# Molecular dissection of nucleolin's role in growth and cell proliferation: new insights

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ABSTRACT **Cells require optimum protein synthetic activity in order to support cell proliferation, maintain homeostatic and metabolic integrity, and repair damage. Since growth depends on protein synthesis through ribosome biogenesis, the control of biosynthesis of ribosomes is necessarily a key element for control of growth. Nucleolin is a major nucleolar protein of exponentially growing eukaryotic cells, which is directly involved in the regulation of ribosome biogenesis and maturation. The highly conserved nucleolin contains three major domains through which it controls the organization of nucleolar chromatin, packaging of pre-RNA, rDNA transcription, and ribosome assembly. Numerous reports have implicated the involvement of nucleolin either directly or indirectly in the regulation of cell proliferation and growth, cytokinesis, replication, embryogenesis, and nucleogenesis. Nucleolin, an RNA binding protein, is also an autoantigen, a transcriptional repressor, and a switch region targeting factor. In addition, nucleolin exhibits autodegradation, DNA and RNA helicase activities, and DNA-dependent ATPase activity. An interesting aspect of nucleolin action is that it is a target for regulation by proteolysis, methylation, ADP-ribosylation, and phosphorylation by CKII, cdc2, PKC-ξ, cyclic AMP-dependent protein kinase, and ecto-protein kinase. For these and other reasons, nucleolin is fundamental to the survival and proliferation of cells. Considerable progress has been made in recent years with the identification of new nucleolin binding proteins that may mediate these many nucleolin-dependent functions. Nucleolin also functions as a cell surface receptor, where it acts as a shuttling protein between cytoplasm and nucleus, and thus can even provide a mechanism for extracellular regulation of nuclear events. Exploration of the regulation of this multifaceted protein in a remarkable number of diverse functions is challenging.—Srivastava, M., Pollard, H. B. Molecular dissection of nucleolin's role in growth and cell proliferation: new insights. *FASEB J.* 13, 1911–1922 (1999)**

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NUCLEOLI OF RAPIDLY dividing cells are functionally hyperactive compared to nondividing cells, and increases in nucleolar activities, such as ribosome biogenesis, are prerequisites for cell proliferation (1). Certain nucleolar components appear to coordinate the phenomena of dynamic changes that occur in the nucleolar structure between interphase and the mitotic phase of the cell cycle (2, 3). Among these candidate nucleolar components, nucleolin, with a unique multiple domain structure, plays a fascinating and fundamental role in linking nucleolar activities to cell proliferation and mitotic dynamics.

Nucleolin is a ubiquitous, nonhistone nucleolar phosphoprotein of exponentially growing eukaryotic cells and is present in abundance at the dense fibrillar and granular regions of nucleolus (4, 5). Intact nucleolin is the major species and represents 5% of nucleolar protein in actively dividing cells. In nondividing cells, degraded forms of various molecular size are predominantly expressed due to autodegradation (6–9). The first comprehensive review of nucleolin was published more than 10 years ago and focused on the problem of ribosomal RNA transcription, maturation, and assembly (10) principally because the expression of this major nucleolar phosphoprotein was directly correlated with ribosomal DNA (rDNA) transcription (10–14). The focus of nucleolin research has widened to include chromatin decondensation (15), cytoplasmic nucleolar transport of ribosomal components and preribosomal particles (12), and nucleogenesis (14). In particular, nucleolin has been shown to be a component of B cell-specific transcription factor (16, 17), an autoantigen (18, 19), a DNA/RNA helicase (20), DNA-dependent ATPase (21), and a transcriptional repressor (22). The protein would therefore appear to be involved in fundamental aspects of transcriptional regulation, cell proliferation, and growth. Indeed, an increasingly complicated literature suggests that these new functions are just the tip of the

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iceberg; this review is designed to be a map through the phenomenology of this amazing protein.

## STRUCTURE-FUNCTION RELATIONSHIPS

Nucleolin belongs to a large family of RNA binding proteins that includes heterogeneous nuclear ribonucleoproteins (RNPs), mRNA polyadenylate binding protein, SS-B/La ribonucleoproteins, small nuclear RNPs (23), the human alternative splicing factors ASF and SF2 (24, 25), and the mammalian splicing factors U2AF and SC35 (26, 27). To date, more than 200 consensus sequence type RNA binding domains (CS-RBDs) containing proteins have been found, including proteins with various functions in RNA splicing, processing, or translation. These RNA binding proteins have a common structure that is used for nucleic acid binding, with additional modules attached for various other functions and for determination of specificity. Since the proteins described above have different functions and bind to distinctly different RNA molecules, it is not surprising that sequences are substantially different in regions outside the consensus sequences. In this respect, nucleolin appears to be more closely related to hnRNP protein A1 than to the poly(A) binding protein. This is consistent with the role of hnRNP protein A1 in packaging newly synthesized RNA compared to the poly(A) binding protein's association with cytoplasmic RNA.

### The amino-terminal domain controls rDNA transcription

Nucleolin is composed of 707 amino acids, and analysis of nucleolin cDNA has revealed the presence of three major domains (6, 28). Due to the high content of negatively charged amino acids in the amino-terminal domain, nucleolin runs in sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels with an apparent molecular mass of 105 kDa, although the actual calculated mass is 77 kDa from the cDNA sequence (6, 28). The amino-terminal third of nucleolin contains alpha helical domains comprising four lengthy acidic stretches, similar to those of certain high-mobility group proteins. This domain has an analogous high-mobility group function in inducing nucleolar chromatin decondensation through ionic interaction of the acidic amino acids with histone H1 (10, 15, 29). These acidic regions, which are variable and less conserved in different species (6, 28, 30), bind to nontranscribed spacer regions in DNA that separate the rRNA gene repeats (31–33). This process organizes nucleolar chromatin in a way that confers specificity for transcription of rDNA by RNA polymerase 1 (34). These regions also contain *in vivo* phosphorylation sites for casein kinase II (CKII), cdc2, and protein kinase interspersed with basic lysine residues susceptible to proteolysis (14, 35, 36). The importance of these sites lies in the fact that transcription of rDNA genes starts only when the serine residues are phosphorylated by casein kinase II and the proteolytic sites in nucleolin are cleaved (34). The phosphorylation of nucleolin seems to enhance its degradation by proteases concordantly, suggesting that the stability of nucleolin is dependent on phosphorylation (37, 38).

As is well known, the transcription of rDNA genes by RNA polymerase 1 gives rise to 47S RNA, which is rapidly cleaved to yield the mature 18S, 28S, and 5.8S rRNA species. Several processing sites have been mapped during this maturation process at the vicinity of 5′-end of the external transcribed spacer (ETS) region of pre-RNA. The 5′-end of pre-RNA is assembled into a large ribonucleoprotein complex involving nucleolin, fibrillarin, and different snoRNPs (small ribonucleoproteins). The specific interaction of nucleolin with the rRNA substrate might be the first step in the processing event. Nucleolin could then recruit other factors, including U3 snoRNP, required for the cleavage reaction in formation of the processing complex (39, 40). In this complex, nucleolin interacts with RNAs as well as other proteins (41, 42). Thus, nucleolin's involvement in rDNA transcription, pre-RNA processing, ribosome assembly, and maturation mediated through the interaction of nucleolin with rDNA (nontranscribed spacer, 5′ and 3′-external nontranscribed spacer, internal transcribed spacer, nascent 45S pre-RNAs, RNA polymerase 1, 18S, 28S rRNAs) and ribosomal proteins could be an efficient way for the cell to regulate the production of large amount of ribosomes needed throughout its life.

### The central globular domain controls pre-RNA processing

While the amino-terminal region interacts with chromatin and is involved in modulating nucleic acid binding activity, the central globular domain is involved in pre-RNA recognition, condensing, and packaging (43–45). The central domain of nucleolin exhibits alternating hydrophobic and hydrophilic stretches, and also contains four CS-RBDs (46). The latter domains consist of ~80 amino acid residues, each containing two highly conserved regions, the so-called RNP motifs (47). Nucleolin interacts specifically with an RNA stem-loop structure (NRE) and with *in vitro*-selected RNAs containing UCCCGA through its first two RNA binding domains (CS-RBDs 1 and 2) (32, 48, 49). This property may account for nucleolin's association with pre-rRNA in the nucleolus. The 45 kDa fragment of nucleolin (exon 5–14,

containing the central and carboxyl-terminal domains) binds strongly to G-rich DNA and ATP. This ATP binding is important for its ATPase activity. Furthermore, this region of nucleolin directly binds GTP, dATP, and dGTP but not dCTP, dTTP, or dUTP (50). In addition, the adenosine analog DRB (5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole), an inhibitor of hnRNA synthesis, modifies the stability and the amount of nucleolin, and thus the nucleolar morphology (51). This implicates nucleolin as a key player in nucleolar structure and function.

### The carboxyl-terminal domain controls nucleolar localization

The extended carboxyl-terminal domain is rich in glycine residues and is interspersed with dimethylarginine and phenylalanine. The function of this domain is to control unstacking of bases and the unfolding of RNA secondary structure (52–55). These events permit RNAs access to the RNA binding motifs located in the central region of nucleolin (44). Furthermore, the intrinsic protease activity of nucleolin for autodegradation has been mapped to the carboxyl-terminal two-thirds of the nucleolin molecule (8). Nucleolin is accumulated within the nucleolus by virtue of its binding to other nucleolar components (probably rRNA) via the two RNA recognition motifs and the glycine-rich domains of the carboxyl-terminal region (45, 56). Hints as to how the mammalian nucleolin is involved in the formation and maturation of preribosomal particles have come from studies of homologous proteins in yeast. In particular, the glycine- and arginine-rich carboxyl-terminal domain of nucleolin is shared homologously by the yeast genes nucleolar phosphoprotein 1/fibrillarin, SSB1, NSR1, Gly/Arg-rich (GAR1), and GAR2. Disruption of the GAR2 gene in yeast affects normal cell growth and leads to an accumulation of 35S pre-RNA and a decrease of mature 18S and 40S rRNA (57, 58). Deletion of NSR1 causes a severe growth defect at cold temperatures and leads to a rapid decrease in 27S, 20S, and 7S rRNA precursors in the nsr1 strain (59). In addition, this domain may mediate protein/protein and/or protein/nucleic acid interactions (60). A recent report has shown that nucleolin interacts directly with a subset of ribosomal proteins through its carboxyl-terminal domain (61).

### Nucleolin is a shuttling protein between the plasma membrane, cytoplasm, and nucleus

Nucleolin has been shown to be a shuttling protein between the cytoplasm and nucleus and contains only an import signal (62). In addition, nucleolin possesses binding affinity for some ribosomal proteins and interacts with a subset through its carboxyl-terminal domain (61), suggesting that nucleolin may play a role in the assembly of ribosomal subunits by bringing together both ribosomal proteins and RNA. Through its nucleocytoplasmic shuttling property, nucleolin may act as a carrier either during the import of ribosomal proteins to the nucleus or during the export of ribosomal subunits to the cytoplasm (12, 56, 63). Nucleolin thus serves as an 'adaptator' for specific binding to rRNA. The bipartite nuclear localization signal, situated between the amino-terminal domain and RNA binding domain of nucleolin, is necessary for nucleolin to enter the nucleus (64). In the developing *Xenopus* embryo, nucleolin has been shown to be localized cytoplasmically up to the midblastula stage, but thereafter accumulates in the nucleus. The nucleolar accumulation event starts at the gastrulation stage (65). In addition, massive phosphorylation by cdc2 or CKII kinases localizes nucleolin to the cytoplasm, and nuclear translocation of nucleolin accompanies dephosphorylation (66). Laminin alters the distribution of nucleolin in intestinal epithelial (IEC-6) cells, which may be an early signal for cell proliferation (67).

As mentioned earlier, the yeast NSR1 gene bears a structural similarity to nucleolin. Like nucleolin, NSR1 in yeast is directly involved in pre-RNA processing and regulates the nuclear entry of ribosomal proteins required for proper assembly of pre-rRNA particles (68). Shuttling in the opposite direction begins as soon as the 47S pre-RNA is assembled into the 80S particle. At this point, nucleolin might be involved in the transport of these newly assembled ribosomal subunits into the cytoplasm. Thus, nucleolin's binding to both chromatin and the 5′-ETS region of the nascent pre-RNA suggests that it may play a key role in the assembly and/or processing of preribosomes and transport of ribosomal proteins across the nuclear envelope (10–12).

## GENE STRUCTURE

Nucleolin is encoded as a single copy gene of ~9 kb with 14 exons and has the characteristic GC-rich promotor sequences found in housekeeping genes. Each of the four RNA binding domains are encoded by two separate exons, and a splice junction interrupts the 11 residue RNP consensus sequence in each case. This suggests that in evolution, the occurrence of an intron within an ancestral gene encoding the RNA binding domain preceded the duplication event leading to the repetition of this domain (30, 69). The human nucleolin gene is localized on chromosome 2q12-qter (69), which is not syntenic with any other RNA binding proteins so far localized.

Several small nucleolar RNAs (snoRNAs), have been shown to be essential for processing steps that

lead to the production of 28S, 18S, and 5.8S rRNA. These antisense snoRNAs have been shown to play a chaperone role in the processing of the pre-rRNA (70). U20 snoRNA contains an extended region (21 nucleotides) of perfect complementarity to the phylogenetically conserved sequence in 18S rRNA (71). The detection of U20 snoRNA in intron 11 of the mammalian nucleolin gene supports the notion that this particular genomic organization has some relevance to those functions of nucleolin that are involved in the production of ribosomes. Perhaps this observation might provide the basis for regulatory linkages during ribosome biogenesis or function. Since these snoRNAs are no longer present in ribosomes, they must have been removed during or after processing of pre-RNA. Nucleolin is a good candidate to perform this function, since nucleolin has DNA/RNA helicase activity, and the number of base pairs in these sequences (up to 21) is fully compatible with the length of RNA duplexes that nucleolin could unwind *in vitro* (20). In addition, the presence of a second intron encoded snoRNA, termed U22, in the nucleolin gene may reflect the multiplicity of regulatory circuits for ribosome production mediated by this multifunctional protein throughout the cell cycle and in different conditions of cell growth (6, 14, 36).

## REGULATION OF NUCLEOLIN FUNCTION

There is evidence that the multiple activities of nucleolin may be regulated by covalent modifications, most notably phosphorylation (14, 36, 72), proteolysis (34, 73), autodegradation (7, 8), and ADP-ribosylation (74). For example, the amino-terminal domain contains sites for modification by phosphorylation and proteolysis, whereas the carboxyl-terminal domain has sites for methylation and proteolysis. The idea that nucleolin function is coupled to growth control by phosphorylation is supported by the observation that active rRNA transcription is positively correlated with highly phosphorylated nucleolin (75–78). Treatment of human fibroblasts and keratinocytes with okadaic acid, calyculin A (79), polyamines, or histones induces hyperphosphorylation of N60, a proteolytic product of nucleolin (80–82). Therefore, a diversity in susceptibility to such modifications can underlie regulation.

### Serine phosphorylation of nucleolin occurs during interphase

Cell growth requires CKII-mediated phosphorylation of several cytosolic and nuclear substrates (69, 83–85). In growing cells, CKII phosphorylates nucleolin, topoisomerase 1 (Topo 1), and RNA polymerase 1. It may not be coincidental that all three proteins are localized on chromosomes containing rDNA. These chromosomes are decondensed and transcribed in daughter cells, and thus appear to be involved in nucleolar structural organization in the G1 phase (86, 87). Phosphorylation by CKII also enhances nucleolin as a substrate for protease to produce active 30 and 72 kDa proteins. These latter fragments then trigger rDNA transcription by RNA polymerase 1 (37). Both CKII activity and phosphorylation of nucleolin are enhanced at day 12 of gestation (72), after stimulation with mitogens (70), in regenerating rat liver after partial hepatectomy (88), and in tumor cells (72). In addition, CKII phosphorylation of nucleolin and rRNA synthesis have been reported to be dependent on hormones, such as dexamethasone (78, 89) and androgen (38), and on growth factors such as epidermal growth factor (90) and fibroblast growth factor 2 (91).

Insulin action may also be mediated by phosphorylation of nucleolin. Insulin induces serine phosphorylation, possibly via CKII, at subnanomolar concentrations, whereas insulin effectively promotes dephosphorylation of nucleolin at the micromolar concentration range. This dose-response relationship is identical to insulin-induced effects on the RNA efflux from nuclei, suggesting that the phosphorylation state of nucleolin might be a regulator of (ribosomal) RNA transport through the nuclear membrane (92). The interaction of CKII with the FK506 binding protein (FKBP) results in the phosphorylation of nucleolin, which itself leads to the regulation of cell growth (93). FKBP is the cellular receptor for the immunosuppressive drugs FK506, cyclosporin, and rapamycin. These findings strongly suggest that the signals that affect CKII also change nucleolin's function, which in turn affects rDNA transcription and ribosome biogenesis.

### Threonine phosphorylation of nucleolin occurs during mitosis

Whereas serine phosphorylation is related to nucleolar function in the control of rDNA transcription, threonine phosphorylation is linked to mitotic reorganization through condensation of nucleolar chromatin (94–97). During mitosis, cdc2 kinase phosphorylates threonine in the TPKK motifs. These TPKK motifs occur nine times in the amino-terminal domain of nucleolin. For example, mitogenic stimulation of resting T cells and lipopolysaccharide induction of mitosis in resting splenic cells have been shown to lead to the induction of phosphorylation of nucleolin. The degree of phosphorylation is therefore closely correlated to the degree of cell proliferation (50, 75). The cdc2 sites play a dual role by

enhancing nuclear translocation exclusively in their dephosphorylated state and promoting cytoplasmic localization when phosphorylated, thereby providing a powerful cell-cycle-dependent regulatory element of the nuclear localization signal. Evidence that nucleolin is extensively phosphorylated during mitosis by cdc2 kinase further implies that it controls the mitotic changes in nucleolar structure by condensing chromosomes. It is possible that sequential cdc2 and CKII phosphorylation could modulate nucleolin function in controlling nucleolar structure and activities between interphase and the mitotic phase during cell growth.

### Phosphorylation of nucleolin by PKC-ξ and ecto-kinases: implications for global function

Nucleolin is a nuclear target protein for cyclic AMP and PKC-ξ. Cyclic AMP-dependent protein kinase influences the phosphorylation of nucleolin. This is exemplified during proliferative stimulation (transition from G1 to S phase) of the rat parotid gland by isoprenaline (98). Recently, it was reported that it is also a substrate for PKC-ξ, which is required for nerve growth factor- (NGF) induced differentiation of PC12 cells. It was suggested that nucleolin might serve to relay NGF signals from cell surface to nucleus in PC12 cells (99). It has been shown that ecto-protein kinase also phosphorylates nucleolin, which suggests that nucleolin expressed on the cell surface is regulated by a cell surface kinase (100). Inasmuch as nucleolin functions on the cell surface as a default LDL receptor in HepG2 cells, it has been speculated that nucleolin might use this property to verify that sufficient exogenous lipids are available to support cell growth (101). Being a regulated shuttling nuclear protein, nucleolin could thus transfer cytoplasmic signals between the cell surface, the cytoplasm, and the nucleus. Phosphorylation of nucleolin by CKII and cdc2 kinase has been shown to regulate its helicase activity (102), and phosphorylation by CKII, cdc2 kinase, PKC-ξ, cyclic AMP-dependent protein kinase, and ecto-protein kinase may regulate nucleolin's functional abilities in chromatin organization, rRNA packaging, rDNA transcription, or ribosome assembly.

### Modification of nucleolin function by proteolysis: apoptosis and autodegradation

Nucleolin is also a central player in the process of T lymphocyte-mediated apoptotic cell death. Nucleolin in the target cell is physiologically cleaved by granzyme A (7, 8, 103). The mechanism involves secretion of perforin and serine proteases by cytotoxic T cell lymphocytes and natural killer cells. Perforin forms pores in the target cells, enabling granzyme A to access nucleolin. Cleaved nucleolin activates autolytic endonucleases, which fragment DNA to cause apoptosis (104, 105). Recently, nucleolin was identified as an apoptosis-associated protein involved in anti-immunoglobulin M antibody-mediated apoptosis in the human Burkitt lymphoma cell line (106). For some unknown reason, nucleolin has been passed over by the apoptosis reagent supply companies, but this may change. Nucleolin is stable in actively dividing cells. In nondividing cells, nucleolin autocatalyzes its own degradation, which can be inhibited by nuclear extracts prepared from proliferative cells (7, 107). In proliferating cells, nucleolin is regulated by the expression of a proteolytic inhibitor that prevents the self-degradation activity of nucleolin (7). The ability to stabilize nucleolin could also be of use to cancer cells.

## NUCLEOLIN, HSP70, AND NUCLEAR TRANSPORT

The transport of nucleolar components between the cytoplasm and nucleolus is a major part of the nucleocytoplasmic traffic during liver regeneration. Nucleolin and the hsp90 and hsp70 proteins (108) appear to have similar ATP binding domains, contain serum responsive elements in their promoters, and encode small nucleolar RNAs in their introns. Their gene expressions are both coactivated during prereplicative stages of hepatocytes (109). In addition, both nucleolin and hsp70 contain regions that resemble the 'nucleolar targeting' sequence of HIV tat protein (110). Recently, nucleolin has been suggested to be functional as potential receptor in the HIV binding process by virtue of its capacity to interact with the V3 loop of gp120 (111). Hsp 70 acts during mitochondrial transport to help maintain nascent precursors in the extended conformation required for translocation (112). A large number of ribosomal proteins, which are karyophilic proteins, have the propensity to aggregate. They may require hsp70 and nucleolin to prevent hydrophobic interactions and translocate them into the nucleolus. Therefore, it is conceivable that hsp70 and nucleolin could play a role in nuclear transport and contribute to the efficiency of ribosomal biosynthesis.

## NUCLEOLIN AND CELL GROWTH

### A role for nucleolin in cancer, the cell cycle, and embryogenesis

As mentioned earlier, the synthesis of nucleolin is positively correlated with increased rates of cell division. It is not surprising, therefore, that nucleolin levels are highest in tumors or other rapidly dividing cells (113). Indeed, nucleolin is

used in studies of different cancer cell lines as a useful marker for cell proliferation (114–117). Nucleolin is present at low levels in nondividing cells and is preferentially associated with chromatin (117). The amount of nucleolin is low in serum-deprived cells; nucleolin expression is induced by v-src in mid and late G1, and thus is likely to be necessary for cell cycle progression into G1 (118). In ABAE cells undergoing the Go-G1 transition, basic FGF enters the nucleolus and stimulates nucleolin, which in turn activates the transcription of ribosomal genes (13). Even in plants, Nucms1 (a plant homologue of nucleolin) expression is induced in the G1 phase on mitogenic stimulation of Go-arrested leaf cells. No expression has been reported in cells that have exited the cell cycle and are undergoing differentiation or polar growth (119). In addition, retinoic acid and dibutyryl cyclic AMP increase neurite outgrowth during differentiation of human neuroblastoma cells and have an inhibitory effect on cell proliferation. Simultaneously, the expression of N-myc, nucleolin, and hsp70 are down-modulated, indicating a possible association of expression of these three genes (120). In cancer, the relationship of nucleolin (a major nucleolar Ag-NOR protein) and cell proliferation represents a reliable parameter predicting the tumor growth rate

Nucleolin expression is regulated post-transcriptionally and transcriptionally in the developing embryo. For example, mRNA levels in *Xenopus*, are high in adult tissues, where protein expression is exceedingly low (14). Ribosome synthesis is activated during oogenesis and embryogenesis. These data indicate that nucleolin participates in nucleogenesis (14). Nucleolin appears before rDNA transcription and ribosome synthesis start. Maximal accumulation of nucleolin coincides at gastrulation with nucleolar reformation.

During mouse embryogenesis, CKII and nucleolin increase concomitantly at day 12 of gestation (72, 121). In chicken embryos, transcriptional down-regulation of nucleolin has been observed between days 3 and 11, with a decrease in both mRNA and protein levels of nucleolin. Nucleolin mRNA progressively decreases during spermatogenesis. Nucleolin message is present only up to the round spermatid stage and is absent from mature sperm (122).These findings suggest that nucleolin is needed for cell proliferation and nucleogenesis and is down-modulated during differentiation (123) along with N-myc and hsp70.

## NUCLEOLIN BINDING PROTEINS AND LIGANDS

### Many nuclear proteins bind to nucleolin

One approach to studying nucleolin function and regulation is to examine its physical association with other cellular proteins or ligands. For example, as mentioned, nucleolin associates with CKII, which phosphorylates nucleolin on serine residues. This strong association may be important for regulating rDNA transcription (124). The interaction of CKII with nucleolin is promoted by spermine and inhibited by heparin. The nature of the interaction shows a similarity to CKII association with p53, DNA topoisomerase II, and HSP90 (37). Interaction of the 540–628 bp region of nucleolin with the 194–239 bp region of B23, an additional nucleolar protein, may represent a nucleolar-targeting mechanism for nucleolin to enter nucleolus in which B23 acts as a nucleolar localization signal binding protein (125). Topo 1 is involved in the regulation of DNA supercoiling, gene transcription, and rDNA recombination. The interaction of the 166–210 aa region of Topo 1 with nucleolin may be needed for Topo 1 to enter the nucleus. Thereafter, nucleolin could target Topo 1 to sites of transcription (126). It has recently been reported that the Gar2 gene (nucleolin-like protein) from *Schizosaccharomyces pombe* results in a mutant that is defective in cytokinesis and nuclear division (127). Nucleolin fragments (70, 48, 47 kDa) and heterogeneous nuclear ribonucleoprotein C (39, 38 kDa) specifically interact with the 3′-untranslated region of amyloid precursor protein (APP) mRNA and stabilize the APP's mRNA. This is a mechanism for post-transcriptional regulation (128). Midkine and heparin binding growth-associated molecules bind to nucleolin, which then translocates these associated molecules to the nucleus in PYS-2, 3T3, and L cells (129). Furthermore, nucleolin is proposed to be a nuclear matrix binding protein (130). In addition, nucleolin binds to a 222-nucleotide motif within the nonstructural unit of the viral (minus) strand of minute virus of the mouse. This suggests that nucleolin may be involved in the regulation of the parvoviral life cycle (131)

### Nucleolin is a cell surface receptor

Although nucleolin is localized predominantly in the nucleolus, it has also been shown to be localized in a phosphorylated form on the cell surface of different cells (132). Cell surface expression of nucleolin has been further substantiated by other investigators. Nucleolin is the protein that specifically binds apoB- and apoE-containing lipoprotein to the surface of the HepG2 cells (101). Nucleolin serves as a substrate for an ecto-protein kinase on the cell surface of HeLa cells (100). The neurite-promoting IKVAV site of laminin-1 (basement membrane protein) binds to nucleolin on the cell surface and has been found to promote the differentiation of primary neurons and a variety of neural cell lines (133). The significant levels of nucleolin in mature brain and in differen-

tiating neural cells suggest that nucleolin may not only function in signaling by extracellular matrix molecules, but may also be important in differentiation and maintenance of neural tissue (134).

Viral infection of host cells depends primarily on binding of the virus to a specific cell surface protein. Recently, human nucleolin has been shown to interact with the amino-terminal domain of hepatitis delta antigens and modulate hepatitis delta virus replication (135). Although polio and Sendai viruses do not bind to nucleolin, Coxsackie B viruses does bind to nucleolin specifically on the cell surface (112). The fructosyllysine-specific binding protein from cell membrane of the monocyte-like cell line U937 is identical to nucleolin (136). These findings indicate that nucleolin itself or putative splicing products might function as cell surface receptors or binding proteins and thus could act as mediators to provide a potential mechanism for extracellular regulation of nuclear events

### Nucleolin binds to lipopolysaccharides, immunoglobulin switch repeats, matrix attachment region, and telomeres

In mitogenically stimulated murine splenocytes with bacterial lipopolysaccharides, nucleolin has been found to be up-regulated in its DNA and ATP binding properties as a result of increased stability and synthesis of nucleolin. Nucleolin also exhibits binding specificity for the immunoglobulin isotype switch DNA repeats (16, 50). Because nucleolin specifically binds to switch region DNA, it might act as a switch region targeting factor in the B cell-specific DNA recombination complex called SWAP (16, 17). It has also been shown that transcription at switch regions is important for recombination (137, 138). The fact that nucleolin is capable of binding to switch repeats indicates it could potentially play a role in active or passive repression of transcription at switch regions. Such actions may attenuate or inhibit switch recombination processes. As a positive regulator of recombination, nucleolin, with its four nucleic acid binding domains, can bind and hold the cleaved strands together during the process of scission and ligation. As a negative regulator, it can play a role as an inhibitor by preventing the recognition of switch repeats. Alternatively, it can protect switch regions from premature or extensive degradation during or prior to recombination. Nucleolin has been shown to be a transcriptional repressor for the alpha-1 acid glycoprotein gene (22). In addition, nucleolin also binds to single-stranded T-rich regions of the matrix attachment region, to the pre-mRNA 3′-splice site sequence r(UUAG/G), and to the human telomeric DNA sequence d(TTAGGG)n (139). These findings suggest that nucleolin may function not only in the control of rRNA transcription and ribosome assembly in the nucleolus, but may also be involved in processes taking place outside the nucleolus.

## AUTOIMMUNITY AND VIRAL INFECTIONS

Rabbit anti-nucleolin antibody specifically depletes autoreactive 110K nucleolin from detergent lysates of human cells derived from patients with autoimmune disorders and viral infections (140, 141). Autoimmune serum from systemic lupus erythematosus patients recognize the 387–461bp domain of nucleolin (19). In scleroderma-like chronic graft-vs.-host disease, three cases have been reported to have antibodies to nucleolar antigen nucleolin. Four cases are reported to possess antibodies to topoisomerase 1. Two cases have antibodies to PM-Sc1. One case has antibodies to La/SSB (142). Recently, human nucleolin has been shown to interact with the amino-terminal domain of hepatitis delta antigens and modulate the hepatitis delta virus (a satellite virus of hepatitis virus associated with fulminant hepatitis and chronic liver necrosis) replication (135)

The development of combination of autoantibodies in a given disease has been explained by the assembly of multiple autoantigens into dynamic particles that which drive the immune response (143). Many proteins that form dynamic particles and become targets of high titer immunoglobulin G autoantibodies share amino acid sequence similarities with nucleolin, e.g., U2 snRNP-B, nuclear RNP (U1 snRNP-70K and A), SS-A/Ro (60K-Y RNA), SS-B/La (48 kDa phosphoprotein complexed with nascent RNA polymerase III transcripts), and fibrillarin (U3 RNP-34K) (143, 144). In addition, nucleolin itself forms dynamic particles with several autoantigens, including histone H1, topoisomerase 1, RNA polymerase 1, and DNA (10). These correlations predict that *1)* the RNA recognition motif in the nucleolin may be an autoantibody epitope, *2)* anti-nucleolin autoantibodies may form 'sets' with certain other anti-nuclear autoantibodies, and *3)* nucleolin can share epitopes with other viral proteins. In addition, multiple endocrine neoplasia type 2A (MEN 2A) encodes a 415 aa protein similar in sequence to nucleolin (145). The correlation observed between anti-nucleolin antibodies and disease activity points to its important role in disease.

## CONCLUSION

Discoveries of the last few years have revealed a plethora of novel functions for nucleolin. Originally, nucleolin was seen as a simple RNA binding protein

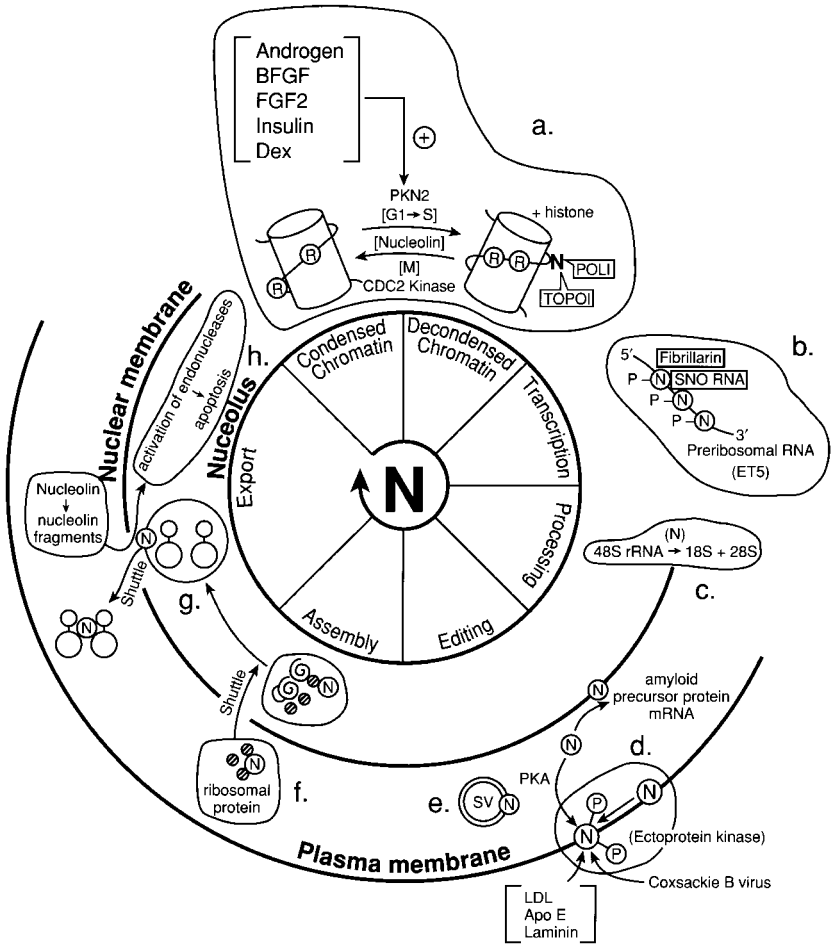


**Figure 1.** Nucleolin control of cell cycle and ribosomal biogenesis. a.) Chromatin condensation and decondensation. Nucleolin decondenses the chromatin by replacing histones. Androgen, basic fibroblast growth factor (bFGF), FGF-2, dexamethasone, and insulin regulate the expression and phosphorylation state of nucleolin by protein kinase NII (PKN2), leading to increased rDNA transcription by RNA polymerase 1 in the G1 to S phase of the cell cycle. During mitosis, nucleolin is phosphorylated by cdc2 kinase and condensates the chromatin. b.) Nucleolin and pre-RNA. Nucleolin binds to the 5′-end of the external transcribed spacer region of pre-RNA and participates in the pre-RNA processing along with fibrillarin and snoRNA. c.) Maturation of 48S RNA. The involvement of nucleolin during the maturation process of 48S RNA, which is rapidly cleaved to yield the mature 18S, 28S, and 5.8S rRNA species during the transcription of rDNA by RNA polymerase 1, is shown. d.) Cell surface functions. Nucleolin is expressed on the cell surface and binds to LDL, apoE, laminin, and Coxsackie B virus, and is phosphorylated by cell surface-specific ecto-protein kinase. e.) Secretory vesicle expression. Nucleolin is expressed in small secretory vesicles. f.) Shuttling activities. Nucleolin acts as a shuttling protein to carry ribosomal protein from cytoplasm to nucleus during the assembly of ribosomes. g.) Export activities. Nucleolin might be involved in exporting the assembled ribosomes from nucleus to cytoplasm. h.) Cell death and apoptosis. Nucleolin is involved in the process of cell death. Nucleolin is fragmented by granzyme A, which activates autolytic endonucleases that fragment DNA to cause apoptosis.

directly involved in the organization of nucleolar chromatin, packaging of pre-RNA, rDNA transcription, and ribosome assembly by shuttling between the nucleus and cytoplasm. The new data now show that nucleolin is involved either directly or indirectly in modulating transcriptional processes, cytokinesis, nucleogenesis, signal transduction, apoptosis, induction of chromatin decondensation, and replication. In addition, it autocatalyzes its own degradation and acts as a DNA and RNA helicase and DNA-dependent ATPase. However, in addition to several functions in the nucleolus, nucleolin functions cytoplasmically and on the cell surface to provide a shuttling mechanism for cytoplasmic and extracellular regulation of nuclear activities. For example, nucleolin is a target of PKC-ξ that serves to relay NGF signals from cell surface to nucleus in PC12 cells and is a substrate for ecto-protein kinase on the cell surface.

All experimental evidence gathered to-date supports an essential role for nucleolin in cell proliferation. Control of nucleolin expression is very complex, phosphorylation being only one of several regulatory elements in the nucleolin tool box. Extensive phosphorylation of nucleolin by a casein kinase (CKII) in interphase and by cdc2 kinase during mitosis suggests that phosphorylation may be a mechanism for regulating nucleolin function during the cell cycle. More recently we have come to

realize that molecular regulators of nucleolin function can contribute further to the complexity of nucleolin regulation by interaction with nucleolin protein. Recent data concerning the structure and cell biology of nucleolin suggest that as an autoantigen, nucleolin is ideally positioned to play a central role in the development of other autoantibodies. In addition, it is a switch region-targeting factor in the recombination complex of a B cell-specific transcription factor, LR1. To illustrate these many functions, we have prepared the diagram shown in **Fig. 1**.

Clearly, more investigation into these correlations is required to identify the mechanisms by which nucleolin performs these disparate tasks. The multifunctional nature of nucleolin could be due to the specialized domains within nucleolin performing different functions. Although it is energetically favorable for a cell to use a single protein for unrelated different functions, understanding the basis of multifunctionality of nucleolin will have to await the phenotype of the knockout mice and its interaction with other macromolecules. From this knowledge, profoundly important insights to cell biology and medicine will undoubtedly be derived. FJ

## REFERENCES


1. Sommerville, J. (1986) Nucleolar structure and ribosome biogenesis. *Trends Biochem. Sci.* **11,** 438–442
2. Warner, J. R. (1990) the nucleolus and ribosome formation. *Curr. Opin. Cell Biol.* **2,** 521–527
3. Scheer, U., and Benavente, R. (1990) Functional and dynamic aspects of mammalian nucleolus. *Bioassays* **12,** 14–21
4. Bugler, B, Caizergues-Ferrer, M., Bouche, G., Bourbon, H., and Amalric, F. (1982) Detection and localization of a class of proteins immunologically related to a 100-kDa nucleolar protein. *Eur. J. Biochem.* **128,** 475–480
5. Shaw, P. J., and Jordan, E. G. (1995) The nucleolus. *Annu. Rev. Cell Div. Biol.* **11,** 93–121
6. Lapeyre, B., Bourbon,, H., and Amalric, F. (1987) Nucleolin, the major nucleolar protein of eukaryotic cells: an unusual protein structure revealed by the nucleotide sequence. *Proc. Natl. Acad. Sci. USA* **84,** 1472–1476
7. Chen, C. M., Chiang, S. Y., and Yeh, N. H. (1991) Increased stability of nucleolin in proliferating cells by inhibition of its self-cleaving activity. *J. Biol. Chem.* **266,** 7754–7758
8. Fang, S. H., and Yeh, N. H. (1993) The self-cleaving activity of nucleolin determines its molecular dynamics in relation to cell proliferation. *Exp. Cell Res.* **208,** 48–53
9. Olson, M. O., Kirstein, M. N., and Wallace, M. O. (1990) Limited proteolysis as a probe of the conformation and nucleic acid binding regions of nucleolin. *Biochemistry* **29,** 5682–5686
10. Jordon, G. (1987) At the heart of the nucleolus. *Nature (London)* **329,** 489–490
11. Herrera, A. H., and Olson, M. O. J. (1986) Association od protein C23 with rapidly labeled nucleolar RNA. *Biochemistry* **25,** 6258–6264
12. Borer, R. A., Lehner, C. F., Eppenberger, H. M., and Nigg, E. A. (1989) Major nucleolar proteins shuttle between nucleus and cytoplasm. *Cell* **56,** 379–390
13. Bouche, G., Gas, N., Prats, H., Baldin, V., Tauber, J. P., Teissie, J., and Amalric, F. (1987) Basic fibroblast growth factor enters the nucleolus and stimulates the transcription of ribosomal genes in ABAE cells undergoing Go-G1 transition. *Proc. Natl. Acad. Sci.* **84,** 6770–6774
14. Caizergues-Ferrer, M., Mariottini, P., Curie, C., Lapeyre, B., Gas, N., Amalric, F., and Amaldi, F. (1989) Nucleolin from Xenopus laevis: cDNA cloning and expression during development. *Genes Dev.* **3,** 324–333
15. Erard, M., Lakhdar-Ghazal, F., and Amalric, F. (1990) Repeat peptide motifs which contain beta-turns and modulate DNA condensation into chromatin. *Eur. J. Biochem.* **191,** 19–26
16. Hanakahi, L. A., Dempsey, L. A., Li, M.-J., and Maizels, N. (1997) Nucleolin is one component of the B-cell specific transcription factor and switch region binding protein, LR1. *Proc. NatI. Acad. Sci. USA* **94,** 3605–3610
17. Borggrefe, T., WabI, M., Akhmedov, A. T., and Jessberger, R. (1998) A B-cell-specific DNA recombination complex. *J. Biol. Chem.* **273,** 17025–17035
18. Minota, S., Jarjour, W. N., Suzuki, N., Nojima, Y., Roubey, R. A., Mimura, T., Yamada, A., Hosoya, T., Takaku, F., and Winfield, J. B. (1991) Autoantibodies to nucleolin in systemic lupus erythematosus and other diseases. *J. Immunol.* **146,** 2249–2252
19. Valdez, B. C., Henning, D., Busch, R. K., Srivastava, M., and Busch, H. (1995) Immunodominant RNA recognition motifs of human nucleolin/C23. *Mol. Immunol.* **32,** 1207–1213
20. Tuteja, N., Huang, N. W., Skopac, D., Tuteja, R., Hrvatic, S., Zhang, J., Ponger, S., Joself, G., Faucher, C., and Amalric, F. (1995) Human DNA helicase IV is nucleolin, an RNA helicase modulated by phosphorylation. *Gene* **160,** 143–148
21. Tuteja, N., Phan, T.-N, Tuteja, R., Ochem, A., and Falaschi, A. (1997) Inhibition of DNA unwinding and ATPase activities of human DNA helicase II by chemotherapeutic agents. *Biochem. Biophys. Res. Commun.* **236,** 636–640
22. Yang, T. H., Tsai, W. H., Lei, H. Y., Lai, M. Y., Chen, D. S., Yeh, N. H., and Lee, S. C. (1994) Purification and characterization of nucleolin and its identification as a transcription repressor. *Mol. Cell. Biol.* **14,** 6068–6074
23. Chan, E. K., Sullivan, K. F., and Tan, E. M. (1989) Ribonucleoprotein SS-B/La belongs to a protein family with consensus sequences for RNA-binding. *Nucleic Acids Res.* **17,** 2233–2244
24. Ge, H., Zuo, P., and Manley, J. L. (1991) Primary structure of the human splicing factor ASF reveals similarities with *Drosophila* regulators. *Cell* **66,** 373–382
25. Kranier, A. R., Mayeda, A., Kozak, D., and Binns, G. (1991) Functional expression of cloned human splicing factor SF2: homology to RNA-binding proteins, U1 70K, Drosophila splicing regulators. *Cell* **66,** 383–394
26. Fu, X. D., and Maniates, T. (1992) Isolation of a complementary DNA that encodes the mammalian splicing factor SC35. *Science* **256,** 535–538
27. Zamore, P. D., Patton, J. G., and Green, M. R. (1992) Cloning and domain structure of the mammalian splicing factor U2Af. *Nature (London)* **355,** 609–614
28. Srivastava, M., Fleming, P. J., Pollard, H. B., and Burns, A. L. (1984) Cloning and sequencing of the human nucleolin cDNA. *FEBS Lett.* **250,** 99–105
29. Goyette, M., Petropoulos, C. J., Shank, P. R., and Fausto, N. (1984) Regulated transcription of c-Ki-ras and c-myc during compensatory growth of rat liver. *Mol. Cell. Biol.* **4,** 1493–1498
30. Bourbon, H. M., Lapeyre, B., and Amalric, F. (1988) Structure of the mouse nucleolin gene. The complete sequence reveals each RNA binding domain is encoded by two independent exons. *J. Mol. Biol.* **200,** 627–638
31. Olson, M. O., and Thompson, B. A. (1983) Distribution of proteins among chromatin components of nucleoli. *Biochemistry* **22,** 3187–3193
32. Ghisolfi-Nieto, L., Joseph, G., Puvion-Dutilleul, F., Amalric, F., and Bouvet, P. (1996) Nucleolin is a sequence-specific RNA-binding protein: characterization of targets on pre-ribosomal RNA. *J. Mol. Biol.* **260,** 34–53
33. Egyhazi, E., Pigon, A., Chang, J. H., Ghaffari, S. H., Dressen, T. D., Wellman, S. E., Case, S. T., and Olson, M. O. (1988) Effects of anti-C23 (nucleolin) antibody on transcription of ribosomal DNA in Chironomus salivary gland cells. *Exp. Cell Res.* **178,** 264–272
34. Bouche, G., Caizergues-Ferrer, M., Bugler, B., and Amalric, F. (1984) Interrelations between the maturation of a 100 kDa nucleolar protein and pre rRNA synthesis in CHO cells. *Nuc. Acids Res.* **12,** 3025–3035
35. Belenguer, P., Caizergues-Ferrer, M., Labbe, J. C., Doree, M., and Amalric, F. (1990) Mitosis-specific phosphorylation of

nucleolin by p34cdc2 protein kinase. *Mol. Cell. Biol.* **10,** 3607–3618
36. Peter, M., Nakagawa, J., Doree, M., Labbe, J. C., and Nigg, E. A. (1990) Identification of major nucleolar proteins as candidate mitotic substrates of cdc2 kinase. *Cell* **60,** 791–801
37. Warrener, P., and Petryshyn, R. (1991) Phosphorylation and proteolytic degradation of nucleolin from 3T3–F442A cells. *Biochem. Biophys. Res. Commun.* **180,** 716–723
38. Tawfic, S., Goueli, S. A., Olson, M. O., and Ahmed, K. (1994) Androgenic regulation of phosphorylation and stability of nucleolin in rat ventral prostate. *Prostate* **24,** 101–106
39. Kass, S., Tyc, K., Steitz, J. A., and Sollner-Webb, B. (1990) The U3 small nuclear ribonuclear protein functions at the first step of pre-ribosomal RNA processing. *Cell* **60,** 897–908
40. Azum-Gelade, M. C., Noaillac-Depeyre, J., Caizergures-Ferrer, M., and Gas, N. (1994) Cell cycle redistribution of U3 snRNA and fibrillarin. Presence in the cytoplasmic nucleus remnant and in the prenucleolar bodies at telophase. *J. Cell Sci.* **107,** 463–475
41. Caville J., Nicoloso, M., and Bachellerie, J.-P. (1996) Targeted ribose methylation of RNA *in vivo* directed by tailored antisense RNA guides. *Nature (London)* **383,** 732–736
42. Ginisty, H., Amalric, F., and Bouvet, P. (1998) Nucleolin functions in the first step of ribosomal RNA processing. *EMBO J.* **17,** 1476–1486
43. Sapp, M., Richter, A., Weisshart, K., Caizergues-Ferrer, M., Amalric, F., Wallace, M. O. Kirstein, M. N., and Olson, M. O. J. (1989) Characterization of a 48-kDa nucleic acid binding fragment of nucleolin. *Eur. J. Biochem.* **179,** 541–548
44. Ghizolfi, L., Kharrat, A., Joseph, G., Amalric, F., and Erard, M. (1992) Concerted activities of the RNA recognition and the glycine rich C-terminal domains of nucleolin are required for efficient complex formation with preribosomal RNA. *Eur. J. Biochem.* **209,** 541–548
45. Creancier, L., Prats, H., Zanibellato, C., Amalric, F., and Bugler, B. (1993) Determination of the functional domains involved in nucleolar targeting of nucleolin. *Mol. Biol. Cell* **4,** 1239–1250
46. Bugler, B., Bourbon, H., Lapeyre, B., Wallace, M. O., Chang, J. H., Amalric, F., and Olson, M. O. J. (1987) RNA binding fragments from nucleolin contain the ribonucleoprotein consensus sequence. *J. Biol. Chem.* **262,** 10922–10925
47. Query, C. C., Bently, R. C., and Keene, J. D. (1989) A common RNA recognition motif identified within the defined U1 RNA binding domain of the 70K U1-snRNP protein. *Cell* **57,** 89–101
48. Bouvet, P., Jain, C., Belasco, J. G., and Ama Amalric, F. (1997) RNA recognition by joint action of two nucleolin RNA-binding domains: genetic analysis and structural modeling. *EMBO J.* **16,** 5235–5246
49. Ghisolfi-Nieto, L., Joseph, G., Puvion-Dutilleul, F., Amalric, F., and Bouvet, P. (1996) Nucleolin is a sequence-specific RNA-binding protein: Characterization of targets on pre-ribosomal RNA. *J. Idol. Biol.* **260,** 34–53
50. Miranda, G. A., Chokler, I., and Aguilera, R. J. (1995) The murine nucleolin protein is an inducible DNA and ATP binding protein which is readily detected in nuclear extracts of lipopolysaccharide-treated plenocytes. *Exp. Cell Res.* **217,** 294–308
51. Noaillac-Depeyre, J., Dupont, M. A., Tichadou, J. L., and Gas, N. (1989) The effect of adenosine analogue (DRB) on a major nucleolar phosphoprotein nucleolin. *Biol. Cell* **67,** 27–35
52. Sipos, K., and Olson, M. O. (1991) Nucleolin promotes secondary structure in ribosomal RNA. *Biochem. Biophys. Res. Commun.* **177,** 673–678
53. Heine, M. A., Rankin, M. L., and DiMario, P. J. (1993) The Gly/Arg-rich (GAR) domain of Xenopus nucleolin facilitates *in vitro* nucleic acid binding and *in vivo* nucleolar localization. *Mol. Biol. Cell* **4,** 1189–1204
54. Ghistolfi, L., Joseph, G., Amalric, F., and Erard, M. (1992) The glycine-rich domain of nucleolin has an unusual supersecondary structure responsible for its RNA-helix-destabilizing properties. *J. Biol. Chem.* **267,** 2955–2959
55. Najbauer, J., Johnson, B. A., Young, A. L., and Aswad, D. W. (1993) Peptides with sequences similar to glycine, arginine-rich motifs in proteins interacting with RNA are efficiently recognized by methyltransferase (s) modifying arginine in numerous proteins. *J. Biol. Chem.* **268,** 10501–10509
56. Schmidt-Zachmann, M. S., Dargemont, C., Kuhn, L. C., and Nigg, E. A. (1993) Nuclear export of proteins: the role of nuclear retention. *Cell* **74,** 493–504
57. Kondo, K., and Inouye, M. (1992) Yeast NSR1 that has structural similarity to mammalian nucleolin is involved in pre-RNA processing. *J. Biol. Chem.* **267,** 16252–16258
58. Gulli, M. P., Girard, J. P., Zapetakis, D., Lapeyre, B., Melese, T., and Caizergues-Ferrrer, M. (1995) gar2 is a nucleolar protein from *Schizosaccharomyces pombe* required for 18S rRNA and 40S ribosomal subunit accumulation. *Nucleic Acids Res.* **23,** 1912–1918
59. Kondo, K., Kowalski, L. R., and Inouye, M. (1992) Cold shock induction of yeast NSR1 protein and its role in pre-RNA processing. *J. Biol. Chem.* **267,** 16259–16265
60. Bandziulis, R. J., Swanson, M. S., and Dreyfuss, G. (1989) RNA-binding proteins as developmental regulators. *Genes Dev.* **3,** 431–437
61. Bouvet, P., Diaz, J. J., Kindbeiter, K., Madjar, J. J., and Amalric, F. (1998) Nucleolin interacts with several ribosomal proteins through its RGG domain. *J. Biol. Chem.* **273,** 19025–19029
62. Nigg, E. A. (1997) Nucleocytoplasmic transport: signals, mechanisms and regulation. *Nature (London)* **386,** 779–787
63. Xue, Z., and Melese, T. (1994) Nucleolar proteins that bind NLSs: A role in nuclear import or ribosome biogenesis. *Trends Cell Biol.* **4,** 414–417
64. Xue, Z., Shan, X., Lapryre, B., and Melese, T. (1993) The amino terminus of mammalian nucleolin specifically recognizes SV40 T-antigen type nuclear localization sequences. *Eur. J. Cell Biol.* **62,** 13–21
65. Messmer, B., and Dreyer, C. (1993) Requirements for nuclear translocation and nucleolar accumulation of nucleolin of *Xenopus laevis*. *Eur. J. Cell* **61,** 369–382
66. Schwab, M. S., and Dreyer, C. (1997) Protein phosphorylation sites regulate the function of the bipartite NLS of nucleolin. *Eur. J. Cell Biol.* **73,** 287–297
67. Yu, D., Schwartz, M. Z., and Petryshyn, Ru (1998) Effect of laminin on the nuclear localization of nucleolin in rat intestinal epithelial IEC-6 cells. *Biochem. Biophys. Res. Commun.* **247I**, 86–192
68. Lee, W. C., Zabetakis, D., and Melese, T. (1992) NSR1 is required for pre-RNA processing and for the maintenance of steady-state levels of ribosome subunits. *Mol. Cell. Biol.,* **12,** 3865–3871
69. 69. Srivastava, M., McBride, O. W., Fleming, P. J., Pollard, H. B., and Burns, A. L. (1990) Genomic organization and chromosomal localization of the human nucleolin gene. *J. Biol. Chem.* **265,** 14922–14931
70. Bachellerie, J. P., Michot, B., Nicoloso, M., Balakin, A., Ni, J., and Fournier, M. J. (1995) Antisense snoRNAs: a family of nucleolar RNAs with long complementarities to rRNA. *Trends Biochem. Sci.* **20,** 261–264
71. Nicoloso, M., Caizergues-Ferrer, M., Michot, B., Azum, M. C., and Bachellerie, J. P. (1994) U20, a novel small nucleolar RNA, is encoded in an intron of the nucleolin gene in mammals. *Mol. Cell. Biol.* **14,** 5766–5776
72. Schneider, H. R., Reichert, G. U., and Issinger, O. G. (1986) Enhanced casein kinase II activity during mouse embryogenesis. *Eur. J. Biochem.* **161,** 733–738
73. Bourbon, H. M., Bugler, B., Caizergues-Ferrer, M., and Amalric, F. (1983) Role of phosphorylation on the maturation pathways of a 100 kDa nucleolar protein. *FEBS Lett.* **155,** 218–222
74. Leitinger, N., and Wesierska-Gadek, J. (1993) ADP-ribosylation of nucleolar proteins in HeLa tumor cells. *J. Cell. Biochem.* **52,** 153–158
75. Geahlen, R. L., and Harrison, M. L. (1984) Induction of a substrate for casein kinase II during lymphocyte mitogenesis. *Biochim. Biophys. Acta* **804,** 169–175
76. Schneider, H. R., Mieskes, G., and Issinger, O. G. (1989) Specific dephosphorylation by phosphatases 1 and 2A of a nuclear protein structurally and immunologically related to nucleolin. Possible influence on the regulation of rRNA synthesis. *Eur. J. Biochem.* **180,** 449–455
77. Suzuki, N., Matsui, H., and Hosoya, T. (1985) Effects of androgen and polyamines on the phosphorylation of nucleolar proteins from rat ventral prostates with particular reference to 110-kDa phosphoprotein. *J. Biol. Chem.* **260,** 8050–8055

78. Suzuki, N., Saito, T., and Hosoya, T. (1987) *In vivo* effects of dexamethasone and cycloheximide on the phosphorylation of 110-kDa proteins and protein kinase activities of rat liver nucleoli. *J. Biol. Chem.* **262,** 4696–4700
79. Suganuma, M., Fujiki, H., Furuya-Suguri, H., Yoshizawa, S., Yasumoto, S., Kato, Y., Fusetani, N., and Sugimura, T. (1990) Calyculin A, an inhibitor of protein phosphatases, a potent tumor promoter on CD-1 mouse skin. *Cancer Res.* **50,** 3521–3525
80. Fujiki, H., Suganuma, M., Yoshizawa, S., Nishiwaki, S., Winyar, B., and Sugimura, T. (1991) Mechanisms of action of okadaic acid class tumor promoters on mouse skin. *Environ. Health Perspect.* **93,** 211–214
81. Guy, G. R., Cao, X., Chua, S. P., and Tan, Y. H. (1992) Okadaic acid mimics multiple changes in early protein phosphorylation and gene expression induced by tumor necrosis factor or interleukin-1. *J. Biol. Chem.* **267,** 1846–1652
82. Suzuki, N., Suzuki, T., and Hosoya, T. (1993) Limited proteolysis of rat liver nucleolin by endogenous proteases: effects of polyamines and histones. *Biochem. J.* **289,** 109–115
83. Luscher, B., Kuenzel, E. A., Krebs, E. G., and Eisenman, R. N. (1989) Myc oncoproteins are phosphorylated by casein kinase II. *EMBO J.,* **8,** 1111–1119
84. Glineur, C., Bailly, M., and Ghysdael, J. (1989) The c-erbA-encoded thyroid hormone receptor is phosphorylated in its amino terminal domain by casein kinase II. *Oncogene* **4,** 1247–1254
85. Meek, D., Simon, S., Kikkawa, U., and Eckhart, W. (1990) The p53 tumor suppressor protein is phosphorylated at serine 389 by casein kinase II. *EMBO J.* **9,** 3253–3260
86. Schneider, H. R., and Issinger, O. G. (1988) Nucleolin (23), a physiological substrate for casein kinase II. *Biochem. Biophys. Res. Commun.* **156,** 1390–1397
87. Caizergues-Ferrer, M., Belenguer, P., Lapeyre, B., Amalric, F., Wallace, M. O., and Olson, M. O. J. (1987) Phosphorylation of nucleolin by a nucleolar type NII protein kinase. *Biochemistry* **26,** 7876–7883
88. Ballal, W. R., Kung, Y. J., Olson, M. O. J., and Busch, H. (1975) changes in nucleolar proteins and their phosphorylation patterns during liver regeneration. *J. Biol. Chem.* **250,** 5921–5925
89. Suzuki, N., Suzuki, T., Uchida, A., Thompson, E. A., and Hosoya, T. (1992) Effect of dexamethasone on nucleolar casein kinase II activity and phosphorylation of nucleolin in lymphosarcoma P1798 cells. *J. Steroid Biochem. Mol. Biol.* **42,** 305–312
90. Suzuki, N., Kobayashi, M., Sakata, K., Suzuki, T., and Hosoya, T. (1992) Synergetic stimulatory effect of glucocorticoid, EGF and insulin on the synthesis of ribosomal RNA and phosphorylation of nucleolin in primary cultured rat hepatocytes. *Biochim. Biophys. Acta* **1092,** 367–375
91. Bouche, G., Baldin, V., Belenger, P., Prats, H., and Amalric, F. (1994) Activation of rDNA transcription by FGF-2: key role of protein kinase II. *Cell Mol. Biol.* **40,** 547–554
92. Csermely, P., Schnaider, T., Cheatham, B., Olson, M. O., and Kagn, C. R. (1993) Insulin induces the phosphorylation of nucleolin. A possible mechanism of insulin-induced RNA efflux from nuclei. *J. Biol. Chem.* **268,** 9747–9752
93. Jin, Y. J., and Burakoff, S. J. (1993) The 25-kDa FK506-binding protein is localized in the nucleus and associates with casein kinase II and nucleolin. *Proc. Natl. Acad. Sci. USA* **90,** 7769–7773
94. Kharrat, A., Derancourt, J., Doree, M., Amalric, F., and Erard, M. Synergistic effect of histone H1, and nucleolin on chromatin condensation in mitosis: role of a phosphorylated heteromer. *Biochemistry* **30,** 10329–10336
95. Weisenberger, D., and Scheer, U. (1995) A possible mechanism for the inhibition of ribosomal RNA gene transcription during mitosis. *J. Cell Biol.* **129,** 561–575
96. Xu, X., Cooper, L. G., DiMario, P. J., and Nelson, J.W, (1995) Helix formation in model peptides based on nucleolin TPAKK motifs. *Biopolymers* **35,** 93–102
97. Erard, M. S., Belenguer, P., Caizergues-Ferrer, M., Pantaloni, A., and Amalric, F. (1988) A major nucleolar protein, nucleolin, induces chromatin decondensation by binding to histone H1. *Eur. J. Biochem.* **175,** 525–530
98. Hoffman, J., and Schwoch, G. (1989) Co-ordinated changes in the cyclic AMP signalling system and the phosphorylation of two nuclear proteins of Mr 130,000 and 110,000 during proliferative stimulation of the rat parotid gland by isoprenaline. Possible identity of the two proteins with pp135 and nucleolin. *Biochem. J.* **263,** 785–793
99. Zhou, G., Seiberhener, M. L., and Wooten, M. W. (1997) Nucleolin is a protein kinase C-zeta substrate: Connection between cell surface signaling and nucleus in PCl2 cells. *J. Biol. Chem.* **272,** 31130–31137
100. Jordon, P., Heid, H., Kinzel, V., and Kubler, D. (1994) Major cell surface-located protein substrates of an ecto-protein kinase are homologs of known nuclear proteins. *Biochemistry* **33,** 14696–14706
101. Semenkovich, C. F., Ostlund, R. E., Jr., Olson, M. O., and Yang, J. W. (1990) A protein partially expressed on the surface of HepG2 cells that binds lipoproteins specifically is nucleolin. *Biochemistry* **29,** 9708–9713
102. Tuteja, N., Huang, N. W., Skopac, D., Tuteja, R., Hrvatic, S., Zhang, J., Pongor, S., Joseph, G., Faucher, C., Amalric, F., and Falaschi, A. (1995) Human DNA helicase IV is nucleolin, an RNA helicase modulated by phosphorylation. *Gene* **160,** 143–148
103. Pasternack, M. S., Bleier, K. J., and McInerney, T. N. (1991) Granzyme A binding to target cell proteins. Granzyme A binds to and cleave nucleolin *in vitro. J. Biol. Chem.* **266,** 14703–14708
104. Arends, M. J., Morris, R. G., and Wyllie, A. H. (1990) Apoptosis: the role of the endonuclease. *Am. J. Pathol.* **136,** 593–608
105. Smith, M. J., Browne, K. A., Thia, K. Y., Apostolidis, V. A., Kershaw, M. H., and Trapani, J. A. (1994) Hypothesis: cytotic lymphocyte granule serine proteases activate target cell endonucleases to trigger apoptosis. *Clin. Exp. Pharmacol. Physiol.* **21,** 67–70
106. Brockstedt, E., Rickers, A., Kostka, S., Laubersheimer, A., Dorken, B., Wittmann-Liebold, B., Bommert, K., and Otto, A. (1998) Identification of apoptosis-associated proteins in a human Burkitt lymphoma cell line. Cleavage of heterogeneous nuclear ribonucleoprotein A1 by caspase 3.J. *Biol. Chem.* **273,** 28057–28064
107. Belenguer, P., Baldin, V., Mathieu, C., Prats, H., Bensaid, M., Bouche, G., and Amalric, F. (1989) Protein kinase NII and regulation of rDNA transcription in mammalian cells. *Nucleic Acids Res.* **17,** 6625–6635
108. Ohmori, H., Murakami, T., Furutani, A., Higashi, K., Hirano, H., Gotoh, S., Kuroiwa, A., Masui, A., Nakamura, T., and Amalric, F. (1990) Simultaneous activation of heat shock protein (hsp 70) and nucleolin genes during *in vivo* and *in vitro* prereplicative stages of rat hepatocytes. *Exp. Cell Res.* **189,** 227–232
109. Shi, Y., and Thomas, J. O. (1992) The transport of proteins into the nucleus requires the 70-kilodalton heat shock protein or its cytosolic cognate. *Mol. Cell. Biol.* **12,** 2186–2192
110. Dang, C. V., and Lee, W. M. F. (1989) Nuclear and nucleolar targeting sequences of c-erb-A, c-myc, N-myc, p53, HSP70, and HIV tat proteins. *J. Biol. Chem.* **264,** 18019–18023
111. Callebaut, C., Blanco, J., Benkirane, N., Krust, B., Jacotot, E., Guichard, G., Seddiki, N., Svab, J., Dam, E., Muller, S., Briand, J. P., and Hovenessian, A. G. (1998) Identification of V3 loop-binding proteins as potential receptors implicated in the binding of HIV particles to CD4(+) cells. *J. Biol. Chem.* **273,** 21988–21997
112. Becker, J., and Craig, E. A. (1994) Heat-shock proteins as molecular chaperones. *Eur. J. Biochem.* **219,** 11–23
113. Derenzini, M., Sirri, V., Trere, D., and Ochs, R. S. (1995) The quantity of nucleolar proteins nucleolin and protein B23 is related to cell doubling time in human cancer cells. *Lab. Invest.* **73,** 497–502
114. De Verdugo, U. R., Selinkga, H. C., Huber, M., Kramer, B., Kellermann, J., Hofschneider, P. H., and Kandolf, R. (1995) characterization of a 100-kilodalton binding protein for the six serotypes of Coxsackie B viruses. *J. Virol.* **69,** 6751–6757
115. Roussel, P., and Hernandez-Verdun, D. (1994) Identification of Ag-NOR proteins, markers of proliferation related to ribosomal gene activity. *Exp. Cell Res.* **214,** 465–472
116. Roussel, P., Sirri, V., and Hernandez-Verdun, D. (1994) Quantification of Ag-NOR proteins using Ag-NOR staining on Western blots. *J. Histochem. Cytochem.* **42,** 1513–1517
117. Sirri, V., Roussel, P., Trere, D., Derenzini, M., and Hernandez-Verdun, D. (1995) Amount variability of total and individual

Ag-NOR proteins in cells stimulated to proliferate. *J. Histochem. Cytochem.* **43,** 887–893
118. Gillet, G., Michel, D., Crisanti, P., Guerin, M., Herault, Y., Pessac, B., Calothy, G., Brun, G., and Volovitch, M. (1993) Serum factors and v-src control two complementary mitogenic pathways in quail neuroretinal cells in culture. *Oncogene* **8,** 564–574
119. Bogre, L., Jonak, C., Mink, M., Meskiene, I., Trass, J., Ha, D. T., Swoboda, I., Plank, C., Wagner, E., Heberle-Bors, E., and Hirt, H. (1996) Developmental and cell cycle regulation of alfalfa nucMs1, a plant homolog of the yeast Nsr1 and mammalian nucleolin. *Plant Cell* **8,** 417–428
120. Murakami, T., Ohmari, H., Tsuda, T., and Higashi, K. (1991) The effect of changes of N-myc products on the gene expression of heat-shock protein (hsp 70) and nucleolin during differentiation of neuroblastoma cells. *Prog. Clin. Biol. Res.* **366,** 65–70
121. Biggiogera, M., Kaufmann, S. H., Shaper, J. H., Gas, N., Amalric, F., and Fakan, S. (1991) Distribution of nucleolar proteins B23 and nucleolin during mouse spermatogenesis. *Chromosoma* **100,** 162–172
122. Maridor, G., Krek, W., and Nigg, E. A. (1990) Structure and developmental expression of chicken nucleolin and NO38: coordinate expression of two abundant non-ribosomal nucleolar proteins. *Biochim. Biophys. Acta* **1049,** 126–133
123. Mehes, G., and Pajor, L. (1995) Nucleolin and fibrillarin expression in stimulated lymphocytes and differentiating HL-60 cells. *Cell Prolif.* **28,** 329–336
124. Li, D., Dobrowolska, G., and Krebs, E. G. (1996) The physical association of casein kinase 2 with nucleolin. *J. Biol. Chem.* **271,** 15662–15668
125. Li, Y. P., Busch, R. K., Valdez, B. C., and Busch, H. (1996) C23 interacts with B23, a putative nucleolar-localization-signal-binding protein. *Eur J. Biochem.* **237,** 153–158
126. Bharti, A. K., Olson, M. O. J., Kufe, D. W., and Rubin, E. H. (1996) Identification of a nucleolin binding site in human topoisomerase 1. *J. Biol. Chem.* **271,** 1993–1997
127. Leger-Silvestre, I., Guelli, M. P., Noaillac-Depeyre, J., Faubladier, M., Sicar, H., Caizergues-Ferrer, M., and Gas, N. (1997) Ultrastructural changes in the *Schizosaccharomyces pombe* nucleolus following the disruption of the gar2+ gene, which encodes a nucleolar protein structurally related to nucleolin. *Chromosoma* **105,** 542–552
128. Zaidi, S. H. E., and Malter, J. (1995) Nucleolin and heterogeneous nuclear ribonucleoprotein C proteins specifically interact with the 3′-untranslated region of amyloid protein precursor mRNA. *J. Biol. Chem.* **270,** 17292–17298
129. Take, M., Tsutsui, J., Obama, H., Ozawa, M., Nakayama, T., Maruyama, I., Arima, T., and Muramatsu, T. (1994) Identification of nucleolin as a binding protein for midkine (MK) and heparin-binding growth associated molecule (HB-GAM). *J. Biochem.* **116,** 1063–1068
130. Dickinson, L. A., and Kohwi-Shigematsu, T. (1995) Nucleolin is a matrix attachment region DNA-binding protein that specifically recognizes a region with high base-unpairing potential. *Mol. Cell. Biol.* **15,** 456–465
131. Barrijal, S., Perros, M., Gu, Z., Avalosse, B. L., Belenguer, P., Amalric, F., and Rommelaere, J. (1992) Nucleolin forms a specific complex with a fragment of the virus (minus) strand of minute virus of mice DNA. *Nucleic Acids Res.* **20,** 5053–5060
132. Pfeiffe, J., and Anderer, F. A. (1983) Isolation and characterization of phosphoprotein pp105 from simian virus 40-transformed mouse fibroblasts. *Biochim. Biophys. Acta***762,** 86–93
133. Kleinman, H. K., Weeks, B. S., Cannon, F, B., Sweeney, T. M., Sephel, G. C., Clement, B., Zain, M., Olson, M. O. J., Jucker, M., and Burrous, B. A. (1991) Identification of a 110-kDa nonintegrin cell surface laminin-binding protein which recognizes an A chain neurite-promoting peptide. *Arch. Biochem. Biophys.* **290,** 320–325
134. Kibbey, M. C., Johnson, B., Petryshyn, R., Jucker, M., and Kleinman, H. K. (1995) A 110-hD nuclear shuttling protein, nucleolin, binds to the neurite-promoting IKVAV site of laminin-1. *J. Neurosci. Res.* **42,** 314–322
135. Lee, C. H., Chang, S. C., Chen, C. J., and Chang, M. F. (1998) The nucleolin binding activity of hepatitis delta antigen is associated with nucleolus targeting. *J. Biol. Chem.* **273,** 7650–7656
136. Krantz, S., Salazar, R., Brandt, R., Kellerman, J., and Lottspeich, F. (1995) Purification and partial amino acid sequencing of a fructosyllysine-specific binding protein from cell membranes of the monocyte-like cell line U937. *Biochim. Biophys. Acta* **1266,** 109–112
137. Zhang, J., Bottaro, A., Li, S., Stewart, V., and Alt, F. W. (1993) A selective defect in IgG2b switching as a result of targeted mutation of the Ig 2b promoter and exon EMBO J. **12,** 3529–3537
138. Bottaro, A., Lansford, R., Xu, L., Zhang, J., Rothman, P., and Alt, F. W. (1994) S region transcription per se promotes basal IgE class switch recombination but additional factors regulate the efficiency of the process. *EMBO J.* **13,** 665–674
139. Ishikawa, F., Matunis, M. J., Dreyfuss, G., and Cech, T. R. (1993) Nuclear proteins that bind the pre-mRNA 3′ splice site sequence r(UUAG/G) and the human telomeric DNA sequence d(TTAGGG)n. *Mol. Cell. Biol.* **13,** 4301–4310
140. Jarjour, W. N., Minota, S., Roubey, R. A., Mimura, T., and Winfield, J. B. (1992) Autoantibodies to nucleolin cross-react with histone H1 in systematic lupus erythematosus. *Mol. Biol.* **16,** 263–266
141. 141. Soulard, M., Lagaye, S., Della Valle, V., Danon, F., Larsen, C. J., and Barque, J. P. (1989) Nucleolar proteins identified in human cells as antigens by sera from dogs with autoimmune disorders. *Exp. Cell Res.* **182,** 482–498
142. Bell, S. A., Faust, H., Mittermuller, J., Kolb, H. J., and Meurer, M. (1996) Specificity of antinuclear antibodies in scleroderma-like chronic graft-versus-host disease: clinical correlation and histocompatibility locus antigen association. *Br. J. Dermatol* **134,** 848–854
143. Tan, E. M. (1989) Antinuclear autoantibodies: diagnostic markers for autoimmune diseases and probes for cell biology. *Adv. Immunol.* **44,** 93
144. Bautz, E. K. F., Kalden, J. R., Homma, M., and Tan, E. M. (1989) *Molecular and Cell Biology of Autoantibodies and Autoimmunity,* Vol. 118, Springer-Verlag, Berlin Heidelberg
145. McDonald, H., Smailus, D., Jenkins, H., Adam, K., Simpson, N. E., and Goodfellow, P. J. (1992) Identification and characterization of a gene at D10S94 in the MEN2A region. *Genomics* **13,** 344–348