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ORIGINAL ARTICLE

Expression and translocation of major nucleolar proteins in relation to the transcriptional activity of the nucleolus

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Summary
Changes in the expression and cellular localization of abundant nucleolar proteins: C23/nucleolin, upstream binding factor (UBF), B23/nucleophosmin, and fibrillarin were examined in human lymphocytes subjected to the control of nucleolar activity by phytohemagglutinin and actinomycin D. Data suggest that the up-regulation of ribosomal RNA transcription induced by phytohemagglutinin was accompanied by a significant increase in the nucleolar content of C23/nucleolin, UBF, B23/nucleophosmin, whilst the nucleolar content of fibrillarin had a relatively low-variable. An unraveling of the multicopy ribosomal gene accompanying the mitogenic stimulation was detected through the immunofluorescence of UBF permanently associated with rDNA. Down-regulation of RNA polymerase I activity induced by actinomycin D, 24 hrs after initiating stimulation, did not influence the expression of C23/nucleolin, UBF, and fibrillarin, and up-regulated the expression of B23/nucleophosmin. This inhibition resulted in the translocation of chaperons C23/nucleolin and B23/nucleophosmin to the nucleoplasm, while UBF and fibrillarin persisted in the nucleolus. The re-clustering of dispersed transcription units of rDNA, induced by actinomycin D, despite the persistence of UBF in the nucleolus, contradicts the hypothesis that neo-synthesis of UBF is the main drive for unraveling a multicopy rDNA gene. The translocations of C23/nucleolin and B23/nucleophosmin are discussed in relation to the cellular stress response caused by the genotoxic activity of actinomycin D. It is suggested that the reannealing activity of nucleolin and nucleophosmin helps to drive the genotoxic agent to the nucleolus, diminishes the diversity of genotoxic damage and inhibits the growth-division activity of cells.

Keywords: UBF – C23 – nucleolin – B23 – nucleophosmin – fibrillarin –stimulated lymphocytes

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INTRODUCTION

The nucleolus is a key organelle which coordinates the synthesis and assembly of ribosomal subunits and forms in the nucleus around the repeated ribosomal gene clusters. Because the production of ribosomes is a major metabolic activity, the

function of the nucleolus is tightly bound to cell growth and proliferation (Pederson 1998, Hannan et al.1998a, Leary and Huang 2001, Moss and Stefanovsky 2002, Grummt 2003, Moss 2004, Raška et al. 2004, Russell and Zomerdijk 2005). The up-regulation of ribosomal biogenesis which accompanies the stimulation of cellular growth and mitogenic activation is promoted by increasing the nucleolar content of proteins engaged in the transcription, processing and co-transcriptional packaging of the pre-rRNA (Hannan et al. 1995, Derenzini 2000, Kruger et al. 2000, Sirri et al. 2000, Brandenburger et al. 2003). Numerous data suggest that the nucleolus is implicated in various aspects of cell biology which include functions such as cell-cycle regulation, gene silencing, senescence and stress responses (Olson et al. 2000, 2002, Iliakis et al. 2004, Andersen et al. 2005, Bicknell et al. 2005, Olson and Dundr 2005). Structural and localization studies have revealed that nucleolar components move about in a variety of ways during ribosomal maturation, at the beginning and at the end of mitosis and that many non-ribosomal regulatory proteins pass through or are retained by the nucleolus (Scott et al. 2001, Olson and Dundr 2005). Some of them are accompanied by multifunctional nucleolar proteins commonly engaged in ribosomal precursor processing. Cell specific translocations of nucleolin and nucleophosmin induced by the necessity of transient extra-nucleolar action represent the subject of recent studies (Liu and Yung 1999, Daniely et al. 2002, Gao et al. 2005).

The stimulation of human T lymphocytes with phytohemagglutinin is an established model for studying the (in vitro) transformation of quiescent cells into primitive blast-like cells which then undergo mitosis (Nowell 1960, Cooper et al. 1963, Marshall and Roberts 1963, Raška and Smetana 1978). This process, accompanied by remarkable structural changes in rDNA chromatin, monitored through fibrillar center distribution (Hozák et al. 1989, Ochs and Smetana 1989), is supported by the strong promotion of the transcription of ribosomal rRNA (Čabart and Kalousek 1998) governed by the transcriptional machinery of RNA polymerase I. Numerous studies examining the opposite effect, i.e. the extra-cellular stimuli dependent down-regulation of ribosomal transcription in rapidly growing and proliferating cells have also been published (Zatsepina et al. 1993, Mahajan 1994, Ghoshal and Jacob 1996, Perlaky et al. 1997, Jordan and Carmo-Fonseca 1998, Zhai et al. 1998, Chen and Mikecz 2000, Mayer et al. 2004, 2005). The estimable volume of data demonstrates the complexity and diversity of the action mechanism, as well as the cell and agent specificity of various anti-cancer drugs and the participation of multifunctional nucleolar proteins in cell responsive processes. In this report, changes in the amount and in the subcellular localization of four abundant nucleolar proteins: nucleolin, UBF, nucleophosmin and fibrillarin were examined in relation to the phytohemagglutinin (PHA) induced promotion and actinomycin D (ActD) induced attenuation of the nucleolar activity.

MATERIAL AND METHODS

Cell cultures: Human lymphocytes were isolated from the peripheral blood of healthy volunteers using a discontinuous density gradient (Böyum 1968) of Histopaque 1077 (Sigma Chemicals, St Louis, MO, USA). Quiescent lymphocytes were suspended at $1x10^6$ cells/ml in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and stimulated with 10 µg/ml of PHA (Sigma) at 37°C in a 5% CO_2 humidified atmosphere for 48 hrs. To selectively inhibit RNA polymerase I, low doses (final concentration 10 nM) of ActD (Fluka, Chemie AG, Busch, Germany) were added to the cultures. To estimate the replication activity of stimulated lymphocytes the incorporation of 5-bromo-2´deoxyuridine (BrdU) into DNA was monitored using BrdU proliferation ELISA (Amersham Biosciences GmbH, Freiburg, Germany) according to the manufacturer's protocol.

Nuclear Run-On transcription of primary rRNA transcript: Nuclei, isolated from $1x10^7$ lymphocytes, as described by Marzluff and Huang (1987) were incubated with 20 µCi of α-^{32}P-GTP (MP Biochemicals, Irvine, CA, USA) in buffer containing 25 mM TRIS-HCl (pH 8.0), 12.5% glycerol, 2.5 mM Mg-Acetate, 4 mM $MgCl_2$, 60 mM KCl, 1 mM of ATP, CTP, UTP and 0.1 mM GTP, 0.05 mM EDTA, 2.5 mM DTT, 10 µl/ml of protease inhibitor cocktail (Sigma) and 1 unit/µl of human placental RNase inhibitor (Amersham Biosciences) in a total volume of 100 µl 60 min at 25°C. 10 nM ActD was added optionally. Radio labeled RNA was isolated using an RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany) and used in hybridizations. Two micrograms of both plasmid pB, containing 5.8 kbp EcoRI fragment B of human ribosomal gene cloned into pBR322 and pBR322 alone as negative control, were denaturated and immobilized on the Hybond-N membrane (Amersham Biosciences). Specific ribosomal RNA transcripts were detected on nylon strips subjected to hybridization in accordance with the manufacturer's protocol for RNA probes. A film-less autoradiography was performed on Fujifilm imaging plates. After 4 hrs of strip exposure, transcription signals were visualized and quantified by scanning autoradiograms (PhosphorImager FLA 2000, Fuji Medical Systems Inc., Stamford, CT, USA).

Immunoblots: To examine the content of nucleolin, UBF, nucleophosmin and fibrillarin in whole cell lysates and nuclear lysates from quiescent, PHA stimulated, and ActD treated lymphocytes, nuclei from $2x10^7$ cells were isolated (Marzluff and Huang, 1987). Whole cells as well as nuclei were then boiled in 50 µl of Laemelli buffer. After overnight centrifugation at 40,000 RPMI and 4^oC on the Beckman ultracentrifuge, supernatants were subjected to 10% SDS PAGE and the separated proteins were blotted, using a semi-dry system (Sigma), onto Hybond nitrocellulose membranes (Amersham Biosciences). Blots were blocked for 4 hrs with 5% non-fat dried milk in PBS-0.05% Tween 20, 0.01% NaN_3 then primary antibodies i.e. rabbit anti-UBF (Kalousek and Křížková 2000), rabbit anti-nucleolin, anti-nucleophosmin or anti-fibrillarin (SantaCruz Biotechnology Inc, SantaCruz, CA, USA) were added to the final dilution 1:2000, and blots were incubated overnight. After washing three times for 5 min with PBS-0.05% Tween, the HRP-conjugated secondary antibody (Amersham Biosciences) diluted 1:100,000 in PBS-0.05% Tween was added for 2 hrs. After another three washes, the blots were developed using the ECL^+ technique. Chemiluminiscent signals were visualized and quantified using the Fluor-SMultiImager System (Bio-Rad Laboratories, Hercules, CA, USA).

Immunofluorescent microscopy: Lymphocytes from PHA and ActD treated cultures were cytocentrifuged on glass slides at 2000 rpm for 4 min and fixed and permeabilized with acetone / methanol 1:1 for 10 min at 20^0C. The cells were subsequently incubated with rabbit antibodies against UBF, nucleolin, nucleophosmin or fibrillarin diluted 1:20 for 45 min at 37^0C. After washing (3 × 5 min) in PBS, the cells were incubated with a FITC-conjugated secondary antibody (Sigma) diluted 1:50 in PBS for 30 min at 37^0C. After repeated washing in PBS, the cells were mounted in a Vectashield medium (Vector Laboratories Inc., Burlingame, CA, USA). Samples were analyzed using Olympus IMT-2 apparatus (Olympus America Inc., Melville, NY, USA). Photographs were taken and images edited using an Olympus digital camera C-3030 and Camedia Suite Olympus Software.

Statistical analysis: Signals from film-less autoradiography and ECL^+ chemiluminiscent signals were evaluated as integrated peak intensities using Software AIDA (Raytest GmbH Straubenhardt, Germany). The experiments were repeated three times, and the means of integrated peak intensities were calculated. The means of triplicate values with SEM error bars are demonstrated in diagrams. Significance levels (p values) were determined by a t-test carried out using Software InStat (GraphPad Software Inc., San Diego, CA, USA). A value of 0.05 or lower was considered a statistically significant difference between the groups compared.

RESULTS

Replication activity in relation to PHA and ActD treatment: Incorporation of BrdU into the replicating DNA (Fig. 1) indicated that quiescent lymphocytes (column 1) and lymphocytes stimulated with PHA for 12 hrs prior to adding ActD did not enter the synthetic phase (column 2). A low fraction of lymphocytes stimulated with PHA started to replicate their DNA 24 hrs after adding PHA (column 3). Replication culminated after 48 hrs of stimulation (column 4) and practically all cells were committed to G1-S transition despite the presence of 10 nM ActD added 24 hrs after initiating stimulation (column 5).

Transcriptional activity of ribosomal genes in relation to PHA and ActD treatment: As indicated in Fig. 2 PHA induced transformation of quiescent lymphocytes (column 1) to lymphoblast-like cells was accompanied by an increased activity of the transcription of ribosomal RNA, demonstrated by the increasing value of the Run-On transcription signal 48 hrs after adding PHA (column 2). This resulted in low doses of ActD, after a temporary increase (statistically significant) of the Run On Signal 8 hrs after adding ActD (column 3), in profound decrease of RNA polymerase I activity, 16 hrs after adding the drug (column 4).

Expression of nucleolar proteins in relation to PHA and ActD treatment: Amounts of nucleolar proteins (nucleolin (C23 in Fig. 3), UBF1, nucleophosmin (B23 in Fig. 3), and fibrillarin) in lymphocytes subjected to PHA stimulation and ActD treatment were examined in Western blots of whole cell and nuclear lysates, raised with specific antibodies. A statistical evaluation of data, shown in Fig. 3, demonstrate no significant differences between cellular and nuclear amounts of examined proteins (statistically not significant) and it is likely that the majority of the proteins are localized in the nuclei. The data in the white and gray columns demonstrate the cellular and nuclear levels of nucleolin, UBF and nucleophosmin significantly augmented following 48 hrs of PHA stimulation (statistically significant). The increase in the cellular and nuclear levels of fibrillarin was less significant (statistically significant). Lymphocytes stimulated with PHA for 48 hrs in cultures added with 10 nM ActD 24 hrs after initiating stimulation (black columns) gained just about the same cellular and nuclear contents of nucleolin, UBF and

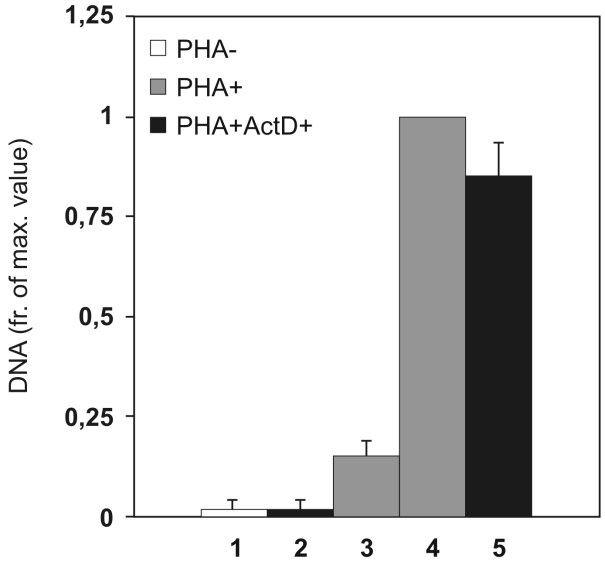

Fig.1. **Replication activity of PHA stimulated lymphocytes and the commitment of lymphocytes stimulated with PHA for 24 hrs to G1-S transition.** (1) quiescent cells, (2) 48 hrs in the presence of PHA and ActD was added 12 hrs after initiating stimulation, (3) 24 hrs of stimulation, (4) 48 hrs of stimulation, (5) 48 hrs in the presence of PHA and ActD was added 24 hrs after initiating stimulation.

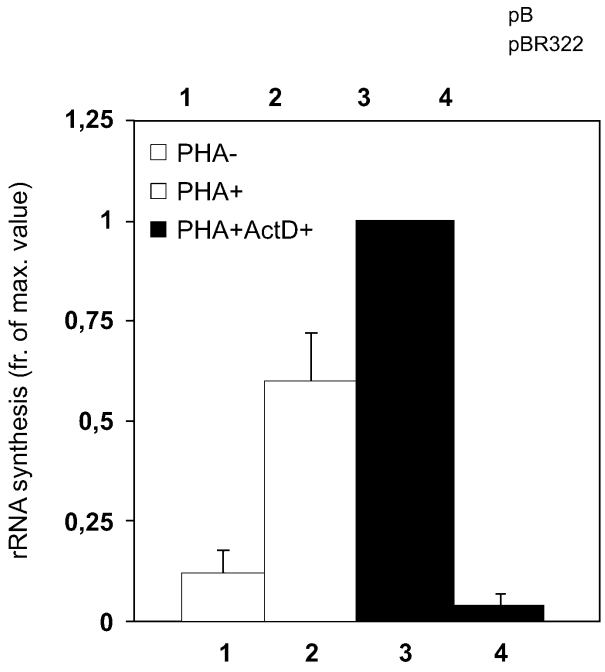

Fig. 2. **Changes in the activity of the ribosomal gene transcription in PHA stimulated lymphocytes and stimulated lymphocytes treated with 10 nM ActD.** ^{32}P-GTP labeled rRNA synthesized on isolated nuclei in Run-On Assays was hybridized to rDNA gene (pB) immobilized on a nylon strip. Plasmid pBR322 was used as negative control. (1) quiescent lymphocytes, (2) lymphocytes stimulated with PHA for 48 hrs in the absence of ActD, (3) lymphocytes stimulated with PHA for 48 hrs in the presence of ActD for the last 8 hrs of PHA treatment, (4) lymphocytes stimulated with PHA for 48 hrs in the presence of ActD for the last 16 hrs of PHA treatment.

fibrillarin as lymphocytes growing in ActD free cultures (gray columns) (statistically not significant). In contrast to the indifference expressed by nucleolin, UBF and fibrillarin to the presence of a low concentration of ActD, the cellular and nuclear content of nucleophosmin significantly increased (statistically significant) in lymphocytes subjected to the presence of 10 nM ActD 24 hrs after initiating stimulation.

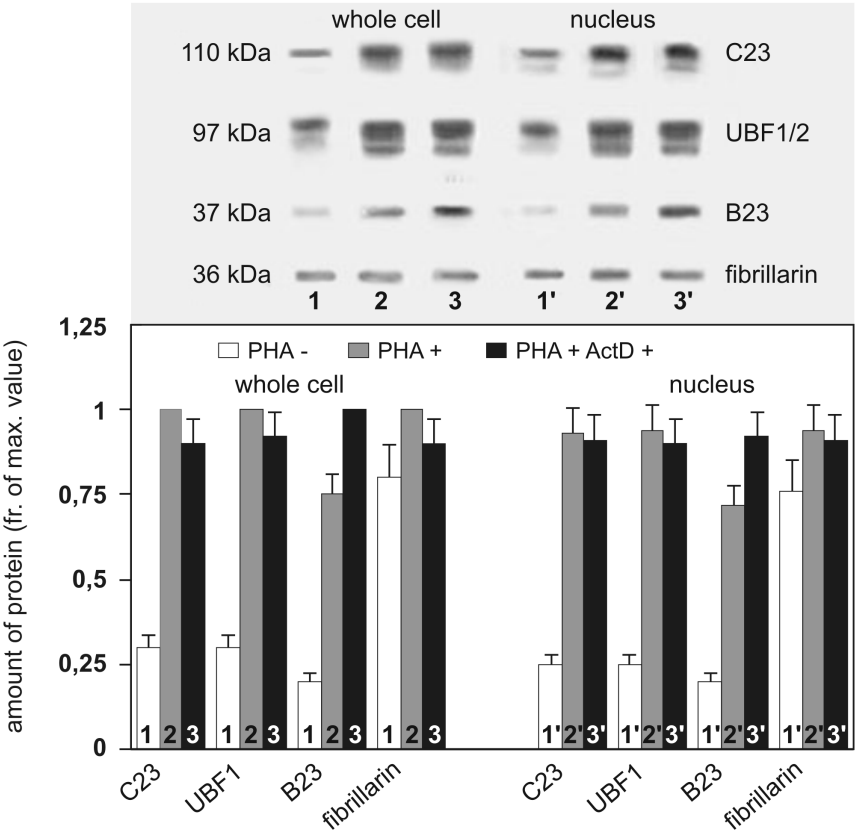

Fig.3. **Expression and nuclear localization of major nucleolar proteins in PHA stimulated lymphocytes and stimulated lymphocytes treated with 10 nM ActD.** Whole cells lysates (1, 2, 3) and nuclear lysates (1´ 2´ 3´) were separated by SDS PAG electrophoresis, blotted on nitrocellulose membrane and blots raised by specific antibodies. Immunocomplexes in lanes 1 and 1´ (white columns in the graph) harbour proteins originating from quiescent cells, lanes 2 and 2´ (gray columns in the graph) proteins from lymphocytes stimulated with PHA for a period of 48 hrs and lanes 3 and 3´ (black columns in the graph) proteins from PHA stimulated lymphocytes growing for 48 hrs in cultures added with 10 nM ActD 24 hrs after initiating stimulation.

Translocation of nucleolar proteins in relation to PHA and ActD treatment: The data obtained from immunofluorescent microscopy, collected in Fig. 4 demonstrate that the increase of nucleolar activity of quiescent lymphocytes (row quiescent) induced by mitogenic stimulation with PHA (row PHA) was accompanied by the accumulation of nucleolin (C23 column), UBF (UBF column), nucleophosmin (B23 column) and fibrillarin (FIBRILLARIN column) in the nucleolus. The process gave rise to the significant enlargement of immunofluorescence spots of nucleolin and nucleophosmin. UBF, which is associated with one or two fibrillar centers in quiescent lymphocytes, was dispersed subsequent to PHA stimulation and the structure of a great number of separated spots appeared. In comparison with other proteins, the nucleolar transformation monitored by the anti-fibrillarin antibody was hardly remarkable. The significant diminution of nucleolar activity induced with a low concentration

of ActD (row PHA + ActD) gave rise to the translocation of a part of nucleolin and a huge part of nucleophosmin outside of the nucleolus. No significant translocation of fibrillarin was found and an accumulation of a great number of small UBF positive spots into configuration frequently demonstrating three, four or five immunofluorecent signals was observed.

DISCUSSION

In this report, changes in the amount and in the subcellular localization of four abundant nucleolar proteins were examined in relation to the promotion and attenuation of nucleolar activity. From various experiments, it is known that the mitogenic stimulation of quiescent lymphocytes with PHA is accompanied by a significant increase in RNA polymerase I activity (Dauphinais 1981, Doggett et al. 1986, Ochs and Smetana 1989, Čabart and Kalousek 1998, Gorczyca et al. 2001) and an estimable number of articles demonstrating the inhibitory effect of ActD on ribosomal RNA elongation have been published. Our data from the Run-On assay which demonstrate a five-fold increase in the rate of rRNA synthesis within a period of 48 hrs after adding PHA and a subsequent twenty-fold decrease 16 hrs after adding 10 nM ActD, authorize the use of PHA stimulated and ActD treated lymphocytes as a convenient model system of controlled nucleolar activity. Our findings show that by adding 10 nM ActD to the lymphocyte culture, you get a non-monotonic effect on the rate of ribosomal transcription which is surprising in the context of the known molecular mechanism of the ActD induced elongation arrest (Sobell 1985, Miller 1987). The antibiotic intercalates into double-stranded DNA between successive G-C base-pairs, and premelted DNA present within the elongation complex is the preferred structure (Sobell 1985, Yu and Bender 1990). The simple mechanism of elongation arrest is a plausible interpretation of the concentration dependent inhibition of rRNA synthesis; however, the premature increase in the transcriptional rate caused by ActD requires another explanation. Hadjiolova et al. (1995) revealed that ActD stimulates the transcription of rRNA minigenes and suggested that the intercalation of ActD changes the conformation of DNA in the promoter region in a manner which stimulates the transcription. Bazett-Jones and co-workers proposed that the RNA polymerase I transcription is activated by HMG boxes of the architectural factor UBF, which loops rDNA in the promoter region into the structure of enhancesome (Bazett-Jones et al. 1994, Pelletier et al. 2000, Stefanovsky et al. 2001). We speculate that the structure of DNA imposed by UBF on the G-C rich promoter region is stabilized by G-C dependent ActD intercalation. We suggest that the early activation to the transcription of "low-damaged" rDNA is the result of a more efficient formation of the initiation complex.

Our results confirmed the findings of other laboratories that major nucleolar proteins accumulate in the nucleolus as a consequence of stimulating the ribosomal transcription (Feuerstein et al. 1988, Mehes and Pajor 1995, Hannan et al. 1995, 1996, 1998b, Čabart and Kalousek 1998, Sirri et al. 1997, 2000, Gorczyca et al. 2001, Dergunova et al. 2002). Our data demonstrating an increase of UBF content in the nuclei of stimulated lymphocytes and the findings of other authors (Zatsepina et al. 1993, Perlaky et al. 1997) indicating an exclusive localization of UBF in nucleoli, despite the arrest of rRNA transcription, suggest that UBF is associated both with transcriptionally active and inactive rRNA genes. The treatment of stimulated cells with 10 nM ActD had a distinct effect on each of the proteins investigated. Although UBF and fibrillarin did not leave the nucleolus, nucleolin and nucleophosmin translocated from the nucleolus to nucleoplasm. Our data from anti-UBF immunofluorescent microscopy mirror the effect of the unraveling of the multicopy ribosomal gene during its activation as described by Ochs and Smetana (1989). They demonstrated, using antibodies against RNA polymerase I, that, in time, following PHA stimulation of quiescent lymphocytes, each fibrillar center "unwinds" to form linear arrays of smaller fibrillar centers until their number approaches the ribosomal gene copy number. The molecular forces which drive this process are still obscure. The hypothesis that neo-synthesized UBF may play an active role is relevant due to the revelation that a part regulatory function of UBF may be executed by modifying a three-dimensional DNA structure (Kermekchiev et al. 1997, Stefanovsky et al. 1996, 2001) and, due to the finding of O'Sullivan et al. (2002), that UBF binds across the entire intergenic spacer and transcribed region. Recently, Chen et al. (2004) revealed large-scale chromatin decondensation induced by UBF association. However, our data suggesting that the presence of ActD induces the re-clustering of dispersed transcription units despite the persistence of UBF in the nucleolus contradicts this hypothesis. From this point of view, the nucleolar accumulation of negatively charged nucleolin (Roussel and Hernandez-Verdun 1994) linked with rDNA through the nascent rRNA transcript (Roger et al. 2003), and leaving the nucleolus after ActD intervention seems to be a better candidate.

Our finding that fibrillarin persisted in the nucleoli of human lymphocytes independently of ActD treatment is similar to that of Puvion-

Dutilleul et al. (1992) which revealed the persistent presence of U3 RNA and fibrillarin in the nucleoli of HeLa and mouse 3T3 cells after ActD induced pre-rRNA depletion. They suggested that the dense fibrillar component could represent an anchorage site for U3 snRNPs, before entering another cycle of pre-rRNA processing reactions. In contrast, Chen and Jiang (2004) demonstrated that 50 nM ActD induced dislocation of fibrillarin from the nucleoli of human HEp-2 cells and concluded that the persistence of fibrillarin in the nucleoli of cells treated with ActD appears to have a cell type-specific effect. Our Run-On data indicated that the treatment of human lymphocytes with ActD attenuated the ribosomal transcription very profoundly but did not stop it. Immunoblotting data on quiescent lymphocytes demonstrated a relatively high level of fibrillarin in the quiescent nucleoli. Thus the possible ability of low amounts of transcript or unfinished sequences to retain fibrillarin within the nucleolus should be taken into account.

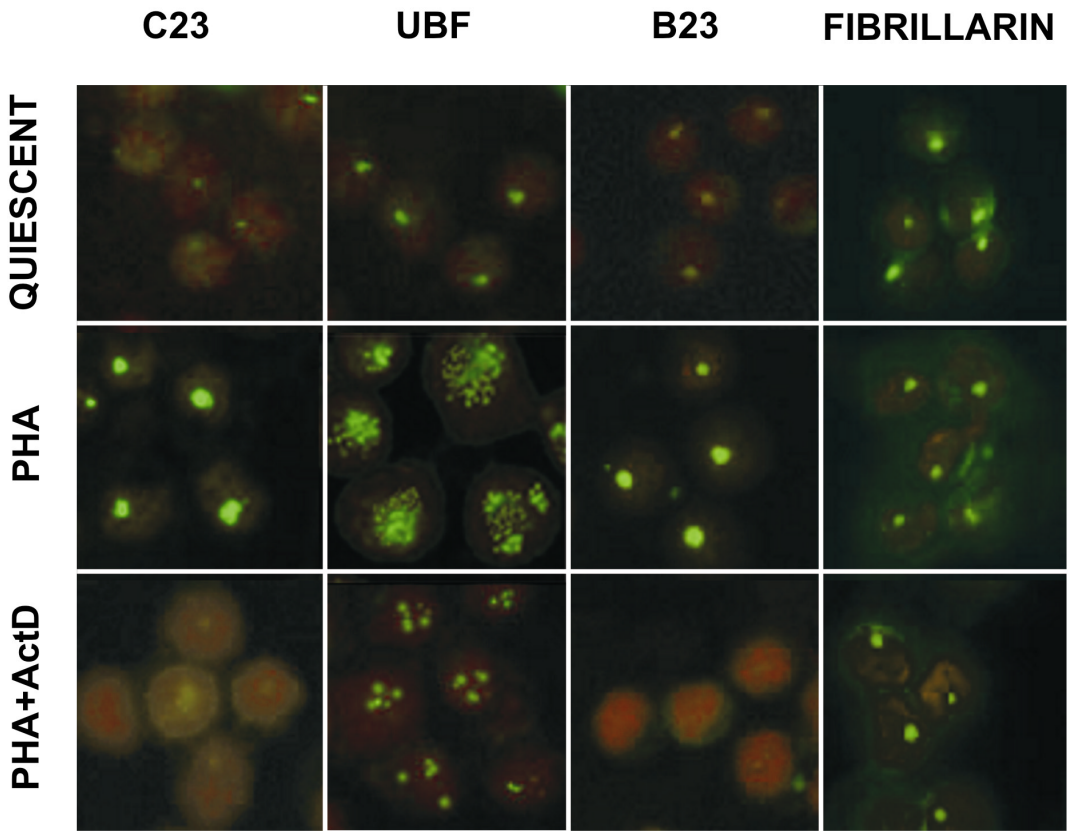

Fig.4. **PHA stimulated nucleolar accumulation and ActD induced translocation of major nucleolar proteins in human lymphocytes.** Nucleolin (column C23), UBF (column UBF), nucleophosmin (column B23) and fibrillarin (column FIBRILLARIN). The immunofluorescent microscopy of quiescent cells (row QUIESCENT), cells treated for a period of 48 hrs with PHA (row PHA) and cells cultured for 48 hrs in a medium containing PHA and supplemented with 10 nM ActD 24 hrs after adding PHA (row PHA + ActD).

Our data suggest that in human lymphocytes cultured in the presence of 10 nM ActD the nucleolin and nucleophosmin translocate from the nucleolus to nucleoplasm. The nucleolar persistence of nucleolin and nucleophosmin or their translocation to nucleoplasm induced by various stimuli including UV or ionizing irradiation, heat shock and genotoxic injury have been discussed in a number of papers (Daniely et al. 2000, Wang et al. 2001, Daniely et al. 2002, Yang et al. 2002, Iliakis et al. 2004, Kim et al. 2005). The collection of data suggests that both proteins function as stress-responsive proteins and participate in a complex multi-pathway mechanism of transient replication inhibition and DNA repair. Additional repair-related functions for nucleolin and nucleophosmin were suggested by biochemical data revealing how both proteins

stimulate DNA strand annealing (Borggrefe et al. 1998, Hanakahi et al. 2000). We speculate that the intercalation of ActD which stabilizes premelted structures (Sobell 1985) may be impaired by the DNA strand reannealing activity of nucleolin and nucleophosmin and that targeted translocation of both proteins to nucleoplasm may contribute to a preferential and growth inhibiting uptake of a gene-damaging agent by nucleolus.

A translocation of nucleolin to nucleoplasm induced by ActD was described by Liu and Yung (1999), Chen et al. (1999) and Shav-Tal et al. (2005) in HeLa cells during the segregation of nucleolar components and the formation of nucleolar caps. The nucleolar persistence of nucleolin was indicated by Zhang et al. (2004) in murine 3T3 fibroblasts in the presence of 50 nM ActD. The disagreement between the findings of Zhang and our data demonstrating the translocation of nucleolin to nucleoplasm in human lymphocytes cultured in the presence of 10 nM ActD supports the hypothesis of Chen and Jiang (2004) that protein persistence in the nucleoli of cells treated with ActD has a cell type-specific effect.

A migration of nucleophosmin out of the nucleolus induced by ActD was described earlier in human HeLa cells, Lo leukemia cells and MCF-7 cells (Yung et al. 1985, Chan 1992, Perlaky et al. 1997, Zatsepina 1997, Chan et al. 1996, 1999, Mukharyamova 1999, Smetana et al. 2001) and murine P388D1 cells (Finch and Chan 1992). The enhancement of DNA repair and the up-regulation of PCNA are associated with nucleophosmin over-expression (Wu et al. 2002). Nucleophosmin interactions with tumor suppressor protein p53 and its down-regulator HDM2 protects p53 (Takemoto et al. 2000, Maiguel et al. 2004) and up-regulates a number of important cell cycle-modulating genes, including the p21 and GADD45 protein family (Kurki et al. 2004a, 2004b). Gadd45, identified as stress inducible proteins (Vairapandi et al. 2002) involved in G2/M arrest does not contain a nuclear localization signal and their nuclear translocation is mediated by nucleophosmin (Gao et al. 2005). Thus, our data indicating the translocation of nucleolin and translocation and increased expression of nucleophosmin in stimulated lymphocytes, which overcame the G1 check point despite the inactivation of the ribosomal gene by ActD, may mirror an engagement of both proteins in the process of deciding the fate of the cell. Our preliminary, unpublished data suggest that the number of mitotic cells in stimulated cultures treated with ActD was five times less than the number of mitotic cells in ActD untreated cultures and that the presence of ActD very significantly up-regulates the expression of proapoptotic protein p53. More studies, however, are required in this direction.

CONCLUSIONS:

The mitogenic transformation of human T lymphocytes is supported by a rapid increase of ribosomal RNA precursor production. This process requires a very significant increase of neo-synthesis of transcription (UBF) and processing (nucleolin and nucleophosmin) regulatory factors and their accumulation in the nucleolus. The nucleolar level of fibrillarin has a relatively low-variable. The inhibition of ribosomal synthesis with the DNA damaging agent, actinomycin D, induces the up-regulation of neo-synthesis of nucleophosmin, translocation of both nucleolin and nucleophosmin to nucleoplasm and, it is likely, an involvement of both proteins in processes of suppression of damaged lymphocytes division. The re-clustering of dispersed transcription units, induced by ActD, despite the persistence of UBF in the nucleolus contradicts the hypothesis that neo-synthesis of UBF is the main drive for unraveling a multicopy rDNA gene. It is suggested that the reannealing activity of nucleolin and nucleophosmin may help drive the genotoxic agent to the nucleolus and diminish the diversity of genotoxic damage as well as inhibit growth-division activity.

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