

The Dynamic Functions of the Nucleolus

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Abstract The nucleolus is the largest nuclear organelle and is the primary site of 3 ribosome subunit biogenesis in eukaryotic cells. It is assembled around arrays of 4 ribosomal DNA genes, forming specific chromosomal features known as nucleolar 5 organizing regions (NORs) which are the sites of ribosomal DNA transcription. 6 While the nucleolus main activity involve different steps of ribosome biogenesis, 7 the presence of proteins with no obvious relationship with ribosome subunit 8 production suggests additional functions for the nucleolus, such as regulation of 9 mitosis, cell cycle progression, stress response and biogenesis of multiple ribonu- cleoprotein complexes. The many novel factors and separate classes of proteins 11 identified within the nucleolus support this view that the nucleolus may perform 12 additional functions beyond its known role in ribosome subunit biogenesis. Here we 13 review our knowledge of the nucleolar functions and will provide a detailed picture 14 of how the nucleolus is involved in many cellular pathways. 15

Keywords Nucleolus • Ribosome biogenesis • rRNA transcription

16

1 Introduction

17

Nucleoli are present in almost every eukaryotic cell type and represent the most 18 prominent compartment of the cell nucleus. The primary function of the nucleolus 19 consists in ribosomal RNA (rRNA) transcription, rRNA processing and ribosome 20 subunit assembly (Hernandez-Verdun et al. 2010; Pederson 2011; Raska 21 et al. 2006). Nucleoli assemble at the end of mitosis around the tandemly repeated 22 clusters of rDNA genes forming a subnuclear compartment that locally recruits the 23 specific transcription and processing machineries that are responsible for generating 24 ribosome subunits (Hernandez-Verdun 2011; Raska et al. 2006). The process of 25 assembling a ribosome subunit requires the initial transcription of the ribosomal 26 DNA (rDNA) genes by the RNA polymerase I. Because these rDNA genes are 27

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28 arranged in arrays of tandem repeats, it results in local concentration of proteins
29 involved in different aspect of transcription, processing and assembly of rRNA into
30 ribosomes. In higher eukaryotes, three sub-nucleolar compartments can be distin-
31 guished by their distinct morphology using electron and light microscopy: The
32 fibrillar centres (FC) are surrounded by the dense fibrillar component (DFC) and the
33 granular component (GC), in which the FC and DFC are embedded. The compo-
34 sition of these sub-compartments is tightly linked to sequential steps in ribosome
35 biogenesis [reviewed in (Olson and Dundr 2005)]. The FC contains unengaged
36 RNA polymerase I transcription factors, whereas the DFC contains mostly
37 pre-RNA processing factors, indicating specialization of these compartments.
38 Transcription occurs at the boundary of the FC and DFC (Raska et al. 2006), and
39 the transcribed rRNA is then moving to the GC compartment for further maturation
40 and assembly into ribosomes (Figs. 1 and 2).

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41 In many cell types, only a subset of rDNA genes are transcriptionally active,
42 even though inactive rDNA are still assembled into nucleoli. The initial 47S
43 ribosomal RNA (rRNA) precursor transcript transcribed by RNA pol I is subse-
44 quently cleaved to form the mature 28S, 18S and 5.8S rRNAs which is then post-
45 transcriptionally modified through interaction with small nucleolar ribonucleopro-
46 teins (snoRNPs) and additional protein processing factors (Henras et al. 2015).
47 Finally, the processed and modified rRNAs are assembled with the many ribosomal
48 proteins, prior to interaction with the export machinery and transport to the
49 cytoplasm.

50 While the nucleolar function in ribosomal biogenesis is well characterized,
51 many additional functions of the nucleolus besides ribosomal biogenesis have
52 been uncovered, suggesting many important cellular functions for the nucleolus
53 (Boisvert et al. 2007; Pederson and Powell 2015). This was made particularly
54 evident following several reports of proteomic analyses to characterize the
55 nucleolar proteome in human, mouse, as well as other organisms (Andersen
56 et al. 2002, 2005; Kar et al. 2011; Pendle et al. 2005; Scherl et al. 2002).
57 Nucleolar proteins identified in these studies have shown that over 70% are not
58 involved in the production of ribosome subunits, consistent with the nucleolus
59 performing additional cellular functions (Boisvert et al. 2007; Lam and Trinkle-
60 Mulcahy 2015; Pederson and Powell 2015). A further dimension to these studies
61 has been added by recent studies that have characterized the dynamic protein
62 composition of the nucleolus following treatment with actinomycin D (Andersen
63 et al. 2005), etoposide (Boisvert et al. 2010) or through the cell cycle
64 (Ly et al. 2014).

65 Additionally, the nucleolus has been linked to multiple forms of diseases
66 involving a wide range of mechanisms including cancer (Tsai and Pederson
67 2014), viral infections (Hiscox 2007) and neurodegenerative diseases (Parlato and
68 Kreiner 2013), affecting either ribosome biogenesis, nucleolar structure or other
69 functions.

70 This chapter provides an overview of the characteristics of nucleolar organisa-
71 tion, discusses non-ribosomal functions of the nucleolus, and will present some of

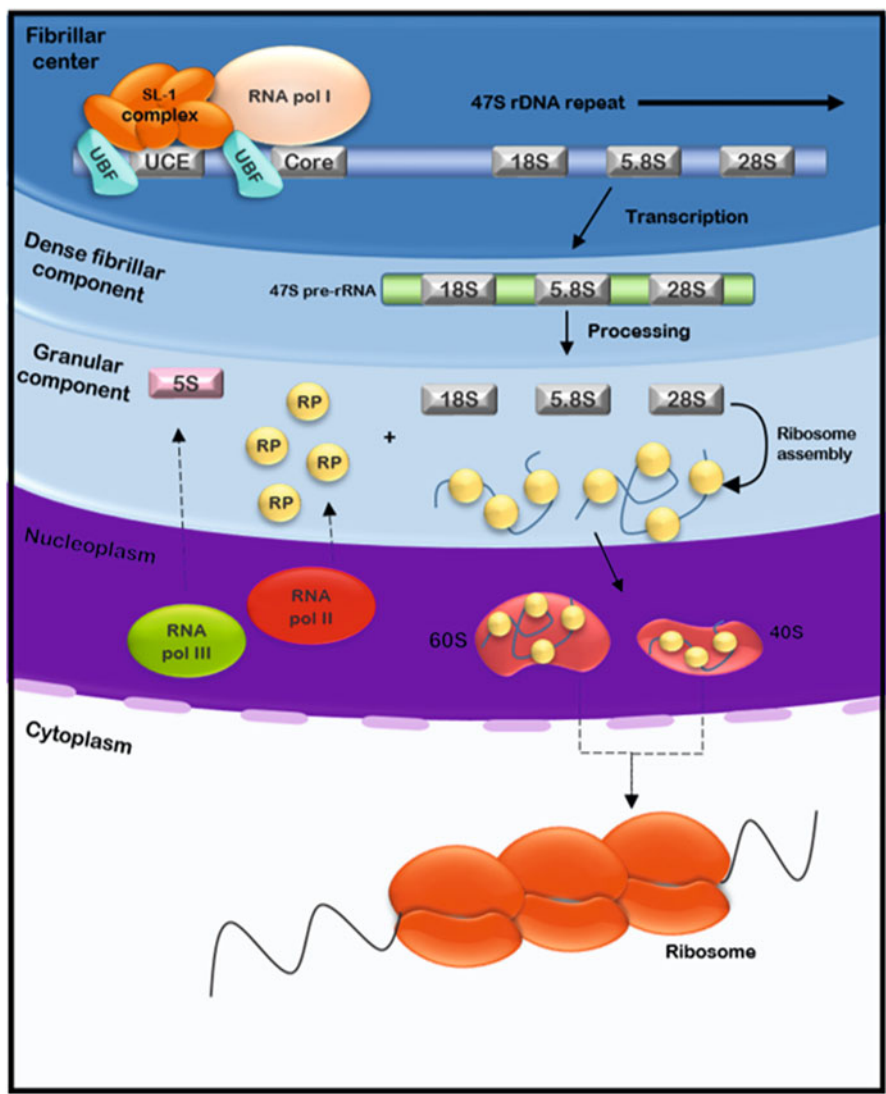
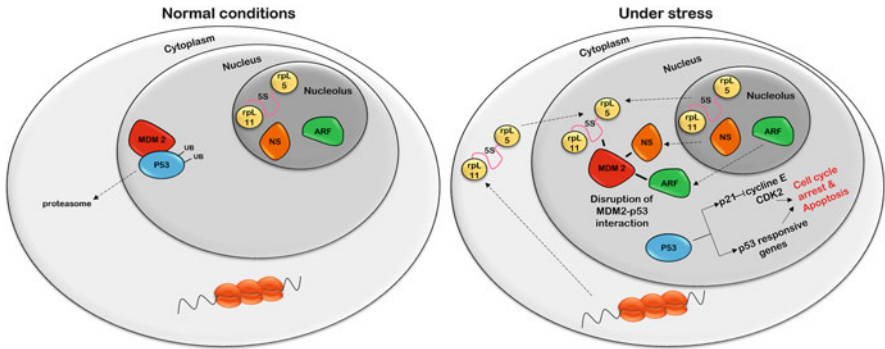


Fig. 1 Ribosome biogenesis. Transcription of ribosomal DNA by RNA polymerase I occurs at the fibrillar centres or at the boundary with the dense fibrillary component. The 47S pre-ribosomal RNA transcripts are then further processed by specific cleavages and post-transcriptional modifications by small nucleolar ribonucleoproteins (snRNPs) in the dense fibrillary component. Assembly of the rRNA with the ribosomal proteins then occurs in the granular component of the nucleolus prior to export of the 40 and 60S subunits to the cytoplasm for final assembly into the functional ribosome

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Fig. 2 Nucleolus under stress. The nucleolus plays a central role during the cellular response to stress. Under normal conditions, p53 is kept at very low level by proteasomal mediated degradation through ubiquitination by MDM2. Following activation of stress responses, oncogene activation or DNA damage, the p14ARF tumor suppressor normally located in the nucleolus associates with HDM2 and sequesters it within the nucleolus. This segregation prevents the ubiquitination of p53 mediated by HDM2 resulting in increased levels of cellular p53 resulting in cell cycle arrest and apoptosis

72 the latest findings regarding their regulation and dynamic behaviour, as well as its
 73 implications in cancers and diseases.

74 2 The Nucleolar Organisation

75 2.1 Nucleolar Organiser Regions (NORs)

76 The rDNA genes are arranged in arrays of head-to-tail tandem repeats, termed
 77 nucleolar organizer regions (NORs). In the human genome, approximately 400 cop-
 78 ies of 43-kb repeat units are distributed along all acrocentric chromosomes (chro-
 79 mosomes 13, 14, 15, 21 and 22) to form NORs (Henderson et al. 1972), which are
 80 assumed to have resulted from interchromosomal recombination (Gonzalez and
 81 Sylvester 1997; Worton et al. 1988). While the rDNA repeats are mostly arranged
 82 into canonical head-to-tail repeats, around a third of them are arranged into
 83 palindromic or non-canonical repeats (Caburet et al. 2005). During metaphase,
 84 these clusters contain so called r-chromatin (for ribosomal genes complexed with
 85 proteins involved in rDNA transcription) and are constituted of 60–80 nm fibers
 86 showing a twisted loop organization as visualized by electron tomography (Heliot
 87 et al. 1997). These RNA Polymerase I associated transcription factors remain
 88 associated with NORs throughout mitosis as well (Roussel et al. 1996).

89 Each of the 43 kb repeats includes a 13–14 kb segment coding for the rRNA
 90 sequence and are separated from the next transcription unit by 30 kb intergenic
 91 spacers (IGS). These spacers contains regulatory elements such as the gene promoter,
 92 repetitive enhancer elements and terminator sequences. Polar replication fork barriers

(RFBs) within the transcription termination element between the individual repeats ensure the stalling of the bidirectionally running replication fork opposite to the direction of transcription (Brewer and Fangman 1988; Gerber et al. 1997; Hernandez et al. 1993; Little et al. 1993; Wiesendanger et al. 1994). Additional elements located within the IGS including small, non-coding RNAs such promoter RNAs (pRNAs) have been recently identified. These 150–250 nucleotides long RNAs are transcribed from a promoter within the IGS and involved in epigenetic mechanisms acting on the rDNA locus (Mayer et al. 2006, 2008; Santoro et al. 2010).

In many cell types, only a subset of rDNA genes are transcriptionally active, even though inactive rDNA are still assembled into nucleoli. (Akhmanova et al. 2000; Strohner et al. 2001; Sullivan et al. 2001). The difference results from different chromatin states of rDNA repeats that correspond with the state of the nucleosomes within the rDNA (Dammann et al. 1993; Langst et al. 1998; Li et al. 2006b; Sogo et al. 1984). Inactive rDNA has been shown to be inaccessible for psoralen crosslinking and exhibits regularly spaced nucleosomes, while active regions of rDNA is accessible for crosslinking and lack nucleosomes (Conconi et al. 1989; Dammann et al. 1993). These different chromatin states are usually stable throughout the cell cycle (Conconi et al. 1989). The rate of production of rDNA can therefore be regulated either by increasing the transcription rate of active genes and/or by activation of silent genes. In general, rapid changes in rRNA expression, i.e., in response to nutrient status or growth factor signalling, will result in a change in the transcription rate of rDNA genes that are already active (Grummt and Pikaard 2003; Russell and Zomerdijk 2005; Stefanovsky et al. 2006), whereas slower changes such as during development or differentiation results from a change in the number of genes that are actively transcribed (Haaf et al. 1991).

2.2 rDNA Transcription

The RNA Polymerase I does not require a TATA box sequence in the promoter, but instead relies on regulatory sequences that are divided in two functional elements: a core element (CORE) next to the transcription start site located between –45 to +20, and an upstream control element (UCE) located between –200 to –107 (Haltiner et al. 1986; Learned et al. 1986). Transcription of rRNA genes requires the formation of a preinitiation complex (PIC) that is composed of the RNA Polymerase I, the upstream binding factor (UBF) and the promoter selectivity factor (SL1) at the rDNA promoter [reviewed in (Grummt and Pikaard 2003; Moss et al. 2007; Russell and Zomerdijk 2005)]. Upon dimerization, the upstream binding factor UBF binds the UCE and the CORE element to recruit the SL1 protein complex composed of the TATA-binding protein (TBP) and TBP-associated factors (TAFs), TAF₁ 110/95; TAF₁ 68; TAF₁ 48 (Comai et al. 1992; Heix et al. 1997; Zomerdijk et al. 1994). The UBF dimer allows the UCE and CORE elements to come into contact by introducing loops in the upstream region into a structure called the enhanceosome to allow the binding of SL1 and formation of a stable PIC

134 (Bazett-Jones et al. 1994; Bell et al. 1988). By interacting with UBF and the RNA
135 Pol I component TIF-1A, TAFs recruit RNA Pol I to the rDNA promoter (Miller
136 et al. 2001; Moorefield et al. 2000). UBF not only binds to the promoter region, but
137 also throughout the whole rDNA repeat (Mais et al. 2005; O'Sullivan et al. 2002)
138 where it acts as a transcriptional activator of RNA Pol I, modulator of elongation in
139 response to growth factor signalling and as an anti-repressor by replacing histone
140 H1 (Kermekchiev et al. 1997; Kuhn and Grummt 1992). Thus, regulation of UBF is
141 central to the regulation of rDNA transcription by RNA Polymerase I. Important
142 insight into the role of UBF in ribosomal chromatin remodelling and nucleolar
143 formation has come from the studies of pseudoNORs [reviewed in (Prieto and
144 McStay 2008)]. PseudoNORs are artificial, high-affinity UBF-binding arrays that
145 are transcriptionally silent due to the lack of a promoter, but behave like active
146 NORs. For example, they exhibit consistent under condensation throughout the cell
147 cycle and are highly enriched in the RNA Pol I machinery through UBF (Mais
148 et al. 2005; Prieto and McStay 2007) that stays associated with rDNA after nucleoli
149 disassembly in mitosis (Roussel et al. 1993). Therefore, UBF is believed to estab-
150 lish a chromatin structure that allows rapid re-initiation of rRNA transcription after
151 mitosis and thereby promotes nucleolar formation. This is also supported by studies
152 that show rDNA gene silencing upon UBF deletion, suggesting a regulatory role for
153 UBF in determining the number of active rDNA genes (Sanij and Hannan 2009;
154 Sanij et al. 2008).

155 As RNA Polymerase I starts the elongation process of the rRNA, UBF and SL1
156 remain associated with the promoter region to allow recruitment of another RNA
157 Polymerase I so that each rDNA gene can be transcribed multiple times simulta-
158 neously, contrary to RNA Polymerase II genes. This gives rise to the Christmas tree
159 like structure as visualized by electron microscopy (Miller and Beatty 1969).
160 Termination occurs through the binding of TTF-I at the 3' end of the transcribed
161 region, bending the termination site and, with the help of the transcript-releasing
162 factor PTRF, will induce RNA Polymerase I to dissociate from the DNA and the
163 new transcript.

164 2.3 *Processing of rRNA and Ribosome Assembly*

165 The production of human 40S and 60S ribosomal subunits prior to export into the
166 cytoplasm is a complex mechanism that requires a large proportion of the cellular
167 energy for production (Warner 1999). Indeed, the size and organisation of the
168 nucleolus reflects the scale of this process (Sirri et al. 2008). The regulation of
169 ribosome biogenesis is crucial for cellular growth and proliferation (Tschochner
170 and Hurt 2003) and it is upregulated in the majority of cancers to accommodate the
171 increase rate of cellular growth and metabolism (Ruggero and Pandolfi 2003). The
172 two ribosomal subunits includes 79 major ribosomal proteins (RPs) as well as four
173 mature ribosomal RNAs (Yusupova and Yusupov 2014). The smaller ribosomal
174 subunit (40S) includes the 18S rRNA, whereas the large subunit (60S) includes the

28S, 5.8S as well as the RNA Polymerase III transcribed 5S rRNA (Fedoriw et al. 2012).

The rRNA precursor is transcribed by RNA Pol I at the FC/DFC border as one long 47S transcript (~13 kb in humans), which includes the sequences for the 18S, 5.8S and 28S rRNA in that order. The additional sequences are called external transcribed spacers called ETS at both ends of the rRNA (5'-ETS and 3'-ETS), as well as internal transcribed spacers (ITS1 and ITS2) within the rRNA precursor. Sequential cleavages at specific sites termed A', A0, 1, 2 and 3 will generate the 18S rRNA and requires distinct small nucleolar RNAs (snoRNAs) (Henras et al. 2015). Cleavage at the T1 site by the U8 snoRNA will remove the 3'-ETS. Site 3 cleavage near the end of ITS1 between the 18S and 5.8S rRNAs will generate a 32S intermediate which will be further processed (Preti et al. 2013). Cleavage at the 4' site will generate the 28S rRNA and will results in a 5.8S rRNA with a longer 3'-end that will be further processed. The maturing rRNAs will be assembled within a 90S pre-ribosome complex which accumulates in the DFC (Henras et al. 2015; Tschochner and Hurt 2003). The pre-ribosome contains the 47S rRNA, 5S rRNA, ribosomal proteins and ~150 non-ribosomal proteins, including factors involved in processing and maturation, for example endo- and exonucleases, pseudouridine synthases, methyltransferases, RNA chaperones, GTPases and AAA-ATPases helicases (Tschochner and Hurt 2003). The pre-ribosome is subsequently separated into pre-40S and pre-60S subunits in the GC. These subunits are exported to the cytoplasm, where they undergo further processing to form the mature small 40S and large 60S ribosome subunits. The 40S subunit contains 18S rRNA and ~33 ribosomal proteins, whereas the 60S subunit is composed of the 28S and 5.8S rRNAs, the RNA Pol III-transcribed 5S rRNA and ~49 ribosomal proteins. Interestingly, around two thirds of the ribosomal protein genes are duplicated in humans. Although it has been assumed that these copies are largely redundant, recent work has suggested that these copies exhibit functional specificity (Komili et al. 2007).

3 Other Functions for the Nucleolus

3.1 RNA Complexity in the Nucleolus

While the nucleolus is known to have a major role in coordinating the processing and maturation of rRNAs, there is now extensive literature demonstrating that the nucleolus is also involved in the processing and maturation of several different families of RNA. For example, the nucleolus has been suggested to be a site of covalent RNA modifications and protein assembly of multiple ribonucleoprotein complexes, such as the spliceosomal small nuclear RNPs, telomerase and several other small RNAs transcribed by RNA polymerase III, such as 5S rRNA, some

213 tRNAs, RNase P RNA, the signal recognition particle (SRP) RNA and now also
214 miRNA (Gerbi et al. 2003; Lam and Trinkle-Mulcahy 2015).

215 The signal recognition particle (SRP) is a ribonucleoprotein complex responsi-
216 ble for the recognition of the N-terminal signal peptide sequence on nascent pro-
217 teins and for proper targeting of proteins onto a receptor on the cytoplasmic face of
218 the endoplasmic reticulum (Walter and Johnson 1994). This complex is formed by
219 6 proteins and a ~300 nucleotide RNA (Walter and Blobel 1982). Recent studies
220 have shown that both the RNA and proteins from the SRP can be found to transit
221 through the nucleolus of mammalian cells prior to SRP export to the cytoplasm
222 (Jacobson and Pederson 1998). These results suggest that the nucleolus is the site of
223 assembly and processing of the SRP complex prior to their cytoplasmic export, and
224 that the RNA could be modified/matured within the nucleolus.

225 In addition to the above roles for the nucleolus in RNA modification and
226 maturation, there are several other observations linking the nucleolus to RNA
227 processing. The RNase P RNA, a component of the pre-tRNA processing enzyme
228 RNase P, has been found in both the nucleolus and nucleoplasm (Jacobson
229 et al. 1997), suggesting that some pre-tRNA processing happens within the nucle-
230 olus. An alternative possibility is that the nucleolus plays a role in the assembly of
231 RNase P ribonucleoprotein complex. The nucleolus contains all the trans-acting
232 factors that are responsible for the synthesis of the eight 2'-O-methylated nucleo-
233 tides and three pseudouridine residues carried by the mammalian U6 spliceosomal
234 small nuclear RNA (Ganot et al. 1999). These findings demonstrate a trafficking
235 pathway in which the U6 spliceosomal RNA cycles through the nucleolus to
236 undergo nucleolar RNA-directed processing. Interestingly, like 5S rRNA, these
237 RNA (tRNA, RNase P RNA and U6 spliceosomal small nuclear RNA) are all
238 transcribed outside the nucleolus by RNA polymerase III and subsequently transit
239 through the nucleolus. This suggest a possible common maturation process shared
240 by RNA pol III transcripts that could occur within the nucleolus. ADAR1 and
241 ADAR2 are editing enzymes that deaminate adenosine to inosine in long double
242 stranded RNA duplexes and specific pre-mRNA transcripts. Live microscopy
243 experiments demonstrate that ADAR1 and ADAR2 are in constant flux in and out
244 of the nucleolus (Desterro et al. 2003). Furthermore, it was shown that ADAR2- but
245 not ADAR1-mediated RNA editing occurs within the nucleolus, indicating a role
246 for the nucleolus in the regulation of RNA editing (Vitali et al. 2005).

247 More recently, evidence has started to emerge demonstrating a role for the
248 nucleolus in the regulation of small interfering RNA (siRNA). The finding that
249 many proteins involved in siRNA processing, including RDR2, DCL3, AGO4, and
250 NRPD1b (the largest subunit of RNA Pol IVb) were identified with siRNAs within
251 the nucleolus in plant cells suggest that processing of endogenous nuclear siRNAs,
252 and possibly RISC storage or sequestration, occurs within the nucleolus
253 (Li et al. 2006a; Pontes et al. 2006). It was also reported in mammalian cells that
254 a microRNA (miR-206) had been found to co-localize with the 28S rRNA in the
255 granular component of the nucleolus, implying that this miRNA associates early
256 with the ribosome subunits (Politz et al. 2006). Several other miRNAs have also
257 been identified within the nucleolus, further supporting these observations (Bai

et al. 2014a, b; Li et al. 2013). It will be interesting to determine whether multiple forms of miRNAs arise within the nucleolus and whether they either function in nucleolar processes or leave the nucleolus to regulate downstream cellular events, such as protein translation.

3.2 Mitosis and Cell Cycle Regulation

The nucleolus is a dynamic structure which is disassembled/re-assembly at each cell division. The first step in prophase is initiated by the phosphorylation of components of the rDNA-transcription machinery by cyclin B1 and CDK1 (Heix et al. 1998). The phosphorylation of these components triggers a repression of rRNA transcription but the machinery such as UBF remains localized to the NORs (Dundr et al. 2000). In contrast, during the disassembly of nucleolus, the rRNA-processing machinery does not remain associated with nucleolar regions and move to the cytoplasm or become attached to the surface of condensed chromosomes at the perichromosomal region (PR) (Gautier et al. 1992). During anaphase, the processing proteins remain attached to the PR while proteins within the cytoplasm become packaged into nucleolar-derived foci (NDF). Cyclin B1/CDK1 levels decrease during late anaphase and early telophase, which results in the reactivation of rRNA transcription by loss of hyperphosphorylation of the transcription machinery (Sirri et al. 2000). During the G1 phase, the rRNA-processing proteins are released in a specific order starting with proteins such as fibrillarin which plays a role early in the rRNA processing followed by proteins involved in late stage of processing (Leung et al. 2004). The dynamic of nucleoli during cell cycle involves several stages which are tightly regulated and still not fully understood. Interestingly, the rDNA-transcription and rRNA-processing proteins might be regulated independently during the cell cycle.

During the interphase, the nucleoli remain dynamic. The nucleolar proteome has been shown to contain approximately 4000 proteins which can change localization (Boisvert et al. 2010). This pool of proteins is not all involved in ribosome biogenesis and several proteins have been shown to interact with the nucleolus at different stages of the cell cycle, further underlining a potential role in cell cycle regulation. In fact, the nucleolus and cell cycle regulation are influenced mutually. The most striking situation where the cell cycle can alter the ribosome biogenesis is in response to DNA damage (Jordan and Carmo-Fonseca 1998). Two proteins involved in DNA damage response, DNA-PK and PARP1, have been identified to be responsible for this inhibition (Calkins et al. 2013). Additionally, the RNA Polymerase I can be inhibited by DNA lesions pathway dependent of ATM, NBS1 and MDC1 (Kruhlak et al. 2007). The nucleolus can also regulate key cell cycle checkpoints as well. One of the mechanisms is by modulation of post-translational modifications such as sumoylation and phosphorylation of proteins involved in the cell cycle. For example, SENP5, a SUMO-specific protease found within the nucleolus is involved in sumoylation of proteins that affect progression

299 through cell division (Di Bacco et al. 2006). Another example is the sequestration in
300 nucleoli of the telomerase reverse transcriptase, the RNP enzyme that adds
301 telomeric sequences (Wong et al. 2002). The telomerase is retained in the nucleolus
302 until the telomeres are replicated at stages of S phase.

303 3.3 Stress Sensor

304 The ribosome biogenesis is the mechanism that requires the largest amount of cell's
305 energy, consuming ~80 % of total cell's energy (Schmidt 1999). Considering the
306 large amount of effort needed for the biogenesis of ribosomes, it is surprising that
307 one of the strategies used to preserve energy homeostasis is decreasing ribosome
308 biosynthesis under stress conditions. In response to stress, such as nutrient deprivation,
309 oxidative stress or drastic change in temperature, several steps in ribosome
310 biogenesis is altered. These stresses can cause a change in the localization of
311 nucleolar proteins, downregulation of RNA polymerase I activity, pre-rRNA
312 processing and transport, or changes in chromatin structure (Grummt 2013).
313 Many drug have also been shown to alter the integrity and the activities of the
314 nucleolus. For example, UV irradiation leads to segregation of nucleolar components
315 and ends with the complete disintegration of the nucleolus (Govoni
316 et al. 1994).

317 The alteration of any of the steps in ribosome biogenesis, such as transcription,
318 processing and assembly of the 40S and 60S subunits, results in the activation of
319 nucleolar stress pathways leading to senescence or apoptosis. The activation of this
320 pathway culminates in the stabilization of the p53 protein (Rubbi and Milner 2003)
321 by disruption of its interaction with MDM2, which can no longer add ubiquitin on
322 p53 and target it for proteasomal degradation. MDM2 can be targeted by three
323 proteins in the nucleolus, alternative reading frame protein p14^{ARF} (ARF),
324 nucleostemin (NS) and ribosomal proteins (RPs), in response to nucleolar stresses.
325 ARF is a tumor suppressor protein usually weakly expressed (Brady et al. 2004).
326 Under different cellular stress conditions, an increase in the expression of ARF
327 results in binding to MDM2 and disruption of the interaction between MDM2 and
328 p53 (Weber et al. 1999). Nucleostemin is a nucleolar protein and its function in this
329 structure is not well understood. However, following stress in the nucleolus, the
330 protein delocalizes to the nucleoplasm and bind MDM2 to prevent the association
331 with p53 (Tsai 2011). Finally, several ribosomal proteins have been found to be
332 involved in p53 stabilization such as rpL11, rpL23, rpL5, rpL7 and rpL26. However
333 recent study demonstrate that only rpL5 and rpL11 are essential for this function
334 (Fumagalli et al. 2012). In addition, the 5s rRNA is also involved in the formation of
335 the rpL5-rpL11-MDM2 complex (Donati et al. 2013). The change of localization of
336 ribosomal proteins has long been considerate passive and a result of nucleolar
337 disruption. However, the protein rpL11 can move to the nucleoplasm without loss
338 of nucleolar integrity (Fumagalli et al. 2009), indicating that the relocalization of
339 ribosomal proteins may be a regulated mechanism. For example, the localization of

rpL11 is regulated by NEDDylation, a ubiquitin-like molecule (Sundqvist et al. 2009). Another method to retain the ribosomal proteins within the nucleolus is through their interaction with PICT1 who is degraded in response to nucleolar stress (Maehama et al. 2014; Sasaki et al. 2011). It is clear that cells react in response to environmental stress by decreasing ribosome biogenesis to decrease energy consumption and preserve homeostasis but the nucleolus is also directly involved in the regulation of cellular response to different type of stresses.

4 The Nucleolus and Diseases

4.1 Cancer and Genomic Instability

Several years before the discovery of the role of the nucleolus in the synthesis of ribosomes, cytological analysis had already made a connection between the size of nucleoli and cancer (Pinease et al. 1896). It was observed that tumor cells had an increase in the number and size of nucleoli. Thereafter, numerous studies confirms these observations and concluded that these abnormalities could be used as a marker for the aggressiveness of malignancies (Derenzini et al. 2009) and was directly related to cell grow rate (Derenzini et al. 1998, 2000). In addition, the biogenesis of ribosome is under control of key cellular growth and proliferation signaling pathways which are known to be frequently mutated in several types of cancer such MYC, RAS, phosphatidylinositol-3-kinase (PI3K), tumor suppressors including TP53 (p53), retinoblastoma protein (Rb) and PTEN (Moss 2004). Irregular shape and deregulation of ribosome synthesis rate has long been considered a consequence of cancer. However, new data suggest that dysfunction of rRNA synthesis is not only a consequence cellular transformation but is also required for the survival of tumor cells and can even initiate the tumor transformation. This is support by two evidences: first, perturbation in ribosome biogenesis activated stress pathways and second, the specific inhibition of RNA polymerase I transcription leads to tumor cell death.

The first evidence is that the cell were demonstrated to monitors closely ribosome synthesis and disruption of this process leads to the activation of a ribosomal surveillance pathway (Zhang et al. 2003). This stress pathway leads to the accumulation of p53 through a non-genotoxic activation, as described in the section stress sensor. The second evidence is based on the observation that deregulation of ribosome synthesis is essential for the survival of cancer and small molecule inhibitors of RNA Polymerase I transcription can, in tumors cells, selectively activate the nucleolar stress pathway (Bywater et al. 2012). Many drugs already used in cancer therapy affect, at least in part, ribosome biogenesis or rRNA processing such as doxorubicin, flavopiridol and roscovitine (Burger et al. 2010). However, these treatments induce a general nucleolar disruption and affect many other pathways resulting in several side effects. By contrast, a new generation of

379 small molecules is being developed that selectively inhibit RNA Polymerase I
380 transcription. The CX-3543 (first generation) and CX-5461 (second generation)
381 bind GC-rich sequence, found in large proportion in rRNA and prevents RNA
382 Polymerase I transcription (Balasubramanian et al. 2011). This treatment specifi-
383 cally induces p53 non-genotoxic stress pathway activation by release of ribosomal
384 proteins from the nucleolus and only affect cancer cells and not in normal cells
385 (Bywater et al. 2012). These targeted transcription therapies are less genotoxic for
386 normal cells reducing risk of secondary cancer associated with classical chemo-
387 therapeutic agent (Godley and Larson 2008). Recent studies also show another
388 possible pathway by which RNA Polymerase I transcription inhibitors are also
389 effective independent of p53 activation (Peltonen et al. 2014). While the mecha-
390 nisms involved remain unknown, these data underline the potential for selective
391 RNA Polymerase I inhibition for cancer treatment whether p53 is present or
392 mutated.

393 4.2 Viral Infections

394 Viruses are obligate intracellular parasites and used host cell for genome replica-
395 tion, protein expression and assembly of new virus particles. Several types of virus
396 involved the nucleolus for effective infection. In fact, the RNA virus, retroviruses
397 and DNA viruses interact with/or alter the nucleolus when they infect cells (Hiscox
398 2002). The infection results in change of morphology and in the proteome of the
399 nucleolus (Dove et al. 2006). The association between viral proteins and the
400 nucleolus results from three type of direct interaction : with rDNA, with nucleolar
401 RNA (consisting mainly of rRNA) or with nucleolar protein components (Carmo-
402 Fonseca et al. 2000). The viruses affect the nucleosome homeostasis at many level.
403 First, the viral components can co-localize with the nucleolus. Second, the virus can
404 use nucleolar proteins to allow his own proliferation and finally, viral infection can
405 result in changes in the localization of nucleolar proteins.

406 Following infection with certain different viruses, such as coronavirus and
407 arterivirus, some normally nuclear proteins are found within the nucleolus (Hiscox
408 et al. 2001; Rowland et al. 1999). Changes in the protein distribution can be either
409 through specific nucleolar-trafficking signal present in the viral proteins (Rowland
410 and Yoo 2003) or viral proteins trafficking through the nucleolus can associate with
411 cellular proteins and recruit them to a different localization such as the hepatitis
412 delta antigen which requires nucleolin association for its nucleolar localization (Lee
413 et al. 1998). The nucleolar localization of viral proteins is also important for an
414 effective infection. For example the disruption of nucleolar localization of Semliki
415 Forest virus non-structural protein nsP2 results in a reduction in neurovirulence
416 (Fazakerley et al. 2002).

417 The three nucleolar proteins that have been most studied during viral infection
418 are B23, fibrillarin and Nucleolin. The B23 protein acts in several functions
419 associated with the nucleolus, such as ribosome assembly, nucleocytoplasmic

shuttling, possibly regulating transcription of rDNA and recent study have indicated 420
is implication in the p53 regulation (Boulon et al. 2010; Hiscox 2002). During 421
infection with HIV, B23 facilitates the nuclear import of Rev proteins promoting 422
virus mRNA trafficking (Szebeni et al. 1997). B23 protein can also stimulates the 423
replication of adenovirus as well (Okuwaki et al. 2001). Fibrillarin is involved in 424
pre-rRNA processing, pre-rRNA methylation, and ribosome assembly (Tollervey 425
et al. 1993). During adenovirus and coronavirus infection, fibrillarin redistribution 426
leads to decrease in RNA Polymerase I transcription (Puvion-Dutilleul and 427
Christensen 1993). Nucleolin has been involved in the regulation of gene expres- 428
sion, chromatin remodeling, DNA recombination and replication, RNA synthesis, 429
rRNA processing, mRNA stabilization, cytokinesis and apoptosis (Mongelard and 430
Bouvet 2007). During infection with HIV, nucleolin promotes DNA replication 431
process and stimulates IRES-mediated translation of the poliovirus genome (Izumi 432
et al. 2001). Nucleolin has also a membrane fraction which is involved in viral 433
infection by facilitating the virus attachment (Nisole et al. 2002). 434

Finally, viral infection can results in relocalization of nucleolar protein to other 435
cellular compartments. Nucleolin, B23 and fibrillarin change their localization after 436
cell are infected with adenovirus or HIV (Matthews 2001). The mechanisms that 437
are responsible for this delocalization are unknown, but the displacement of nucle- 438
olar proteins changes the nucleolar, nuclear or cytoplasmic pool of these proteins. 439
The interaction between the nucleolus and viral components is thus an interesting 440
potential therapeutic target. These associations can be used for the development of 441
new therapies against viral infection. For example, the HIV Rev protein localizes in 442
the nucleolus and is involved in viral mRNA trafficking (Dundr et al. 1995). A 443
nucleolar localizing Rev response element (RRE) decoy in infected cells results in a 444
significant inhibition of the replication of HIV-1 in cell culture (Michienzi 445
et al. 2006). 446

4.3 Neurodegenerative Disorders 447

While the increased size of the nucleolus is associated with cell proliferation and a 448
potential marker for cancer, the reduction in size of nucleoli is correlated with 449
completely different disorders. It was reported patients affected by Alzheimer's 450
disease and Parkinson's disease show a reduction in nucleolar size in different part 451
of the brain (Mann et al. 1988; Mann and Yates 1982). In fact, the dysregulation of 452
different nucleolar aspects is associated with several human neuropathological 453
conditions (Kinderman and LaVelle 1976). 454

In Alzheimer's disease, not only the size of the nucleolus is affected but the 455
organization of NOR is also altered (Donmez-Altuntas et al. 2005). The aberrant 456
structure is associated with a diminution of mature rRNA products and is found as 457
an early event in patients affected (Ding et al. 2005). This is further confirmed by 458
recent studies which demonstrate that rRNA promoter region is hypermethylated in 459
early Alzheimer's disease, leading to a diminution in transcription of rRNA 460

461 (Pietrzak et al. 2011). Reducing nucleolar transcription may participate in the
462 decline of the rRNA component of brain ribosomes contributing to Alzheimer
463 associated synapse loss and dementia. In Parkinson's disease, nucleolar integrity
464 was also reported to be disrupted in the dopaminergic neurons of patients (Rieker
465 et al. 2011). In support of this observation, two proteins which are associated with
466 Parkinson's disease, Alpha-synuclein and DJ-1, are capable of interacting with
467 nucleolin (Jin et al. 2007), although the exact function of this interaction is not
468 known and could also be a consequence of an accumulation of DNA damage and
469 activation of oxidative stress (Markesbery and Lovell 2006; Rieker et al. 2011).

470 The Huntington's disease and spinocerebellar ataxias belong to a group of poly-
471 glutamine disease which are cause by additional repetition of CAG in particular
472 genes. The repeat of these nucleotides alters the function of the affected proteins
473 leading to toxicity (Orr and Zoghbi 2007). However, the toxicity is not only caused
474 by the gain of function of the proteins. Recent study showed that mutant transcripts
475 can also contribute to the adverse effect (Tsoi et al. 2012). The mutant transcripts
476 can alter rDNA transcription and induced apoptosis by activating nucleolar stress
477 pathways. In amyotrophic lateral sclerosis or frontotemporal dementia, an abortive
478 transcripts of C9orf72 gene is produce with several repetitions of the
479 hexanucleotide repeat region GGGGCC (Haeusler et al. 2014). This abortive
480 transcript migrates to the nucleolus and binds Nucleolin. This association results
481 in a mislocalized Nucleolin and likely contributes to the nucleolar stress activation
482 observed (Kwon et al. 2014).

483 5 Summary

484 It has become increasingly apparent that the cellular function for the nucleolus goes
485 well beyond ribosome subunit production. Multiple lines of investigation have
486 demonstrated a wide range of function including cell cycle regulation, stress
487 responses and maturation and biogenesis of a wide range of ribonucleoprotein
488 complexes. Several recently developed technologies are driving forward our under-
489 standing of the extent and relevance of the nucleolus in these cellular processes, and
490 the dynamic nature of the nucleoli underline the importance of studying the
491 structure and function of this nuclear organelle under a wide range of conditions.
492 Considering the large number of proteins identified in the nucleolus in proteomic
493 experiments that have no known functions or that are still poorly characterized,
494 there will be likely further functions that will be uncovered in the future associated
495 with the nucleolus.

496 Cell growth and proliferation is critically dependent on an efficient supply of
497 ribosomes to maintain protein synthesis levels. Therefore, the nucleolus is emerg-
498 ing as a key centre of cell growth regulation and it is not surprising that its activity is
499 influenced by a wide range of signaling events that can modulate the efficiency of
500 rRNA expression and ribosome subunit assembly and transport. Several examples
501 where disruption of nucleolar components and activities result in human disease,

including inherited genetic disorders and predisposition to cancer, directly reflecting the importance on cell function of disrupting mechanisms that occur in the nucleolus. The link between the nucleolus and regulation of such important cellular function demonstrate the potential as a therapeutic target for cancer treatment, viral infection and neurodegenerative diseases.

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