compartment specialized in ribosome production. The primary focus will be on nucleoli of human cells. However, we will also document results from yeast and other species where equivalent data have not yet been determined for human cells.

Relating ribosome production to nucleolar structures

Morphologically, nucleoli consist of three basic components, which have been implicated in rRNA metabolism: the fibrillar center (FC) – innermost, lightly stained component with fine fibrillar appearance, the dense fibrillar component (DFC) – dense substructure usually forming a rim around the FC, and the granular component (GC) – peripheral region of closely packed 15 nm granules (Fig. 1). Nevertheless, the overall structure of a nucleolus is largely determined by its activity in ribosome biogenesis (Melese and Xue, 1995; Berciano et al., 2007). A good example of this is found in human lymphocytes. Peripheral blood (dormant) lymphocytes contain usually a single small nucleolus containing just one FC. Upon cell activation with phytohemagglutinin, the nucleoli become much larger and exhibit many FCs (Fig. 2; Raska et al., 1983a,b). However, the higher nucleolar activity in large-sized rat neurons is accompanied by the formation of a giant FC (Lafarga et al., 1989; Casafont et al., 2007). Striking changes occur when nucleolar transcription is inhibited e.g. by low doses of actinomycin D. In this case, the major nucleolar components redistribute into clearly segregated zones and nucleoli disintegrate later on (Busch and Smetana, 1970; Hadjiolov, 1985).

It is generally thought that ribosome biogenesis is a vectorial process which may be envisaged as an assembly line, beginning in the fibrillar part, i.e. FC and DFC, and continuing into the surrounding granular

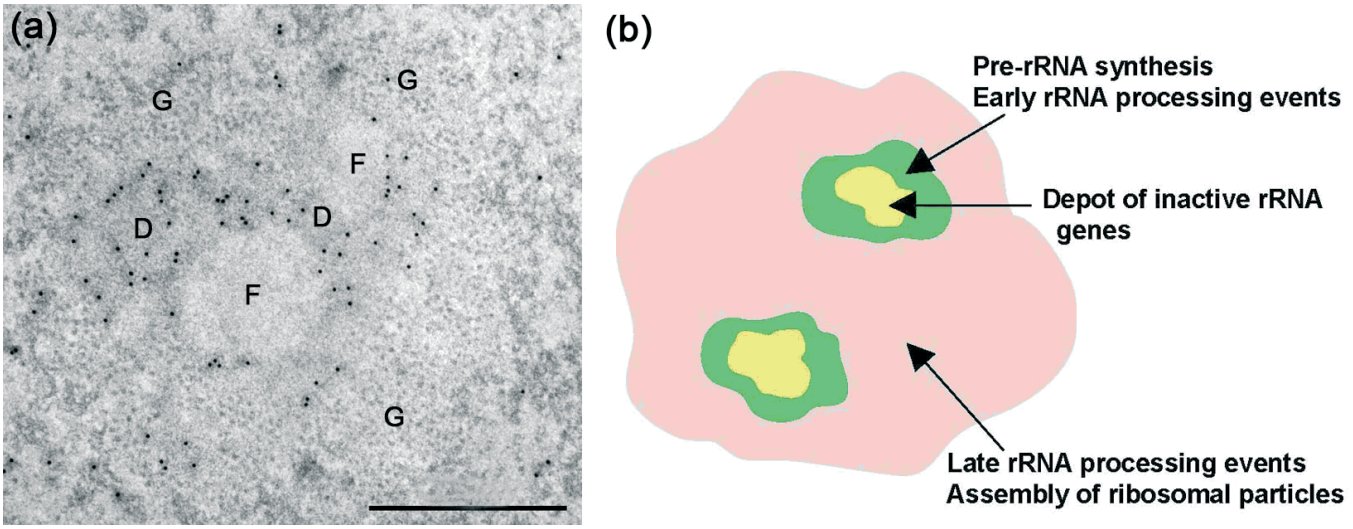

Fig. 1. A. Electron microscopical mapping of nucleolar transcription on resin sections of HeLa cells. Hypotonically incorporated BrU was revealed by labeling with a monoclonal antibody to BrU and a secondary antibody coupled with 10 nm colloidal gold. Gold particles within the nucleolus are confined to the DFCs together with the DFC/FC border. Bar: 0.2 µm. Reproduced from Koberna et al. (2002). Copyright 2002 The Rockefeller University Press. **B.** Schematic representation of nucleolar ultrastructure. The respective ribosomal functions are ascribed to the FCs (yellow), DFC (green), and GC (pink) of the nucleolus. Reproduced with permission, from Raska et al. (2004). © Portland Press Ltd.

portion of the nucleolus. In this way, the fibrillar part represents sites of nucleolar location of the rRNA genes (rDNA) and rDNA is the organizing center for the pol I transcriptional process. The product of transcription, pre-rRNA, is the initial material from which ribosomes are generated. The affinities of proteins and RNP factors for the pre-rRNA ultimately provide the basis for the structures of the subnucleolar compartments, and the nucleolus is built around relatively stable macromolecules i.e. rRNA genes and nascent transcripts (Scheer and Hock, 1999; Olson and Dundr, 2005; Raska et al., 2006a,b). It seems that pol I driven transcription organizes and maintains the typical steady-state nucleolar structure. However, Gonda et al. (2003) have shown in *Xenopus* that the integrity of nucleolar morphology can be uncoupled from RNA polymerase I (pol I)-driven transcription. RNA binding proteins FRGY2a and FRGY2b reversibly disassemble the nucleolus, probably through their non-sequence-specific rRNA binding, which may prevent the interaction of rRNA with other RNAs or proteins, although pol I transcription continues. Therefore, transcription activity of pol I is not sufficient for the maintenance of nucleolar structure, which, in addition, must depend on the complex intermolecular interaction of nucleolar components.

Only a subset of the rDNA repeats is actively transcribed at any given time (Grummt, 2003; McStay, 2006; Preuss and Pikaard, 2007). Recent studies clearly indicate that the transcriptional state of rDNA is mediated by an epigenetic network with a close relationship to nucleolar architecture (Li et al., 2006; Espada et al., 2007). The interplay of DNA methylation, histone post-translational modification and chromatin-remodeling activities establish silencing at the rDNA locus in higher eukaryotes, including humans (Grummt and Pikaard, 2003; Santoro, 2005). Consistent with the existence of active and silenced rDNA repeats there are probably two classes of NORs. Some NORs are fully repressed in a heterochromatic nucleosomal state and are not associated with any transcription factors. The remaining NORs are apparently in a more or less open euchromatic state in which they can be, or are, transcribed (Huang et al., 2006). The silent NORs are associated with methylation at cytosine bases located 5` to a guanosine in a CpG dinucleotide of rDNA promoter, as well as with the ATP-dependent nucleolar remodeling complex that interacts, at the promoter level, with the DNA methyltransferase 1 and with the histone deacetylase HDAC1 (Santoro et al., 2002). Although increasing evidence implicates a number of factors in the regulation of ribosomal chromatin, including pol I, transcription upstream binding factor (Mais et al., 2005; Huang et al., 2006; Stefanovsky et al., 2006) and non-coding functional elements from intergenic spacers (Gonzales and Sylvester, 2001; Caburet et al., 2005; Lebofsky and Bensimon, 2005; Mayer et al., 2006), the mechanisms that control the transition between heterochromatin and euchromatin states of NORs and the maintenance of each state are not completely

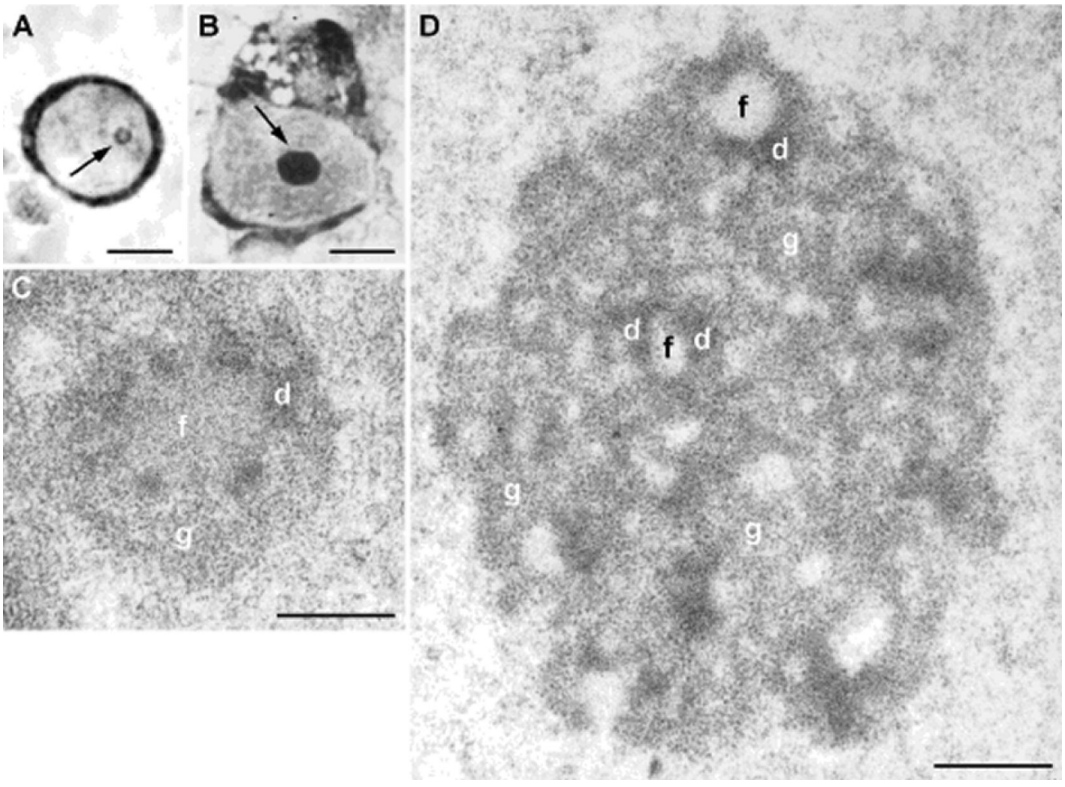

Fig. 2. Dormant and phytohemagglutinin stimulated human peripheral blood lymphocytes. **A** and **B** show light micrographs, **C** and **D** show equivalent cells at ultrastructural level. **A.** Lymphocyte, stained for RNA containing structures with toluidine blue, contains a small nucleolus (arrow) with RNA enriched structures only at its periphery. **B.** After 48 h stimulation, lymphocytes contain large nucleoli (arrow). **C.** Prior to stimulation, one large FC is seen in the nucleolus of the dormant cell. **D.** After 48 h stimulation several tiny FCs are scattered in the large nucleolus of the stimulated cell. Bars: A, B, 5 μm; C, D, 0.5 μm. Reproduced with permission, from Raska et al. (2004). © Portland Press Ltd.

understood (Preuss and Pikaard, 2007).

The active rRNA genes in the forms of Christmas trees (CTs) are seen in electron micrographs of isolated and highly loosened chromatin spreads (Miller and Beatty, 1969). These spectacular preparations enable the ribosomal DNA axis engaged with many pol I molecules and the attached nascent transcript to be visualized. The terminal knobs that can be seen on the nascent rRNA transcripts correspond to rRNA processing complexes. These RNP complexes base-pair with sequences near the 5' end of pre-rRNA.

It is emphasized that CTs are visualized in a standard way only using certain cell types, typically yeast cells and maturing amphibian or insect oocytes. Due to the presence of interfering chromatin structures in mammalian somatic cells, a complete ribosomal genes repeat has never been visualized and the few pictures obtained of ribosomal genes show either CTs with maximal pol I loading or inactive ribosomal genes covered by nucleosomes (Raska, 2003; Raska et al., 2006a,b).

The CTs have not yet been truly seen in ultrathin sections of embedded cells in electron microscopy preparations. For this reason we do not yet have direct proof of the precise localization of nucleolar transcription. However, a strong indication, using mainly ultrastructural localization of halogenated nucleotides incorporated into nascent rRNA of mammalian and plant cells, shows that CTs are in the DFC, including the border region between the DFC and the FC (Melcak et al., 1996; Koberna et al., 1999, 2002; Cmarko et al., 2000; Gonzalez-Melendi et al., 2001; Raska, 2003; Raska et al., 2004; Casafont et al., 2006).

In vivo activation of the human rRNA promoter requires protein-protein interaction between DNA-binding protein, upstream binding factor and the promoter selectivity factor (SL1) for the targeting of pol I and stable preinitiation complex formation (Russell and Zomerdijk, 2006). In combination with several other pol I transcription factors, this confers pol I promoter specificity to produce a long single pre-rRNA transcript (Grummt and Pikaard, 2003). Pre-rRNA initially accumulates in DFC, and its first processing steps take place in this region. These processing modifications encompass methylations and pseudouridylations before the three mature rRNAs (18S, 5.8S and 28S rRNA) are produced by nuclease cleavage. Each modification is made at a specific position in the pre-rRNA. These positions are specified by "guide RNAs" called small nucleolar RNAs (snoRNAs) (Lestrade and Weber, 2006; Reichow et al., 2007). The most studied of these is U3 snoRNA, the most abundant snoRNA. The U3 snoRNA is essential for rRNA processing leading to formation of 18S rRNA (Borovjagin and Gerbi, 2004; Granneman and Baserga, 2005).

The transcription of rDNA and pre-rRNA processing are considered to be tightly coordinated in yeast (Granneman and Baserga, 2005; Schneider et al., 2007). Moreover, electron micrographs of yeast chromatin in the form of CT have shown that the pre-rRNA processing takes place frequently co-transcriptionally, before transcription of the entire gene is complete (Osheim et al., 2004). It seems that coordination between the nucleolar transcription and RNA processing takes place also in mammalian cells (Kopp et al., 2007; Prieto and McStay, 2007). Furthermore, Kopp et al. (2007), using immunoprecipitation experiments and microinjection of plasmid DNA that contained only the pol I promoter or the promoter with various lengths of rDNA, suggested a model for sequential recruitment of pol I and pre-rRNA processing factors, rather than co-recruitment to sites of active transcription.

An essential step in ribosomal biogenesis is the association of processed rRNAs with 5S rRNA that is transcribed by RNA polymerase III in the nucleoplasm outside the nucleolus (Haeusler and Engelke, 2006), and with up to 80 ribosomal proteins (r-proteins) to form the RNP precursors, which mature into the 40S and 60S subunits of the ribosome (Rundra and Warner, 2004; Borovjagin and Gerbi, 2005). The genes encoding r-proteins are transcribed by RNA polymerase II, and newly translated r-proteins move into the nucleus and nucleolus. Interestingly, r-proteins accumulate in human nucleoli nearly 4 times more rapidly than any other nucleolar factors and, typically, 40-80% of their nucleolar content corresponds to protein synthesized within the previous 2 hr in HeLa cells (Lam et al., 2007). Moreover, photobleaching and mass-spectroscopy experiments showed that only a subset of r-proteins is assembled with rRNAs to form ribosomes (Lam et al., 2007). Free r-proteins in excess of the requirement for ribosome subunit production will continue to shuttle into the nucleoplasm, where they are probably ubiquitinylated and exposed to proteasome-mediated degradation (Matsumoto et al., 2005; Andersen et al., 2005; Lam et al., 2007). This model has some support from study of Stavreva et al. (2006) where the importance of ubiquitin and the proteasome in ribosome biogenesis was shown. The data by Lam et al. (2007) collectively point out a post-translation mechanism that, although strikingly inefficiently, controls the participation of r-proteins in the ribosome subunit assembly in human cells, and that r-protein level is never rate limiting for this process (Granneman and Tollervey, 2007).

Ultrastructural localization, as well as live-cell imaging of several early binding r-proteins, illustrated their occurrence in the nucleolar GC (Kruger et al., 2007). This indicates that pre-ribosomal assembly does not take place in the DFC, as was generally believed (Chooi and Leiby, 1981; Hadjiolov, 1985; Gerbi and Borovjagin, 2004), and also provides a link between r-proteins association with rRNA and the emergence of distinct granules at the DFC-GC interface (Leary et al., 2004; Kruger et al., 2007). Accordingly, the GC probably consists, at least in part, of pre- ribosomal subunits. However, rRNA deficient domains are also seen within the nucleolar GC (Politz et al., 2002). Such domains can contain tumor cell-specific protein nucleostemin (Politz et al., 2005) or tumor suppressor

protein ARF (Ma and Pederson, 2007). This suggests that the GC is not only involved in ribosome synthesis but also contains adjacent zones populated by proteins or protein complexes that can serve nonribosomal functions (Politz et al., 2005). According to these data, Pederson (2007) proposed a hypothesis that disruption of normal ribosome assembly in the nucleolus triggers a direct negative effect on the coresident nonribosomal mechanisms, especially on the cell cycle machinery (see also Dez and Tollervey, 2004). It should be mentioned that the DFC also exhibit structure-function difference: fibrillarin, a specific marker of the DFC, is located close to r-genes during transcription, probably associated with nascent transcripts, but is located apart from the r-genes during their replication (Raska et al., 1991; Pliss et al., 2005).

Individual steps of ribosome biogenesis, including nuclear export, have been studied most extensively in yeast (Fatica and Tollervey, 2002; Ferreira-Cerca et al., 2005; Bernstein et al., 2006; Panse et al., 2006; Seiser et al., 2006; Rosado et al., 2007; Zemp and Kutay, 2007). Proteomic methods have revealed several distinct, successive pre-ribosomal particles, and refined the model for the maturation of both 40S and 60S ribosomal subunits. They also greatly expanded the inventory of nonribosomal factors that participate in the ribosomal assembly. Once ribosomal subunits are assembled into nearly mature 60S and 40S preribosomal particles, they are exported to the cytoplasm independently of one another (Zemp and Kutay, 2007). Nuclear export to the cytoplasm of the vast majority of both subunits, via the nuclear pores, requires Crm1, the major export karyopherin, as well as RanGTPase system and subset of nucleoporins (Gadal et al., 2001; Trotta et al., 2003). Ribosomal subunits undergo a few final maturation steps before achieving translation competence in the cytoplasm (Zemp and Kutay, 2007).

It has been shown, in human cells, that a diffusion-based mechanism is responsible for the nucleoplasmic transport of pre-ribosomal subunits (Politz et al., 2003). However, it has been proposed that the double mechanism, diffusive and active, based on actin and myosin-driven movement might be also involved (Cisterna et al., 2006; Percipalle, 2007).

Implications and future research direction

The existence of the nucleolus depends on the activity of ribosomal genes and the generation of ribosomes. It has been claimed that the nucleolus is formed by the act of building a ribosome (Melese and Xue, 1995). Nevertheless, it should be noted that not only transcriptionally active NORs are present in the nucleolus, but also most silent NORs (Kalmarova et al., 2007). Thus, even non active genes may play a structural role with regard to the nucleolar integrity.

The ribosome assembly is a vectorial process starting in the DFC and finishing in the GC, from which almost mature ribosomal subunit particles leave the nucleolus. However, much of the protein and RNA movement in the nucleolus is not directed and is apparently dependent on diffusion and relative affinities for other nucleolar components (Politz et al., 2003; Handwerger et al., 2005; Olson and Dundr, 2005). Nucleolar factors dynamically change their localization within the nucleolus, as well as between the nucleolus and nucleoplasm, depending on the vast cellular stimuli, and the steady state structure represents the product of such dynamic processes (Olson and Dundr, 2005; Handwerger and Gall, 2006; Raska et al., 2006b).

In addition, it seems that nucleoli, as the ribosome factory, also creates the domains of molecular machinery that can be of benefit to other functions such as cell-cycle control, stress response, senescence and coordination of the biogenesis of other classes of functional RNPs (Pederson, 1998; Olson et al., 2002; Gerbi et al., 2003; Dez and Tollervey, 2004; Kobayashi, 2008). It must be emphasized that this fact necessarily makes any simple tripartite structural model of the nucleolus an oversimplification. But why is the nucleolus involved in so many other activities even if ribosome biogenesis represents a huge investment of resources and energy? One explanation that has been suggested for the nucleolar localization of apparently unconnected factors is that the nucleolus is a subnuclear compartment that has a markedly different composition and structure from the rest of the nucleus and, within the nucleolus, most nuclear proteins can be met (Olson and Dundr, 2005; Boisvert et al., 2007). Along this line, the nucleolus is able to take advantage of a myriad of possibilities for protein-protein interactions and may use them for sequestration of various factors away from the nucleoplasm, and their regulated release when required. An alternate explanation for the involvement in maturation of diverse RNAs (Politz et al., 2000; Pendle et al., 2005; Sommerville et al., 2005) is that the nucleolus simply profit from the high concentration of RNA-editing proteins. It is possible that these proteins may use closely related processes on different RNA, including rRNA. It should also be noted, that the nucleolus as a rich protein and RNA depot, is also exploited by viruses to usurp host-cell function and recruit nucleolar proteins to facilitate virus life cycle (Hiscox, 2007).

Our knowledge of nucleolar ribosome biogenesis, as well as many new nucleolar non-ribosomal processes has expanded dramatically in the past few years, but we are still lacking detailed *in situ* structural information about the cascade of steps in ribosomal biogenesis, as well as about the unconventional functions. Challenging tasks for today's molecular cell biology are therefore to expand our information concerning correlation of established nucleolar metabolic processes and the observed nucleolar structures. This endeavour should be facilitated by means of recently developed sophisticated light (Simpson, 2006; Hell, 2007; Lang et al, 2007) and electron microscopical methods, together with tomography and correlative techniques (Grabenbauer et al., 2005; Koster and Zandberger, 2005; Yakusheva et al., 2007; van Donselaar et al., 2007).

Acknowledgements. Our work is funded by grants from the Ministry of Education, Youth and Sports MSM0021620806 and LC535, from the Grant Agency of the Czech Republic 304/06/1662 and 304/06/1691, from the Academy of Sciences of the Czech Republic AV0Z50110509, and from Wellcome Trust 075834/04/Z.

References

Andersen J.S., Lyon C.E., Fox A.H., Leung A.K., Lam Y.W., Steen H., Mann M. and Lamond A.I. (2002). Directed proteomic analysis of the human nucleolus. Curr. Biol. 12, 1-11.

Andersen J.S., Lam Y.W., Leung A.K., Ong S.E., Lyon C.E., Lamond A.I. and Mann M. (2005). Nucleolar proteome dynamics. Nature 433, 77-83.

Berciano M.T., Novell M., Villagra N.T., Casafont I., Bengoechea R., Val-Bernal J.F. and Lafarga M. (2007). Cajal body number and nucleolar size correlate with the cell body mass in human sensory ganglia neurons. J. Struct. Biol. 158, 410-420.

Bernstein K.A., Granneman S., Lee A.V., Manickam S. and Baserga S.J. (2006). Comprehensive mutational analysis of yeast DEXD/H box RNA helicases involved in large ribosomal subunit biogenesis. Mol. Cell. Biol. 26, 1195-1208.

Boisvert F.M., van Koningsbruggen S., Navascues J. and Lamond A.I. (2007). The multifunctional nucleolus. Nat. Rev. Mol. Cell. Biol. 8, 574-585.

Borovjagin A.V. and Gerbi S.A. (2004). Xenopus U3 snoRNA docks on pre-rRNA through a novel base-pairing interaction. RNA 10, 942-953.

Borovjagin A.V. and Gerbi S.A. (2005). An evolutionary intra-molecular shift in the preferred U3 snoRNA binding site on pre-ribosomal RNA. Nucleic Acids Res. 33, 4995-5005.

Busch H. and Smetana K. (1970). The nucleolus. Academic Press, NewYork.

Caburet S., Conti C., Schurra C., Lebofsky R., Edelstejn S. and Bensimon A. (2005). Human ribosomal RNA gene arrays display a broad range of palindromic structures. Genome Res. 15, 1079-1085.

Casafont I., Navascues J., Pena E., Lafarga M. and Berciano M.T. (2006). Nuclear organization and dynamics of transcription sites in rat sensory ganglia neurons detected by incorporation of 5'-fluorouridine into nascent RNA. Neuroscience 140, 453-462.

Casafont I., Bengoechea R., Navascues J., Pena E., Berciano M.T. and Lafarga M. (2007). The giant fibrillar canter: A nucleolar structure enriched in upstream binding factor (UBF) that appears in transcriptionally more active sensory ganglia neurons. J. Struct. Biol. 159, 451-461.

Chedin S., Laferte A., Hoang T., Lafontaine D.L.J., Riva M. and Carles C. (2007). Is ribosome synthesis controlled by pol I transcription? Cell Cycle 6, 11-15.

Chen D., Dundr M., Wang C., Leung A., Lamond A., Misteli T. and Huang S. (2005). Condensed mitotic chromatin in accessible to transcription factors and chromatin structural proteins. J. Cell Biol. 168, 41-54.

Chooi W.Y. and Leiby K.R. (1981). An electron microscopic method for localization of ribosomal proteins during transcription of ribosomal DNA: a method for studying protein assembly. Proc. Natl. Acad. Sci. USA 78, 4823-4827.

Cisterna B., Necchi D., Prosperi E. and Biggiogera M. (2006). Small ribosomal subunits associate with nuclear myosin and actin in transit to the nuclear pores. FASEB J. 20, E1257-1263.

Cmarko D., Verschure P.J., Rothblum L.I., Hernandez-Verdun D., Amalric F., van Driel R. and Fakan S. (2000). Ultrastructural analysis of nucleolar transcription in cells microinjected with 5-bromo-UTP. Histochem. Cell Biol. 113, 181-187.

Conconi A. (2005). The yeast r-DNA locus: a model system to study DNA repair in chromatin. DNA Repair (Amst.) 4, 897-908.

Coute Y., Burgess J.A., Diaz J.J., Chichester C., Lisacek F., Greco A. and Sanchez J.C. (2006). Deciphering the human nucleolar proteome. Mass Spectrom. Rev. 25, 215-234.

DiMario P.J. (2004). Cell and molecular biology of nucleolar assembly and disassembly. Int. Rev. Cytol. 239, 99-178.

Dez C. and Tollervey D. (2004). Ribosome synthesis meets the cell cycle. Curr. Opin. Microbiol. 7, 631-637.

Espada J., Ballestar E., Santoro R., Fraga M.F., Villar-Garea A., Nemeth A., Lopez-Serra L., Ropero S., Aranda A., Orozco H., Moreno V., Juarranz A., Stockert J.C., Langst G., Grummt I., Bickmore W. and Esteller M. (2007). Epigenetic disruption of ribosomal RNA genes and nucleolar architecture in DNA methyltransferase 1 (Dnmt1) deficient cells. Nucleic Acids Res. 35, 2191-2198.

Fatica A. and Tollervey D. (2002). Making ribosomes. Curr. Opin. Cell Biol. 14, 313–318.

Ferreira-Cerca S., Poll G., Gleizes P.E., Tschochner H. and Milkereit P. (2005). Roles of eukaryotic ribosomal proteins in maturation and transport of pre-18S rRNA and ribosome function. Mol. Cell 20, 263-275.

Gadal O., Strauss D., Kessl J., Trumpower B., Tollervey D. and Hurt E. (2001). Nuclear export of 60s ribosomal subunits depends on Xpol p and requires a nuclear export sequence-containing factor, Nmd3p, that associates with the large subunit protein Rp110p. Mol. Cell. Biol. 21, 3405-3415.

Gerbi S.A. and Borovjagin A.V. (2004). Pre-ribosomal RNA processing in multicellular organisms. In: The nucleolus. Olson M.O.J. (ed). Kluwer Academic / Plenum Publishers. pp 170-198.

Gerbi S.A., Borovjagin A.V. and Lange T.S. (2003). The nucleolus: A site of ribonucleoprotein maturation. Curr. Opin. Cell Biol. 15, 318-325.

Gonda K., Fowler J., Katoku-Kikyo N., Reed P., Tamada H. and Kikyo N. (2003). Reversible disassembly of somatic nucleoli by the germ cell proteins FRGY2a and FRGY2b. Nat. Cell Biol. 5, 205-210.

Gonzalez I. and Sylvestr J. (2001). Human rDNA: evolutionary patterns within the genes and tandem arrays derived from multiple chromosomes. Genomics 73, 255-263.

Gonzalez-Melendi P., Wells B., Beven A.F. and Shaw P.J. (2001). Single ribosomal transcription units are linear, compacted Christmas trees in plant nucleoli. Plant J. 27, 223-233.

Gorski S.A., Dundr M. and Misteli T. (2006). The road much traveled: trafficking in the cell nucleus. Curr. Opin. Cell. Biol. 18, 284-290.

Grabenbauer M., Geerts W.J., Fernandez-Rodrigues J., Hoenger A., Koster A.J. and Nilsson T. (2005). Correlative microscopy and electron tomography of GFP through photooxidation. Nat. Methods 2, 857-862.

Granneman S. and Baserga S.J. (2003). Probing the yeast proteome for RNA-processing factor. Genome Biol. 4, 229.

Granneman S. and Baserga S.J. (2005). Crosstalk in gene expression: coupling and co-regulation of rDNA transcription, pre-ribosome assembly and pre-rRNA processing. Curr. Opin. Cell Biol. 17, 281-286.

Granneman S. and Tollervey D. (2007). Building ribosomes: even more

expensive than expected? Curr. Biol. 17, R415-417.

Grummt I. (2003). Life on a planet of its own: Regulation of RNA polymerase I transcription in the nucleolus. Genes Dev. 17, 1691-1702.

Grummt I. and Pikaard C.S. (2003). Epigenetic silencing of RNA polymerase I transcription. Nat. Rev. Mol. Cell. Biol. 4, 641-649.

Hadjiolov A.A. (1985). The nucleolus and ribosome biogenesis. Springer, NewYork.

Haeusler R.A. and Engelke D.R. (2006). Spatial organization of transcription by RNA polymerase III. Nucleic Acids Res. 34, 4826-4836.

Handwerger K.E., Cordero J.A. and Gall J.G. (2005). Cajal bodies, nucleoli, and speckles in the Xenopus oocyte nucleus have a low-density, sponge-like structure. Mol. Biol. Cell 16, 202-211.

Handwerger K.E. and Gall J.G. (2006). Subnuclear organelles: new insights into form and function. Trends Cell Biol. 16, 19-26.

Hell S.W. (2007). Far-field optical nanoscopy. Science 316, 1153-1158.

Hernandez-Verdun D. (2006). Nucleolus: from structure to dynamics. Histochem. Cell. Biol. 125, 127-137.

Hinsby A.M., Kiemer L., Karlberg E.O., Lage K. Fausboll A., Juncker A.S., Andersen J.S., Mann M. and Brunak S. (2006). A wiring of the human nucleolus. Mol. Cell 22, 285-295.

Hiscox J.A. (2007). RNA viruses: hijacking the dynamic nucleolus. Nat. Rev. Microbiol. 5, 119-127.

Huang J., Brito I.L., Villen J., Gygi S.P., Amon A. and Moazed D. (2006). Inhibition of homologous recombination by a cohesin-associated clamp complex recruited to the rDNA recombination enhancer. Genes Dev. 20, 2887-2901.

Huh W.K., Falvo J.V., Gerke L.C., Carroll A.S., Howson R.W., Weissman J.S. and O´Shea E.K. (2003). Global analysis of protein localization in budding yeast. Nature 425, 686-691.

Kalmarova M., Smirnov E., Masata M., Koberna K., Ligasova A., Fidlerova H. and Raska I. (2007). Positioning of NORs and NOR-bearing chromosomes in relation to nucleoli. J. Struct. Biol. 160, 49-56.

Kobayashi T. (2008). A new role of the rDNA and nucleolus in the nucleolus-rDNA instability maintains genome integrity. BioEssays 30, 267-272.

Koberna K., Malinsky J., Pliss A., Masata M., Vecerova J., Fialova M., Bednar J. and Raska I. (2002). Ribosomal genes in focus: new transcripts label the dense fibrillar components and form clusters indicative of “Christmas trees” in situ. J. Cell Biol. 157, 743-748.

Koberna K., Stanek D., Malinsky J., Eltsov M., Pliss A., Ctrnacta V., Cermanova S. and Raska I. (1999). Nuclear organization studied with help of a hypotonic shift: its use permits hydrophilic molecules to enter into living cells. Chromosoma 108, 325-335.

Kopp K., Gasiorowski J.Z., Chen D., Gilmore R., Norton J.T., Wang C., Leary D.D., Chan E.K.L., Dean D.A. and Huang S. (2007). Pol I transcription and pre-rRNA processing are coordinated in a transcription-dependent manner in mammalian cells. Mol. Biol. Cell 18, 394-403.

Koster A.J. and Zandbergen H. (2005). Electron microscopy: cutting the cost of high-resolution microscopy. Nat. Mater. 4, 885-886.

Kruger T., Zentgraf H. and Scheer U. (2007). Intranucleolar sites of ribosome biogenesis defined by the localization of early binding ribosomal proteins. J. Cell Biol. 21, 573-578.

Lafarga M., Berciano M.T., Hervas J.P. and Villegas J. (1989). Nucleolar organization in granule cell neurons of the rat cerebellum. J. Neurocytol. 18, 19-26.

Lam Y.W., Trinkle-Mulcahy L. and Lamond A.I. (2005). The nucleolus. J. Cell Sci. 118, 1335-1337.

Lam Y.W., Lamond A.I., Mann M. and Andersen J.S. (2007). Analysis of nucleolar protein dynamics reveals the nucleolar degradation of ribosomal proteins. Curr. Biol. 17, 1-12.

Lang M.C., Engelhardt J. and Hell S.W. (2007). 4Pi microscopy with linear fluorescence excitation. Opt. Lett. 32, 259-261.

Leary D.J., Terns M.P. and Huang S. (2004). Components of U3 snoRNA-containing complexes shuttle between nuclei and the cytoplasm and differentially blocalize in nucleoli: implications for assembly anf function. Mol. Biol. Cell 15, 281-293.

Lebofsky R. and Bensimon A. (2005). DNA replication origin plasticity and perturbed fork progression in human inverted repeats. Mol. Cell. Biol. 25, No. 15, 6789-6797.

Lestrade L. and Weber M.J. (2006). snoRNA-LBME-db, a comprehensive database of human H/ACA and CÚD box snoRNAs. Nucleic Acids Res. 34, D158-162.

Li J., Langst G. and Grummt I. (2006). NoRC-dependent nucleosome positioning silences rRNA genes. EMBO J. 25, 5735-5741.

Ma H. and Pederson T. (2007). Depletion of the nucleolar protein nucleostemin causes G1 cell cycle arrest via the p53 pathway. Mol. Biol. Cell 18, 2630-2635.

Mais C., Wright J.E., Prieto J.L., Raggett S.L. and McStay B. (2005). UBF-binding site arrays form pseudo-NORs and sequester the RNA polymerase I transcription machinery. Genes Dev. 19, 50-64.

Matsumoto M., Sakamoto M., Hayashi H. and Benno Y. (2005). Novel phylogenetic assignment database for terminal-restriction fragment length polymorphism analysis of human colonic microbiota. J. Microbiol. Methods 61, 305-319.

Mayer C., Schmitz K.-M., Li J., Grummt I. and Santoro R. (2006). Intergenic transcripts regulate the epigenetic state of rRNA genes. Mol. Cell 22, 351-361.

McStay B. (2006). Nucleolar dominance: a model for rRNA gene silencing. Genes Dev. 20, 1207-1214.

Melcak I., Risueno M.C. and Raska I. (1996). Ultrastructural nonisotopic mapping of nucleolar transcription sites in onion protoplasts. J. Struct. Biol. 116, 253-263.

Melese T. and Xue Z. (1995). The nucleolus: an organelle formed by the act of building a ribosome. Curr. Opin. Cell Biol. 7, 319-324.

Miller Jr. O. L. and Beatty B. R. (1969). Visualization of nucleolar genes. Science 164, 955-957.

Olson M.O. and Dundr M. (2005). The moving parts of the nucleolus. Histochem. Cell Biol. 123, 203-216.

Olson M.O., Hingorani K. and Szebeni A. (2002). Conventional and nonconventional roles of the nucleolus. Int. Rev. Cytol. 219, 199-266.

Osheim Y.N., French S.L., Keck K.M., Champion E.A., Spasov K., Dragon F., Baserga S.J. and Beyer A.L. (2004). Pre-18S ribosomal RNA is structurally compacted into the SSU processome prior to being cleaved from nascent transcripts in *Saccharomyces cerevisiae*. Mol. Cell 16, 943-954.

Panse V.G., Kressler D., Pauli A., Petfalski E., Gnadig M., Tollervey D. and Hurt E. (2006). Formation and nuclear export of preribosomes are functionally linked to the small-ubiquitin-related modifier pathway Traffic 7, 1311-1321.

Pederson T. (1998). The plurifunctional nucleolus. Nucleic Acids Res. 26, 3871-3876.

Pederson T. (2001). Fluorescent RNA cytochemistry: tracking gene transcripts in living cells. Nucleic Acids Res. 29, 1013-1016.

Pederson T. (2007). Ribosomal protein mutations in Diamond-Blackfan anemia: might they operate upstream from protein synthesis?

FASEB J. 21, 3442-3445.

Pendle A.F., Clark G.P., Boon R., Lewandowska D., Lam Y.W. Andersen J., Mann M., Lamond A.I., Brown J.W. and Shaw P.J. (2005). Proteomic analysis of the Arabidopsis nucleolus suggests novel nucleolar functions. Mol. Biol. Cell 16, 260-269.

Percipalle P. (2007) Genetic connection of the actin cytoskeleton and beyond. BioEssays 29, 407-411.

Pliss A., Koberna K., Vecerova J., Malinsky H., Masata M., Fialova M., Raska I. and Berezney R. (2005). Spatio-temporal dynamics at rDNA foci: global switching between DNA replication and transcription. J. Cell. Biochem. 94, 554-565.

Politz J.C., Yarovoi S., Kilroy S.M., Gowda K., Zwieb C. and Pederson T. (2000). Signal recognition particle components in the nucleolus. Proc. Natl. Acad. Sci. USA 97, 55-60.

Politz J.C., Lewandowski L.B. and Pederson T. (2002). Signal recognition particle RNA localization within the nucleolus differs from the classical sites of ribosome synthesis. J. Cell Biol. 159, 411-418.

Politz J.C., Tuft R.A. and Pederson T. (2003). Diffusion-based transport of nascent ribosomes in the nucleus. Mol. Biol. Cell 14, 4805-4812.

Politz J.C., Polena I., Trask I., Bazett-Jones D.P. and Pederson T. (2005). A nonribosomal landscape in the nucleolus revealed by the stem cell protein nucleostemin. Mol. Biol. Cell 16, 3401-3410.

Preuss S. and Pikaard C.S. (2007). Rrna gene silencing and nucleolar dominance: Insights into a chromosome-scale epigenetic on/off switch. Biochim. Biophys. Acta 1769, 383-392.

Prieto J.L. and McStay B. (2007). Recruitment of factors linking transcription and processing of pre-rRNA to NOR chromatin is UBF dependent and occurs independent of transcription in human cells. Genes Dev. 21, 2041-2054.

Raska I. (2003). Oldies but goldies: searching for Christmas trees within the nucleolar architecture. Trends Cell Biol. 13, 517–525.

Raska I., Armbruster B.L., Frey J.R. and Smetana K. (1983a). Analysis of ring-shaped nucleoli in serially sectioned human lymphocytes. Cell Tissue Res. 234, 707-711.

Raska I., Rychter Z. and Smetana K. (1983b). Fibrillar centres and condensed nucleolar chromatin in resting and stimulated human lymphocytes. Z. Mikrosk. Anat. Forsch. 97, 15-32.

Raska I., Michel LS., Jarnik M., Dundr M., Fakan S., Gasser S., Gassmann M., Hubscher U., Izaurralde E. and Martinez E. (1991). Ultrastructural cryoimmunocytochemistry is a convenient tool for the study of DNA replication in cultured cells. J. Electron Microsc. Tech. 18, 91-105.

Raska I., Koberna K., Malinsky J., Fidlerova H. and Masata M. (2004). The nucleolus and transcription of ribosomal genes. Biol. Cell 96, 579-594.

Raska I., Shaw P.J. and Cmarko D. (2006a). Structure and function of the nucleolus in the spotlight. Curr. Opin. Cell Biol. 18, 325-334.

Raska I., Shaw P.J. and Cmarko D. (2006b). New insights into nucleolar architecture and activity. Int. Rev. Cytol. 255, 177-235.

Reichow S.L., Hamma T., Ferre-D´Amare A.R. and Varani G. (2007). The structure and function of small nucleolar ribonucleoproteins. Nucleic Acids Res. 35, 1452-1464.

Rosado I.V., Kressler D. and Cruz J.D. (2007) Functional analysis of *Saccharomyces cerevisiae* ribosomal protein Rpl3p in ribosome synthesis. Nucleic Acids Res. 35, 4203-4213.

Rudra D. and Warner J.R. (2004). What better measure than ribosome synthesis? Genes Dev. 18, 2431-2436.

Russell J. and Zomerdijk J.C. (2006). The RNA polymerase I transcription machinery. Biochem. Soc. Symp. 73, 203-216.

Santoro R. (2005). The silence of the ribosomal RNA genes. Cell. Mol. Life Sci. 62, 2067-2079.

Santoro R., Li J. and Grummt I. (2002). The nucleolar remodeling complex NoRC mediates heterochromatin formation and silencing of ribosomal gene transcription. Nat. Genet. 32, 393–396.

Scheer U. and Hock R. (1999). Structure and function of the nucleolus. Curr. Opin. Cell Biol. 11, 385-390.

Scherl A., Coute Y., Deon C., Calle A., Kindbeiter K., Sanchez J.C., Greco A., Hochstrasser D. and Diaz J.J. (2002). Functional proteomic analysis of human nucleolus. Mol. Biol. Cell 13, 4100-4109.

Schneider D.A., Michel A., Sikes M.L., Vu L., Dodd J.A., Salgia S., Osheim Y.N., Beyer A.L. and Nomura M. (2007). Transcription elongation by RNA polymerase I is linked to efficient rRNA processing and ribosome assembly. Mol. Cell 26, 217-229.

Schuemie M., Chichester C., Lisacek F., Coute Y., Roes P.J., Sanchez J.C., Kors J. and Mons B. (2007). Assignmant of protein function and discovery of novel nucleolar proteins based on automatic analysis of MEDLINE. Proteomics 7, 921-931.

Seiser R.M., Sundberg E., Wollam B.J., Zobel-Thropp P., Baldwin K., Spector D.M. and Lycan D.E. (2006). Ltv1 is required for efficient nuclear export of the ribosomal small subunit in Saccharomyces cerevisiae. Genetics 174, 679-691.

Simpson G.J. (2006). Biological imaging: The diffraction barrier broken. Nature 440, 879-880.

Sommerville J., Brumwell C.L., Politz J.C., and Pederson T. (2005). Signal recognition particle assembly in relation to the function of amplified nucleoli of Xenopus oocytes. J. Cell Sci. 118, 1299-1307.

Sirri V., Urcuqui-Inchima S., Roussel P. and Hernandez-Verdun D. (2008). Nucleolus: the fascinating nuclear body. Histochem. Cell. Biol. 129, 13-31.

Stavreva D.A., Kawasaki M., Dundr M., Koberna K., Mueller W. G., Tsujimura-Takahashi T., Komatsu W., Hayano T., Isobe T., Raska I., Misteli T., Takahashi N. and McNally J.G. (2006). Potential roles for ubiquitin and the proteasome during ribosome biogenesis. Mol. Cell. Biol. 26, 5131-5145.

Stefanovsky V., Langlois F., Gagnon-Kluger T., Rothblum L.I. and Moss T. (2006). Growth factor signaling regulates elongation of RNA polymerase I transcription in mammals via UBF phosphorylation and r-chromatin remodeling. Mol. Cell 21, 629-639.

Trotta C.R., Lund E., Kahan L., Johnson A.W. and Dahlberg J.E. (2003). Coordinated nuclear export of 60S ribosomal subunits and NMD3 in vertebrates. EMBO J. 22, 2841-2851.

Tschochner H. and Hurt E. (2003). Pre-ribosomes on the road from the nucleolus to the cytoplasm. Trends Cell Biol. 13, 255-263.

van Donselaar E., Posthuma G., Zeuschner D., Humbel B.M and Slot J.W. (2007). Immunogold labeling of cryosections from high-pressure frozen cells. Traffic 8, 471-485.

Vollmer M., Horth P., Rozing G., Coute Y., Grimm R., Hochstrasser D. and Sanchez J.C. (2006). Multi-dimensional HPLC/MS of the nucleolar proteome using HPLC-chip/MS. J. Sep. Sci. 29, 499-509.

Yakusheva T.A., Shaikh A.G., Green A.M., Blazquez P.M., Dickman J.D. and Angelaki D.E. (2007). Purkinje cells in posterior cerebellar vermis encode motion in an inertial reference frame. Neuron 54, 973-985.

Zemp I. and Kutay U. (2007). Nuclear export and cytoplasmic maturation of ribosomal subunits. FEBS Lett. 581, 2783-2793.

Accepted April 16, 2008