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Nucleolar Adaptation in Human Cancer

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Abstract

While the nucleolus was first observed over two hundred years ago, its role in human cancers is only now being appreciated. Long thought to be a static, ribosome-producing, subnuclear organelle, recent investigations have shown a more dynamic and adaptable side of the nucleolus. Containing not only proteins for the production of ribosomes but also newfound nucleolar oncogenes and tumor suppressors, mechanistic links between the nucleolus and cancer are now more evident. In this regard, much of the work from the past decade has focused on the ability of these proteins to promote and suppress tumorigenesis from the nucleolus. In this review, we will discuss how historical measurements of the nucleolus are being translated into contemporary studies of nucleolar dysfunction in human cancer.

Keywords

Nucleolus; Tumor suppressor; ARF; Cell cycle; Ribosome biogenesis

INTRODUCTION

A Brief History of the Nucleolus

The nucleolus is a unique cellular organelle rich in history, with its origin of discovery in 1781 by Fontana who noted its occurrence in the slime of an eel and reported it as a simple afterthought in his treatise on the venom of vipers.[1] To date, the most exhausting review of the nucleolus came in 1898 when Montgomery published a 300-page review in the *Journal of Morphology* containing over 700 references of the work that had been done in the 100 years following Fontana's discovery.[2] The Montgomery review became the basis for most of the research focused on nucleolar function throughout the early twentieth century. Montgomery himself had studied nucleoli in the oocytes of over 175 different species and had arrived at three remarkable conclusions that still hold true today: 1) There is no constant number of nucleoli per cell; 2) cells that are actively growing typically have more and larger nucleoli; and 3) generally, the larger the cell the larger its nucleolus.[2] However, the most noteworthy scientific contribution would come some 30 years later when two prominent scientists, Emil Heitz and Barbara McClintock, would begin work that would define their careers and forever change the way we think about the nucleolus.

The early period of the twentieth century was a fascinating time for cell biologists, with the study of the nucleolus and its relation to chromosomes serving as the focal point for much of the research of this era. In fact, the genetecist Eduard Zacharias came close to defining the role of nucleic acids and their relation to cell growth in 1883, only to be challenged by histochemists.[3] They concluded that his combinations of dyes used to visualize chromosomes

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were too nonspecific, and forced the scientific community to wait nearly 50 years to appreciate the role of nucleic acids in cell growth. However, on the basis of these methods, Zacharias correctly concluded that nucleoli and chromosomes were distinct substances, albeit ones that localized in overlapping subcompartments of the nucleus, and sparked renewed enthusiasm into nucleolar investigations. Building on the cytogenetic methods of Zacharias, Emil Heitz took an imaginative approach to studying the formation of nucleoli with relation to chromosome location. Using **s**ine **a**cid **t**hymonucleinico (SAT), he identified chromosome satellites that correlated with the positions of nucleoli, and later punning his staining technique and **sat**ellites, called these chromosomal regions SATs.[4] Using this technique, Heitz followed the formation of nucleoli around these SATs during the early phases of the mitotic cycle, providing the first evidence of nucleolar organization. This novel finding was translated further by the work of Barbara McClintock who, using the maize plant, was studying x-ray-induced chromosomal rearrangements. In a decisive piece of work, McClintock was able to show that the maize nucleolus was organized around a specific SAT of chromosome 6 and that when this region was broken in two through rearrangement, both rearranged chromosomal segments could give rise to separate nucleoli.[5] McClintock termed the locus the ‘‘nucleolar organizer,’’ and as her analysis was rapidly transferred to animals, the phrase was modified to ‘‘**n**ucleolar **o**rganizer **r**egions’’ or ‘‘NORs.’’ The 70 years since her discovery have been occupied with the ventures of many scientists into the understanding of nucleolar function. In this review, we will highlight their accomplishments and explore the ever-evolving involvement of the nucleolus in cell growth.

Structural Components

In a cellular organelle that is not bound by a membrane, the nucleolus is organized around the 5 pairs of chromosomes that contain the ribosomal RNA genes (analogous to McClintock’s NORs), the ribosomal RNA (rRNA) processing machinery (over 100 proteins), and the rRNA itself (Figure 1A).[6] Using electron microscopy, the nucleolus is shown to have 3 distinct regions (Figure 1B and C).[7] The contiguous granular region covers the largest part of the nucleolus and contains maturing ribosomes while the dense fibrillar component (Figure 1C, blue) and the fibrillar centers (Figure 1C, red) contain sites of actively transcribed rRNA genes and nontranscribed rRNA genes, respectively.

As a cell approaches prophase and chromosomes begin to condense, the nucleolus begins to dissociate into small nucleolar bodies consisting of numerous nucleolar proteins such as RNA polymerase I, topoisomerase I, and nucleo-lin.[8–10] Metaphase marks the completion of nucleolar dissociation, leaving solitary NORs located at the ribosomal RNA genes on 5 pairs of chromosomes.[11] With the onset of telophase, the nucleolus begins to reform at NORs, a process requiring active transcription of rRNA genes.[12–16] As the cell progresses through the ensuing G1 phase of the cell cycle, the NORs begin to coalesce into coherent nucleoli.[17] As first described by Montgomery,[2] the size, shape, and number of nucleoli can vary from cell type to cell type[18] and, as discussed below, all of these nucleolar criteria can be indicators of proliferation and tumorigenesis.

AgNOR AS A CLINICAL MARKER FOR CANCER

By the early 1970s, histologists began standardizing nucleolar morphology measurements, eventually agreeing on a method that utilized a novel property of several nucleolar proteins. The technique exploited the finding that a handful of nucleolar proteins, when properly fixed, actively bound to silver salts.[19] As the proteins themselves could not precipitate the silver, the interactions were readily visualized with the addition of reducing agents. These highly acidic nucleolar proteins were termed argyrophilic proteins (Greek, meaning ‘‘silver loving’’) and were found to associate with NORs throughout the cell cycle, making them an accurate measure of nucleolar structures.[19] Silver-stained N ORs, known as AgNORs (Ag is the

symbol for silver element) have been shown to localize to both the fibrillar and granular components of the interphase nucleolus.[20,21] Nucleophosmin (also known as B23 or numatrin), nucleolin (C23), UBF, and subunits of RNA polymerase I were all initially localized to AgNORs and, subsequently, found to be the argyrophilic proteins responsible for the silver-staining properties of nucleolar structures.[22,23] Recalling from the work of Montgomery, that rapidly growing cells typically exhibited larger and more enumerated nucleoli, cancer biologists quickly made the conceptual connection between AgNOR staining and cell proliferation.[24]

Although AgNOR staining was first described in 1975,[19] it only has been in the past 10 years that a standardized procedure has been defined and agreed upon in the cancer biology community.[25] In fact, entire scientific conferences in the mid-1990s were devoted to the debate of a unified AgNOR staining technique. As reviewed by Treré,[26] there are several factors, such as fixative used, and staining time and temperature, that can influence the size, intensity, and appearance of AgNORs in any given cell. In a study examining these parameters on the same tumor tissue, Derenzi and Treré[27] were able to show that AgNOR staining results varied greatly depending on the technique employed, underscoring the misinterpretation of early studies reporting the ability of AgNOR staining to predict tumor pathology. In addition to the staining techniques employed, the manner in which AgNORs are quantified also has been in flux until recently.[28] While counting AgNORs in several fields at high (100–1000×) magnification is widely used, the most reproducible process is the morphometric method.[29] This computational technique utilizes image analysis software to evaluate AgNOR morphology from CCD camera-captured images.[30,31] Employing this method to examine 40 breast carcinomas, a 2-fold increase in reproducibility of the morphometric method over counting was established.[29] Combining the morphometric method with the standardizing of staining techniques, AgNOR analysis can be a powerful prognostic marker of tumor pathology.

Pich and colleagues[32] undertook a multivariate analysis to assess the ability of AgNOR to predict tumor pathology, with the hypothesis that a more independent AgNOR variable would provide a greater prognostic value. Indeed, when comparing AgNOR to other prognostic indicators such as age of the patient, tumor grade, and tumor cell mass (and numerous others), AgNOR was consistently the best indicator of survival for pharyngeal carcinoma, multiple myeloma, male breast cancer, and prostate carcinoma. Pich also reasoned that the independence of AgNOR staining from other variables could be used to predict patient responses to chemotherapeutic agents. Again, AgNOR staining was the best indicator for response to treatment in adult patients with acute myelogenous leukemia. Moving a step further, Pich verified that AgNOR staining could be used as an effective independent prognostic factor in prospective studies. Not surprisingly, increases in AgNOR staining correlates well with other disease prognostic markers such as increased DNA content in breast cancer,[30,33,34] bladder cancer,[35] and ovarian cancer,[36] as well as markers of proliferation including BrdU staining, [37–39] tritiated thymidine uptake,[24] and percentage of S-phase cells.[40,41] The list of AgNOR quantified cancers is nearly exhaustive, as Pich has noted,[32] with the common theme being the invaluable definitive measure of tumor pathology provided by proper AgNOR staining and quantification. However, mechanistic insight into why there is a correlation between nucleolar staining and aberrant cell proliferation has remained elusive.

RIBOSOMES: TRANSLATIONAL CONTROL IN CANCER

The Ribosome Machinery

As described in the 1960s, the nucleolus is the center of ribosome biogenesis, containing much of the machinery required for protein synthesis in the cell. [42,43] An increase in AgNOR score correlates well with hallmarks of tumorigenic growth, including an increase in protein translation.[44] However, the question of cause and effect has been poorly understood in this

context. Are changes in nucleolar structure and function a result of tumorigenesis or a cause of it? While the simple answer for decades was the former, new findings are causing many to revisit this age-old question. [45]

Transcriptional control has always been thought of as the primary means of controlling the cellular proteome. In particular, oncogenic factors stimulate cell cycle progression through the induction of cyclins, largely through increased transcription, and the activities of their catalytic cyclin-dependent kinase (CDK) partners.[46] However, more recent data have shown that in both tumorigenic growth and development, cells can respond to external stimuli through a change in the profile of mRNAs being translated.[47–51] This type of molecular switch is a very robust and efficient way of altering the proteome of a cell. Instead of using all its resources to activate gene transcription—which does happen—the cell can achieve a quick change in protein production by altering the translation of a selective subset of mRNAs. While the mechanism behind this process is still being defined, there are specific mRNAs that are preferentially translated in response to oncogenic stimulation.[52] To accomplish this specificity, individual mRNAs encoding ribosomal proteins contain a terminal oligopyrimidines tract (TOP) in the 5′ untranslated region (UTR) allowing preferential translation in response to growth signals.[47–49,53] Also using ribonomics, a combination of im-munoprecipitation of mRNA binding proteins, and expression array analysis, a shift in the translated pool of mRNAs has been demonstrated in response to growth stimuli.[50,51,54,55] This leads to the intriguing possibility that altered ribosomal protein control can lead to a tumorigenic phenotype.

To this end, there have been ribosomal proteins that have been shown to play a direct role in tumorigenesis.[56] Germline mutation of DKC1, the gene altered in Dyskeratosis congenita, has a direct affect on ribosome assembly, and in humans has been associated with an increased risk of cancer.[57,58] A psuedouridine synthase, DKC1 mediates the posttranscrip-tional conversion of uridine to psuedouridine, which is required for proper rRNA folding and eventual ribosome biogenesis. Thus, an altered ribosome could alter the mRNA pool that is translated, preferentially increasing the translation of oncoproteins or decreasing the translation of tumor suppressing proteins. In either case, experiments supporting or disproving these possibilities are lacking. However, in mouse models of Dyskeratosis congenita, the fact that over 50 percent of mutant mice develop tumors during their life span[57] points to a more direct role of the ribosome in tumorigenesis than previously thought. Additionally, the small ribosomal subunit protein S19 has been shown to be mutated in Diamond-Blackfan anemia, a condition associated with an increased susceptibility to haematopoetic malignancies.[59] While the direct mechanism of S19-induced tumorigenesis is not known, its mutation once again provides direct evidence of a ribosomal defect promoting cancer susceptibility.[60] Several primary tumors, including leukemias as well as tumors of the liver and colon, have been shown to overexpress numerous large and small subunit ribosomal proteins,[61–68] but unlike mutations in DKC1 and S19, it is not clear if these other ribosomal protein alterations are a cause or result of tumorigenesis. Clearly there is a link between ribosome biogenesis and cancer; however, more studies are required to delineate the mechanisms behind alteration of the ribosome and uncontrolled cell proliferation.

Signaling to the Ribosome

In addition to the dysregulation of ribosome biogenesis, aberrant modifications in the nonribosomal proteins that regulate translation have been shown to lead to cancer. One of these proteins, the mammalian target of rapamycin (mTOR), is currently the target of several anticancer therapies.[69–71] Initially described as the cellular sensor for nutrient availability, mTOR activates protein translation through several downstream targets.[72] First, mTOR can activate the translation initiation factor eIF4E, increasing global mRNA translation in the

cell.[73] Overexpression of the translation initiation factor eIF4E has been found in several cancers including those of the esophagus, neck, and breast.[74] Second, mTOR can increase overall translation through the increased production of ribosomal proteins themselves.[52] The translation elongation factor, eEF1A2, a preferential mTOR target, has been shown to be amplified in primary ovarian tumors and, consistent with the idea that its amplification may be tumorigenic, overexpression of eEF1A2 in fibroblast cell lines promotes their ability to form tumors in nude mice.[75] Negative regulators of mTOR, TSC1/2 (tuberous sclerosis complex 1/2) and PTEN (phosphatase with tensin homolog deleted from chromosome 10) are bona fide tumor suppressors, with their loss occurring frequently in numerous human cancers.[76–78] While we are only beginning to understand the many connections of ribosome synthesis to tumorigenesis, the identification of oncoproteins and tumor suppressors that regulate these processes provides us with a foundation of basic mechanisms.

TUMOR SUPPRESSION IN THE NUCLEOLUS

The Nucleolar ARF-Mdm2-p53 Feedback Loop

The human INK4a/ARF locus, encoding both the $p16^{INK4a}$ and $p14^{ARF}$ tumor suppressors, exhibits an unparalleled efficiency of organization within a mammalian genome. Specifically, $p16^{INK4a}$ (**In**hibitor of CD**K4**) and $p14^{ARF}$ contain distinct promoters and first exons, yet splice into a shared second exon that is translated in **a**lternative **r**eading **f**rames (ARF).[79,80] While both proteins clearly contribute to tumor surveillance in mice and humans, they appear to play coordinate yet independent roles within the cell cycle. In human cancers, the frequency of INK4a/ARF loss is second only to mutation of *p53*, providing critical evidence of this locus' role in preventing tumorigenesis.[81,82] While much controversy exists over which protein, $p16^{INK4a}$ or ARF, is more important in preventing human cancer, there are enough instances of their independent loss of function during tumor formation to indicate that both are extremely important.[83–86] While ARF levels are nearly undetectable in normal tissues,[87–91] oncogenic signals, such as those emanating from the Ras and myc oncoproteins, result in dramatic increases in ARF protein levels within the nucleolus.[87,92,93] In this manner, ARF is a critical sensor of hyperproliferative signals. ARF accumulation in the nucleolus inhibits cell cycle progression through a direct interaction with the *p53*-ubiquitin ligase, Mdm2 oncoprotein (originally isolated from mouse tumors on amplified **m**ouse **d**ouble **m**inute chromosomes).[93,94] The ARF-Mdm2 interaction is quite unique in that one of the Mdm2 binding domains within ARF is its own nucleolar localization signal.[95] Upon binding to Mdm2, a dramatic conformational change reveals a cryptic nucleolar localization signal embedded within Mdm2, mobilizing and sequestering the ARF-Mdm2 complex in the nucleolus.[95–97] This results in the nucleoplasmic stabilization of the *p53* tumor suppressor, allowing *p53* to induce the expression of downstream negative regulators of proliferation, such as $p21^{CIP1}$.[98,99]

Recent evidence supports the idea that ARF also can act independent of *p53* to prevent tumorigenesis. In mice lacking only *p53*, T-cell lymphomas dominate the tumor spectrum (approx. 70 percent).[100] However, in ARF-null mice, lymphomas make up only 25 percent of tumors, while the majority of tumors consist of sarcomas (approx. 50 percent).[98,101] When crossed, p53/ARF double-null animals develop multiple tumor types and often develop multiple primary tumors,[102] arguing against a strictly linear ARF-*p53* pathway with the potential of revealing other ARF targets. Again, if the ARF-*p53* pathway is strictly linear, then tumor analysis should provide an inverse correlation between loss of ARF and *p53* mutation (an either/or setting), a relationship that has not been borne out in clinical human tumor studies.[103–107] In addition to the genetic data, cells devoid of *p53* and Mdm2 are susceptible to growth arrest by ARF induction in response to hyperproliferative signals.[108] These data indicate that while ARF can regulate the cell cycle through *p53*, there exists a *p53*-independent arm of the ARF pathway.

As a prelude to identifying *p53*-independent ARF targets, Charles Sherr and Martine Roussel demonstrated that nucleolar ARF also can exert its growth inhibitory effects through the inhibition of ribosomal RNA maturation,[109] an important finding given that cell cycle arrest has been shown to reduce rRNA synthesis and protein translation.[110,111] These data tie ARF's tumor suppressive capabilities to its topological location in the nucleolus and begin to point work on this novel tumor suppressor back to the early hypotheses of Montgomery, Heitz, and McClintock.

The ARF-NPM Network

Nucleophosmin (NPM), an abundant nucleolar phosphoprotein that has been shown to be a requirement for rRNA processing,[112,113] is one of the argyrophilic proteins of AgNORs.[114] In addition to its proposed role in ribosome biogenesis, recent studies have shown that NPM is phosphorylated by the cyclin E-cdk2 holoenzyme and that this modification of NPM is required for centrosome duplication and subsequent DNA replication.[115] NPM is a potent oncogene[116] and has been shown to be a major target for chromosomal translocation in acute myeloid leukemias, forming oncogenic fusion proteins with ALK, MLK1, and RARα.[117–122]

Providing a direct link to historical nucleolar function, recent reports from Sherr and Roussel as well as Yanping Zhang and our own lab have shown that NPM and ARF physically interact within the nucleolus.[123–125] While rRNA processing is negatively affected by the formation of ARF-NPM complexes within the nucleolus, additional data indicate that NPM nucleocytoplasmic shuttling is key to its ability to promote cell proliferation.[125] What we do know is that ARF interacts with a major argyrophilic nucleolar protein to prevent ribosome production and tumorigenesis, again underscoring the oncogenic potential of the nucleolus. Without an intact ARF checkpoint, nucleolar proteins, such as NPM, might adapt to their newfound freedom, promoting tumorigenesis through their numerous nucleolar functions. It appears that the cell has adopted a defense mechanism against unwarranted nucleolar activity and that the tumor suppressive role of ARF is to prevent the very nucleolar morphology first observed by Montgomery in rapidly proliferating cells. In keeping with this modus operandi, cells devoid of ARF adapt altered nucleolar morphology (Figure 2), one that concurs with Montgomery, committing cells to increased ribosome and subsequent protein production as they continue down the road to tumorigenesis.

ADAPTATIONS OF THE NUCLEOLUS

Proteomics

To gain insights into the link between tumorigenesis and nucleolar function, a molecular blueprint of the nucleolus is required. Angus Lamond recently provided the scientific community with a nucleolar proteome, a complete set of expressed proteins.[126,127] From purified nucleoli, Lamond extracted and identified 350 proteins that reside in tnucleolus of HeLa tumor cells with up to an additional 130 proteins suggested by later observations of others.[127] In just a short year, the number of nucleolar proteins identified has increased to 692 aided by refinements in mass spectrometry sensitivity.[128] A remarkable number of nucleolar proteins, all positively identified by Lamond, have been conserved from yeast to man (Figure 3), suggesting that the nucleolus has maintained a conserved function throughout evolution. Analysis of the nucleolar differences between species could reveal which proteins were added to the nucleolar proteome as the organelle adopted new properties needed for increased regulation in higher eukaryotes. Specifically, many of proteins that reside in the nucleolus throughout the cell cycle (residents) tend to mobilize proteins that normally reside outside the nucleolus (drifters) to the nucleolus. This phenomenon of nucleolar mobilization

or sequestration is most frequently seen in mammalian cells and could represent an evolutionary step in nucleolar function.

Of the 121 human proteins previously identified by biochemical and immunoflourescent techniques to be localized to the nucleolus, over 90 percent were identified multiple times by mass spectrometric analysis of isolated nucleoli. [126] In addition, approximately 31 percent of the identified proteins in the analysis were of unknown function,[127] leaving many to wonder what these nearly 110 novel proteins are actually doing in the nucleolus. Interestingly, the authors were able to detect a subset of proteins that localized to the nucleolus only after inhibition of RNA synthesis through actinomycin D treatment,[126] indicating the dynamic nature of the nucleolus. The only caveat to these experiments is that they were performed in a tumorigenic cell line[126,128,129] and it will be of increasing importance to determine if the proteome of a nontumorigenic cell's nucleolus is vastly different from Lamond's tumor cell proteome. The fact that the present nucleolar proteome consists of nearly 110 proteins of unknown function illustrates how little currently is understood of its biological function.

A Sensor of Stress

While the nucleolus has been shown to be the center of ribosome biogenesis with the cell, expending between 3 0 and 50 percent of its transcriptional activity on rRNA genes,[130] several lines of evidence suggest that the nucleolus is an integrating center of various signals that direct cellular fate. The *p53* tumor suppressor protein is the primary responder to cellular stresses, such as irradiation, hypoxia, transcriptional inhibition, depletion of nucleotides, viral infection, heat shock, and oncogenic signaling.[131,132] While it is clear that *p53* exerts its tumor suppressive properties in response to these damaging events, there is no central theme as to how these perturbations are directly relayed to *p53*. One hypothesis offered by Jo Milner,[133] and later reviewed by Karen Vousden,[134] is that the henucleolus provides a sensitive integration point for different cellular stresses. In response to cellular stress, the nucleolus dissociates, leaving only NORs and scattered nucleolar proteins throughout the nucleoplasm.[133] Importantly, Milner showed that this nucleolar disruption occurred separate from DNA damage and was required for induction of the *p53* response as induction of DNA damage without nucleolar disruption did not activate *p53*. Interestingly, this model also explains why *p53* levels fluctuate throughout the cell cycle. When the nucleolus is present and rRNA synthesis is at its peak, *p53* levels are low; however, when the nucleolus dissociates in mitosis, *p53* levels begin to increase.[135] Further proof of this concept was observed in cells treated with the mitotic inhibitor nocodazole, which induced a *p53* response correlating with the prevention of nucleoli from reforming,[132] a finding that reflects the dynamics and regulation of nucleolar organization described by McClintock nearly 70 years earlier.

Understanding how stress signals are interpreted by the nucleolus and which proteins are involved in this process will be central to determining how the nucleolus adapts to stress and subsequent cell cycle arrest or transformation. One potential protein, BOP1 (**B**lock **o**f **P**roliferation 1) was first identified through a cDNA screen designed to isolate growth suppressors.[136] However, the clone identified was only a partial cDNA known as BOP1 Δ. which acts as a dominant negative mutant inhibiting BOP1's proproliferative actions.[136] BOP1 expression is regulated by the cell cycle and peaks at mid G1 phase,[137] concomitant with increased nucleolar function. It is localized to the granular region of the nucleolus and has been shown to play a positive role in rRNA synthesis.[138] Interestingly, inhibition of normal BOP1 function, through overexpression of BOP1Δ, triggers a *p53* response, suggesting that BOP1 might be a potential nucleolar integrator of stress signals.[137] Moreover, inhibition of *p53* function results in the attenuation of cell cycle arrest induced by BOP1Δ.[139] Further identification of nucleolar proteins involved in sensing cellular stress will shed light on the

way in which the nucleolus not only adapt to variant signals within the cell, but also relay these messages to proper downstream targets in (ARF) and out (*p53*) of the nucleolus.

CONCLUSIONS

Initially described as an organelle contained within specific chromosomal regions, the nucleolus has advanced through the past century imparting much knowledge about cell biology and cancer along the way. Once hindered with the label of being the static center of ribosome biogenesis, a newfound appreciation for this uniquely visible organelle has been established in recent years. With the adoption of AgNOR staining as a reliable marker for tumorigenic growth and a prognostic indicator for cancer patient response to therapy and survival, the nucleolus successfully has bridged clinical and basic science. A home for not only the ribosomal synthesis machinery but also tumor suppressors and oncogenes, the nucleolus is a monitor of cellular well being, consistently adapting to cellular stress through not only ribosome production and subsequent protein translation but also through activation of downstream growth suppressors such as ARF and *p53*. Nucleolar adaptation may play an important role in tumorigenesis with several new studies providing glimpses of nucleolar influences in cancer biology and what will surely develop into a burgeoning area of scientific research.

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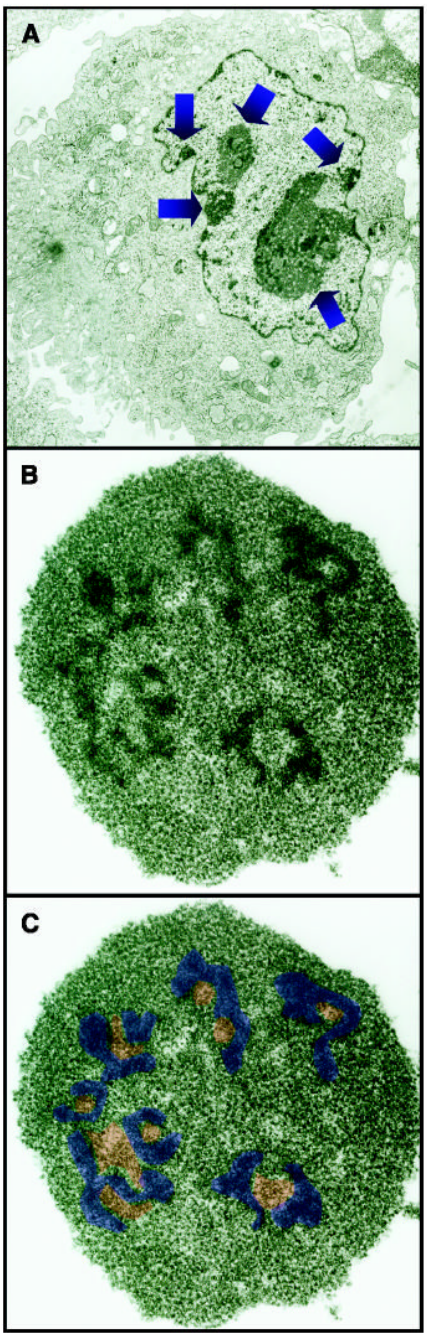

FIG. 1.

Electron micrograph (EM) of the nucleolus in a cell. A) EM of a primary mouse embryo fibroblast (MEF) depicting several nucleoli of various morphologies (blue arrows) contained within a single nucleolus. B) EM of a single nucleolus isolated from a primary MEF displaying varying grades of density. C) Coloring of the EM shown in (B) with the dense fibrillar component shown in blue (containing actively transcribed rRNA genes) and the fibrillar center shown in red (containing nontranscribed rRNA genes). Surrounding both is the contiguous granular region of the nucleolus which contains the growth promoters NPM and BOP1 as well as the ARF tumor suppressor.

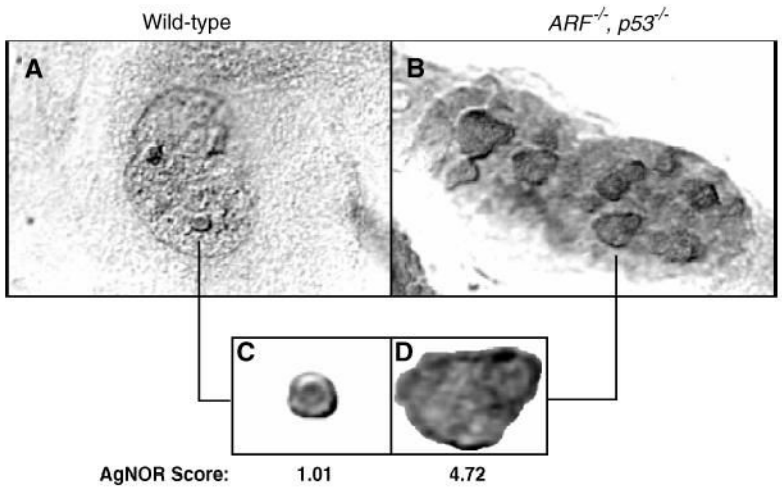

FIG. 2.

AgNOR staining depicting nucleolar adaptation. A,C) Wild-type MEFs that retain a normal ARF tumor suppressive response exhibit low AgNOR staining indicating nucleolar conformity. B,D) Loss of the ARF and *p53* tumor suppressors results in significant increases in AgNOR staining and a marked increase in overall AgNOR score (using the morphometric method). Nucleoli are large and irregular in shape and tend to bind greater amounts of silver salts. MEFs lacking ARF and *p53* proliferate faster than their wild-type counterparts, but are not tumorigenic, indicating an adaptation of the nucleolus prior to tumorigenesis.

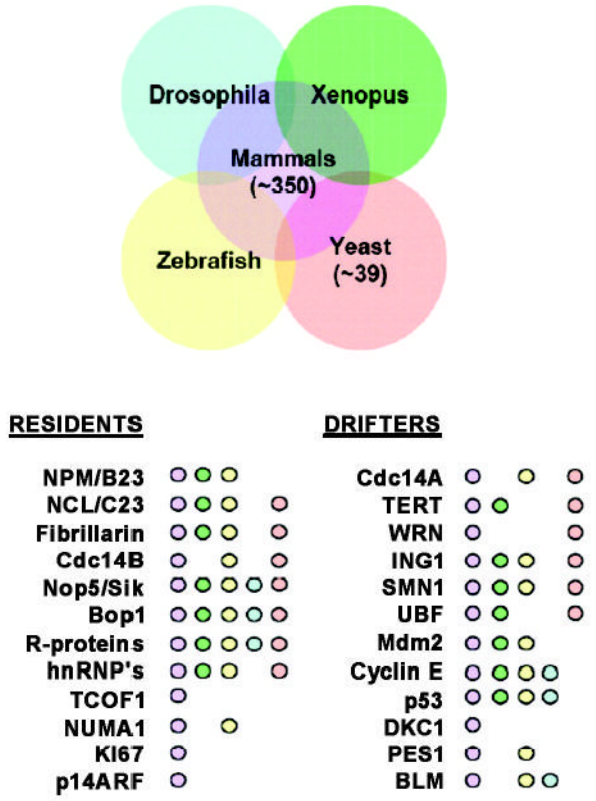

FIG. 3.

Evolutionary conserved components of the nucleolus. Resident nucleolar proteins are found in the nucleolus of all interphase cells, while drifters move in and out of the nucleolus in a dynamic fashion in response to various cellular signals. Residents contained in higher eukaryotes, such as ARF, actively recruit and sequester protein drifters into the nucleolus. This process typically is observed in mammalian cells and not in lower organisms and may account for some of the proteome additions acquired by mammalian nucleoli.