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REVIEW

M. O. J. Olson · Miroslav Dundr

The moving parts of the nucleolus

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Abstract The cell nucleolus is the subnuclear body in which ribosomal subunits are assembled, and it is also the location of several processes not related to ribosome biogenesis. Recent studies have revealed that nucleolar components move about in a variety of ways. One class of movement is associated with ribosome assembly, which is a vectorial process originating at the sites of transcription in the border region between the fibrillar center and the dense fibrillar component. The nascent preribosomal particles move outwardly to become the granular components where further maturation takes place. These particles continue their travel through the nucleoplasm for eventual export to the cytoplasm to become functional ribosomes. In a second kind of motion, many nucleolar components rapidly exchange with the nucleoplasm. Thirdly, nucleolar components engage in very complex movements when the nucleolus disassembles at the beginning of mitosis and then reassembles at the end of mitosis. Finally, many other cellular and viral macromolecules, which are not related to ribosome assembly, also pass through or are retained by the nucleolus. These are involved in nontraditional roles of the nucleolus, including regulation of tumor suppressor and oncogene activities, signal recognition particle assembly, modification of small RNAs, control of aging, and modulating telomerase function.

Keywords Nucleolus · Ribosome assembly · Mitosis · Nucleolar assembly · Macromolecular mobility

M. O. J. Olson (✉)
Department of Biochemistry, The University of Mississippi Medical Center, 2500 N State Street, Jackson, 39216, MS, USA
E-mail: molson@biochem.umsmed.edu
Tel.: +1-601-9841500
Fax: +1-601-9841501

M. Dundr
National Cancer Institute, National Institutes of Health, Bethesda, 20892, MD, USA

Introduction

For several decades, the typical textbook illustration of the nucleolus has been the very prominent, densely stained, structure seen in an electron micrograph (Fig. 1). This static image is difficult to remove from one's mind, and it makes it difficult to think of the nucleolus as a dynamic subnuclear body. In fact, recent work has revealed that what we see in the micrograph is just a snapshot of multiple nucleolar components in rapid motion. These movements are not only related to the process of ribosome biogenesis, but they are also associated with recently discovered nontraditional functions of the nucleolus.

The major task of actively growing and dividing cells is to make proteins. For that to happen, ribosomes must be continuously produced and replenished. The nucleolus is the provider of ribosomes to satisfy the cell's voracious requirement for proteins. It was established in the 1960s that the nucleolus is responsible for ribosome assembly (Busch and Smetana 1970); however, in the late 1990s, many other unexpected functions for the nucleolus began to appear (Olson 2004a). Coincidentally, it was also about that time that new information about the dynamic nature of nucleolar components emerged.

Eukaryotic ribosome assembly may be summarized as a series of a few major operations (Fig. 2) (Olson 2004b): (1) transcription of preribosomal RNA (pre-rRNA) from the multiple copies of rDNA, (2) association of proteins and small nucleolar RNAs (snoRNAs) with the pre-rRNA during and after transcription, (3) modification of the RNA by pseudouridylation and methylation, (4) processing of the pre-rRNA into the three major ribosomal RNAs, (5) incorporation of ribosomal proteins and 5S rRNA into the maturing preribosomal particles at various stages in the process, and (6) export of the mature preribosomal particles to the cytoplasm. At first glance, these operations seem relatively simple, but in reality, the ribosome biogenesis

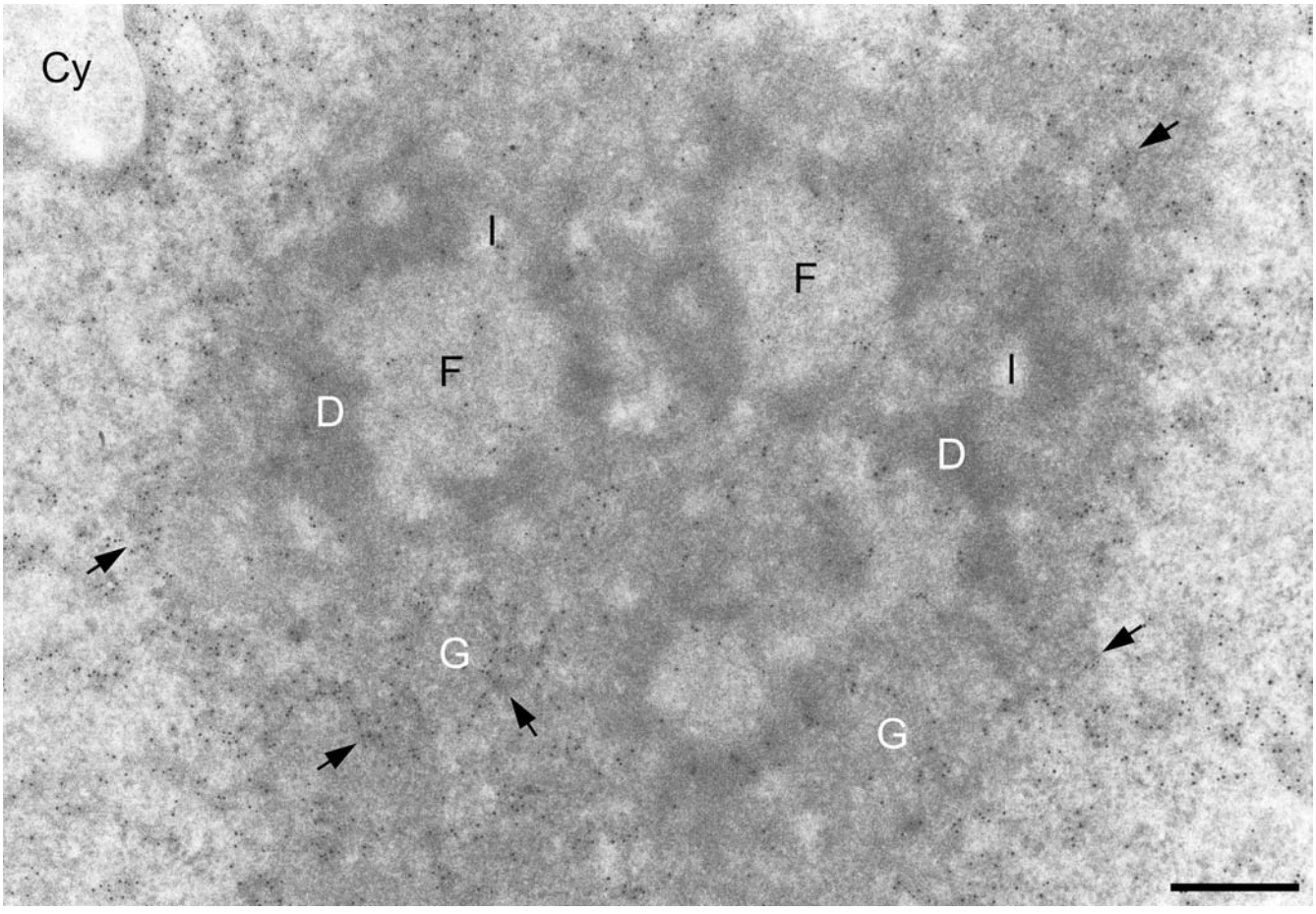

Fig. 1 Ultrastructural organization of the mammalian nucleolus. HeLa cells were embedded into Lowicryl, thin-sectioned, and visualized by electron microscopy. The relatively large opaque fibrillar centers (*F*) in this HeLa cell nucleolus are surrounded by highly contrasted fine strands of the dense fibrillar component (DFC) (*D*). The DFCs are the early intermediates of preribosomal particles, which later form the granular components (*G*), which fill out the peripheral parts of the nucleolus. The DFCs are occasionally interrupted by small regions of low electron density termed nucleolar interstices (*I*). The perinucleolar and intranucleolar clumps of condensed chromatin (*arrows*) detected by terminal deoxynucleotide transferase labeling are visualized by immunogold particles. *Cy* cytoplasm. Bar, 500 nm

process is highly complex with many intermediate stages using a large number of proteins and small nucleolar RNAs (snoRNAs) as assembly factors. To date, over 350 different proteins have been found in mammalian nucleoli (see the nucleolar protein database compiled by the Angus Lamond Laboratory at http://www.dundee.ac.uk/lifesciences/lamonddatabase/flash.htm; (Anderson et al. 2002; Scherl et al. 2002), and there may be 200 or more species of snoRNAs (Bachellerie et al. 2002). About half of the proteins in the catalog seem to be related to ribosome biogenesis, but at least 40% have unknown or "other" functions. The well-characterized nonribosomal proteins are nucleases, topoisomerases, RNA binding proteins, RNA modifying enzymes, helicases, and a variety of apparent ribosome assembly factors of poorly understood function. The snoRNAs provide guidance to many of the operations, including methylation, pseudouridylation, and RNA cleavage (Bertrand and Fournier 2004).

Dynamics of ribosome assembly

Because an active nucleolus is busy making ribosomes, it is inherently dynamic, with preribosomes at various stages of completion engaging in directed movements down a pathway that is analogous to an assembly line. In addition, the many ribosomal and nonribosomal components move as they are incorporated into or removed from the preribosomal particles. As a result of these dynamics, the overall structure of a nucleolus is largely determined by its ability to assemble ribosomes; a nucleolus probably would not exist if it did not have that capacity (Melese and Xue 1995). In fact, nucleolar size is an indication of the rapidity of cellular proliferation (Derenzini et al. 2000). By the same token, nucleolar ultrastructure depends on the rate at which the cell is making ribosomes. A good example of this is found in lymphocytes. Dormant lymphocytes, which have low ribosome biogenesis activities, have relatively small nucleoli with narrow shells of dense fibrillar components (DFCs). When these cells are stimulated

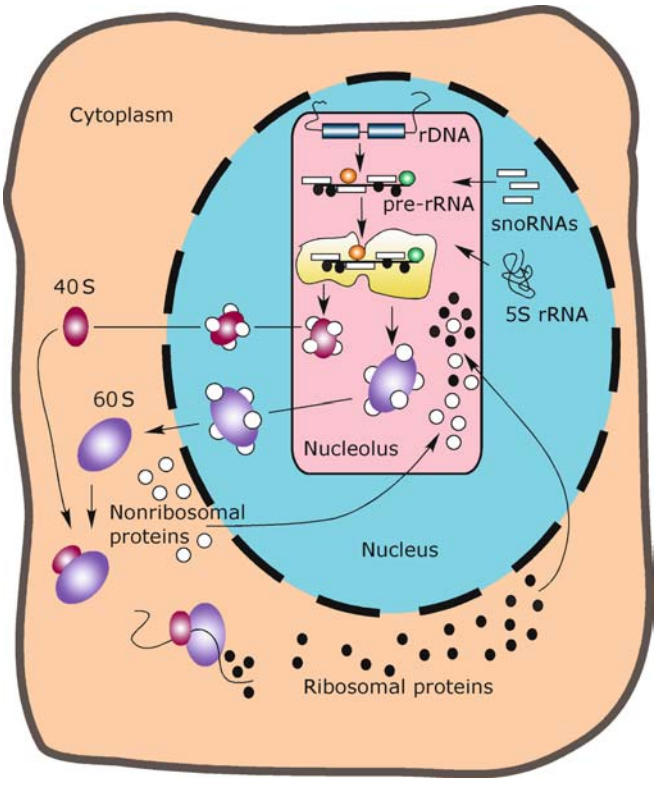

Fig. 2 Major steps in eukaryotic ribosome biogenesis. The process starts with transcription of preribosomal RNA (pre-rRNA) from multiple copies of the genes for pre-rRNA (rDNA). Nonribosomal proteins (*open circles*) and small nucleolar RNAs (snoRNAs; *open rectangles*) associate with the nascent transcript. The pre-rRNA is methylated and pseudouridylated under the guidance of the snoRNAs. 5S rRNA, a component of the 60S subunit, is added to the maturing complex. The pre-rRNA undergoes a series of cleavages ultimately resulting in 18S, 5.8S, and 28S (25S in yeast) rRNAs. The complex is split into the two precursor particles for the small (40S) and large (60S) ribosomal subunits. Ribosomal proteins (*black circles*) are added to the precursor complexes at various stages of assembly. The nearly mature subunits are exported to the cytoplasm through the nuclear pore complexes with the aid of adaptor proteins. The small and large subunits are eventually incorporated into ribosomes in the cytoplasm. Figure modified from Olson (2004b)

with phytohemagglutinin or interferon, the nucleoli and their DFCs become much larger (Mosgoeller 2004; Raska et al. 2004). Opposite changes occur when RNA polymerase I (RNA pol I) transcription is inhibited; the structure of the nucleolus is drastically altered, and its major components redistribute into clearly segregated zones (Hadjiolov 1985; Thiry and Goessens 1996). Rapid redistribution of components can be seen after treatment of cells with actinomycin D, which induces massive rDNA chromatin condensation and segregation of nucleolar subcompartments (Fig. 3). The latter phenomenon occurs because ribosome assembly is a vectorial process in which the preribosomal particles move away from the sites of transcription to the granular components (GC), then into the nucleoplasm, and eventually into the cytoplasm (de la Cruz et al. 2004). The maturation process is carried out as the preribosomes move along this pathway. Disruption at any stage traps assembly intermediates and distorts the overall nucleolar structure. Thus, the steady-state movement of nucleolar components during ribosome assembly determines the normal structure of an active nucleolus.

The movements associated with ribosome biogenesis start with transcription of the rDNA by RNA polymerase I (RNA pol I), but there is rapid motion of the enzyme and its associated factors even before transcription is initiated. Dundr et al. (2002) prepared constructs that expressed GFP tagged RNA pol I subunits and several other RNA pol I transcription factors. After transfecting each of these into monkey kidney cells and demonstrating their functionality, they examined the motions of these proteins, employing photobleaching techniques. Using kinetic modeling, which compared the exit rates of the RNA pol I subunits from the nucleolus with their entry rates onto the rDNA promoter, they were able to determine the probability that a given subunit would associate with the promoter. For all subunits tested, there was about a 50% chance of this happening. However, even with this relatively high probability of finding the promoter, the chance of initiating and elongating was only between 1% and 11%. Thus, the assembly of all the components of an engaged RNA pol I holoenzyme seems to be highly inefficient although some order may be provided by sequentially building the complex with a series of metastable intermediates. The major feature of this model is that the assembled RNA pol I complex is not recycled as an intact unit but the components reassemble after each round of transcription.

The idea that the RNA pol I complex is not always a uniform entity is strongly supported by recent findings that different subunits vary in their association kinetics with nucleolar organizer regions (NORs) in metaphase chromosomes. Chen et al. (2005) and Leung et al. (2004) have shown that the recruiting subunit RPA43-GFP along with preinitiation factor UBF are present at NORs, but RPA16, RPA20, RPA39/40, and RPA194 are not. These studies indicate that the RNA pol I complex is not retained at the transcriptionally silenced NORs as a preassembled holoenzyme during metaphase but that it disassembles during mitosis.

Although the pol I subunit exchange model is based on observations in living cells, recent work using biochemical techniques suggests that there may be alternative interpretations. Schneider and Nomura (2004) used mutant strains of yeast, in which RNA pol I was either engaged or not in transcription of rDNA, to study the state of association of the enzyme subunits. They found that the apparent size of the enzyme did not change appreciably when it was disengaged from transcription, as measured by glycerol gradient ultracentrifugation, although there was some accumulation of slower sedimenting A190 subunit when transcription by RNA pol I was inactivated. In addition, two of the largest subunits (A135 and A190) did not dissociate from one another between multiple rounds of

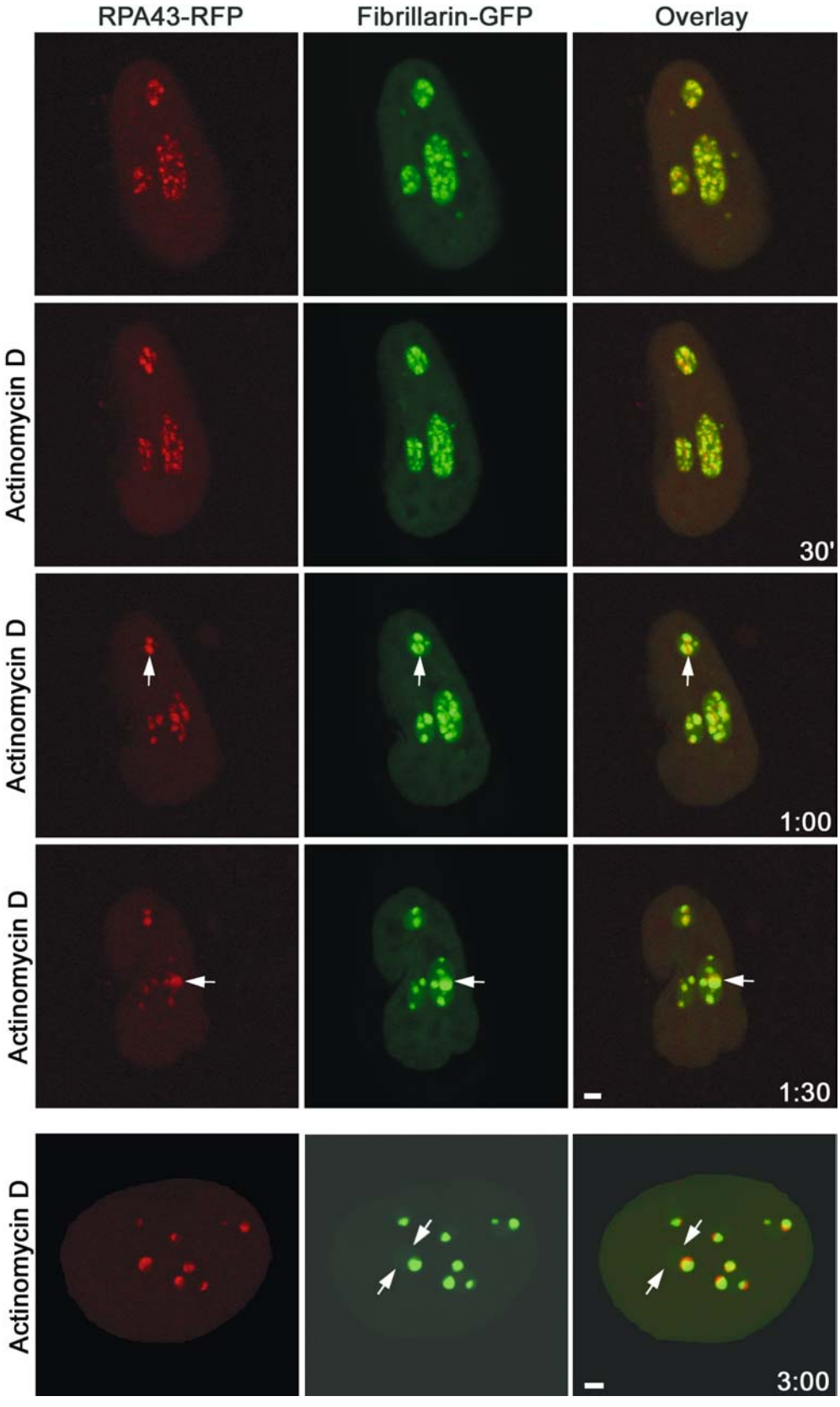

Fig. 3 Dynamic changes in nucleolar organization after inhibition of RNA polymerase I transcription by actinomycin D (AMD). HeLa cells transfected with constructs expressing RPA43-red fluorescent protein (RPA43-RFP) and fibrillarin-green fluorescent protein (fibrillarin-GFP) were treated with 0.5 μg/ml of AMD and monitored by live time-lapse microscopy. The images are maximum projections of z-stacks of focal plane sections through entire nuclear volumes. The RPA43-RFP and ffibrillarin-GFP label the fibrillar centers (FCs) and dense fibrillar components (DFCs), respectively (*top panels*). AMD intercalates into the A-T rich regions of ribosomal genes and induces the condensation of chromatin. After 30 min of AMD treatment, the strands of the DFCs labeled with fibrillarin-GFP are more pronounced, and the nucleoli have lost their irregular shapes. After 1 h, the numerous DFCs have fused into a few domains due to massive chromatin condensation; at the same time, the FCs labeled by RPA43-RFP and the DFCs labeled by fibrillarin-GFP have undergone polar separation (*arrows*). After 1 h 30 min, the nucleoli become less elongated and contain a few round DFCs with caps of FCs (*arrows*). After 3 h, the nucleoli are spherical bodies with the three nucleolar substructures completely segregated; each nucleolus contains a single granular component (GC) (*arrows*) with one or more round DFCs with caps representing the FCs. Bars, 2 μm

transcription. In contrast to pol I subunits in mammalian cells, these subunits also did not separate over several cell divisions. The study concluded that yeast RNA pol I is a stable protein complex and not especially dynamic. Unfortunately, with the exception of the A190 in yeast or RPA194 in mammals, the two groups did not

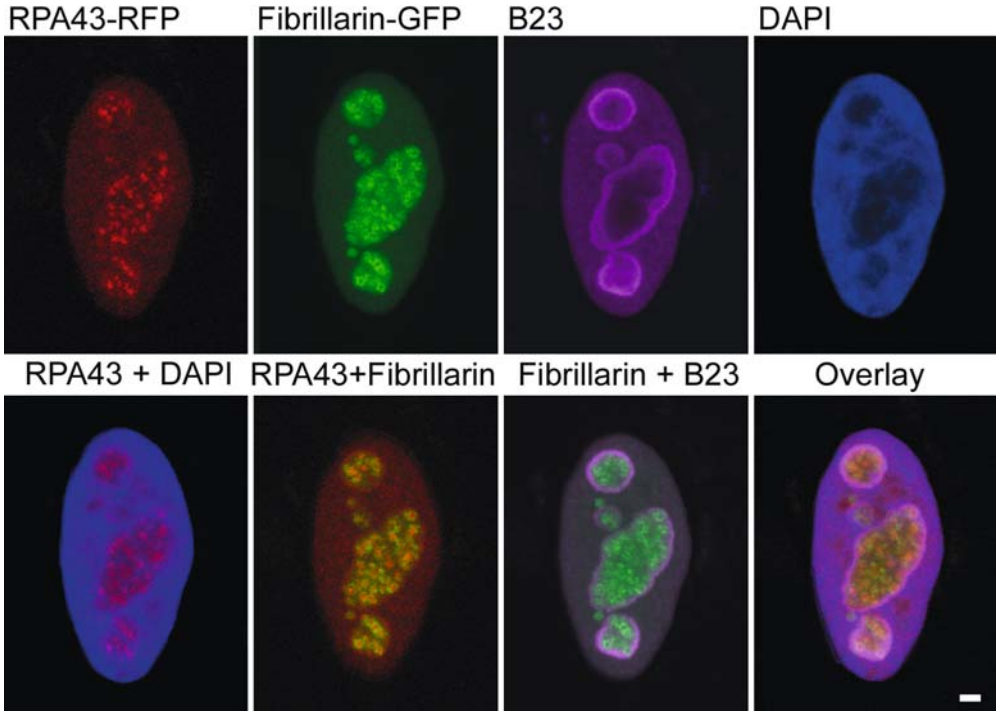

Fig. 4 Organization of nucleoli. HeLa cells were transiently cotransfected with constructs expressing the RNA-polymerase-I-recruiting subunit RPA43 (Peyroche et al. 2000) fused with the monomeric red fluorescent protein (RPA43-RFP) (Shaner et al. 2004) and fibrillarin-green fluorescent protein (fibrillarin-GFP) (Dundr et al. 2000) and stained with an antibody against protein B23 detected by a secondary antibody conjugated with Cy5. All proteins were detected in a z-stack throughout the nuclear volume and visualized as maximum projections. RPA43-RFP is localized in a distinct punctate pattern scattered through the nucleolar volume representing fibrillar centers and the inner portions of the dense fibrillar components (DFCs). Fibrillarin-GFP, a methyltransferase that catalyzes the 2′-*O*-methylation of pre-rRNA (Omer et al. 2002), is primarily localized in regions of DFCs, which surround the punctate localization of RPA43-RFP. This demonstrates the structural link between RNA pol I transcription and early modification and processing steps of pre-rRNA maturation (overlay of RPA43-RFP and fibrillarin-GFP). In contrast, protein B23, which is involved in later steps of pre-rRNA processing and the assembly of ribosomal subunits, is primarily detected at the periphery of the nucleolus (overlay of fibrillarin-GFP and protein B23). Bar, 2 μm

study the exchange properties of comparable RNA pol I components. Also, the experiments of the two groups were done with very different organisms and used completely different approaches.

Once transcription has started, the vectorial movement of the growing preribosomal particles also begins. Although the precise location of transcription has been debated for several decades (reviewed by Huang 2002; Raska 2003; Raska et al. 2004), it is most likely in the border between the FC and the DFC. Regardless of where transcription begins, the pre-rRNA initially accumulates in the inner region of the DFC and then migrates outward, eventually making its way to the GC (Cheutin et al. 2002). Using BrUTP labeling, this movement of the pre-rRNA can be traced from the inner portion of the nucleolus to its periphery (Thiry et al. 2000). During a time course of labeling, there is a radial flow of the newly synthesized RNA in the form of concentric rings, which eventually fuse as they move toward the nucleolar periphery. Figure 4 illustrates that concentric rings are also formed by proteins that serve as markers for the FC (RNA pol I subunit RPA43), the DFC (fibrillarin), and the GC (protein B23).

Is it possible that the preribosomal particles are actively propelled as they move outwardly from the DFC? The finding of the motor proteins, actin (Funaki et al. 1995) and myosin I (Pestic-Dragovich et al. 2000), in the nucleolus opened the possibility that they might aid in that process. Very recently, it was shown that both nuclear actin and myosin I are associated with the rDNA chromatin and with the RNA pol I transcription machinery (Philimonenko et al. 2004). Both of these proteins are required for optimal transcription by RNA pol I, with nuclear myosin I being important for initiation and actin necessary for both elongation and initiation. The current interpretation of this study is that these proteins are acting like RNA pol I transcription factors rather than as motor proteins. Thus, there is no evidence that actin and myosin actually facilitate the movement of the pre-rRNP complexes through the nucleolus.

Very early in the transcription process, proteins and snoRNAs associate with the nascent pre-rRNA. In fact, electron-dense ‘‘terminal balls’’ can be seen near the leading ends of the transcripts while they are still attached to the template (Mougey et al. 1993). These contain processing and remodeling components, including U3 snoRNA and a number of proteins. Some ribosomal proteins may also be added during transcription (Chooi and Leiby 1981). However, most of the

ribosomal proteins seem to be assembled into more mature pre-rRNP particles (de la Cruz et al. 2004).

An important early step in the assembly process is the incorporation of 5S rRNA into the nascent pre-rRNP particle, which is preceded by a complex series of movements. In vertebrates, 5S rRNA is synthesized in the nucleoplasm and then becomes a component of a 7S RNP particle, which is exported to the cytoplasm (Steitz et al. 1988). There, the 7S particle picks up ribosomal protein L5, and this complex is reimported into the nucleus. The association of rpL5 with 5S rRNA occurs during translation; the RNA apparently acts as a chaperone for the folding of rpL5 (Lin et al. 2001). Recently, another protein called NVL2 was found to be associated with rpL5 during its nucleolar lifetime (Nagahama et al. 2004). This protein belongs to the AAA class of ATPases and is essential for optimal production of 60S ribosomal subunits. The nucleolar localization of the NVL2 protein is also dependent on its interaction with rpL5. This illustrates the interdependence of multiple ribosomal components and assembly factors in ribosome biogenesis, which in this case relies on both export from and reimportation to the nucleus.

Ultimately, the nearly mature preribosomal particles leave the nucleolus, enter the nucleoplasm, and are transported to the cytoplasm. In yeast, preribosomes have been observed by electron microscopy to line up on tracks in the nucleoplasm as they wait their turn to be exported through the nuclear pore complex (Léger-Silvestre et al. 1997). However, more recent work in

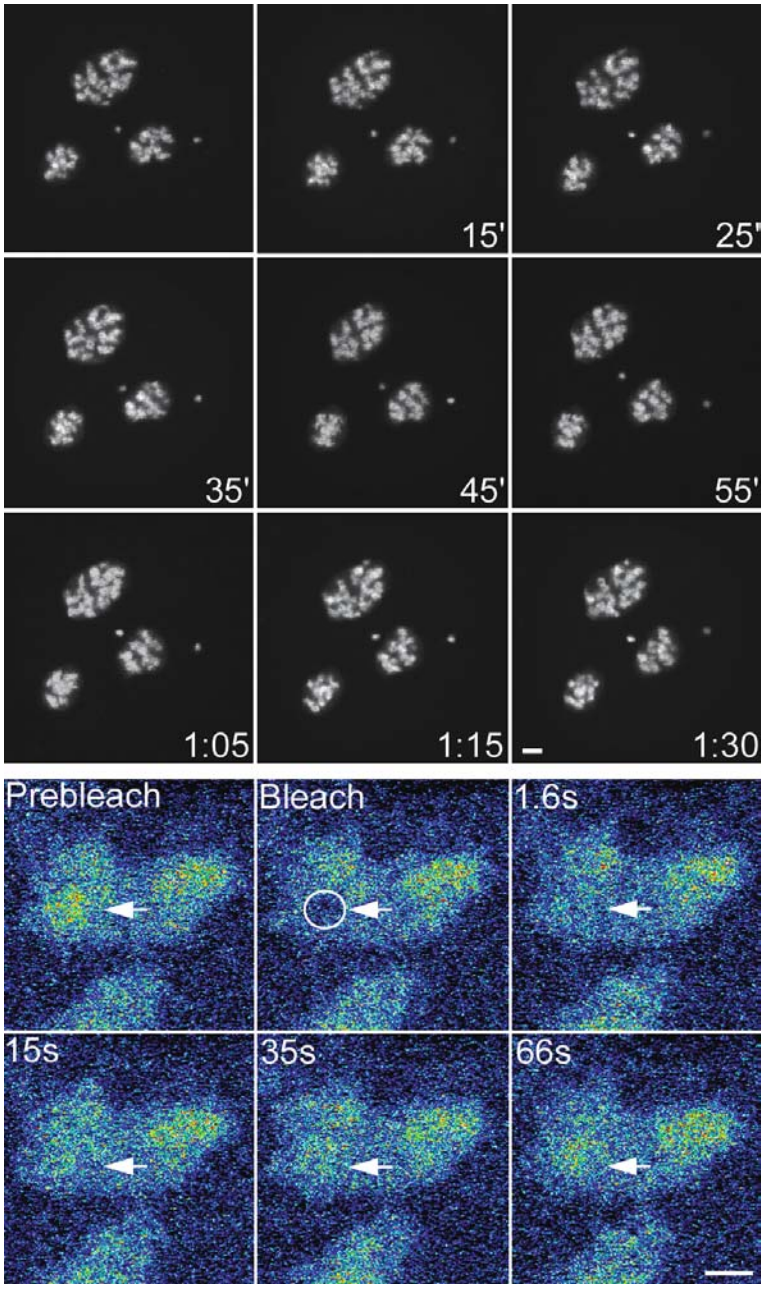

Fig. 5 The nucleolus is a stable structure with rapidly exchanging components. HeLa cells were transfected with fibrillarin-green fluorescent protein (fibrillarin-GFP), which was visualized as a maximum projection of z-stack of focal plane sections through an entire nuclear volume and monitored for 90 min by a time-lapse microscopy. The pattern of the dense fibrillar components visualized by fibrillarin-GFP fluorescence does not show major morphological changes over a 90-min period, indicating that the nucleolus is a stable structure. The small round structures near the nucleoli are Cajal bodies also labeled with fibrillarin-GFP. The *lower colored panels* show the recovery kinetics of fibrillarin-GFP in a HeLa cell nucleolus. A small portion of a nucleolus was bleached (*arrow*), and the recovery of the signal was monitored by time-lapse microscopy. The fluorescent signal in the bleached area recovers within approximately 1 min, indicating that fibrillarin is rapidly exchanging between the nucleolus and the nucleoplasm. This figure demonstrates that even though the steady-state nucleolar distribution of fibrillarin-GFP makes it appear to be a stable component, the photobleaching experiment reveals that it continuously enters and exits the nucleolus (Dundr et al. 2000; Phair and Misteli 2000; Snaar et al. 2000, Chen and Huang 2001). Bars, 2 μm

mammalian cells has shown that once the large ribosomal subunits have left the nucleolus, they move in all directions with no evidence for movement along a specific pathway in the nucleoplasm (Politz et al. 2003). In the later stages of ribosome biogenesis in yeast, transport and maturation seem to be coupled with three nucleolar proteins, Noc1p, Noc2p, and Noc3p, required for both processes (Milkereit et al. 2001, 2003). Although the Noc proteins seem to be required for the passage of the preribosomal subunits from the nucleolus to the nucleoplasm (Tschochner and Hurt 2003), it is not clear that they have any influence on the pathway between the nucleolus and the nuclear pore.

Once the ribosomal subunits have progressed to the vicinity of the nuclear pore, they are exported by a receptor-mediated process, which is energy-dependent, saturable, and unidirectional. The receptor for export of both subunits is Crm1p; however, the large subunit does not interact directly with this protein (Johnson et al. 2002). Instead, another protein called NMD3 provides the nuclear export signal and serves as an adaptor between the 60S subunit and Crm1p. There is some evidence that NMD3 loads onto the large subunit in the nucleolus (Trotta et al. 2003). The export of the 40S subunit also uses Crm1p for export, probably with the aid of an adaptor, but that protein has not been identified (Rodriguez et al. 2004).

New concepts of macromolecular mobility and nucleolar architecture

As indicated above, the process of ribosome biogenesis is analogous to production on an assembly line in which the type of movements described above are to be expected; i.e., parts and subassemblies are delivered to the nucleolus, components are added, and modifications are made in a linear manner with eventual delivery of the products to the cytoplasm. However, what has been surprising in recent years is the finding that the structures of nucleoli and nuclear bodies in general are inherently dynamic. Because the concepts related to the dynamics of nuclear substructures have been elaborated in detail in several review articles (Misteli 2001; Roix and Misteli 2002; Fakan 2004; Cheutin et al. 2004; Leung and Lamond 2003; Dundr and Misteli 2004), the main points will only be summarized below.

The current ideas about the dynamics of nuclear architecture were developed with the aid of new technologies, including vectors for expressing fluorescently tagged proteins in living cells, high resolution confocal microscopy, devices for rapid image acquisition, and kinetic modeling methods. These advances have been coupled with photobleaching techniques, especially fluorescence recovery after photobleaching (FRAP), to visualize the dynamics of individual proteins in specific compartments of cells. For more details on these methods, see reviews by Phair and Misteli (2001), Dundr and Misteli (2003), and Shav-Tal et al. (2004). The experiments using these methods have revealed that the nucleolus is not a static structure but that its components are constantly exchanging with the surrounding nucleoplasm (Phair and Misteli 2000) (Fig. 5).

The residence times of most nucleolar proteins are on the order of tens of seconds (Dundr et al. 2000; Phair and Misteli 2000; Chen and Huang 2001). With this rapid movement, one might expect that the nucleolus would appear as just a blur in a living cell. However, these movements are slower than biologically inactive molecules (those that are not interacting with other molecules) whose residence times are 10–200 times shorter. In reality, the structure of a nucleolus is the sum total of molecules entering and leaving a given subnuclear space, with the residence times of individual molecules governing its overall organization. The residence times are determined by interactions between nucleolar molecules; i.e., the greater the affinity of one nucleolar component for another, the longer the residence time. Thus, most of the molecules we see in a nucleolus are those which have relatively long residence times. In this regard, it is interesting that ribosomal proteins have much longer residence times than assembly factors in the nucleolus (Chen and Huang 2001). This is presumably because the ribosomal proteins have high affinities for the components of preribosomal particles once they have been appropriately incorporated.

Another tenet of the concept of the dynamic nucleus and nucleolus is that proteins are entering and leaving the nucleolus more or less at random and by simple diffusion. The implication of this is that almost any molecule in the nucleus could pass through the nucleolus, but only those with affinity for nucleolar components would be retained there for longer periods of time (Dundr and Misteli 2002). In other words, it is unlikely that specific components are actively targeted to the nucleolus; it is more probable that they are retained there by interactions with other nucleolar components. This could also provide an explanation of why so many macromolecules not related to ribosome biogenesis are found in the nucleolus (Andersen et al. 2002; Scherl et al. 2002).

The nucleolus is essentially a self-organizing body, the structure of which depends both on function and on the existence of organizing centers. As indicated above, maintenance of nucleolar structure depends on its ability to assemble ribosomes. This process starts with transcription, of which the pre-rRNA product is not only the initial material from which ribosomes are constructed, but it also serves as the backbone for the DFC and subsequently for the GC. The affinities of proteins and snoRNAs for the pre-rRNA transcripts ultimately provide the basis for the structures of these subnucleolar compartments. In a similar manner, the rDNA present in the NORs is the organizing center for the transcriptional process. Thus, the nucleolus is built around more stable macromolecules, but most of its components are rapidly exchanging with the nucleoplasm.

Dynamics of nucleolar components during mitosis

In higher eukaryotes, the nucleolus is disassembled during the early stages of mitosis and reassembles at the end of mitosis. As the cells proceed through the phases of mitosis, the nucleolar components undergo a complex series of movements resulting in the redistribution of the existing nucleolar material between the two daughter cells. Recent detailed reviews of this topic are available (DiMario 2004; Hernandez-Verdun 2004).

Nucleolar disassembly takes place in prophase. During this time, transcription by RNA pol I gradually decreases until it is completely repressed by the end of prophase (Prescott and Bender 1962). Roughly concurrent with the transcriptional repression, the pre-rRNA processing is also suppressed (Fan and Penman 1971; Dundr and Olson 1998). The pre-rRNA processing machinery of the DFC and GC begins to move out of the nucleolus at the end of the G2 phase, and by the end of prophase, the nucleolus is no longer visible (Gautier et al. 1992).

The transcriptional apparatus behaves somewhat differently than the processing machinery in that at least some of the transcription components remain associated

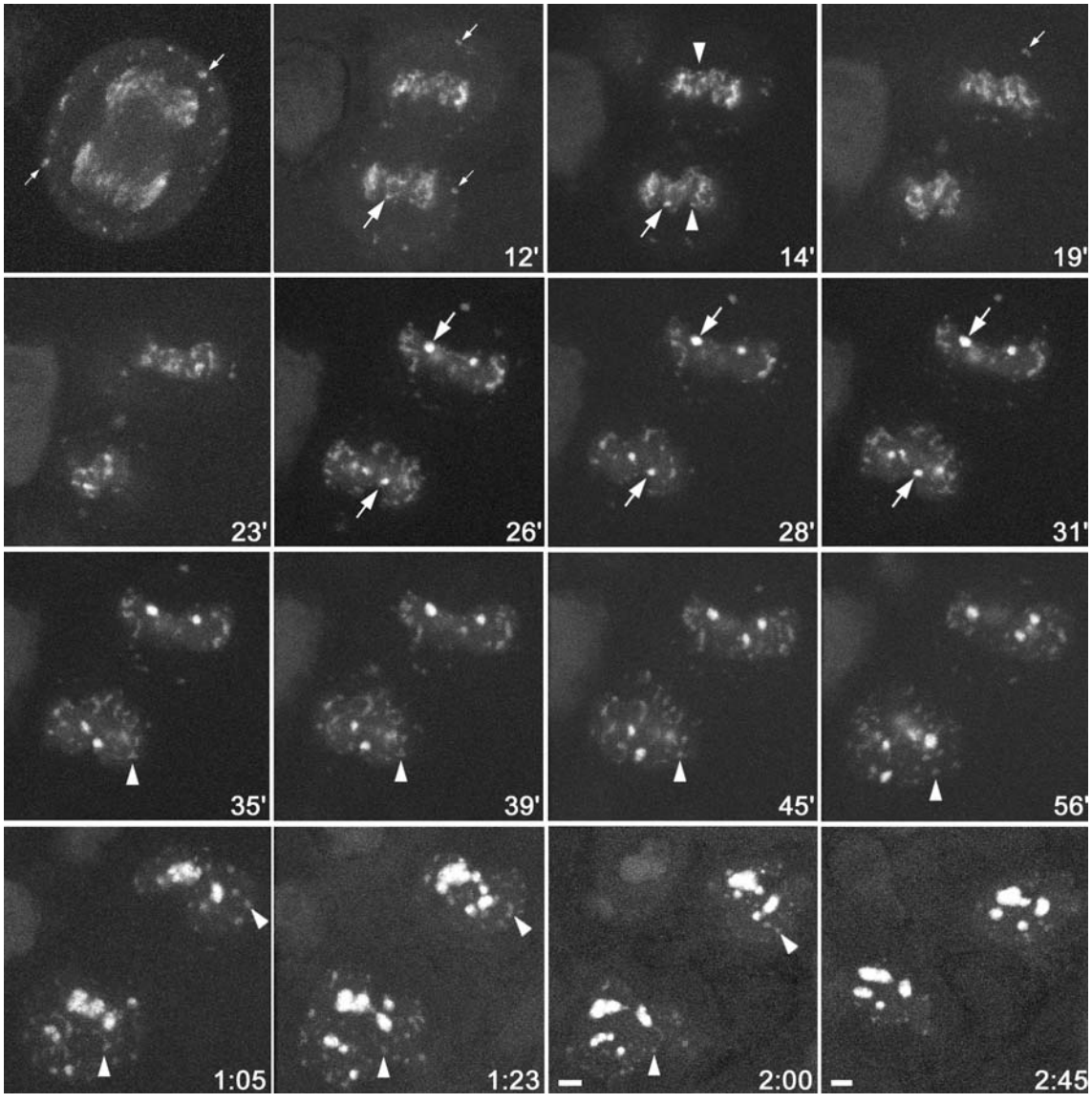

Fig. 6 Dynamics of nucleolar reassembly at the end of mitosis. HeLa cells transfected with fibrillarin-green fluorescent protein (fibrillarin-GFP) were monitored by live time-lapse fluorescence microscopy. The images are maximum projections of z-stacks of focal plane sections through the cell volume. At anaphase fibrillarin-GFP is present in the perichromosomal region of all chromatids and in numerous extrachromosomal particles called nucleolus-derived foci (NDF) (*small arrows*). At the end of anaphase, the ribosomal DNA loci are transcriptionally activated (*larger arrows*) and prenucleolar bodies (PNBs) are formed (*arrowhead*). At early telophase, distinct nucleoli are clearly produced, partially from nucleolar components delivered from PNBs in daughter cells. In the progression through telophase, the nucleoli become enlarged concurrent with the activation of more ribosomal genes while the diminished number of PNBs appear as more compact structures. By early G1 phase, fully functional nucleoli, which contain all three nucleolar substructures, are formed while very few PNBs remain. Bars, 2 μm

with the active NORs during mitosis (Scheer and Hock 1999). It was once thought that the entire transcription machinery stayed with the NORs in all phases of mitosis. Recent studies indicate that UBF, indeed, is present at the NORs in all phases of mitosis (Leung et al. 2004; Chen et al. 2005). However, some RNA pol I subunits, including RPA16, RPA20, RPA39/40, and RPA194, are absent from transcriptionally inactive NORs during metaphase and reappear in late anaphase (Leung et al. 2004; Chen et al. 2005). This suggests that these RNA pol I subunits are not efficiently recruited to the ribosomal promoters at the NORs by the RNA pol I initiation complex. In contrast, RPA43, which is responsible for recruitment of RNA pol I to the promoter through specific interactions with preinitiation factors, is present at the NORs during metaphase (Chen et al. 2005). Curiously, RPA43 has significantly faster exchange kinetics during metaphase than in interphase when it is engaged in transcription. These studies also support the idea that the RNA pol I transcription machinery assembles onto promoters in a sequential manner as opposed to being recruited as a preassembled holoenzyme.

The pathways taken by the processing machinery throughout the course of mitosis are much more complex than those of the transcription system. As the nucleolus disperses in prophase and the nuclear envelope breaks down, the processing components are found both in the cytoplasm and associated with the perichromosomal regions (PRs). This process of disassembly appears to be initiated in the FC, with loss of DFC and GC components occurring simultaneously but somewhat later (Leung et al. 2004). In metaphase, some of this material remains cytoplasmic and in the PRs; however, most of it seems to become associated with the spindle apparatus. In anaphase, these components move to other locations: again, much of this material is cytoplasmic and in the PRs, but we now see the appearance of large cytoplasmic particles called nucleolus-derived foci (NDF). These are large bodies (1–3 μm) that can number as many as 100 per cell during anaphase. As the cells proceed through telophase, the number of NDF per cell decreases to a few or none. At this time, another kind of particle, the prenucleolar body (PNB), appears in the nucleus. The processing components are now distributed among the PNBs, the nucleoplasm, the PRs of the decondensing chromosomes as well as in the cytoplasm.

The material found in the NDF and PNBs contains the processing components in association with partially processed pre-rRNA (Dundr and Olson 1998). This RNA consists primarily of 45S and 46S pre-rRNAs that were derived from 47S pre-rRNA synthesized at the beginning of mitosis. One can only speculate on the role played by the pre-rRNA in these complexes. It could serve as a scaffold for keeping the processing proteins and RNAs in close proximity to one another for rapid utilization once ribosome production restarts. It could also provide a means for orderly assembly of nucleoli at the end of mitosis. It is even possible that the pre-rRNA produced in the previous cell cycle is incorporated into new ribosomes in the reassembled nucleoli.

The bulk of nucleolar reassembly occurs in telophase when rDNA transcription is activated. The time-lapse immunofluorescence micrograph in Fig. 6 shows the redistribution of one of the processing components, fibrillarin, from anaphase into G1 phase. During telophase, the processing components are distributed in several different places, but they seem to relocate in nuclei by two possible routes. First, when the nuclear envelope is formed, some of this material will automatically be present in nuclei by virtue of being carried on the mitotic chromosomes as a perichromosomal layer. A second fraction seems to be imported into nuclei from a cytoplasmic pool, of which the NDF are a part. This material may be transported through the nuclear envelope by the established nuclear import system. The processing components in the nucleus are recruited to prenucleolar bodies (PNBs) and are eventually incorporated into the reforming nucleoli.

Nucleolar reassembly is a stepwise process in which the DFC forms before the GC (Dundr et al. 2000; Savino et al. 2001). For example, fibrillarin, which is predominantly found in the DFC, leaves the PNBs and is incorporated into newly forming nucleoli before protein B23, a GC marker (Dundr et al. 2000; Savino et al. 2001). Recent quantitative analyses of nucleolar reassembly showed that there is an interval of approximately 18 min between the formation of the GC and DFC (Leung et al. 2004). The release of individual processing components approximately follows the same pattern as ribosome biogenesis; e.g., fibrillarin is involved in early processing events and leaves the PNBs early whereas B23, the activity of which is used later in processing (Itahana et al. 2003), leaves the PNBs after fibrillarin (Dundr et al. 2000). Therefore, it seems likely that the complexes of partially processed pre-rRNA and processing components play a role in timing the flow of these components to the developing nucleoli.

Nontraditional functions of the nucleolus

The concept that the nucleolus is a multitasking body emerged during the last decade of the twentieth century. The early clues to this idea came from a series of studies that accidentally uncovered a variety of nucleolar macromolecules that apparently have nothing to do with ribosome biogenesis. A number of the nontraditional nucleolar components have reasonably well-defined relationships to cellular functions; however, for many of them, we have little or no understanding of why they are there. In addition, a variety of viral proteins have nucleolar locations (Hiscox 2002). The nontraditional roles of the nucleolus include regulation of tumor suppressor and oncogene activities, cell-cycle regulation in yeast, signal recognition particle (SRP) assembly, modification of small RNAs, control of aging, and

modulating telomerase function (Olson et al. 2002; Olson 2004a). The majority of the nontraditional components are not permanent residents of the nucleolus; instead, they are temporary visitors that leave after their transactions have been completed. Some of the novel functions bear some relationship to ribosome biogenesis, such as the regulation of p53 activity (see section below), whereas others do not. For example, the assembling SRP components are located in parts of the nucleolus that do not contain assembling ribosomes (Politz et al. 2002), suggesting that this activity is completely independent of ribosome biogenesis. Space does not permit a complete discussion of the growing list of nontraditional nucleolar functions; instead, two examples are presented below.

HIV proteins in the nucleolus

The viral component that has attracted the most attention is the HIV-1 Rev protein, which is predominantly localized to the nucleolus (Kubota et al. 1999; Malim et al. 1989) and interacts with the nucleolar protein B23 (Fankhauser et al. 1991). Figure 7 shows how Rev is primarily located in the nucleolus with lesser amounts in the cytoplasm while the HIV-1 p24-core protein is exclusively cytoplasmic. Rev regulates the splicing pattern of the HIV-1 mRNA by promoting unspliced or partially spliced mRNA export to the cytoplasm (Kjems and Askjaer 2000). Studies by Zolotukhin and Felber (1999) suggest that a complex comprising Rev, two nucleoporin proteins, and the nuclear export factor hCrm1 assemble in the nucleolus, which could facilitate movement of this complex to the nuclear pore. The multimerization pattern of Rev in the nucleolus also suggests that it plays a role in nucleocytoplasmic transport (Daalemans et al. 2004). There is evidence that the HIV-1 mRNA actually passes through the nucleolus during its life cycle (Michenzi et al. 2000). Finally, studies by Michenzi et al. (2002) suggest that the HIV-1 Tat protein also localizes to the nucleolus and that nucleolar trafficking by Tat is essential for viral replication. Thus, there are strong hints that the nucleolus participates in HIV infection in some way, possibly as one station in the HIV mRNA transport process. However, that role is still poorly understood.

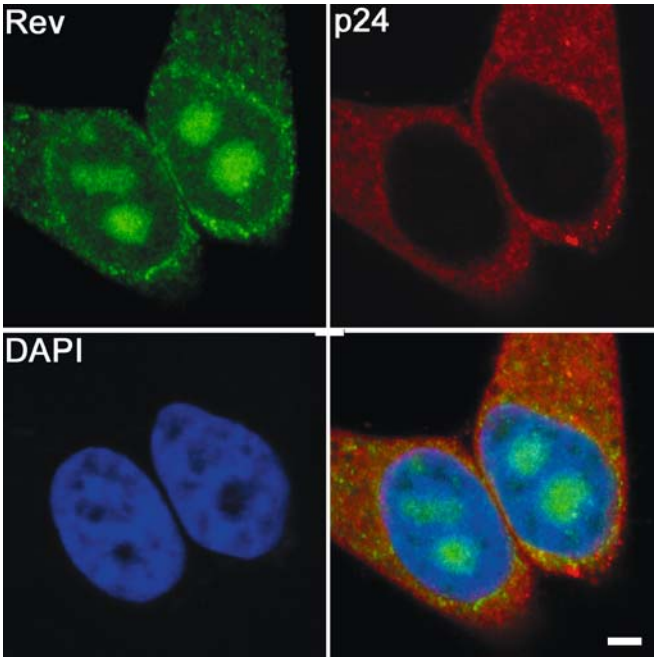

Fig. 7 The HIV-1 Rev protein accumulates in the nucleoli of HIV-1-infected cells. HeLa cells were transfected with the HIV-1 molecular clone PNL4-3, which expresses the Rev protein in a cascade of viral progression. Twenty-four hours after transfection, the HIV-1 Rev protein detected by a specific antibody is localized predominantly in the nucleoli (*green*). Viral replication was monitored by an antibody against the HIV-1 p24 core protein (*red*). The overlay of all three labelings is shown in the *lower right panel*. Bar, 2 μm

The nucleolus as a stress sensor

The movements of proteins in and out of the nucleolus serve as the basis for one of the more intriguing new proposed functions of the nucleolus: regulation of the level of the tumor suppressor p53 [for background, see review by Zhang and Xiong (2001)]. Under cellular stress conditions, p53 protein levels become elevated, which may lead to either growth inhibition or to cell death (Ryan et al. 2001). Although there are several ways that p53 activity is regulated, changing the balance between its synthesis and degradation seems to be one of the most important. Under normal conditions, the p53 protein is continuously synthesized and then quickly degraded to maintain a low level of the protein. Under stress conditions, the cellular amounts increase by inhibition of its degradation. The degradation is promoted by a protein called MDM2, which is an E3 ubiquitin ligase. Ubiquitination of p53 marks it for export to the cytoplasm and/or proteolysis by the proteasome. This mechanism has the advantage that the accumulation of p53 is not dependent on gene activation followed by transcription and translation, thereby facilitating a quick response to stress.

How does the nucleolus enter into this regulatory system? A study by Rubbi and Milner (2003) proposed that the nucleolus is a major stress sensor that transmits signals for the regulation of p53 activity [for a more detailed review of this topic, see Olson (2004c)]. They observed that p53 stabilization correlates with nucleolar disruption by a number of stressors, including DNA-damaging agents, inhibitors of transcription, heat shock, and oxidative stress. This disruption causes the translocation of nucleolar components, such as protein B23, to the nucleoplasm (Chan et al. 1996). In fact, Rubbi and Milner (2003) used this movement of B23 as a measure of nucleolar disruption. What is the signal that is transmitted from the nucleolus to the p53 regulatory system? A strong candidate for this is a protein called

ARF. This protein is a major suppressor of MDM2 activity; more importantly, ARF is predominantly a nucleolar protein (Zhang and Xiong 1999; Weber et al. 2000). ARF also translocates from the nucleolus into the nucleoplasm when cells are treated with inhibitors of cyclin-dependent protein kinases with a concomitant increase in p53 levels (David-Pfeuty and Nouvian-Dooghe 2002). Various models for the control of p53 levels by ARF are described by Zhang and Xiong (2001). The simplest model suggests that release of ARF from the nucleolus allows it to interact with MDM2, thereby reducing the latter protein's activity (See Fig. 8). Another possibility is that ARF sequesters MDM2 in the nucleolus. Finally, it has been proposed that the nucleolus is required for export of a p53-MDM2 complex (Tao and Levine 1999) and that disruption of the nucleolus inhibits that process. Recent studies provide evidence that ARF interacts with MDM2 in the nucleoplasm but that it is highly susceptible to proteasomal degradation in that location (Rodway et al. 2004). The nucleolar location of ARF may provide a means for storage and/or protection from degradation.

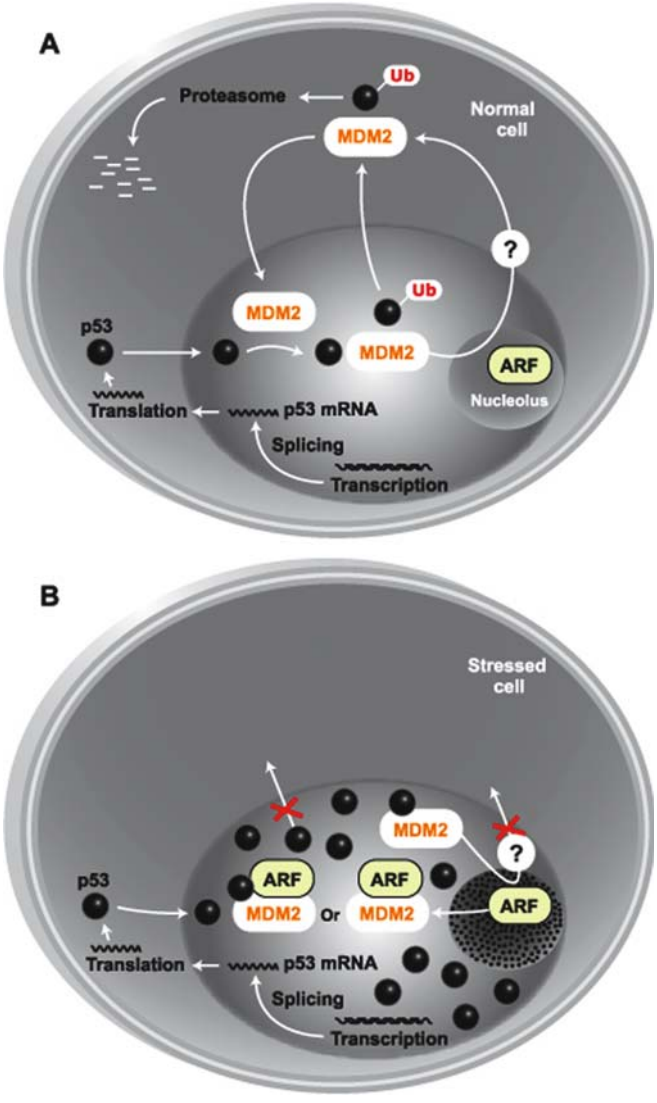

Fig. 8 Proposed role of the nucleolus in stabilization of p53 under stress conditions. The *upper panel* depicts a normal cell in which p53 levels are maintained at low levels via ubiquitination by MDM2, export to the cytoplasm, and degradation by proteasomes. The ubiquitinated p53-MDM2 complex could be directly exported from the nucleoplasm, or the nucleolus could be involved in the export. The ARF protein, which regulates MDM2 activity, is normally located in the nucleolus. Under stress conditions (*lower panel*), the nucleolus is disrupted and ARF is released from the nucleolus to the nucleoplasm where it combines with MDM2 to inhibit its activity, either in a binary complex or a ternary complex containing p53, MDM2, and ARF. Inhibition of the MDM2 activity prevents export to the cytoplasm. An alternative model suggests that passage through the nucleolus is essential for export of the complex, and disruption of the nucleolus prevents nuclear export. Reprinted with permission from Olson (2004c)

In addition to the relationship between ARF and MDM2 activity, there are other connections between the nucleolus and p53 regulation. For example, nucleolar protein B23 interacts directly with p53 and possibly regulates p53 stability (Colombo et al. 2002). Another abundant nucleolar protein, nucleolin, is mobilized from the nucleolus to the nucleoplasm to interact directly with p53 under stress conditions (Daniely and Borowiec 2000). Zhang et al. (2003) showed that ribosomal protein L11 also binds MDM2 and inhibits its activity. Finally, Kurki et al. (2004) showed that B23 interacts with HDM2 to prevent the ubiquitination of p53.

Another predominant nucleolar protein, called nucleostemin, also seems to regulate p53 activity. Nucleostemin is a GTP-binding protein that is found in stem cells and cancer cells but not in committed and terminally differentiated cells (Tsai and McKay 2002). In its GTP-bound form, nucleostemin is present in the nucleoplasm; in contrast, when GTP is not bound, it localizes to the nucleolus. More importantly, nucleostemin is able to bind p53, and its overexpression, especially in the form of mutants lacking the GTP-regulatory domain, prevents cells from entering mitosis and causes p53-dependent apoptosis. A working hypothesis is that in cancer cells or stem cells, the protein is predominantly nucleolar, which allows normal cell progression. However, when cells differentiate, there is loss of nucleostemin expression, which leads to cell-cycle exit. The authors also suggest that the GTP-binding domain plays a role in the dynamic shuttling between the nucleolus and the nucleoplasm. A recent study that surveyed several cancer tissues showed that nucleostemin was present in all of them but absent in terminally differentiated tissues (Liu et al. 2004). This suggests that the protein is important in regulation of cancer growth and could be a target for chemotherapy. Thus, a growing body of literature points to regulation of p53 by movement of nucleolar molecules.

Concluding remarks

There are many ways that nucleolar components move. Some of this motion may be directed, such as the vectorial movement of the assembling preribosomal parti-

cles outward from the sites of transcription. However, much of the movement in the nucleolus is clearly not directed and is only dependent on diffusion and relative affinities for other nucleolar components. This idea forces us to think of the nucleolus as a much more dynamic structure. The concept of a dynamic nucleolus is not entirely surprising, considering the many precedents for this in cellular systems. For example, microtubules engage in a process of "dynamic instability" in which their length is dependent on controlled equilibria between tubulin dimers and polymers (Desai and Mitchison 1997) In fact, most cellular structures could theoretically be defined by the sum total of the association constants among their numerous components. The nucleoli need these dynamics for assembly of ribosomes, for communication with the rest of the cell, and for the ability to disassemble and reassemble during mitosis. A challenge of the future will be to identify the interactions among the nucleolar components and to determine how their dynamics are regulated.

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