A higher concentration of an antigen within the nucleolus may prevent its proper recognition by specific antibodies

E.V. Sheval*, M.A. Polzikov°, M. O.J. Olson#, O.V. Zatsepina*°

*A.N. Belozersky Institute of Physical and Chemical Biology, Moscow State University, Moscow, Russia; °Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAN, Moscow, Russia; #Department of Biochemistry, University of Mississippi Medical Center, Jackson, Mississippi, USA

Transient transfection of HeLa cells with a plasmid encoding the full-length human fibrillarin fused to a green fluorescent protein (GFP) resulted in two major patterns of intensity of the nucleolar labeling for the chimeric protein: weak and strong. Both patterns were maintained in fibrillarin-GFP expressing cells after fixation with formaldehyde. When the fixed fibrillarin-GFP expressing cells were used for immunolabeling with antibodies to fibrillarin, only the nucleoli with a weak GFP-signal became strongly labeled, whereas those with the heavy signals were only lightly stained, if at all. A similar pattern was observed if the cells were immunolabeled with antibodies to GFP. These observations suggest that an increase in antigen accumulation within the nucleolus, which could take place under various physiological or experimental conditions, could prevent the antigen from being recognized by specific antibodies. These results have implications regarding contradictory data on localization of various nucleolar antigens obtained by conventional immunocytochemistry.

Key words: nucleolus, GFP-fusion proteins, immunolabeling, fibrillarin.

Correspondence: Dr. Olga V. Zatsepina,
Shemyakin-Ovchinnikov Institute
of Bioorganic Chemistry RAN, Moscow 117997, Russia.
Tel: +07.095.7792366.
Fax: +07.095.3357103.
E-mail: zatsepin@ibch.ru.

Paper accepted on February 11, 2005

European Journal of Histochemistry
2005; vol. 49 issue 2 (Apr-Jun): 11-18

The nucleolus is a complex nuclear territory where ribosomal RNAs (rRNAs) are synthesized, processed and assembled in pre-ribosomal particles (for review, see Carmo-Fonseca *et al.,* 2000; Olson *et al.,* 2000; Olson 2004). The major molecular constituents of the nucleolus are proteins, which comprise up to 60 % of the nucleolar dry mass. Based on recent mass-spectroscopy data, the nucleolus harbors more than 300 proteins, of which at least one third are involved in ribosome biogenesis, whereas others either are not directly related to ribosome production or their role in cell metabolism still remains obscure (Andersen *et al.,* 2002; Scherl *et al.,* 2002). At the ultrastructural level, the functional nucleolus is composed of three sub-compartments, namely the fibrillar center (FC), the dense fibrillar component (DFC), and the granular component (GC) (Olson *et al.,* 2000; Fakan, 2004). The assignment of a given step of rRNA synthesis and maturation to any one of these compartments has been the subject of intense investigation. The genes for ribosomal RNA (rDNA), RNA polymerase I and its associated factors, the upstream binding factor (UBF) and the promoter selectivity factor (SL1) were found in the FCs, in the DFC or in both compartments by different research groups (Scheer and Rose 1984; Roussel *et al.,* 1993; Zatsepina *et al.,* 1993). The pre-rRNA processing machinery composed mainly of small nucleolar RNAs (snoRNAs) and proteins such as nucleolin, fibrillarin and B23/nucleophosmin, were found in both the DFC and the GC (Azum-Gelade *et al.,* 1994; Cmarko *et al.,* 2000). In many cases there is contradictory data on immunolocalization of nucleolar proteins throughout the literature and the reasons for this remain obscure. In the current study, we choose fibrillarin as a well-studied nucleolar ectopically expressed protein to test an idea that a higher local concentration of an antigen within the nucleolus might prevent its proper recognition by specific antibodies, when conventional cell immuno-

labeling assays are applied. Fibrillarin is a basic nucleolar protein of 34-36 kDa in humans (Aris and Blobel 1991), which is also known as NOP1 in yeasts (Henriquez *et al.,* 1990) and B-36 in primitive plants (Christensen and Fuxa 1988; Pierron *et al.,* 1989; Testillano *et al.,* 1992). Its major function is participation in processing and methylation of newly synthesized 45-47S pre-rRNAs and in ribosome biogenesis (Tollervey *et al.,* 1993). In the nucleolus, fibrillarin is mainly located within the DFC (Ochs *et al.,* 1985; Puvion-Dutilleul and Christensen, 1993; Azum-Gelade *et al.*, 1994) and foci of active rRNA genes, which can be detected by BrUTP incorporation run-on assays (Cheutin *et al.*, 2002; Trentani *et al.*, 2003) or *in situ* immunolocalization of Z-DNA (Cerna *et al.*, 2004). By ultrastructural immunochemistry in HeLa cells fibrillarin was located within the nucleolonema that is composed of fibrillar and granular material of uncertain origin (Mukharyamova *et al.,* 1999). Immunolabeling of cells with specific antibodies is a commonly used assay for elucidation of protein location and potential function(s). Alternatively, these questions are studied by the expression of constructs coding for a protein of interest coupled to green fluorescent protein, GFP. The latter approach was applied, for example, to examine the dynamics of the human full-length fibrillarin in mitosis (Dundr *et al.,* 2000; Savino *et al.,* 2001), and for a protein turnover analysis by fluorescence recovery after photobleaching (Phair, Misteli 2000; Snaar *et al.,* 2000; Chen, Huang 2001). Our recent data showed that transient transfection of HeLa cells with vectors encoding GFP-tagged fibrillarin results in various intensities of cell and nucleolar labeling, which apparently mirror different levels of the plasmid expression. At any time of post-transfection, cells with relatively weak or relatively strong fluorescence of fibrillarin-GFP in the nucleoli can be seen. Similarly, there were large differences in the staining of nucleoli by anti-fibrillarin antibodies in the transfected cells. In the current paperstudies, we tested the idea that augmentation of a protein concentration within the nucleolus might affect its proper recognition by specific antibodies when conventional cell immunolabeling assays are applied. We foundOur results show that an inverse correlation between ectopic expression of GFP-tagged fibrillarin and the intensity of the nucleolar staining by anti-fibrillarin or GFP antibodies is observed in the nucleoli with a higher local concentration of the chimeric protein.

Materials and Methods

HeLa cells were grown on coverslips placed in 35 mm Petri dishes containing DMEM supplemented with 10 % fetal bovine serum, glutamine, and antibiotics at 37°C in an atmosphere containing 5 % CO2. When the cells reached approximately 50 % confluence, they were transfected with 2 μg plasmid DNA coding for the human full-length fibrillarin (321 amino acid residuals) and a green fluorescent protein, eGFP (Dundr *et al.,* 2000) by use of the Invitrogen transfection kit (USA) following the recommendations of the supplier. Cells were further cultured for 32-36 h, and then fixed with 2% paraformaldehyde in 0.1 M PBS (0.14 M NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH2P04, pH 7.2-7.4) at room temperature for 15 min. The cells were then incubated with the anti-fibrillarin autoimmune serum S4 (Reuter *et al.*, 19890chs *et al.*, 1985) or anti-fibrillarin mouse monoclonal antibody 72B9 (Takeuchi *et al.*, 1995) for 40 min. Alternatively, the cells were incubated with a mouse monoclonal antibody anti-GFP antibodies (Molecular Probes, USA) for 40 min or with either of two mouse monoclonal anti-GFP antibodies, kindly donated by Dr. A.Yu. Surovoy (Institute of Bioorganic Chemistry RAS, Moscow, Russia). After washing with PBS, the cells were exposed to anti-mouse or anti-human immunoglobulins coupled with Texas red (both from Jackson ImmunoResearch Lab., USA). Cells were counterstained with 0.1 μg/mL DAPI (Sigma, USA) at room temperature for 10 min, and mounted in Mowiol (Calbiochem, USA) containing an anti-bleaching agent, DABCO (Sigma). Specimens were studied with an epifluorescence microscope Axiovert 200 (Carl Zeiss, Germany) with the objectives PlanNeofluar 10/0.85, PlanApochromat 100/1.3 or Fluar 100/1.25 and an appropriate filter set. Images were acquired with a 13 bit b/w CCD camera CoolSnapcf (Spectroscopy and Imaging, USA), and analyzed with the Adobe Photoshop Version 6.0 (Adobe Systems, USA) and Scion Image Beta software (Version 4.0.2; Scion Corporation, USA).

To study immunoreactivity of antibodies against fibrillarin and GFP, HeLa cells were grown in 60-mm Petri dishes and transfected as described

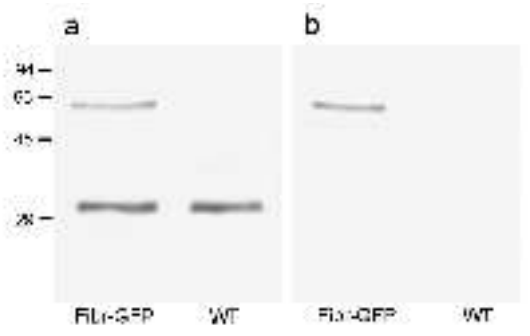

Figure 1. SDS-PAGE analysis of whole cell lysates from HeLa cells transiently transfected with the plasmid encoding fibrillarin-GFP. Electrophoresis and immunoblotting with the corresponding antibody was done 36 h after transfection. WT - lysates from cells exposed to Invitrogen transfection reagent without plasmid DNA. In (a), using the anti-fibrillarin antibody, the WT lane shows one band corresponding to endogenous fibrillarin, the Fibr-GFP lane shows two bands corresponding to endogenous fibrillarin and the fibrillarin-GFP fusions. In (b), probing with the anti-GFP antibody, the WT lane shows no one band and the Fibr-GFP lane shows one band corresponding the fibrillarin-GFP fusions. The molecular masses (in kD) of marker proteins are indicated in the left-hand side of the blots.

above. $3x10^6$ cells were lysed in SDS-PAGE sample buffer (60 mM Tris-HCl, ph 6.8, 2% SDS, 25% glycerol, 14.4 mM β-mercaptoethanol, 0.01% bromphenol blue), boiled for 5 min, resolved in 12% SDS-polyacrylamide gel, and transferred to 0.45 μm nitrocellulose membrane (Millipore, France). The membrane were incubated with the anti-fibrillarin autoimmune serum S4 or a mouse monoclonal anti-GFP antibody taken at appropriate dilutions, then with the relevant secondary antibodies conjugated to alkaline phosphatase (Sigma), and finally in 1 M Tris-HCl, pH 8.6, containing 0.3 mg/mL nitroblue tetrazolium and 50 μg/ml indoxyl phosphate in dimethylformamide. For controls, HeLa cells were incubated with the Invitrogen transfection reagent without DNA, and then used for Western blots following the procedure described above.

Results

Transient transfection of HeLa cells with a plasmid that codes for the human full-length fibrillarin fused to GFP (fibrillarin-GFP) reached the peak of the chimeric protein expression at 32-36 h of post-transfection, when the number of transfectants was equal to 60-70%. When equal aliquots of whole cell extracts from transfected and control cells, were subjected to SDS-PAGE and immunoblotting using autoimmune serum against fibrillarin, endogenous fibrillarin was seen in the both extracts as a 34-36-kD band (Figure 1a). On the same membrane, in transfected cells the fibrillarin-GFP fusion protein was seen as a band of 60-65 kD (Figure 1a). A band with the same electrophoretic mobility was also observed in transfected cell extracts incubated with an antibody against GFP, whereas no signals were present in control cells (Figure 1b). Thus, one may conclude that in transfected cells the anti-fibrillarin serum recognizes the fibrillarin-GFP fusion protein as well as the wild-type fibrillarin. By microscopic analysis, in living or formaldehyde fixed fibrillarin-GFP-expressing cells, two major patterns of cell labeling with GFP were recognized: in approximately 80% cells, only

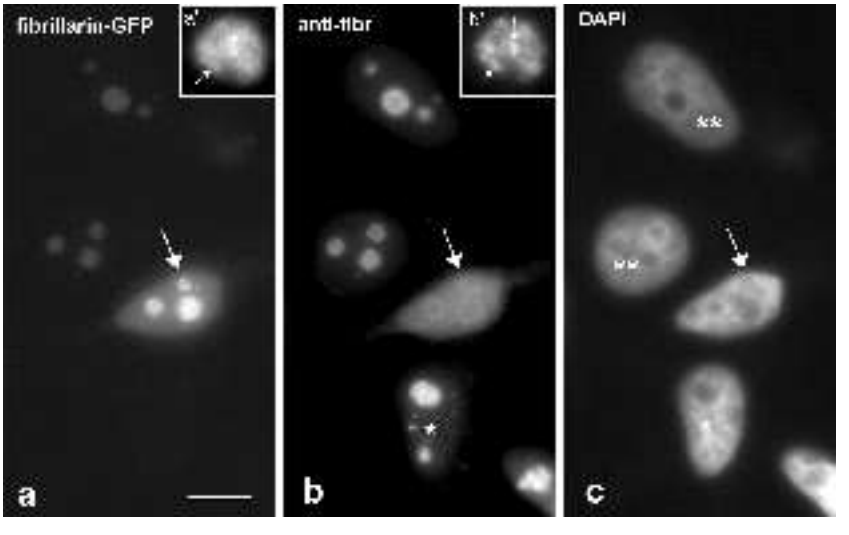

Figure 2. Immunolabeling of fibrillarin-GFP expressing HeLa cells with the anti-fibrillarin monoclonal antibody 72B9 after 36 h of transfection. a – fibrillarin-GFP expression; b – immunolabeling for fibrillarin; c – DAPI staining. In a cell not expressing fibrillarin-GFP (asterisk in b and c) nucleoli are labeled for fibrillarin; the nucleoli, which are weakly labeled for fibrillarin-GFP (double asterisk in c), are accessible for anti-fibrillarin antibodies; the nucleoli that are heavily labeled for fibrillarin-GFP are not labeled for fibrillarin (arrow in a-c). In a' and b', locations for fibrillarin-GFP (a') and endogenous fibrillarin (b') are shown, small arrows indicate intranucleolar fibrillarin-positive foci (rRNA synthesis foci). Bar, 10 μm.

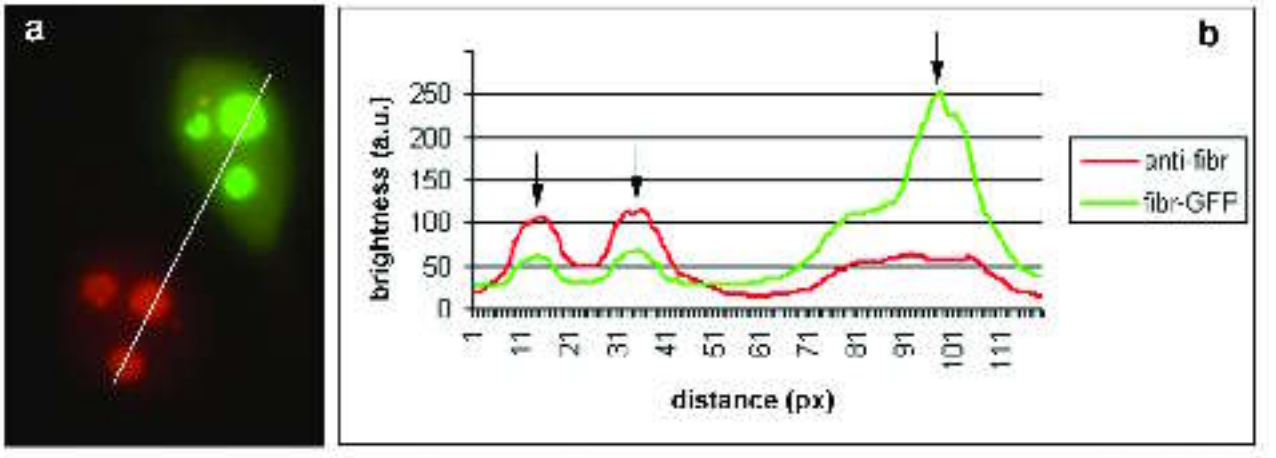

Figure. 3. Variations in intensities of nucleolar labeling for fibrillarin-GFP (green) and fibrillarin (red) as defined by plotting gray values for each channel. a - straight line selection through the nucleoli with a weak (bottom cell) and heavy (upper cell) labeling for fibrillarin-GFP; b - a plot of values along the selected line; arrows indicate the values over the nucleoli (from the top to the bottom). Note, the more intense is the nucleolar fibrillarin-GFP fluorescence, the less is labeling for fibrillarin and vice versa.

the nucleoli were labeled; in others, in addition to the nucleoli, uniform fluorescence of the nucleoplasm was also evident (Figure 2a, c). As judged by visual observations (Figures 2a, c and 4a, c) and also by plotting of nucleolar fluorescence intensities by Scion Image software (Figures 3a and 5a) in the former cells the intensity of the nucleolar fluorescence was weaker than that in the cells where the chimeric protein was located both in the nucleoli and nucleoplasm.

In order to examine whether fibrillarin in weakly and intensely fibrillarin-GFP-labeled nucleoli was equally accessible to anti-fibrillarin antibodies we performed immunolabeling of the GFP-expressing cells with the specific anti-fibrillarin monoclonal antibody 72B9. As shown Figure 2 (a, b), the anti-fibrillarin antibody was able to recognize the protein only in nucleoli of weakly fibrillarin-GFP-labeled cells. The nucleoli, which were heavily labeled with fibrillarin-GFP, remained unlabeled with antibodies. In the fibrillarin-positive nucleoli, the fibrillarin-GFP and endogenous fibrillarin were coincident (Figure 2a′, b′). The same results were also observed with the patient anti-fibrillarin serum S4 (data not shown).

In Figure 3, variations in intensities of labeling for fibrillarin-GFP (*green*) and endogenous fibrillarin (*red*) by plotting gray values for each channel along the same nucleoli (Figure 3a) are shown. The graph in Figure 3b shows that the higher the concentration of fibrillarin-GFP within a nucleolus, the less intense is the labeling of the nucleolus by the antiserum against fibrillarin.

We tested whether the nucleoli of cells, which differ in intensities of fibrillarin-GFP expression, can be equally labeled with antibodies against GFP. Application of all three anti-GFP antibodies (see Materials and methods) provided similar results. As shown in Figure 4 a, b, the nucleoli of cells that do not express fibrillarin-GFP remain unlabeled for GFP (Figure 4 a, c, *asterisk*) thus confirming specificity of the anti-GFP labeling. The nucleoli that accumulate relatively low amounts of fibrillarin-GFP were accessible for anti-GFP antibodies (Figure 4a-c, *double asterisk*), and within such nucleoli fibrillarin-GFP was coincident with anti-GFP signals (Figure 4 a′, b′). In contrast, the nucleoli that were intensely fluorescent for fibrillarin-GFP were not labeled internally with anti-GFP antibodies (Figure 4 a, c, *arrow*). In the latter nuclei, positive labeling of the nucleolar surface and nucleoplasm was only evident (Figure 4a″, b″). Thus, the nucleoli accumulating more fibrillarin-GFP were inaccessible less accessible for labeling either with anti-fibrillarin or with GFP-antibodies.

In Figure 5, variations in intensities of labeling for fibrillarin-GFP (*green*) and GFP (*red*) by plotting gray values for each channel along the same nucleoli (Figure 5a) are shown. The graph in Figure 5b shows that the higher the concentration of fibrillarin-GFP within a nucleolus, the less intense is the labeling of the nucleolar interior by the anti-

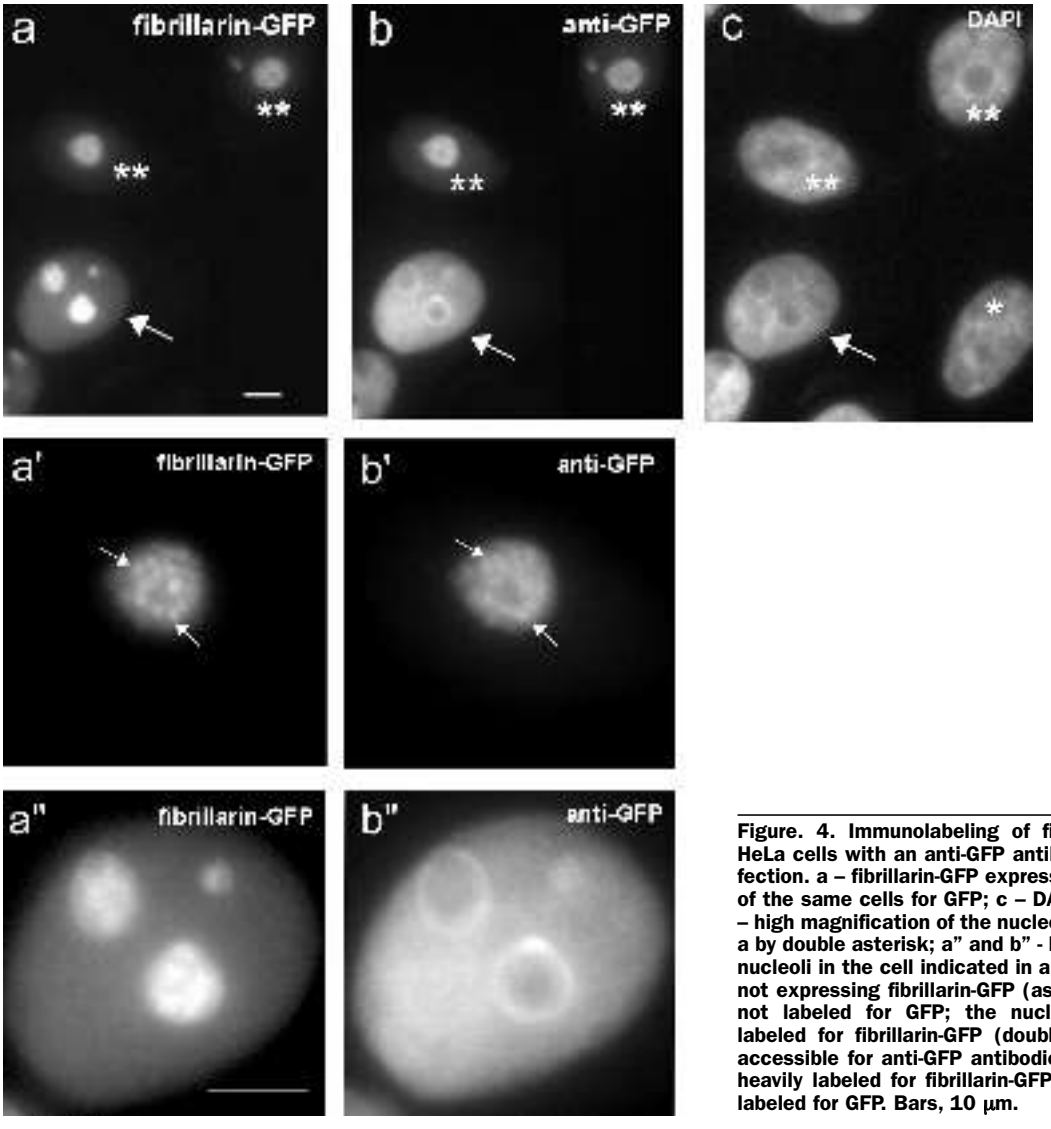

Figure. 4. Immunolabeling of fibrillarin-GFP expressing HeLa cells with an anti-GFP antibody after 36 h of transfection. a – fibrillarin-GFP expression; b – immunolabeling of the same cells for GFP; c – DAPI staining; in a' and b' – high magnification of the nucleolus in a cell indicated in a by double asterisk; a" and b" - high magnification of the nucleoli in the cell indicated in a and b by arrow. In a cell not expressing fibrillarin-GFP (asterisk in c), nucleoli are not labeled for GFP; the nucleoli, which are weakly labeled for fibrillarin-GFP (double asterisk in a- c) are accessible for anti-GFP antibodies; the nucleoli that are heavily labeled for fibrillarin-GFP (arrow in a- c) are not labeled for GFP. Bars, 10 μm.

GFP antibody, relative to the GFP fluorescence.

Thus, the nucleoli accumulating more fibrillarin-GFP were less accessible for labeling either with anti-fibrillarin or with GFP-antibodies.

Discussion

Immunolabeling of the nucleolar antigens with specific antibodies remains a generally useful approach for investigation of the intracellular location(s) of a protein of interest. However, contradictory results obtained by different authors concerning localization of many proteins are well known. Following similar protocols of cell fixation RNA polymerase I and its specific transcription factor, UBF, have been localized within FCs (Scheer and Rose 1984; Cheutin *et al.,* 2002), at the periphery of FCs (Zatsepina *et al.,* 1993), or in the both - FCs and DFC (Rendon *et al.,* 1992; Roussel *et al.,* 1993; Mosgoeller *et al.,* 1998). Fibrillarin, a major nucleolar pre-rRNA processing protein, in many cells was predominantly located in the DFC (Ochs *et al.,* 1985; Raska *et al.*, 1990; Puvion-Dutilleul and Christensen, 1993; Azum-Gelade *et al.*, 1994; Biggiogera *et al.* 2001), but its minor associations with FCs (Ochs *et al.* 1985), or GC regions (Azum-Gelade *et al.*, 1994; Mukharymova *et al.,* 1999) have also been described. At the light microscopic level, a ribosome assembly factor B23/nucleophosmin was described by some authors as rather uniformly distributed throughout the nucleolus (Ochs *et al.,* 1983; Paulin-Levasseur *et al.,* 1995), where-

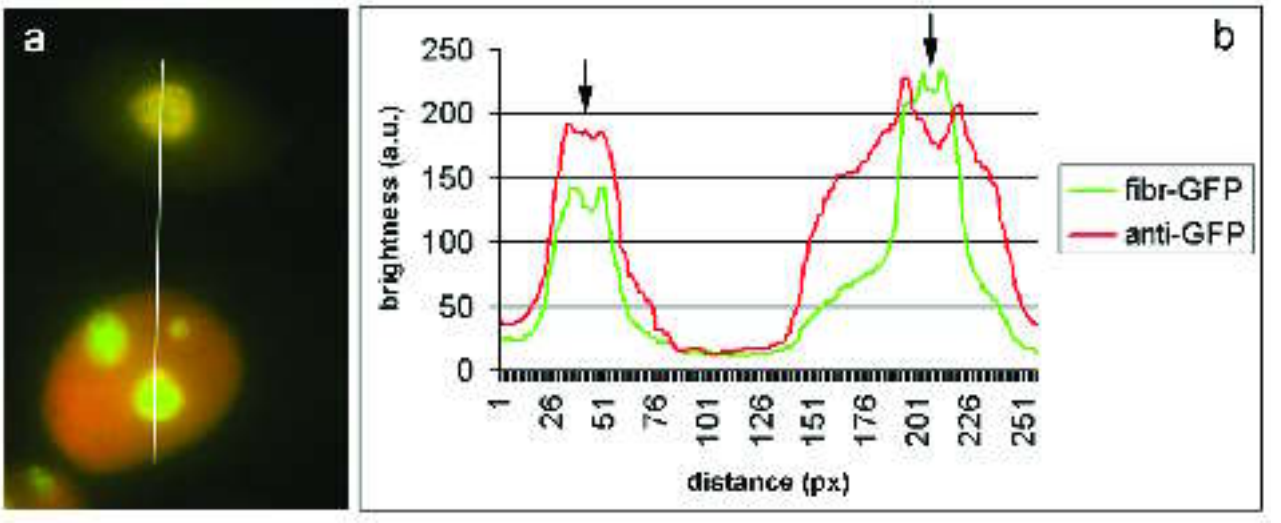

Figure 5. Variations in intensities of nucleolar labeling for fibrillarin-GFP (green) and GFP (red) as defined by plotting gray values for each channel. a - straight line selection through the nucleoli with a weak (upper cell) and heavy (bottom cell) labeling for fibrillarin-GFP; b - a plot of values along the selected line; arrows indicate the values over the nucleoli (from the top to the bottom). Note, the more intense is fibrillarin-GFP fluorescence, the less is labeling for GFP and vice versa.

as others paid attention to a higher local accumulation of the protein at the nucleolus periphery as compared to its interior (Zatsepina *et al.,* 1997). The reasons of such discrepancies remain unclear. In part, they can be explained by some technical reasons, including specificity of probes (antibodies) and by different protocols for cell fixation and labeling. In the current work, for the first time we present experimental data supporting an idea that a higher concentration of an antigen within the nucleolus can prevent the antigen from being properly recognized by specific antibodies. Transient transfection of HeLa cells with a plasmid coding for fibrillarin fused to GFP results in variable efficiencies of chimeric protein expression among individual cells (Figure 2a and 4a). These variations very likely result from fluctuations in the number of plasmids transfected into and expressed in each cell. Generally, two major types of fibrillarin-GFP-positive nucleoli can be recognized based on intensity of their fluorescence, namely weak versus strongly labeled nucleoli. In the intensely labeled nucleoli the threshold of fibrillarin-GFP fluorescence reaches approximately 250, whereas in the weakly labeled nucleoli it is below 150 (Figure 3 and 5). In the both types of nucleoli, fibrillarin-GFP accumulated mainly in numerous discrete foci (Figures 2a and 4a′, a″), which were coincident with endogenous fibrillarin recognized by anti-fibrillarin antibodies (Figure 2a′, b′). However, only in the weakly labeled nucleoli was fibrillarin-GFP accessible to the antibodies against fibrillarin or GFP. The nucleoli that were the most brightly fluorescent and thus accumulated more fibrillarin-GFP, remained unlabeled (Figures 3 and 5). The fact that labeled and unlabeled nucleoli were observed in adjacent cells (Figures 2 and 4) excludes even minor fluctuations in conditions of cell fixation, immunolabeling or image acquisition. Therefore, we assume that a higher local concentration of an antigen within the nucleolus (e.g., fibrillarin-GFP) prevents the proper recognition of the antigen by a specific antibody (i.e., anti-fibrillarin or anti-GFP). Under such conditions the nucleolar labeling will be artificially negative almost as if the antigen is completely absent. These results indicate that although an antigen's concentration within the nucleolus or its subdomains (e.g., the DFC that is the major location of fibrillarin) may change with altered physiological or experimental conditions, negative labeling of the nucleolus may also be the result antigen overexpression. In a line with this conclusion, our recent data show that overexpression of a specific nucleolar protein Surf-6 that is apparently involved in ribosome biogenesis (Magoulas *et al.*, 1998) prevents Surf-6 positive recognition by specific polyclonal antibodies in 3T3 mouse cells (unpublished results). Therefore, caution should be exercised when conclusions about relative protein levels are based on immunocytochemical methods using a single antibody or protocol for cell immunolabeling, especially when proteins are ectopically expressed in cells.

Acknowledgements

The authors are very thankful for Dr. A.Yu. Surovoy (Institute of Bioorganic Chemistry RAS, Moscow, Russia) for anti-GFP antibodies. The work was supported by the Russian Foundation for Basic Researches (project 03-04-48951).

References

Andersen JS, Lyon CE, Fox AH, Leung AK, Lam YW, Steen H et al. Directed proteomic analysis of the human nucleolus. Curr Biol 2002; 12:1-11.

Aris JP, Blobel G. cDNA cloning and sequencing of human fibrillarin, a conserved nucleolar protein recognized by autoimmune antisera. Proc Natl Acad Sci USA 1991; 88:931-5.

Azum-Gelade MC, Noaillac-Depeyre J, Caizergues-Ferrer M, Gas N. Cell cycle redistribution of U3 snRNA and fibrillarin. Presence in the cytoplasmic nucleolus remnant and in the prenucleolar bodies at telophase. J Cell Sci 1994; 107:463-75.

Biggiogera M, Malatesta M, Abolhassani-Dadras S, Amalric F, Rothblum LI, Fakan SJ. Revealing the unseen: the organizer region of the nucleolus. J Cell Sci 2001; 114:3199-205.

Carmo-Fonseca M, Mendes-Soares L, Campos L. To be or not to be in the nucleolus. Nat Cell Biol 2000; 6:107-12.

Cerna A, Cuadrado A, Jouve N, Diaz de la Espina SM, De la Torre C. Z-DNA, a new in situ marker for transcription. Eur J Histochem 2004; 48:49-56.

Chen D, Huang S. Nucleolar components involved in ribosome biogenesis cycle between the nucleolus and nucleoplasm in interphase cells. J Cell Biol 2001; 153:169-76.

Cheutin T, O'Donohue MF, Beorchia A, Vandelaer M, Kaplan H, Defever B et al. Three-dimensional organization of active rRNA genes within the nucleolus. J Cell Sci 2002; 115:3297-307.

Christensen ME, Fuxa KP The nucleolar protein, B-36, contains a glycine and demethylagrinine-rich sequence conserved in several other nuclear RNA-binding proteins. Biochem Biophys Res Com 1988; 155:1278-83.

Cmarko D, Verschure PJ, Rothblum LI, Hernandez-Verdun D, Amalric F, van Driel R et al. Ultrastructural analysis of nucleolar transcription in cells microinjected with 5-bromo-UTP. Histochem Cell Biol 2000; 113:181-7.

Dundr M, Misteli T, Olson MOJ. The dynamics of postmitotic reassembly of the nucleolus. J Cell Biol 2000; 150:433-46.

Fakan S. Ultrastructural cytochemical analyses of nuclear functional architecture. Eur J Histochem 2004; 48:5-14.

Henriquez R, Blobel G, Aris JP. Isolation and sequencing of NOP1. J Biol Chem 1990; 265:2209-15.

Magoulas C, Zatsepina OV, Jordan PW, Jordan EG, Fried M. 1998. The SURF-6 protein is a component of the nucleolar matrix and has a high binding capacity for nucleic acids in vitro. Eur J Cell Biol. 75: 174-83.

Mosgoeller W, Schofer C, Wesierska-Gadek J, Steiner M, Muller M, Wachtler F. Ribosomal gene transcription is organized in foci within nucleolar components. Histochem Cell Biol 1998; 109:111-8

Mukharyamova KS, Doudnik OA, Speransky AI, Zatsepina OV. Double immunolocalization of major nucleolar proteins, fibrillarin and B23, in dividing mammalian cultured cells. Membr Cell Biol 1999; 12:829-43.

Ochs R, Lischwe M, O'Leary P, Busch H. Localization of nucleolar phosphoproteins B23 and C23 during mitosis. Exp Cell Res 1983; 146:139-49.

Ochs RL, Lischwe MA, Spohn WH, Busch H. Fibrillarin: a new protein of the nucleolus identified by autoimmune sera. Biol Cell 1985; 54:123-34.

Olson MO, Dundr M, Szebeni A. The nucleolus: an old factory with unexpected capabilities. Trends Cell Biol 2000; 10:189-96.

Olson MOJ, Ed. The Nucleolus. Landes Bioscience, Georgetown, 2004.

Paulin-Levasseur M, Julien M, Horner M, Chen G. Characterization of the 2H12 antigen as a nonshuttling human isoelectric variant of the nucleolar protein B23. Exp Cell Res 1995; 219:514-26.

Puvion-Dutilleul F, Christensen ME. Alterations of fibrillarin distribution and nucleolar ultrastructure induced by adenovirus infection. Eur J Cell Biol 1993; 61:168-76.

Phair RD, Misteli T. High mobility of proteins in the mammalian cell nucleus. Nature 2000; 404:604-09.

Pierron G, Pedron J, Schelling M, Christensen M. Immunoelectron microscopic localization of the nucleolar protein B-36 (fibrillarin) during the cell cycle of Physarum polycephalum. Biol Cell 1989; 65:119-26.

Raska I, Ochs RL, Salamin-Michel L. Immunocytochemistry of the cell nucleus. Electron Microsc Rev 1990; 3:301-53.

Rendon MC, Rodrigo RM, Goenechea LG, Garcia-Herdugo G, Valdivia MM, Moreno FJ. Characterization and immunolocalization of a nucleolar antigen with anti-NOR serum in HeLa cells. Exp Cell Res 1992; 200:393-403.

Roussel P, Andre C, Masson C, Geraud G, Hernandez-Verdun D. Localization of the RNA polymerase I transcription factor hUBF during the cell cycle. J Cell Sci 1993; 104:327-37.

Savino TM, Gebrane-Younes J, De Mey J, Sibarita JB, Hernandez-Verdun D. Nucleolar assembly of the rRNA processing machinery in living cells. J Cell Biol 2001; 153:1097-110.

Scheer U, Rose KM. Localization of RNA polymerase I in interphase cells and mitotic chromosomes by light and electron microscopic immunocytochemistry. Proc Natl Acad Sci USA 1984; 81:1431-5.

Scherl A, Coute Y, Deon C, Calle A, Kindbeiter K, Sanchez JC et al. Functional proteomic analysis of human nucleolus. Mol Biol Cell 2002; 13:4100-9.

Snaar S, Wiesmeijer K, Jochemsen AG, Tanke HJ, Dirks RW. Mutational analysis of fibrillarin and its mobility in living human cells. J Cell Biol 2000; 151:653-62.

Takeuchi K, Turley SJ, Tan EM, Pollard KM. Analysis of the autoantibody response to fibrillarin in human disease and murine models of autoimmunity. J Immunol 1995; 154:961-971.

Testillano PS, Sanchez-Pina MA, Lopez-Iglesias C, Olmedilla A, Christensen ME, Risueno MC. Distribution of B-36 nucleolar protein in relation to transcriptional activity. Chromosoma 1992; 102:41-49.

Tollervey D, Lehtonen H, Jansen R, Kern H, Hurt EC. Temperature-sensitive mutations demonstrate roles for yeast fibrillarin in pre-rRNA processing, pre-rRNA methylation, and ribosome assembly. Cell 1993; 72:443-57.

Trentani A, Testillano PS, Risueno MC, Biggiogera M. Visualization of transcription sites at the electron microscope. Eur J Histochem 2003; 47:195-200.

Zatsepina OV, Todorov IT, Philipova RN, Krachmarov CP, Trendelenburg MF, Jordan EG. Cell cycle-dependent translocations of a major nucleolar phosphoprotein, B23, and some characteristics of its variants. Eur J Cell Biol 1997; 73:58-70.

Zatsepina OV, Voit R, Grummt I, Spring H, Semenov MV, Trendelenburg MF. The RNA polymerase I-specific transcription initiation factor UBF is associated with transcriptionally active and inactive ribosomal genes. Chromosoma 1993; 102:599-611.