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The Nucleolus



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The Nucleolus

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Preface

The Nucleolus: A Nuclear Body Full of Surprises

The deeper we delve into nature, the more surprises we find. The nucleolus is no exception; as we learn more about the structure and functions of the nucleolus, the more surprising it becomes. It has taken almost two centuries to reach this point. In fact, well over a century passed between the first description of the nucleolus (Wagner 1835) and the publication of definitive experiments that established its primary function as a factory for ribosome biogenesis during the 1960s (summarized by Hadjiolov 1985). In the past four to five decades, research has been largely focused on investigating its structure and ribosome assembly process, defining its component parts and determining how it does and what it does. Still ongoing, these efforts are now at a relatively mature level, taking us out of the “black box” era. The picture that has emerged is a highly complex, multistep vectorial process that utilizes a large number of components. Although there is still much to be learned about the mechanisms of ribosome biogenesis, the field has moved into structural and functional analyses of individual components and larger sub-complexes as well as studies on integration and regulation within the system and by the cell.

With the primary focus of research during the second half of the twentieth century on the elucidation of the role of the nucleolus in ribosome assembly, most researchers did not expect that it could do much else. Consequently, the nucleolus managed to keep its other functions hidden. However, within the past two decades something extraordinary happened; new functions for the nucleolus began to appear. In many cases, some of these were met with skepticism, but several of the new roles have now become established and even found in textbooks. Others are under active investigation. These novel tasks for the old factory have given the field a new vitality, generating renewed excitement and interest. Moreover, the findings have attracted researchers who had little or no previous interest in the nucleolus.

The surprising features of the nucleolus are not limited to its newly discovered functions; they also include aspects of its conventional role. Consequently, almost

two-thirds of this volume is devoted to traditional functions of the nucleolus. There has been a near-explosion of progress in elucidating nucleolar structures, functions, and mechanisms during the past decade. How do we account for these developments? It is best explained by a synergistic effect between the renewed interest in the subject and the continuous development and improvement of technology. As an example, advances in mass spectrometry allowed researchers to identify virtually every protein molecule in the nucleolus. Even with highly sensitive instrumentation, this was only possible because of the availability of genome sequences from several species. To the surprise of most researchers, several thousand polypeptides were found in the nucleolus (see Chap. 2), many of which have no apparent function in ribosome biogenesis. With this finding, questions about the dynamics of these polypeptides arose. Mass spectrometry coupled with isotopic methods has allowed researchers to analyze the dynamics of multiple molecules moving in and out of the nucleolus under various physiological conditions. Complementing this is the availability of laser scanning confocal microscopy coupled with photobleaching techniques to measure the dynamics of individual molecules in living cells. Not only has recent research provided us with new information, but it has changed our perception of the nucleolus; we are now forced to change our mental image of the nucleolus as the static structure shown in textbooks to one in which the components are constantly in motion. Although the details are important, the changing big picture may be more significant. To quote Sir William Bragg, “The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them.”

This subject has matured to the point where every subtopic cannot be covered in one volume. Therefore, we have focused on recent progress in specialized topics within the general subject. We apologize to those researchers whose work is not covered.

The Complex Nucleolus

The acquisition of greater knowledge about the nucleolus has also brought more complexity. Is the complexity surprising? Probably not, if we consider the complex products it assembles, the things it does and how it does them. Prokaryotes get along quite well with a relatively simple system of ribosome assembly. A superficial examination of the general features eukaryotic ribosome biogenesis (Fig. 0.1) suggests that the process is relatively simple. However, when one delves into the details described in the chapters of this volume, the eukaryotic ribosome production system turns out to be exceedingly complex. As eukaryotes evolved the complexity increased and so arose the need for a nucleolus. This came about for a number of reasons. Important insights into this issue occur when the compositions and structures prokaryotic and eukaryotic ribosomes are compared. Eukaryotic ribosomes are about 40% larger than their bacterial counterparts; their RNAs are longer and they have about 25 more proteins. Recent progress in X-ray crystallography also helps us make the comparison. The crystal structure of the prokaryotic ribosome became available about a decade ago (Ramakrishnan and Moore 2001), but recently the structures of the yeast 80S ribosome (Ben Shem et al. 2010) and the *Tetrahymena*

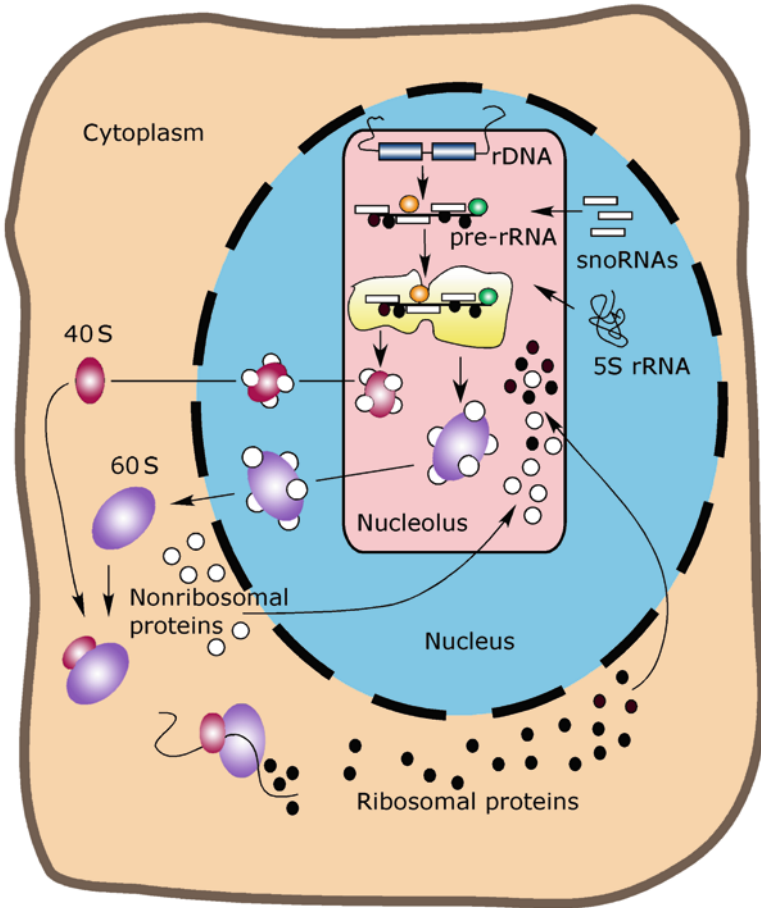


Fig. 0.1 Major steps in eukaryotic ribosome biogenesis. The process starts with transcription of preribosomal RNA (pre-rRNA) from multiple copies of the genes for pre-rRNA (rDNA). Nonribosomal proteins (*open circles*) and small nucleolar RNAs (snoRNAs; *open rectangles*) associate with the nascent transcript. 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 (2004)

40S subunit in complex with initiation factor 1 (Rabl et al. 2011) were published. Although the core structure of the ribosome is conserved across all organisms, the additional components lie at the periphery. The added segments of rRNA and extra proteins appear to play a role in the regulation of translation. Hence, the assembly system had to evolve and become more complex to accommodate these regulatory components. Moreover, longer RNAs offer more opportunities for misfolding and a

precise order of assembly is required to prevent this from happening during ribosome assembly. Of particular importance is the pseudoknot in the 18S rRNA that is required for ribosome function. The formation of this structure is delayed until later in the assembly process by utilization of base pairing with small nucleolar RNAs (snoRNAs) (Hughes 1996). Thus, timing of events and precision in the assembly process adds more complexity.

A second factor is related to the hundreds of RNA modifications found in eukaryotic rRNA, which are largely absent in prokaryotes. The relatively few modifications in the latter are performed by freestanding enzymes. For performing these operations in eukaryotes, the nucleolus contains a multitude of small (snoRNAs), which serve as guides, along with their modifying enzymes and other associated proteins. This system contributes hundreds of components to the nucleolar machinery that are not seen in prokaryotes.

Although ribosome production is regulated in prokaryotes, it is more tightly controlled in eukaryotes to meet the needs of the cell. The various levels of regulation are described in Chaps. 4, 6, 8, 12, and 13. The number of regulatory factors is growing; to a large extent these interact with the transcriptional machinery. This introduces additional components into the nucleolus, many of them only transiently. In addition, there is control of virtually every step of ribosome biogenesis, thereby adding more proteins and RNAs to the mixture.

A major difference between prokaryotes and eukaryotes is that the latter contain multiple copies of the genes for rRNA (rDNA), numbering in the hundreds. This brings us to the fourth reason for the complexity. The genes are tandem repeats, which in themselves increase the complexity. In addition, for efficient utilization of the transcription, processing and assembly machinery the rDNA repeats are carefully packaged within the compact structure of the nucleolus. This is likely to be the primary factor in the development of the nucleolus.

Finally, the nucleolus has several other functions in addition to ribosome production (covered in Part 3 of this volume). These include routine housekeeping tasks e.g., signal recognition particle (SRP) assembly and nucleolar participation in regulation of cell growth and the cell cycle e.g., nucleostemin. These novel functions add another layer of complexity to an already complex nuclear body.

Nucleolar Structure and Organization

Within the cell nucleus, individual chromosomes tend to occupy preferred territories, which form clusters of genes for efficient use of the transcription machinery (Misteli 2011). One of these territories is the nucleolus, which evolved to be an organized structure for efficient production of ribosomal RNA and ultimately, ribosomes. Were it not for the multiple copies of the genes for ribosomal RNA (rDNA) and their clustering at the nucleolar organizer regions (NORs) on chromosomes, the nucleolus would not exist. Without the gene clustering, eukaryotic cells might go about making ribosomes the way that prokaryotes do, in a less organized

manner. However, as described in Chap. 1, nucleolar structure is not just due to gene organization, but is closely related to the process of assembly of pre-ribosomal particles. This is similar to the structural role of RNA in the biogenesis of other nuclear bodies (Shevtsov and Dundr 2011). It remains essentially correct that the nucleolus is “an organelle formed by the act of building a ribosome” (Mélèse and Xue 1995). This phenomenon accounts for at least two of the major components of nucleoli of higher eukaryotes: the dense fibrillar components (DFCs) and the granular components (GCs), which contain pre-ribosomal RNP particles at various stages of assembly. Ribosome assembly flows from transcription at the border between the fibrillar centers (FCs) and the DFCs, continues in the DFCs, and nears completion in the GCs. Curiously, lower eukaryotes and anamniote higher eukaryotes; e.g., turtles, do not have FCs (Thiry and Lafontaine 2005). The FCs are the interphase equivalent to the NORs, which contain the rDNA. The difference appears to be due to the fact that amniotes have much longer spacer regions in the rDNA than anamniotes. How ribosome biogenesis differs with or without FCs is not clearly understood. As also discussed in Chap. 1, the size of the nucleolus depends on the activity of the cell, with rapidly growing cells having larger nucleoli than cells that are less active.

One of the most unexpected findings has been the identification of more than 6,000 polypeptides in nucleoli (see Chap. 2). Only about 30% of these are related to the process of ribosome biogenesis, including ribosomal proteins and the machinery for producing ribosomes. The diverse identities and functions of the remaining 70%, supports the idea that nucleolus engages in many functions other than ribosome assembly. However, many of these polypeptides have no known functions, leaving the field open for further study. Nucleolar proteomics has moved a step further in being able to quantitatively analyze alterations in protein content under changing physiological conditions. For example, it is possible to monitor changes in the nucleolar protein content following inhibition of transcription or DNA damage. This will further our understanding of changes in nucleolar function in response to chemotherapy or the stress response.

To meet the enormous demand for proteins, growing cells have as many as ten million ribosomes (Alberts et al. 2007). Consequently, the nucleolus must have sufficient capacity to produce large numbers of ribosomal subunits at a rapid pace. The process of evolution has scaled up the first source of raw materials by providing multiple copies of the genes for rRNA. The numbers vary from a few hundred in birds and mammals to several thousand in amphibians. These are contained in tandem repeats connected by spacer regions, whose lengths vary according to the species from which they are derived. Chapter 3 provides a detailed description of how these genes are organized at the DNA level and in chromatin. Complexed with histones and other proteins, the rDNA chromatin can adopt at least three different functional states. The genes that are generally permanently inactive are in the form of condensed, heterochromatic chromatin. Of the two other forms, one is less condensed, but inactive and the other is completely active and fully decondensed. With the aid of labeling techniques, these forms can be identified microscopically. As might be expected, the most active forms are found in the DFC and completely inactive rDNA appears as buds on the nucleolar periphery. McKeown and Shaw also describe in Chap. 3 the kinds of proteins associated with the various forms of rDNA.

The different forms of rDNA chromatin have evolved to be responsive to the needs of the cell and at the same time to facilitate conservation of cellular resources. RNA levels can be modulated either by controlling the rate of transcription or by regulating the number of genes available for transcription; cells obviously use both mechanisms. Although regulation of the transcription machinery has been extensively studied over the past three decades, what accounts for switching on and off of individual genes has become an active area of study. This introduces us to a relatively new area of molecular biology, epigenetics, which is the study of heritable changes in gene expression caused by mechanisms other than changes in the underlying DNA sequence. These chromatin alterations may be carried through multiple cell divisions or they may be perpetuated through numerous generations. The epigenetic process involves the placement of “marks” on histones (acetylation and methylation) and DNA (methylation). In addition, in vertebrates, the positioning of the nucleosome seems to determine whether an rRNA gene is active or silent. Chapter 4 focuses on the three forms of rDNA chromatin: active, reversible silent, and stable silent rRNA genes and nicely complements and extends the information in Chap. 3. Central to the silencing process is the nucleolar remodeling complex (NoRC), which associates with newly replicated silent rRNA genes. This complex attracts an assortment of enzymes, which modify the histone and DNA components of chromatin. A surprising aspect of the silencing process is that it requires a noncoding RNA that originates in the intergenic spacer region (IGS). Santoro also discusses the intriguing idea that the silencing of large blocks of rDNA results in their heterochromatinization, which not only contributes to the architecture of the nucleolus, but it is also important in maintaining genomic stability.

It has long been known that the rRNA genes are organized in NORs and that these regions of chromosomes can be identified by silver staining (Goodpasture and Bloom 1975). But what facilitates this organization and other than active epigenetic marks, what signals transcriptional competence? Using *Xenopus* IGSs, McStay and colleagues (Chap. 5) were able to construct what are called pseudo-NORs. These have essentially the characteristics of true NORs including silver staining and recruitment of the transcriptional apparatus. However, the pseudo-NORs are not transcriptionally active, because they lack promoter sequences. Thus, it follows that the IGS region and not the transcribed sequences of the rDNA are responsible for NOR formation. One important protein that is involved in NOR formation and rDNA organization is the upstream binding factor (UBF), which is an abundant transcription factor for RNA polymerase I (Pol I). UBF should be considered to be a multifunctional protein in that it not only plays a major role in enhancing transcription, but it also is an architectural factor that participates in the decondensation of active rDNA chromatin. Because of the manner in which UBF acts, it seems likely that these two roles are not separable.

In summary, a variety of factors contribute to the structure and organization of the nucleolus. Although multiple genes for rRNA may exist in a given cell type, chromatin programming at the DNA and protein levels determines whether they are active in nucleoli. Once that commitment is made, the final structure of the nucleolus depends on the cell type in which it is located and the rate of ribosome production that is required by that cell.

The Role of the Nucleolus in Ribosome Biogenesis

The complex journey of ribosomal RNA on its way to becoming an essential component of a new ribosome begins with transcription by RNA Pol I. Although the transcription of 5S rRNA by RNA Pol III is of equal importance to the cell, it occurs in the nucleoplasm of higher eukaryotes and it is not covered in the volume. At the foundation of Pol I transcription is an elaborate apparatus containing ten catalytic core and four associated subunits in the mammalian enzyme (Chap. 7). By itself, the enzyme is not really functional; it needs nearly a dozen additional factors for initiation, elongation, and termination to operate at optimal efficiency. A surprising feature of the initiation process is that it is highly dynamic; i.e., the individual components move in and out of the nucleolus very rapidly until they become stabilized in the initiation complex (Dundr et al. 2002). Once the polymerase machinery has been assembled it must rapidly move along the rDNA. Although this process is poorly understood, there are several candidate factors, including chromatin remodeling proteins that clear the path for the polymerase to progress down the template. More intriguing is the finding that the apparent driving force for the movement is the combination of nuclear actin and myosin, which function together as a molecular motor. Because ribosome biogenesis is an energy-intensive process, nature has devised multiple mechanisms to conserve energy, but still meet the needs of the cell. Consequently, the activities of nearly all Pol I transcription factors are altered by posttranslational modifications, which in turn, are regulated by numerous signaling pathways. These are triggered in response to metabolic stress, growth factors, nutrient availability, oncogenesis, and phases of the cell cycle. It is now abundantly clear that the level of ribosome biogenesis does not simply depend on the number of rRNA genes available, but that the rate of transcription is fine-tuned to meet the changing conditions in which the cell finds itself.

The steps taken by pre-rRNA during and after transcription are numerous and complex. They have been reviewed in detail recently by Henras et al. (2008); therefore, they are not covered in depth in this volume. However, it is important to highlight a few salient features of the process. How does pre-rRNA make its way from a very long precursor to the 18S, 5.8S, and 28S rRNAs found in ribosomes? Obviously, nucleases are required to do the job, but what determines their ability to precisely generate the ends of the three ribosomal RNAs? It turns out that a subset of the numerous snoRNAs are essential for cleavage. These are not nucleases themselves, but they seem to serve as chaperones or anchors to recruit processing factors and their associated nucleases to the sites to be cleaved. The best known of these is U3 snoRNA as part of a snoRNP complex, which associates with the nascent transcript during transcription. In addition to being an essential factor for pre-rRNA cleavage, U3 also participates in base pairing that facilitates the accurate formation of a pseudoknot in the 18S rRNA.

For ribosomes to function optimally, ribosomal RNA needs to be posttranscriptionally modified. Approximately, 200 sites are modified in vertebrate rRNA with a combination of base methylation, 2'-O-methylation, and pseudouridylation. These

modifications are believed to stabilize secondary and tertiary structures of the RNA; cell growth and viability are optimal when most or all sites are modified. In Chap. 7, Bleichert and Baserga describe the modification process and the machinery that performs this task. Again, the 2'-*O*-methylation and pseudouridylation, but not the base methylation modifications, are precisely directed by snoRNPs. As the multitude of snoRNAs began to be discovered, researchers were surprised to find so many of them, numbering into the hundreds of unique snoRNAs in some species. Now that we know the number of modifications, their locations and the mechanism by which they take place it is clear why the number of snoRNAs is large. Most of these are well characterized and there are crystallographic structures available for a few of the snoRNP complexes (Reichow et al. 2007). In addition, we are beginning to understand how the proteins of these complexes affect the RNA components, facilitating the positioning of the RNA substrates into the active site of the modifying enzymes (Hamma and Ferré-D'Amaré 2010).

As indicated in Chap. 6, the level of transcription by RNA Pol I is adjusted to the cellular growth rate and is also dependent on the phase of the cell cycle of a given cell. But do alterations in ribosome production also affect the cell cycle? There is now evidence for communication between the ribosome biogenesis apparatus and the cell cycle. Chapter 8 provides us with insights into how ribosome biogenesis is monitored during G1 phase and how this influences the G1/S transition. Several studies show that when ribosome biogenesis components are depleted in yeast, the cells accumulate in the G1 phase, although the molecular mechanisms for this have not been determined. In multicellular organisms, deficiencies in certain ribosomal proteins or in factors required for ribosome assembly cause G1 arrest. For these organisms, the G1 arrest is largely mediated by the p53 response (see Chap. 12 for more details on this topic). Depletion of other factors; e.g., nucleophosmin/NPM or nucleolin, causes defects in progression through mitosis. More importantly, several defects in ribosome biogenesis result in diseases, including those labeled as “ribosomeopathies.” These are now beginning to be understood, but much work is needed before treatment strategies can be developed.

The numerous steps in ribosome biogenesis require a multitude of different proteins. Some of the best characterized of these are the abundant proteins nucleolin, nucleophosmin/NPM/B23, and NOPP140, which are covered in Chaps. 9–11, respectively. A surprising common feature of these proteins is that they contain what might be considered extremes in the distribution of positively and negatively charged regions. The already highly acidic segments are also phosphorylated by kinase CK2, which contributes to their characteristically low isoelectric points (pIs around 5). Another unusual feature is that the positively charged segments are interspersed with basic segments. These proteins are also heavily modified by additional posttranslational modifications too numerous to mention. So, if these polypeptides have structural features in common, are their functions also similar? The answer to this is mixed. Although the sequences of these proteins became available several decades ago, the functions have been difficult to elucidate. The one apparently universal function of these three proteins is that they all have chaperone activities of one form or another. Nucleolin is able to assist in nucleosome assembly and

chromatin remodeling through a kind of chaperone activity. NPM also is capable of aiding in nucleosome assembly and it has characteristics very similar to traditional molecular chaperones. NOPP140 acts as a different kind of chaperone by delivering snoRNPs to the nucleolus. However, chaperoning seems to be only part of what these proteins do. For example, nucleolin is essential for Pol I transcription and its RNA binding activity is needed for ribosome assembly. NPM is a ribonuclease that is essential for cleavage of pre-rRNA and it is also involved with centrosome duplication. NOPP140 is a component of Cajal bodies and is also a transcription factor for RNA Pol II. Thus, these are multifunctional proteins that are utilized for many cellular activities.

Novel Functions of the Nucleolus

In the early 1990s clues began to appear that suggested that the nucleolus did other things besides assemble ribosomes. Researchers were surprised to find proteins and RNAs in the nucleolus that had no apparent function in ribosome biogenesis. This idea has been especially reinforced by proteomic studies, which have revealed that a minority of proteins in the nucleolus are involved with its traditional role (see Chap. 2). The list of new functions for the nucleolus is growing and the nucleolus is now established as “plurifunctional” as proposed by Pederson (1998).

Why do multiple functions not related to ribosome biogenesis cluster in the nucleolus? We have a poor understanding of this but there are a few clues that might point us in the right direction. Organisms have evolved to utilize what is available to them and the nucleolus provides an abundance of molecular machinery of which to take advantage. The most obvious example is one involving spliceosomal RNAs, which traffic through the nucleolus to be modified by 2'-*O*-methylation and pseudouridylation (Lange 2004). The nucleolus contains the enzymes and guide snoRNAs to accomplish that task. In the case of another RNP, the SRP, it is less obvious why assembly is partially performed in the nucleolus (see Chap. 15). Although the SRP is a RNP, there is no evidence that it utilizes ribosome biogenesis components for the assembly process and the SRP RNA is not modified in the way that spliceosomal RNAs are. Furthermore, the SRP components are not found in the same locations as are pre-ribosomal particles. We are left with the presumption that in assembling the SRP, the nucleolus provides a platform that is separate from the rest of the cell. What anchors the SRP components in the nucleolus has not been determined.

The primary mediator of the response to cell stress is the tumor suppressor protein p53, which can trigger either apoptosis or inhibition of cell growth cell cycle arrest when its cellular levels are increased (Ryan et al. 2001). p53 is normally kept at low levels by a continuous cycle of syntheses and degradation. About a decade ago, it was discovered that the nucleolus is the location of a few proteins linked to p53 regulation, operating by some poorly understood mechanism (Zhang and Xiong 2001). An important advance in our understanding of the role of the nucleolus in this process came through the work of Rubbi and Milner (2003) who showed that a

number of agents that cause p53 stabilization also disrupt the nucleolus. This suggested that the nucleolus acts as a general stress sensor for the cell. How it performs this task is not entirely clear, but Chap. 12 provides us with an overview of the machinery involved. A key player is the tumor suppressor ARF, which is primarily a nucleolar protein. ARF is an inhibitor of the ubiquitin ligase, MDM2, which marks p53 for degradation by the proteasomal system. This inhibition of MDM2 appears to take place in the nucleoplasm, so it seems possible that ARF is released from the nucleolus by its disruption, although this remains a debatable issue. There is also an intriguing relationship between ARF and ribosome biogenesis; ARF interacts with NPM/B23, which serves as a ribonuclease for the cleavage of at least one site in pre-rRNA. It is interesting that overexpression of ARF stimulates the degradation of NPM, which would obviously cause a defect in the processing of pre-rRNA. As indicated in Chap. 8, unproductive ribosome synthesis can lead to cell cycle arrest. This illustrates the intricate relationships among the cell stress response, ribosome biogenesis, and the cell cycle.

Other nucleolar proteins aid in controlling cell cycle progression. The most well characterized of these are the proteins belonging to the nucleostemin family (see Chap. 13). The protein was named such because of its enrichment in embryonic stem cells (Tsai and McKay 2005). Nucleostemin (NS) is a major factor in controlling cell cycle progression; low levels of it inhibit, intermediate levels promote, and overexpression inhibits progression. These effects are channeled through the p53 system. NS is a GTP-binding protein, with the GTP-bound form preferring the nucleolar location. Conversely, the GTP-unbound form of NS has a nucleoplasmic location, where it interacts with MDM2. This has a stabilizing effect on MDM2 by preventing its ubiquitylation, which ultimately results in a lower transcriptional activity of p53. The current knowledge of NS reinforces the idea that the nucleolus is not only itself regulated by cell growth and division, but that it actively participates in their control.

The nucleolus seems to need additional nucleoplasmic actors to play supporting roles. One of these is the Cajal body (CB). Because of its proximity to and occasional physical association with the nucleolus, it was originally called the nucleolar accessory body by its discoverer, Santiago Ramón y Cajal in 1903. In the 1960s, when electron microscopists examined the CBs, they found that they were composed of aggregates of tangled threads and named the structure the coiled body. Consequently, the major protein component of CBs was given the name, coilin. However, about 10 years ago the name of the CB was changed to Cajal body to honor its discoverer. Although it has been over a hundred years since this nuclear body was first observed, its functions were poorly understood until recently. As described in Chap. 15 maturation of snoRNPs occurs in the CBs; this is part of the supply chain for providing tools to build ribosomes in the nucleolus. Additionally, there is exchange of some nonribosomal proteins between the CBs and the nucleolus. Finally, the nucleolus and CBs share a similar response to stress, probably as a means of coordinating the levels of ribosome production with the availability of snoRNPs.

We have seen that cells take advantage of the nucleolus for performing a variety of functions not related to ribosome biogenesis. In the same vein, invading organisms utilize the nucleolus for crucial parts of their life cycles. This is especially the case with viruses, many of which have components that locate in the nucleolus (see Chap. 14). Viral proteins of many different types, including those from RNA and DNA viruses can be found in the nucleolus. Because viruses carry limited amounts of genetic information, one can understand why they need to hijack cellular structures and components for replication. However, in many cases, it has yet to be determined what the nucleolar locations of these components do for the virus. Of special importance is HIV-1, which has two proteins that are found in nucleoli of infected cells. One of these is the Rev protein whose function is to facilitate the transport of unspliced or partially spliced HIV-1 mRNA to the cytoplasm. The nucleolar location is essential for that function. The second HIV protein that locates partially in the nucleolus is the Tat protein, which binds the HIV-1 mRNA TAR element. As with Rev, the nucleolar trafficking of Tat is essential for HIV-1 replication. The nucleolar location of these viral components is interesting in itself, but even more appealing is the possibility that the nucleolar machinery can be utilized for treatment of HIV-1 infections (Chap. 17). Rossi and his colleagues have developed ribozymes based on snoRNAs that cleave HIV RNA, which results in inhibition of replication (Unwalla et al. 2008). Taking this approach one step further, Rossi and colleagues used siRNA in a TAR decoy to inhibit viral replication (Unwalla and Rossi 2010). What is more important about these pioneering studies is that they are now being translated into clinical trials (DiGiusto et al. 2010) and they offer hope for the development of new therapeutic modalities.

The Future of the Nucleolus

Does the nucleolus hold more surprises or is the field at a level at which major discoveries will be few and far between? As Niels Bohr once said, "Prediction is very difficult, especially about the future." Thus, we can only speculate about the outlook for new discoveries in this subject. Future directions are discussed in most chapters of this volume, but a few issues should be highlighted and expanded. The first of these deals with mechanism at several levels. Although, the component parts of the ribosome biogenesis process have been defined, we are only beginning to understand how they do what they do. For example, we do not really understand how the transcription machinery is propelled along the template and how this is coordinated with the vectorial process of ribosome assembly. In another example, we have a general idea of how the snoRNPs operate to modify rRNA and to aid in the cleavage of pre-rRNA, but our understanding of the mechanism by which it takes place is limited. Expanding this knowledge will require difficult and painstaking work utilizing genetic engineering, enzymology, more X-ray crystallography of complexes, and possibly technologies that have not yet been invented.

A second issue is related to regulation. Much regulation is at the level of transcription and involves communication with the rest of the cell. This is beginning to be understood, but much more detail is needed. But what about regulation of the ribosome biogenesis process itself? Ribosome assembly requires a precise order and timing of events. How are these controlled? Another issue concerns the feedback of ribosome biogenesis with the cell cycle.

For decades, researchers have been attempting to correlate ultrastructure with function in the nucleolus. One of the puzzling features of nucleolar ultrastructure is the fibrillar center. It appeared later in evolution and is not present in some lower eukaryotes. It contains rDNA and RNA Pol I, but transcription occurs only at its periphery. So what is it and what does it do for the cell? This and other poorly understood ultrastructural questions should be answered in the future. Relating ultrastructure to function can be taken a step further by doing it in three dimensions. Ongoing studies using electron tomography are aimed at understanding the three-dimensional organization of nucleolar components (Tchelidze et al. 2008). We look forward to advances in this area.

Although the potential for surprises in the area of ribosome biogenesis may have reached its apex, the chances for finding more novel functions in the nucleolus remain high. Proteomics has shown us that there are hundreds of proteins of unknown function in the nucleolus; these are likely to keep researchers busy for many years. In addition, the roles of many viral components in the nucleolus will continue to intrigue us and hopefully, move beyond the phenomenology that is now the case with many viral components in the nucleolus. More importantly, there is already evidence that we can take advantage of our knowledge of the nucleolus to develop therapeutic strategies. Hopefully, this approach will be extended to viruses in addition to HIV and to other diseases. We may even see a new era of nucleolar translational medical research.

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Acknowledgments

When I was first introduced to the nucleolus in the late 1960s, the state of knowledge on the subject was such that it could be covered adequately in one book. At that time, Harris Busch and Karel Smetana had just completed such a volume (Busch H, Smetana K (1970) *The nucleolus*. Academic Press, New York). The timing of the publication of the latter volume was important in that it followed the decade in which researchers determined that the nucleolus does, in fact, make pre-ribosomal RNA and that the genes for such are located in the nucleolus organizer regions on chromosomes. This book was also the first comprehensive overview of the subject. The next compilation was published in 1982, when Jordan and Cullis gathered material from a symposium at the 200th meeting of the Society for Experimental Biology in 1980 in Oxford, UK, and assembled it into a volume also entitled *The Nucleolus* (Jordan EG, Cullis CA (1982) *The nucleolus*. Cambridge University Press, New York). The latter book covered research from the previous decade concerning the locations and multiplicity of ribosomal RNA and protein genes, transcription and maturation of pre-rRNA, assembly of pre-ribosomal particles, and regulation of ribosome biogenesis. Another comprehensive volume on the nucleolus (Hadjiolov AA (1985) *The nucleolus and ribosome biogenesis*. Springer, New York) is still a useful reference for many aspects of the nucleolus. A book by Thiry and Goessens focuses on nucleolar ultrastructure and also includes a very useful historical overview, presented in outline form (Thiry M, Goessens G (1996) *The nucleolus during the cell cycle*. R. G. Landes Company, Austin). A more specialized volume on rDNA transcription was compiled in 1998 by Marvin Paule (Paule MR (ed) (1998) *Transcription of ribosomal RNA genes by eukaryotic RNA polymerase I*. R.G. Landes Company, Georgetown). Each year, several reviews provide updates on general aspects of the nucleolus as well as one with specialized topics within the subject area. Many of these are cited in the chapters of this volume.

The latest attempt at comprehensive coverage of the field was a book edited by the editor of the current volume (Olson MOJ (ed) (2004) *The nucleolus*. R.G. Landes Company, Georgetown). However, the body of knowledge has exploded to

the point where several volumes would be needed to cover all topics of this field. Therefore, the current collection of chapters focuses on well-established components, systems, and mechanisms operating in the nucleolus. These are covered in greater depth than in previous compilations. This volume is published under the umbrella of *Protein Reviews*; thus, it is reasonable to expect that the emphasis would be on nucleolar proteins, especially those that have been studied extensively. This is not to diminish the crucial importance of nucleic acids, which are covered indirectly in various chapters. A guiding theme of this volume is that the structure and function of the nucleolus ultimately is the result of individual macromolecules interacting with other macromolecules. Thus, we have included chapters on nucleolar ultrastructure, the locations and dynamics of nucleolar components, and on regulatory systems. Because of recent discoveries on novel functions of the nucleolus, about a third of the book is devoted to that topic.

Having worked in this field for about four decades, it has been a pleasure to see it mature to where it stands today. The subject has always been intriguing, but it is even more so now and full of surprises. Therefore, I thank Professor M. Z. Atassi, the Editor of this series, for giving me the opportunity to compile and edit this volume. I also thank all of the authors for their enthusiasm and cooperation in submitting their chapters. I have made numerous friends in this field over the years, too many to name individually; I dedicate this book to all of them.

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Part I
Nucleolar Structure and Organization

Chapter 1

Structural Organization of the Nucleolus as a Consequence of the Dynamics of Ribosome Biogenesis

Danièle Hernandez-Verdun

Abbreviations

CDKs	Cyclin dependent kinases
CK2	Casein kinase 2
DFC	Dense fibrillar component
DRB	5,6-Dichloro-1-ribo-furanosylbenzimidazole
EM	Electron microscopy
FC	Fibrillar center
FRET	Fluorescence resonance energy transfer
GC	Granular component
GFC	Giant FC
NADs	Nucleolus-associated chromatin domains
NDF	Nucleolar derived foci
NOR	Nucleolar organizing region
NPM	Nucleophosmin
NS	Nucleostemin
PAGFP	Photoactivatable GFP
PNB	Preruleolar body
PNC	Perinucleolar compartment
PtK1	Potorous tridactylis kidney
Pol I	RNA polymerase I
rDNA	Ribosomal gene
rRNA	Ribosomal RNA
rProtein	Ribosomal protein
RNP	Ribonucleoprotein

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snoRNP	Small nucleolar RNP
SUMO	Small ubiquitin-like modifier
TIP5	TTF1-interacting protein-5
UBF	Upstream binding factor

1.1 Introduction

The nucleolus is the most prominent visible structure in the nucleus of all eukaryotic cells. Cytologists consequently described it even before it was known to be the site of ribosome biogenesis in eukaryotic cells (Montgomery 1898). Each cell possesses at least one nucleolus that reflects the state of activity or differentiation of that particular cell. It was proposed that the nucleolus is “an organelle formed by the act of building a ribosome” (Mélèse and Xue 1995). Indeed, the size of the nucleolus depends on the level of ribosome production (Smetana and Busch 1974) and the molecular processes occurring in this organelle determine the structural organization of the nucleolus (Hadjiolov 1985). By electron microscopy (EM), this membraneless organelle presents a structural compartmentation corresponding to the major steps of ribosome biogenesis. This compartmentation has been described in higher eukaryotes as composed of three basic “building blocks”: the fibrillar center (FC), the dense fibrillar component (DFC) and the granular component (GC) corresponding to the major steps of the biogenesis of the two ribosome subunits (see below for details) (Goessens 1984; Hernandez-Verdun 1986; Jordan 1991; Mosgoeller 2004). The nucleolus constitutes a model to understand the principles of the organization of the nuclear domains; the dynamics of assembly of these domains after mitosis; and the relationship between nuclear bodies dedicated to related functions, in particular the Cajal body, the PML (promyelocytic leukaemia) body, and the nuclear speckles (for definitions see (Spector 2001)).

The region of the chromosome that carries the ribosomal genes (rDNA) was designated the nucleolar-organizing region (NOR) because the formation of the nucleolus was associated with a particular chromosome translocation in *Zea mays* (McClintock 1934). This hypothesis was verified in an anucleolated *Xenopus laevis* mutant in which rRNAs are not synthesized (Brown and Gurdon 1964). The number of NOR-bearing chromosomes varies in different species, from one chromosome to several chromosome pairs. In humans, the acrocentric chromosomes 13, 14, 15, 21, and 22 are the NOR-bearing chromosomes (Henderson et al. 1972). In haploid budding yeast (*Saccharomyces cerevisiae*), the NOR-bearing chromosome is chromosome XII. In dividing eukaryotes, the nucleoli assemble at the exit from mitosis; they remain functionally active throughout interphase, and disassemble at the beginning of mitosis. Ribosome production varies between G1/S/G2 interphase periods, being maximal in G2 (Sirri et al. 1997, 2000b). Ribosome biogenesis is a multistep process including ribosomal RNA (rRNA) synthesis, modification, and processing, and rRNA assembly with most of the ribosomal proteins (rproteins). The rDNA are repeated genes organized in tandem (Miller and Beatty 1969).