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Review

Ribosomal DNA and the Nucleolus as Keystones of Nuclear Architecture, Organization, and Function

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The multicopy ribosomal DNA (rDNA) array gives origin to the nucleolus, a large nonmembrane-bound organelle that occupies a substantial volume within the cell nucleus. The rDNA/nucleolus has emerged as a coordinating hub in which seemingly disparate cellular functions converge, and from which a variety of cellular and organismal phenotypes emerge. However, the role of the nucleolus as a determinant and organizer of nuclear architecture and other epigenetic states of the genome is not well understood. We discuss the role of rDNA and the nucleolus in nuclear organization and function – from nucleolus-associated domains (NADs) to the regulation of imprinted loci and X chromosome inactivation, as well as rDNA contact maps that anchor and position the rDNA relative to the rest of the genome. The influence of the nucleolus on nuclear organization undoubtedly modulates diverse biological processes from metabolism to cell proliferation, genome-wide gene expression, maintenance of epigenetic states, and aging.

Highlights

rDNA is the basis for nucleolus biogenesis, organization, and function. rDNA is evolutionarily ultra-conserved but displays extensive copy-number variation between individuals.

The nucleolus is a dynamic nonmembrane-bound nuclear organelle that displays structural diversity during the cell cycle, DNA repair, and under stress.

The rDNA and nucleolus are coordinating centers for cellular functions (e.g., heterochromatin maintenance, metabolism) that go well beyond those of ribosome biogenesis.

The rDNA/nucleolus, as an organizer of chromatin territories, exerts influence over X-chromosome inactivation, imprinting, genome-wide gene expression, and epigenetic states of the genome.

rDNA and the nucleolus are re-emerging as crucial determinants underlying or modulating cancer, neurodegeneration, and aging.

A Multifunctional Powerhouse at the Core of the Cell Nucleus

rDNA is the most abundantly transcribed segment of the genome, producing >70% of all cellular RNAs in an eukaryotic cell [1]. Although each transcribed mRNA of the highly expressed ribosomal protein genes is translated into a larger number of ribosomal proteins, each transcribed rRNA molecule is used only once as it is assembled with ribosomal proteins to produce >2000–10 000 ribosomes per minute [1,2]. This imposes a disproportionate demand for rRNAs that must be dutifully supplied during ribosome biogenesis to ensure cell survival and proliferation. Maintenance of remarkably high rRNA transcription and ribosomal assembly rates is dependent upon the nucleolus, a large nonmembrane-bound nuclear organelle that is dedicated to the task of rRNA transcription and ribosome assembly, and that occupies a substantial and disproportionate volume of the cell nucleus.

Despite essential and well-documented roles of the rDNA/nucleolus in ribosome biogenesis, little is known about the influence of the rDNA/nucleolus on nuclear organization and genome function. For instance, nucleolar-associated domains (NADs) were identified about 10 years ago [3,4], but their evolutionary conservation and stability through development and cell stress have remained uncertain. Recent studies have used proximity ligation approaches to identify rDNA contacts within the nucleus, which also need to be reconciled with NAD localization and behavior [5–7]. Furthermore, the copy number of 45S rDNA, an attribute that is relevant to nucleolus function, impacts on cellular senescence and genome stability [8,9], in addition to being associated with the induction and silencing of hundreds to thousands of genes dispersed throughout the genome [10–12]. Similarly, rDNA methylation and nucleolar size vary across individuals and are associated with age and longevity [13–15] but NADs themselves appear to be

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stable through cell senescence [16]. We review here how nucleolar elements are organized, how rDNA arrays are epigenetically regulated, and how both interact and influence the architecture of the nucleus, with implications for cellular function and organismal health.

The rDNA Encodes the Nucleolus

The nucleolus originates from the nucleolus organizer regions (NORs) that are composed of rDNA repeat clusters typically dispersed across several chromosomes (Figure 1). In humans, NORs are present in the short arm of five acrocentric chromosomes (13, 14, 15, 21, and 22), whereas

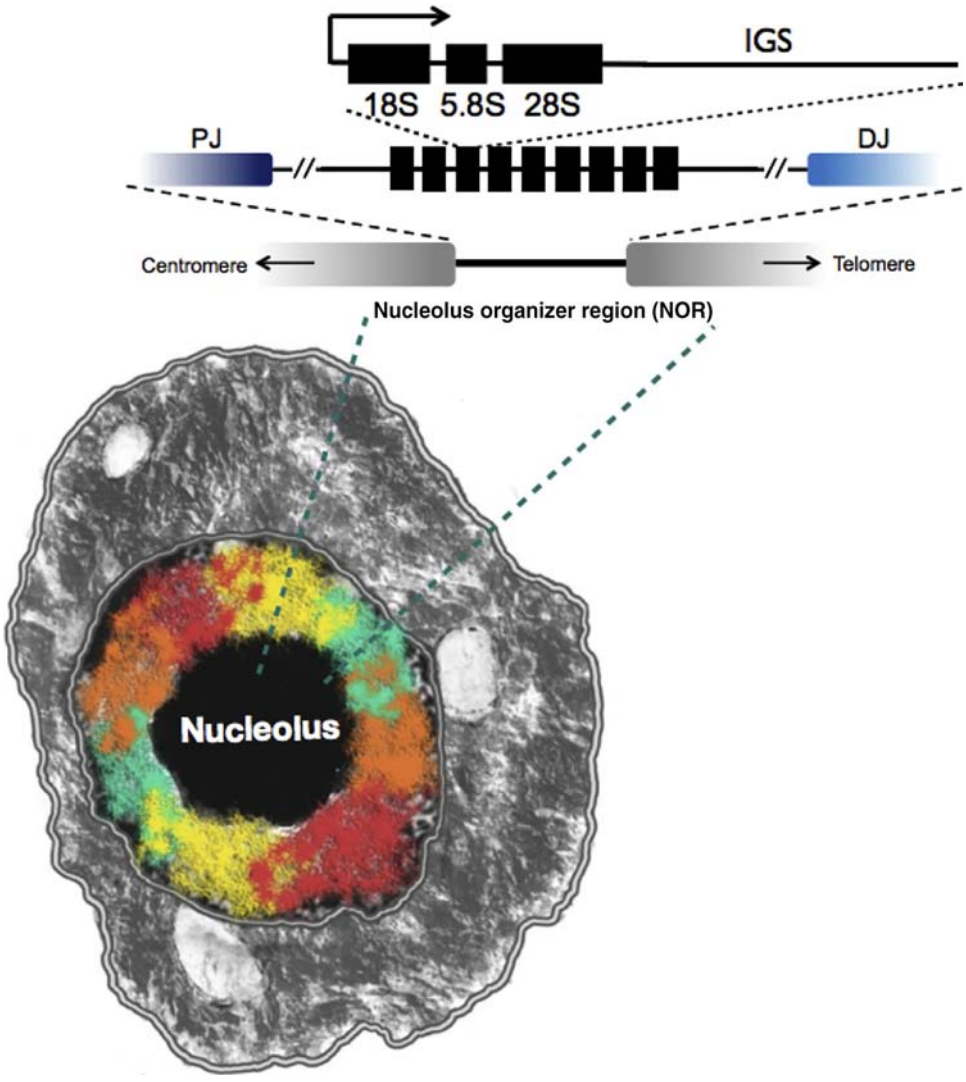

Figure 1. Organization of the Nucleolus Organizer Region (NOR) and the Ribosomal DNA (rDNA) Array. Structure of the rDNA array (IGS, nontranscribed intergenic spacer). Proximal (PJ) and distal (DJ) junctions link the rDNA array to the neighboring genomic region. NORs reside in five human chromosomes and are structurally complex instead of being neatly organized as shown above: repeat units can be truncated or inverted, and can contain small and large indels. NORs give origin to the nucleolus (black circle), a relatively large nonmembrane-bound organelle inside the cell nucleus. The colors represent chromatin from other chromosomes. Each nucleus may contain a single nucleolus or multiple nucleoli.

in laboratory mice they are found on six chromosomes (11, 12, 15, 16, 18, and 19). The fundamental repeat unit consists of a ~13 kb rDNA core that contains the RNA polymerase I (Pol I)-transcribed 45S rRNA genes and ~30 kb intergenic spacers (IGS). The IGS may contain regulatory elements, replication fork barriers, and other RNAs transcribed by Pol II [17]. The core 45S rDNA produces nascent 45S transcripts that are processed into 18S, 5.8S, and 28S rRNA components which, together with the 5S RNA, are loaded into the newly formed ribosomes that are assembled within the nucleolus [18]. The 5S RNA genes, that are typically localized somewhere else in the genome, are transcribed by Pol III in the euchromatic compartment of the nucleus or at the periphery of the nucleolus [6]. In some organisms the 5S rDNA is located inside the 45S rDNA array [19,20]. The evolutionary pressures and functional constraints that contribute to a linked rDNA array with intermixed 45S–5S units (as seen in some plants and fungi), or the separate 45S and 5S arrays (as seen in mammals and *Drosophila*), are poorly understood [5,6].

Dynamic Structural Organization of the Nucleolus

The nucleolus consists of a large granular component (GC), with one or a few dense fibrillar components (DFCs), each of which has a fibrillar center (FC) (Figure 2). In addition, a layer of

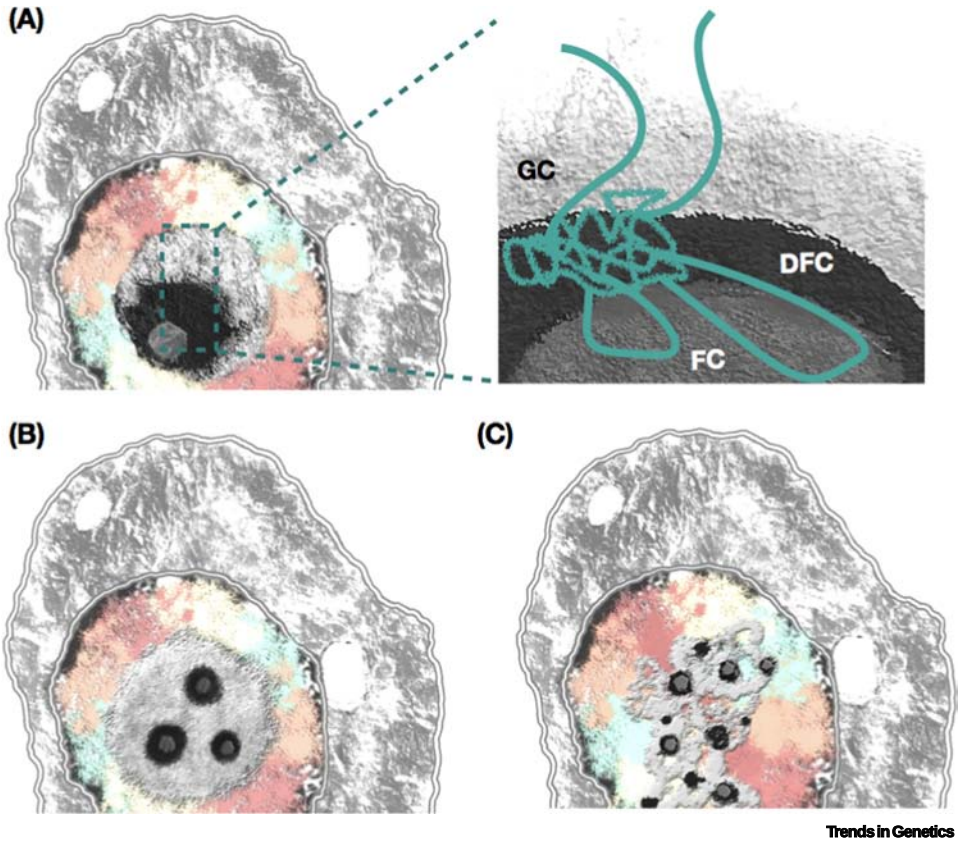

Figure 2. Organization and Diversity of the Nucleolus. The nucleolus is structurally organized into at least three subregions: fibrillar centers (FCs), dense fibrillar components (DFCs), and granular components (GCs). A single nucleolus or multiple nucleoli can be present in each cell. (A) Under one hypothesis, the intergenic spacers (IGS) are localized in DFCs, and transcribed 45S units extend into FCs and possibly into GCs. (A–C) The nucleolus displays morphological diversity across cells and conditions. Note that single or multiple FC and DFC foci can be present in each nucleolus and can occur at the center or near the periphery of the organelle; reticulate nucleoli have also been documented.

heterochromatin surrounds the nucleolus, forming the perinucleolar chromatin [21]. From this shell around the nucleolus, strands of chromatin enter the organelle, forming the intranucleolar chromatin (Figure 2). The organization of nucleolus elements into substructures is not completely understood [18]. There is a consensus, however, that the majority of the rDNA is located in either FCs or DFCs [22,23], as well as in perinucleolar heterochromatin and in loops of rDNA extending from perinucleolar heterochromatin into the interior of nucleolus (also called intranucleolar stretches) [18]. Overall, the rDNA outside the nucleolus is thought to be transcriptionally inactive [24–26], whereas intranucleolar stretches contain both active and silenced genes [25]. One hypothesis is that nascent rRNA transcripts are found in one of the fibrillary parts, mainly in DFCs [27] (Figure 2), and that the nascent rRNA generates the DFC [28]. Another hypothesis is that the rDNA and its transcription localize to FCs. This is supported by early data showing Pol I localization to FCs [29]. However, some have argued that the rDNA and its transcription are located in DFCs [30] where fibrillarin (FBL) is located, and which contributes to keeping chromatin in an open state [31–33].

The nucleolus displays substantial structural diversity during development and across tissues, as well as dynamic organization – with assembly and disassembly of components during the cell cycle, DNA repair, and response to stress (Figures 2 and 3). A variety of nucleolar proteins, chromatin modulators, and transcription regulators such as FBL, nucleophosmin (NPM1), Pol I, and upstream binding factor (UBF) contribute to determining nucleolus structures [34]. Proteomic analysis revealed that >500 proteins localize to the nucleolus [35–37]. UBF plays an essential role in the aggregation of nucleolar proteins [38–40], and ectopically located UBF results in nucleolus-like structures with pseudo-NOR and Pol I machinery [41]. The distal flanking region of the rDNA might also be relevant in nucleolar aggregation and assembly/disassembly of rDNA from different chromosomes. Experimental data showed, for instance, that the distal junction associates with the nucleolus even when it is ectopically positioned [42]; it localizes closer to the periphery of the organelle and is transcriptionally active. However, rDNA association can occur even without the flanking regions [43,44]. Thus, flanking regions might be involved in NOR coalescence but not in its association with other nucleolar components and nucleolus formation [18]. Furthermore, inactive NORs localize outside the nucleolus [25]; the higher the proportion of active NORs on a NOR-

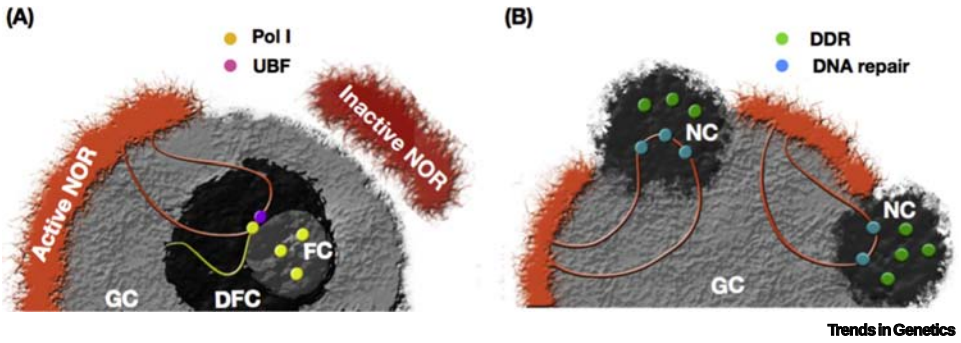

Figure 3. Nucleolar Organization and Reorganization upon DNA Damage. (A) Genomic regions that surround the nucleolus (e.g., nucleolus-associated domains, NADs) preferentially acquire a silenced condensed state. The ribosomal DNA (rDNA) in nucleolus organizer regions (NORs) may invaginate into the internal layers of the nucleolus, forming the intranucleolar stretches that contain both inactive and translated genes. In normal conditions, active NORs form the fibrillar centers (FCs) and dense fibrillar centers (DFCs), inside the granular component (GC). (B) Nucleolar reorganization upon DNA damage. The nucleolar cap (NC) is formed, replacing FCs and DFCs, and the rDNA is dislocated to the periphery of the nucleolus where the DNA repair machinery can access and repair the rDNA. Abbreviations: DDR, DNA damage response; Pol I, RNA polymerase I; UBF, upstream binding factor.

bearing chromosome, the closer it is to the nucleolus, suggesting that transcriptional activity is a factor in nucleolus association [24,45].

During the cell cycle, the nucleolus disassembles as the cell reaches metaphase and subsequently reassembles [43,46]. The assembly process begins in early telophase when pre-rRNA processing factors associate with NORs [47]. At the end of mitosis, nucleolar proteins aggregate, forming prenucleolar bodies (PNBs) [34], which then associate with NORs to form the nucleolus. Although the association with PNBs depends on Pol I, the beginning of the process involving pre-rRNA processing factors does not [47]. The nucleolus is also responsive to DNA damage [48,49], and Pol I transcription is inhibited [50] in response to double-strand breaks (DSBs) both in rDNA [50] and outside the nucleolus [51]. This disruption of the transcription process leads to substantial internal dynamics and modification of nucleolar structures in which FC and DFC go to the GC periphery, together with DNA repair machineries and numerous DNA damage response (DDR) proteins (Figure 3), to form the nucleolar cap [48,50,52,53]. Changes in nucleolar organization upon DNA repair have also been observed, and rDNA moves to the periphery and nucleolar components (nucleolin and nucleophosmin) translocate to and out of the nucleolus in an ATM-dependent manner [54]. Nucleolar/rDNA changes through the cell cycle and during DNA repair are likely to substantially alter the nuclear landscape. All in all, dynamic changes to the nucleolus [18,54,55] during the cell cycle, DNA repair, or tissue differentiation are expected to influence the architecture of the entire nucleus and play a role modulating the epigenetic, transcriptional, metabolic, and other functional states of the cell.

Epigenetic States of rDNA

rDNA is found in three different transcriptional states: active, poised, and inactive. The poised state has open chromatin and unmethylated promoters but an inactive nucleosome conformation – it contains silenced units that are more easily activated [56,57]. These epigenetic states of the rDNA are controlled by multiple elements. One such element is UBF, without which NORs become inactive and unbound to nucleoli [58]. Another important element is the transcription termination factor 1 (TTF1), which binds to terminator elements upstream and downstream of the rDNA. Two factors may bind to TTF1: Cockayne syndrome B protein (CSB) and the nucleolar remodeling complex (NoRC). When TTF1 binds to CSB, it promotes histone methylation, leading to activation of chromatin, whereas TTF1 binding to NoRC promotes both DNA methylation and histone deacetylation, leading to rDNA silencing [59].

CpG methylation is presumed to be an important regulator of rDNA transcription in humans and mice. Methylated DNA in the nucleolus preferentially localizes in the periphery of the organelle [60], and possibly on loops of rDNA (intranucleolar stretches) that extend from the perinucleolar heterochromatin into the interior of nucleolus. rDNA methylation is maintained by DNA methyltransferase 1 (DNMT1): cells lacking DNMT1, but not DNMT3b, show DNA methylation loss at rDNA genes as well as a disrupted and disorganized nucleolus [61]. The data show the relevance of DNMT1 function on epigenetic states of rDNA and the structure of nucleolus. On the other hand, DNMT3a and DNMT3b were not initially localized to nucleoli in NIH3T3 cells [62]; however, more recent data indicate that both DNMT3a and DNMT3b interact with the nucleolar remodeling complex (NoRC) and are recruited to nucleoli [63].

The insulator factor CTCF is a zinc-finger protein that mediates folding and looping of the genome. CTCF is key to both genome-wide nuclear organization and to the epigenetic control of the rDNA [64,65]. The protein binds to thousands of sites along the genome, as well as to specific sites in the rDNA [66]; it contributes to rDNA activation through changes in nuclear and nucleolar structure, as well as by facilitating UBF loading onto the rDNA [65–68]. CTCF binds

both to UBF and a site immediately upstream from the spacer promoter [65], leading to increased Pol I activity. One hypothesis is that CTCF enhances UBF binding to this region, promoting rDNA transcription. Further research has provided additional evidence to support the hypothesis that CTCF promotes rDNA expression because overexpression of CTCF causes increased Pol I-dependent rRNA transcription [68]. CTCF might recruit cohesin, a protein that is primarily known for its role in sister chromatid cohesion during chromosome segregation and DNA repair, but that also plays a key role in epigenetic regulation of the genome [69]. The CTCF–cohesin complex promotes the formation of loops in the rDNA [70], leading to promoter–enhancer interactions [71,72] as well as chromatin insulation [64]. Intriguingly, variation in *CTCF* mRNA abundance across individuals in human populations is negatively associated with rDNA copy number [11]. Regulatory feedbacks that directly link CTCF mRNA/protein abundance to rDNA copy number remain an intriguing possibility that could significantly and globally impact on nuclear architecture.

Nucleolus-Associated Domains

Non-rDNA parts of the genome have long been hypothesized to interact with the nucleolus, such as the centromere of human chromosomes 1 and 9 [73] (Figure 4, Key Figure). Seminal studies on yeast also showed that tRNA genes preferentially localize at the periphery of the nucleolus [74–76]. However, sequencing approaches have enabled agnostic analyses of nucleolus-associated chromatin. Accordingly, genome-wide identification of genomic segments associated with the nucleolus was reported in two seminal studies that conducted deep sequencing of DNA retrieved from isolated human nucleoli [3,4]. The studies identified NADs that preferentially localized around the nucleolus. Both studies found that NADs are enriched in olfactory receptor genes, zinc-finger genes, and immunoglobulin gene families. Another study identified nucleolus-associated regions in *Arabidopsis thaliana*, and found that they are enriched in pseudogenes and tRNA genes [77]. In addition, studies on human cells [3,4] also noted an overlap between NADs and lamina-associated domains (LADs), a finding that is concordant with data showing that genes in LADs may temporarily move to the nucleolar periphery [78]. Because LAD and NAD territories have a disproportionate number of silenced genes [79,80], it has been hypothesized that LADs and NADs regulate tissue- and development-specific gene expression as genes move to and from heterochromatic and euchromatic states. LAD territory in the nuclear periphery is organized by lamin B [79]. Lamin B has in turn also been attributed an organizational role in the nucleolus, according to evidence of its binding to nucleolin and nucleophosmin, as well as of nucleolar disruption in its absence [81]. However, recent studies of NAD-specific genes showed that one third of them do not overlap with LADs [16]. This is expected because some LADs are constitutively maintained in the nuclear periphery [82]. Further detailed analyses of NADs and their dynamic during development, across tissues, and during the cell cycle, DNA repair, and response to stress are certain to provide novel insights on nuclear organization.

A recent study analyzed NADs during cell senescence in fibroblasts [16]. Surprisingly, almost no change in NAD identity was detected [16]. However, satellite repeat clusters in centromeric and pericentromeric regions, which generally localize in perinucleolar heterochromatin, showed impaired interactions with the nucleolus. Furthermore, histone H3 trimethylated on lysine 9 (H3K9me3)-marked heterochromatin, that is typical of centromeric and pericentromeric satellite repeat clusters in nucleolus-associated chromatin, was shown to be lost and rearranged in the nucleus. These changes in nucleolar localization of satellite repeats and histone marks are likely responsible for senescence-associated distension of satellites (SADS), which associates with increased transcription of these regions during aging.

Key Figure

The Nucleolus as an Organizer of the Nuclear Space and Coordinator of Disparate Nuclear Functions and Regulatory Programs.

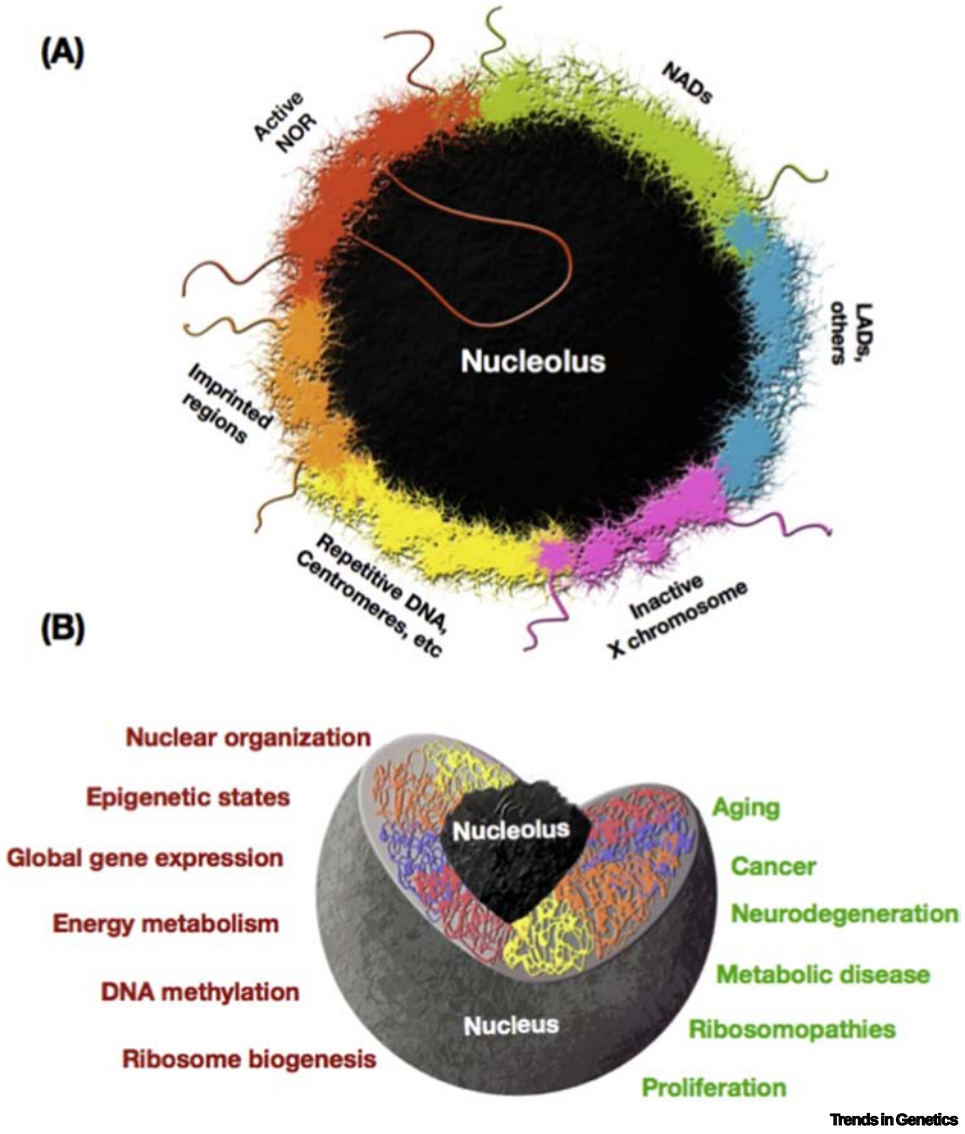

Figure 4. (A) Nucleolus-associated domains (NADs) contain >1000 genes transcribed by RNA polymerase II (Pol II). NADs have a significant overlap with lamina-associated domains (LADs). The inactive X chromosome and some imprinted regions are also localized in the periphery of the nucleolus. Repetitive segments including telomeric and centromeric sequences preferentially associate with the nucleolus and form a layer of condensed heterochromatin around the organelle. Other segments include those harboring developmentally regulated genes and multigene families (e.g., olfactory receptors) that might use proximity to nucleoli to maintain the inactive state of specific members. (B) The nucleolus modulates a variety of biological processes and functional states of the cell, and has been linked to diverse organismal and cellular phenotypes. Abbreviation: NOR, nucleolus organizer region.

The clustering of NADs around the nucleolus emerges from many factors. Research in yeast, for example, has found that repeat elements (which are enriched in NAD regions [3,4]) interact with ETS2 in the nucleolus, locking the chromosomes in a specific conformation around the organelle [83]. The insulator factor CTCF also has a role in tethering genomic regions to the nucleolus periphery in a nucleophosmin 1 (NLP1)-dependent way [84]. This is further supported by the fact that NLP1 binds to centromere regions [85], is enriched in NADs, and that NLP1 depletion triggers nucleolus disruption [86]. Another mechanism suggested is the binding of satellite RNA to centromere-specific proteins, CENPC1 and INCENP, directing them to the nucleolus during interphase and to kinetochore assemblies during mitosis [87]. Some histone modifications, such as H4K20me3, H3K9me3, and H3K27me3, are enriched in NADs and may be a binding site for scaffolding proteins such as heterochromatin protein 1 (HP1) and polycomb group (PcG) proteins [88]. It remains unclear, however, whether these histone modifications drive NAD association with the nucleolus or whether they emerge as a byproduct of NAD localization in the periphery of the nucleolus.

The typical assumption is that perinucleolar localization causes a change in chromatin state leading to gene silencing, at least around large segments of heterochromatic NADs [89]. This hypothesis is corroborated by a recent study in which euchromatin is associated with nuclear speckles, whereas repressive heterochromatin is associated with the nucleolar periphery [90]. Repressive domains are present on rDNA-bearing chromosomes of *A. thaliana* and partially correspond to genomic regions flanking the rDNA arrays [90,91]. Accordingly, NOR-bearing chromosomes 2 and 4 (NOR2 and NOR4) of *A. thaliana* are enriched in NADs. In leaf tissues that exclusively transcribe rDNA repeats from chromosome 4, NOR4 is associated with the nucleolus, whereas chromosome 2 rDNA repeats are transcriptionally repressed and are excluded from the nucleolus. Induced expression of NOR2-derived rDNA, which is normally inactive, leads to its nucleolar association and global reorganization of the short arm of chromosome 2 inside the nucleus [90]. This is accompanied by reduced expression of chromosome 2 genes [91]. Nevertheless, tRNA localization in the nucleolar periphery has been suggested to facilitate tRNA gene expression [74–76]. This needs to be reconciled with observations that proximity to tRNAs and nucleolar localization inhibit Pol II- and Pol III-mediated transcription of some genes [76]. Similarly, it has been suggested that ribosomal protein gene (RPG) localization proximal to the nucleolus could facilitate coordinated expression of RPGs and rRNAs [6], although nucleolar proximity could also instead be used to inactivate specific RPGs. Genes and NORs with tissue- and development-specific expression might therefore be regulated through changes in their position relative to the nucleolus. These ideas are in accordance with findings that targeted modification of rDNA copy number impacts on gene expression across the whole genome [10].

rDNA Contact Maps and Nuclear Architecture

Proximity-ligation technology such as Hi-C (a genome-wide extension of 3C, chromatin conformation capture) has enabled the analysis of chromatin interactions, thereby providing a clearer understanding of chromosomal organization in the interphase nucleus and its relation to functional states of the cell [92,93]. Multiple Hi-C datasets were recently assembled to uncover rDNA array interactions with the genome [5,6]. Accordingly, rDNA contacts preferentially occur at repressed genes, in repetitive or insulator segments, and encompass CTCF binding regions. These observations have also been replicated in 4C (circular chromosome conformation capture) data [7] and are concordant with evidence suggesting that the nucleolus and its periphery have a mostly repressive chromatin environment.

Furthermore, interactions between 5S and 45S rDNA arrays were absent in both Hi-C [5,6] and 4C [7] data despite concerted copy-number variation between these two regions [94].

However, there was considerable overlap between the contact sites of the 5S and 45S rDNA across the genome, probably reflecting some amount of spatial proximity between the arrays. Sites of joint 5S and 45S contact might point to a potential mechanism for concerted copy-number variation and, possibly, for 5S–45S coregulation [6]. Nevertheless, the observation that direct 5S and 45S rDNA contacts were lacking [5–7] needs to be reconciled with reports of 5S rDNA localization within NADs. On the other hand, Hi-C studies observed a large number of contacts between the rDNA and genes encoding proteins that localize to the mitochondria [6]. Differential expression of mitochondrial protein genes had already been linked with rDNA in fruit flies [10] and humans [11]. These intriguing observations suggest an evolutionarily conserved and physiologically relevant relationship between the rDNA arrays and the mitochondria. The 5S rDNA was also enriched in contact with mitochondrial protein genes, and might have an even closer relationship with the mitochondria given that 5S RNAs are imported into the mitochondria [95].

RPGs comprise another class with closer than average rDNA proximity, although the magnitude of the shift is relatively small [6]. RPG–nucleolus proximity could partially reflect coordination between RPG and rRNA expression so as to produce ribosomal components in a stoichiometric manner. On the other hand, the proximity of some RPGs to the nucleolus could reflect a regulatory mechanism to inactivate specific ribosomal protein variants. Indeed, localization to the nucleolar periphery appears to be used for the selective inactivation of specific alleles in some imprinted loci. For instance, the Kcnq1ot long noncoding (nc)RNA is exclusively transcribed from the paternal chromosome, and it drives the targeting of the paternal locus to the nucleolar periphery, where a 1 Mb region containing 10 protein-coding genes is silenced [96,97]. Similarly, expression of the Xist long ncRNA drives X chromosome association to the nucleolar periphery and is necessary to maintain the inactivated X chromosome in a silenced state [98]

Changes in rDNA interactions with the genome during progression to malignancy in a Myc-driven B cell lymphoma model were documented with 4C-seq [7]. The model is particularly interesting because oncogenic Myc localizes to the nucleolus, physically associates with the rDNA, remodels rDNA chromatin looping structures, induces TTF-1, and activates RNA Pol I transcription when quiescent cells re-enter the cell cycle [99,100]. The transition from quiescence to cell-cycle entry and proliferation is mediated by Myc-dependent attachment of rDNA to the nuclear matrix via the rDNA nontranscribed IGS region [100]. Matrix-attached rDNA repeats are hypomethylated, and are presumably active or poised for transcription, whereas hypermethylated silenced rDNA repeats are not recruited to the matrix [100]. Interestingly, genes encoding protein components of the ribosomes displayed decreased contact density with the rDNA and increased mRNA expression upon Myc activation and malignancy progression [7].

In summary, although most studies with deep sequencing of nucleoli and 4C/Hi-C proximity ligation are in fairly good agreement regarding key observations, some discrepancies have remained. These differences likely reflect the different methods employed (NADs identified by deep sequencing of nucleoli vs proximity ligation), but might also emerge from differences in the cell type studied, their stage in the cell cycle, or the DNA repair activity of the cell. Continued studies with common protocols and a greater variety of cell types and conditions will undoubtedly help to elucidate these discrepancies and link the rDNA/nucleolus to specific metabolic states of the cell. Overall, the findings shed light on a crucial component of nuclear organization, reaffirming the role of the nucleolus in modifying and perhaps coordinating genomic expression, cellular function, and pathological alterations in human diseases.

rDNA Contact Maps and Human Diseases

Nucleolar dysfunction has been implicated in a variety of human diseases (Figure 4B). In cancer, structural alterations of the nucleolus have long been documented [101]. In addition, many oncogenes and tumor-suppressor genes have been shown to physically interact with or directly influence the nucleolus and rDNA arrays [102–104]. Furthermore, cancer lineages have expanded 5S rDNA and contracted 45S rDNA arrays relative to normal adjacent tissue from the same individual [105,106]. A smaller rDNA array might in turn enable the release of rDNA-bound proteins such as Myc [107], CTCF [67], and histones [67], which could then be more abundantly supplied to other parts of the genome, possibly altering global nuclear organization and cellular function. Thus, genome-wide alterations in rDNA transcription in cancers could be partially caused by changes in nuclear organization that are mediated by rDNA copy number, as has been suggested in fruitflies [10] and humans [11]. Indeed, recent research on a Myc-driven B cell lymphoma model has shown an UBF-mediated increase in the open chromatin state of the rDNA and altered nucleolar interaction with NADs [7]. Genes associated with B cell differentiation displayed increased interaction with NADs and decreased expression, whereas genes involved with cell growth and metabolism displayed increased expression. These alterations are compatible with a causal role of rDNA-mediated changes in nuclear architecture during carcinogenesis. Because accelerated ribosome biogenesis is presumably necessary because of the higher demand for protein synthesis in rapidly proliferating cells, the decrease of rDNA copy number may be compensated by an upregulation of genes responsible for increased nucleolar activity [106,108,109]. Accordingly, Myc, which is overexpressed in several cancers [110–116], promotes selectivity factor 1 (SL1) binding to DNA consensus elements. This in turn recruits Pol I and increases rRNA transcription, leading to cell-cycle re-entry [107,117]. Recent evidence further supports the hypothesis of rDNA-mediated changes in nuclear organization [7] as well as increased nucleolar activity in cancers [7,106], despite rDNA copy-number loss [105,106]. The data suggest that carcinogenesis is accompanied by conversion of rDNA chromatin to an open state [7], with activation of poised rDNA genes and concomitant changes in nuclear organization and global gene expression.

The nucleolus has also been implicated in neurodegenerative diseases. Nucleolar/rDNA activity is important, for instance, to meet the demands of high protein synthesis during cell regeneration from previous axon damage and in maintaining synaptic plasticity [118]. Inactivation of Pol I, that is responsible for rDNA transcription, has been linked to neurodegeneration [119]. Defects in rDNA transcription due to dysfunctional UBF have been linked to Huntington disease pathology [120]. Moreover, neurons from brains affected by either Alzheimer's disease (AD) or mild cognitive impairment show smaller nucleoli and reduced or defective ribosomes [121]. Likewise, alterations in AIDA-1, a synaptonuclear factor that increases nucleoli number and protein synthesis in an activity-dependent way, results in synaptic defects in mice. Genome-wide studies have linked *AIDA1* variants to neuropsychiatric disorders such as schizophrenia, bipolar disorder, and AD [122,123]. Dementia with Lewy bodies (DLB) neurons had increased rDNA copy number but stable rDNA CpG methylation in parietal cortex [118]. AD showed increased rDNA copy number in frontal and parietal cortex, but this was associated with hypermethylation and, therefore, silencing [124]. There is apparent hypertrophy of nucleoli in old individuals with pathological signatures of AD but who are asymptomatic for the disease [125]. One hypothesis is that increased nucleolus activity protects from cognitive impairment despite AD pathology [125], possibly explaining why the pathologically affected areas in AD and Parkinson disease might also display a higher rDNA copy number because those would be the remaining positively selected cells [118].

Ribosomopathies might also be influenced by epigenetic states of the nucleolus and the impact of the organelle across the whole genome. Diamond–Blackfan anemia is characterized by

haploinsufficiency of ribosome proteins, leading to nucleolar stress and activation of the p53 pathway [126]. Treacher–Collins syndrome is another ribosomopathy. It results from mutation in *TOC1*, a gene whose product is responsible for rRNA processing [127]. Mutations in *TOC1* also lead to apoptosis by nucleolar stress via a p53 mechanism [128,129]. Finally, recent hypotheses link nucleolar dysfunction and autoimmune diseases [130]. Accordingly, stressors during development might lead to nucleolar expansion, thereby altering nuclear architecture and engulfing the inactive X chromosome of females. This process purportedly increases the formation of autoantigens, which generally pass through the nucleolus [131,132]. Polyamines, typically increased in cell stress, might help to stabilize the autoantigens and further contribute to the initiation of an autoimmune response.

Concluding Remarks

The rDNA and the nucleolus have long been viewed as an organelle that is 'merely' responsible for the production of ribosomes and the expression of the vast majority of all RNAs in the cell. Although these processes are essential, and represent in and by themselves the single major source of energy expenditure by the cell, the rDNA/nucleolus is also increasingly recognized as an organizer hub that coordinates and controls seemingly disparate cellular functions and biological processes (Figure 4). These include processes such as maintenance of genomic integrity, DNA repair and recombination, telomere maintenance, heterochromatin stability, control of repeat element expression, maintenance of X chromosome inactivation, allelic exclusion in imprinting, genome-wide transcriptional regulation, and modulation of epigenetic states. The nucleolus is also a keystone in nuclear organization, not only providing a port for anchoring components of heterochromatin and gene silencing but also impacting on epigenetic states and gene expression throughout the whole nucleus. The rDNA/nucleolus has also been implicated in cancer, metabolic diseases, progeria, neurodegeneration, and aging. All in all, much work and many surprises are certain to lie ahead as investigators connect specific disease etiologies and cellular states to changes in nuclear architecture and epigenetic states of the genome that are driven by the rDNA/nucleolus (see Outstanding Questions).

Outstanding Questions

What determines the localization of specific NADs to the nuclear periphery? How dynamic is NAD localization, and how does it change during development, stress response, aging, across tissues, and in specific diseases? Are NADs evolutionarily conserved?

What determines rDNA–genome and rDNA–gene contacts? How dynamic are these contacts? Do they change during development, stress response, aging, across tissues, and in specific diseases? Are rDNA contacts evolutionarily conserved?

How do specific proteins (e.g., CTCF) organize NAD structure and localization as well as rDNA contacts with the rest of the genome.

How does proximity to the nucleolus affect the transcriptional and epigenetic states of specific genes and segments of the genome? What are the mechanisms through which the rDNA/nucleolus impacts on global chromosomal organization and chromatin states in the nucleus?

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