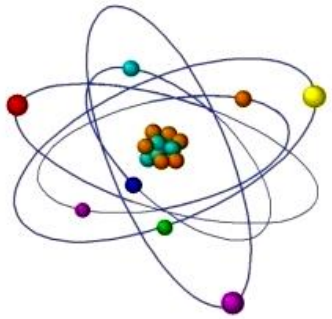
GAMMA-IRRADIATION INDUCES THE NUCLEAR AND NUCLEOLAR CHANGES IN PLANT MERISTEMATICCELLS: AN ULTRASTRUCTURAL AND OPTICAL TOMOGRAPHY APPROACHES



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ABSTRACT: *The ultrastructural analysis combined with optical tomographic approach performed by means of serial semithin sections has been utilized to study 3D nuclear and nucleolar changes induced by acute γ-radiation of onion (Alllium cepa L.) root tips. To discriminate dose-dependent nuclear/nucleolar reaction at the photonic and electron microscope levels we utilized medium (2.5 Gy) and high (5.0 Gy) doses of irradiation. .^{137}Cs was used as a source of γ-radiation. Our results are indicative to profound micro- and ultrastructural changes related to morphological signs of the radiation induced inhibition of entire nuclear/cell metabolism. Therefore, the nucleolar reaction fits perfectly all requests to be served as reliable criterion of plant cells and tissues functional state in postradiation period. Additionally, the intensity of developing postradiation nuclear/nucleolar damages correlates with the dose impact. Most likely these alterations are strictly doze-dependent.*

Key words: γ-radiation, plant cell, nuclear and nucleolar changes

INTRODUCTION The problem of the anthropogenic load on biosystem is assumed to be the key issue of the environmental deteriorations. In living organisms, the radiation induced cellular disorders are the most dangerous. Therefore, there is an acute need forreliablemorphological criteriato dismantle the radiation pathologies in cells and tissues. Traditionally the cell nucleus is considered as the most radiosensitive cellular site due to: i) its large size, and ii) localization of genetic material, while its precisely arranged spatial structure is highly sensitive to overall functional disturbances [1]. In this context the specific (chromonemal) organization of a plant cell nucleus presents especially interesting link between structure, functional activity and restoration capacity of the irradiated cell and tissue.

Importantly, that among morphological features of plant nucleus the large sizes of nucleolus, caused by extremely high dose of ribosomal genes as well as its peculiar ultrastructure depicts itself as rather attractive [2-14]. The nucleolus is a highly dynamic compartment inside the non-random 3D architecture of the cell nucleuswith key function of ribosome biogenesis. Posed as a derivate of the chromosomal nucleolus organizer regions (NORs) the interphase nucleolus in plant [3-15] and animal [15-32] objects appears in light and electron microscopes in form of the most prominent/largest and complex nuclear component. It's well documented that nucleolar micro- and ultrastructure reflect post-mitotic spatial distribution of three major processes involved in ribosome biogenesis, i.e. transcription of ribosomal genes (r-genes), processing of newly synthesized rRNA (pre-rRNA) and assembling of preribosomal particles [16, 17, 19-31]. The nucleolus emerges as a result of compact folding of active r-genes into the structure ranging from one to several micrometers. As a consequence, rRNA synthesis and processing/assembling machineries acquire the strict territorial pattern in the form of five sub-compartments or nucleolar components (NCs). Thusthree basic NCs,

i.e. fibrillar centres (FCs), together with r-gene expression products in the form of dense fibrillar component (DFC) and granular component(GC)are regularly seen in animal and plant objects. Meanwhile, transcriptionally active nucleolar DNA adopts by folding into FCs non-nucleosomal conformation, the inactive part retains nucleosomal organization. Correspondingly, the nucleolus also contains nucleolus-associated DNA domains (naDNA) or intra- and peri-nucleolar condensed chromatin (ICC and PCC, respectively) linked to interstices or nucleolar vacuoles (NV). Furthermore, DFC and GC correspond to early and late processing subcompartments [16-31].

The sensitive nuclear response to changes in ribosomal genes r-genes activity could be of particular interest. Because the function of ribosomal r-genes is ultrastructurally embodied into specific nucleolar organization its changes offer a key to the visualization of their r-genes expression *in situ* [3, 15, 17, 19, 22, 23, 27, 30, 31]. Therefore, the ultrastructure of the nucleolus in highly proliferative plant tissues in response to the less studied action of physical factors (radiation, in particular) looks like very prospective. Hence, the value of the electron microscope (EM) visualization of radiation-induced nuclear/nucleolar damages is obviously high. Indeed, the functional state of the whole cell metabolism that directly depends on the ribosome biogenesis level in postraditionaly damaged plant cell could be easily traced according ultrastructural changes of the prominent nucleolar organization.

Here we propose a plant model focused on the dose-dependent spatial nuclear/nucleolar changes in radiation damaged meristematic cells (*Alllium cepa L.*) root tip. Since the activity of r-genes and related molecular events linked to the ribosome biogenesis is morphologically expressed in quantitative parameters and topography of NCs their rearrangement has been studied in the course of inhibitory effect of the acute γ-radiation. In order to demonstrate 3D nuclear/nucleolar reaction at the resolution of light microscope (LM) we addressed to the optical tomography (8) using 1μm serial semithin sections.

MATERIAL AND METHODS

For onion bulbs irradiation the following technique has been applied. The meristem cells of 1cm onionroot tip has been used as an object for LM and EM observations. Before irradiation, the water sprouted onion bulbs were incubated at the room temperature for 24h. The exposure of plants to γ-irradiation was performed by means of “Gamma-capsule” apparatus, equipped with a holder for ^{137}Cs as a source of γ-rays and producing 1.2 Gy/min. The samples were treated within the appropriate time period in order to reach 2.5 Gy and 5.0 Gy doses. Then bulbs were kept growing at the same conditions for additional 24h before fixation. The same zones of normal and irradiated root type meristeme have been used in further LM and EM examinations. Plant tissue processing for EM (fixation, dehydration and embedding) was carried out according to routine technique. The fixation procedures were done at 4°C, whereas the dehydration and impregnation were performed at room temperature. For fixation, onion root tips were immersed in 2.5% glutaraldehyde (Merck, USA) diluted in PBS (Gibco, USA). Then the tissue samples were postfixed with 2% OsO_4 (Merck) solution diluted in the same buffer. Both, fixation and postfixation were performed for 24h. After dehydration in gradual series of acetone (Merck) and impregnation with acetone and Epon 812 (Embed Kit, EMS) mixture tissue samples were embedded in Epon and polymerized at 60°C for 48h. Tissue blocks were sectioned using glass knives and “Reichert-Young Ultracut” ultramicrotome. Ultrathin sections were counterstained by conventional contrasting procedure using lead citrate by staining sections mounted on Maxtaform Mesh 200 copper grids. Contrasted ultrathin sections were observed and registered with “Hitachi 300” (Japan) EM.

The optical tomography has been performed using 50-100 serial semithin sections (1μm

thick) placed onto polylysin-coated Super Frost slides (Menzel Glasser, USA). The following conditions allow the proper serial sectioning as well as the collection of series on the slide (28). First of all, it is necessary to trim under low magnification of ultramicrotome stereomicroscope the epoxy resin block to obtain the appropriate size of a pyramid and produce ~1x1 mm sections, so that cross sectioned root tip must be completely positioned within the section plane. Moreover, it is necessary to change the knife as often as it possible, in order to prevent scratches as well as other damages usually produced by a glass knife. In order to avoid sticking of section to the knife edge we used high speed cutting mode.Right after producing short series or single section that float in knife bath ribbons of 2-4 serial sections as well as separated single sections can be easily picked-up and than transfered (grouped or one by one) to the slide surface using Magic Loop (EMS, USA). Under low magnification microscope control the series of semithin sections could be accurately mounted on slides. Importantly, that the working surface of pick-up loop must be rinsedin 96% alcohol every time before use. To maintain precise serial consequence the cutting must be stopped after a suitable number of sections have been accumulated in the bath in the form of short ribbon or single sections. In this way each slide can be covered by the more or less oriented rows consisting of 10-20 semithin sections. In order to follow precisely the serie we have numberd each section right after they have been placed on the slide and dried. To perform this, firstly we checked in phase contrast microscope whether serial sections were arranged and labeled in proper way using super fine permanent pen. After drying sections became firmly attached to the polylysin-coated glass surface, that allows their further staining with commercial (ready to use) 1% aqueous solution of Toluidine Blue (EMS, USA). Following extensive washing with water or differentiation using 70^0 ethanol can be also safely performed due to firm adhesion of sections to the polylysin coat.

After the staining the serial semithin sections were examined and imaged in 512x512 pxl format using Carl Zeiss Axiolab Microscope. Under low magnification the suitable zones have been selected for further imaging and 3D reconstruction. The contrast and the brightness of the recorded images were finally adjusted by means of ImageJ software. For 3D reconstruction and modelling of onion root tip meristeamatic cells the basic method described by Chelidze and coauthors [19] has been used. The contours of the cell, nucleus and nucleolus were outlined directly on the screen of PC monitor using digitizer, and then “extracted” from entire images. The resulting files were exported to “AutoCAD” (AutoDesk, USA) software, and the contours of cell, nucleus and nucleolus were matched by color gradation, with following layer-by-layer assembling and triangulation of primary volumes. At the final stage the reconstructed volumes were rendered and rotated using “3D Studio Max” (AutoDesk, USA) software in order to select most appropriate foreshortenings of resulted 3D models.

RESULTS

The following EM images (Fig. 1) visualize nuclear and nucleolar morphology in control cells. On random sections nuclei display all ultrastructural characteristics that are common for many plant species, including onion root tip meristematic cells [4-14]. Nuclei appear as having smooth, round-shaped contours of nuclear membrane and typical for plants chromonemic organization of chromatin. Large nucleoli are prominent not only because their big sizes but also due to extremely dense package of NCs. They are positioned in central or close to central regions within the nuclear volume and can reach reach up to 4-6 μm in diameter. At low magnifications it is clearly visible that nucleolus usually occupies nearly two thirds of nuclear volume. According ultrustructural composition nucleoli of onion root tip meristematic cells may be related to highly active group of compact nucleoli, characterized by

complete absence of reticular network or nucleolonema, and tight package of DFC and GC specifically organized to yield the "core-cortex" composition (Fig. 1, d,e; see also 15) In similar manner, compact nucleoli presented in the object under the test exhibit the predominance of GC that indicates the intensive production of pre-ribosomal particles. Indeed, in all our samples GC was especially well developed (Fig. 1, d,e). Typically for compact nucleoli, GC was localized on the nucleolar periphery while the DFC occupied central region of the nucleolus. Importantly, that to one more specific feature of plant cell the permanent presence within the nucleolus of profound light zones could be attributed. These light intranucleolar zones are clearly visible even at low magnifications (Fig. 1; arrows). One can identify mentioned light zones as FCs (Fig. 1, a-e; arrows) or nucleolar vacuoles (Fig.1, c; upper nucleolus). Since the fibrillar network of FC is made up by undercondensedrDNP clusters with non-nucleosomal conformation the pale staining interior of FC is sharp contrast with the condensed nucleosomal chromatin inside relatively small intersticies or large NVs. Hence, light zones we observed in onion root tip can be definitely attributed to FCs due to less condensed package of the DNP fibrils. Meanwhile, the small discrete foci or more elongated and twisted fibers of condensed chromatin are especially well distinguishable within FCs due to their sharp contrast against light, fibrillar background constituted of ~10 nm fibrils with homogenous appearance. These, so called heterogenous FCs (hFC) according Medina and coauthors (4, 9, 10; see Fig. 1, d,e) are also among the most specific nucleolar features described in plant cells, while no evidences exist in regard to presence of hFC in animal objects.

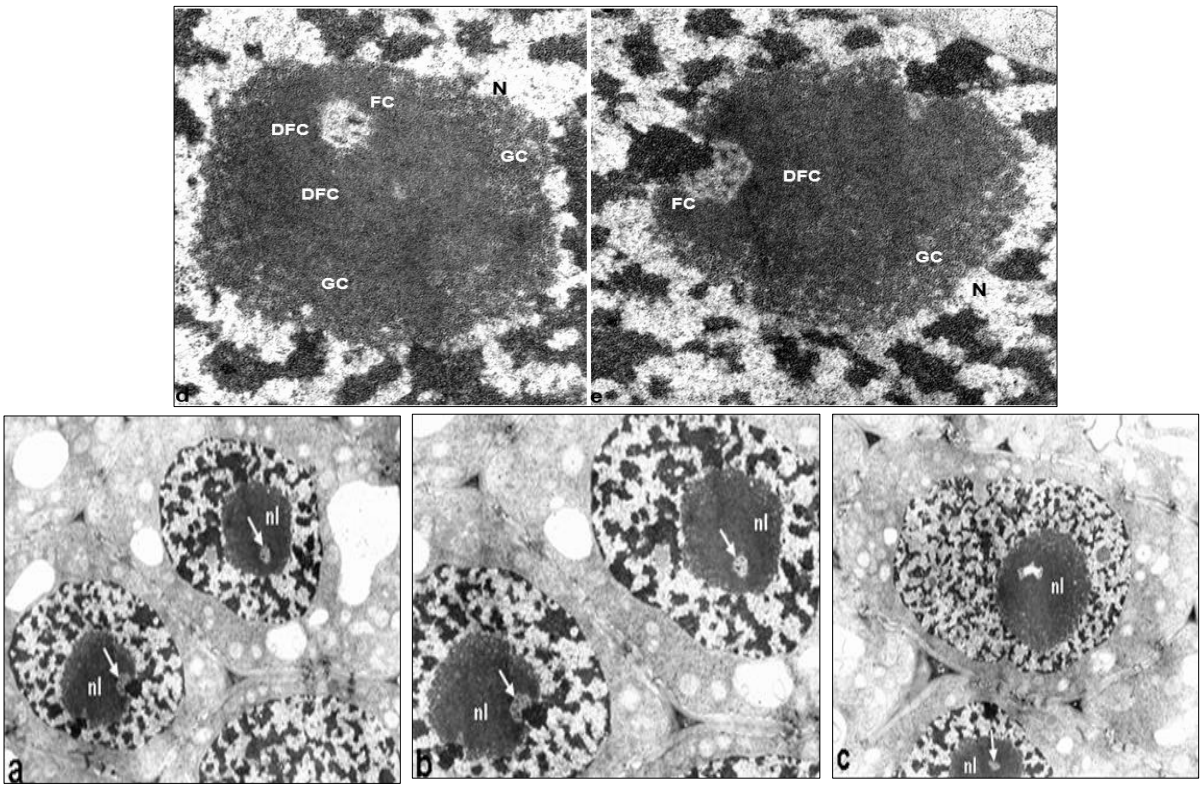

Fig.1. Ultrastructural organization of the normal meristematic cell from onion root tip. Note the large compact with well recognizable FC (arrows). At higher magnification the DFC and GC are organized in "core-cortex" fashion. Magnifications: a – 6.000; b – 8.500; c – 7.300; d,e – 20.000

3D models demonstrating the overall spatial organization of the reconstructed whole cell as well as nuclear/nucleolar volumes in control meristematic cell of onion root tip are displayed on Fig.2 Rotation and tilting of models at different angles reveals strongly polygonal cellular borderlines (Fig.

2,a,b) whereas nucleus is characterized by elliptical shape with smooth surface. As it was alreadymentioned the smooth nuclear outlines were presumed by analysis of random ultrathin section, being finally reproduced by means of 3D models (Fig. 2,c-f). In addition, analyzing 3D models by rotation and tilting it became obvious that large nucleolus takes nearly one third of the nuclear volume.

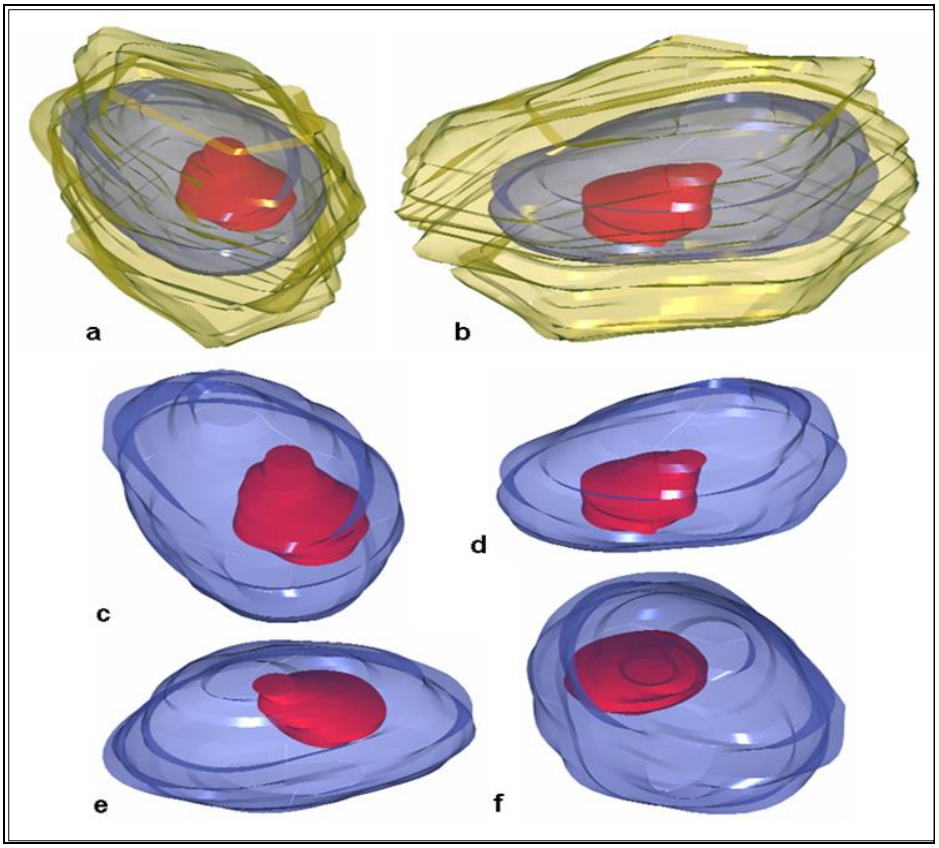

Fig.2. 3-D models demonstrating the global organization of onion root top meristematic cell as revealed by the optical tomography. Note the polygonal shape of the meristematic cell. Nucleolus occupies around one third of nuclear volume.

Exposure of root meristematic cells to the dose of 2.5 Gy provokes drastic ultrustructural reorganization in the global intracellular arrangement. Thus after 24h of irradiation, significant damages were observed, which are more distinctly reflected in the ultrastructure of nucleus and nucleolus (fig. 3). In fact, these changes cover all meristematic cells we have investigated. Obviously, such an influence leads to dramatic cessation of r-genes expression that is distinguished by the morphological alterations of the nucleolus known as nucleolar segregation (3, 17,24, 30-32). Within observed postradiational period nucleoli shift to the nuclear periphery and their sizes decrease sharply (Fig. 3). On the EM images they look like very dense and round-shaped bodies 1-1.5 μm in diameter which are almost completely consisted of DFC which is sharply delineated from the FCs (Fig. 3, a) while territories occupied by GC are visibly reduced. Thus on Fig. 3, a, it became clear that territories occupied by GC are visibly reduced that witnessed of rRNA processing cessation or even blockage. At the same time GC was also clearly separated from DFC. Usually, FCs are localized on nucleolar periphery and contact with the blocks of peri-membrane condensed chromatin blocks (Fig. 3, a,b). Moreover, meristematic cell response to radiation is also evidenced by changes of nuclear envelope. At the low magnification it was clearly visible that nuclei lose their regular round contours while the nuclear envelope permanently revealing quite deep invaginations (Fig. 3, b).

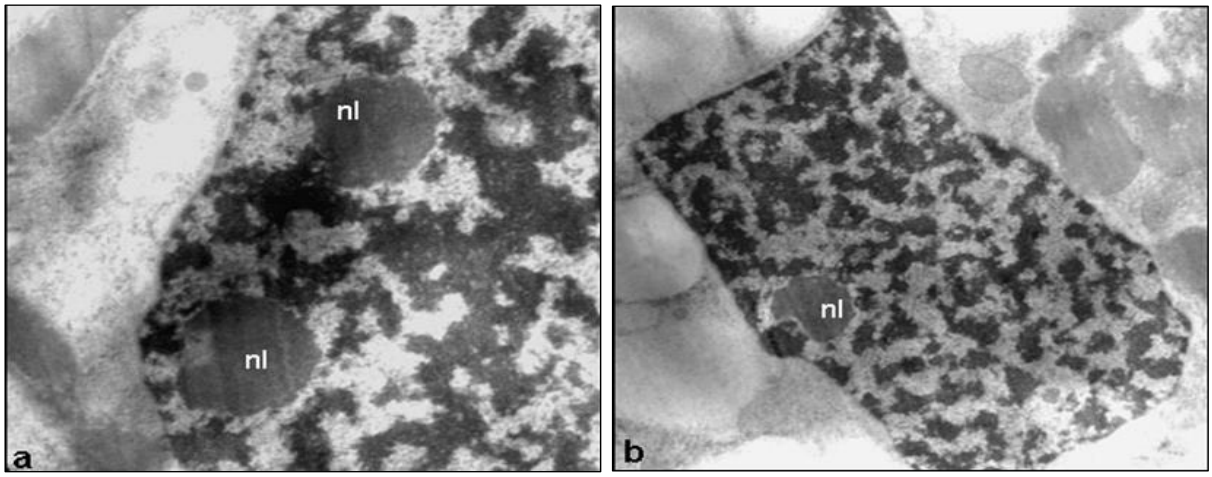

Fig. 3. Segregated nucleoli (Fig. 3,a) appeared as a result of the exposure of onion root tip cells atthe dose of 2.5 Gy. Note the drastic nuclear deformation on Fig 3,b. Magnifications: a – 16.000; b – 12.000.

All these ultrastructural peculiarities which certainly are indicative to the effect of radiation were well pronounced on corresponding 3D models (Fig.4). First of all, deep deformations of the cell wall arose as consequence of irradiation making cellular borderlines largely irregular (Fig. 4, a-c). At appropriate angles the profound deformations of nuclei could be distinctly visualized by rotation and tilting of corresponding 3D models (Fig. 4, d-f). Moreover, such approach made it largely clear that nucleolar territory is dramatically reduced and nucleoli are shifted toward the nuclear envelope.

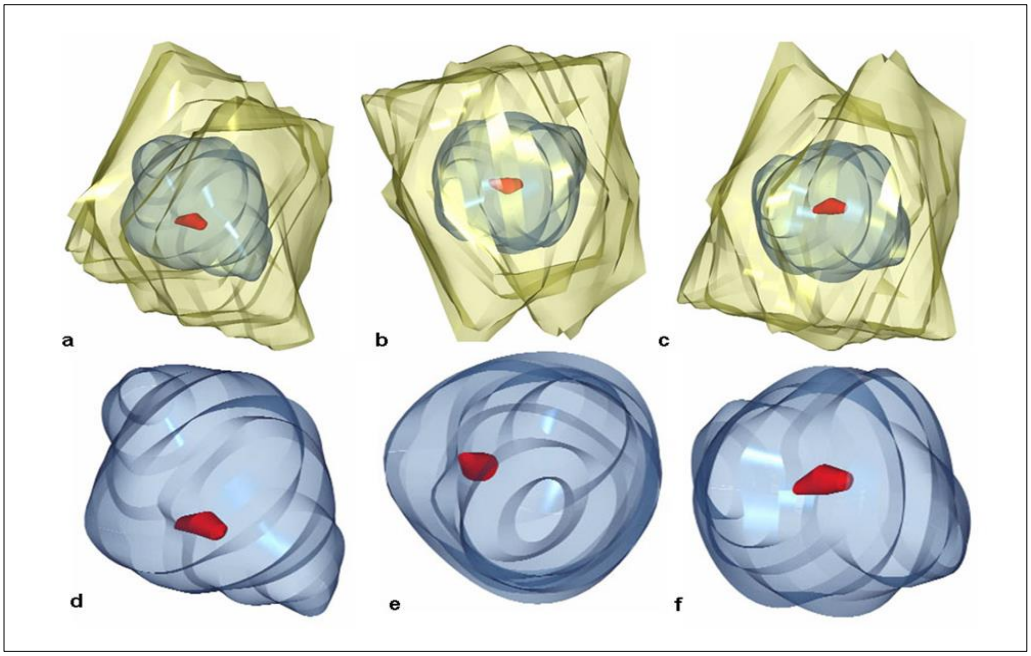

Fig. 4. The deep structural changes e.g. cellular and nuclear deformations are clearly visible during rotation of 3-D models. Nucleolar volume reduces dramatically wile the nucleolus is localized in the peripheral regions of nucleus.

In parallel to the dose enhancement the strong destructive events are observed in majority of meristematic cells. Thus by the treatment of onion roots at a doze of 5.0 Gy morphological signs of nuclear inactivation became much more pronounced (Fig. 5, a-c). For example, the most strictly depicted changes were regarded to the deep nuclear disorganization that was revealed on EM images

as peculiar deformation causing strange shape of nuclei (Fig. 5, c). Beside nuclear pathologies the intensive structural changes of nucleoli that are extremely "non-canonic" were also obvious. Also, the extensive decrease in number of nucleoli containing more or less evident FCs has been noticed. In contrast, the great number of irradiated cells reveals instead FCs the large blocks of intranucleolar condensed chromatin (Fig. 5, a,b).

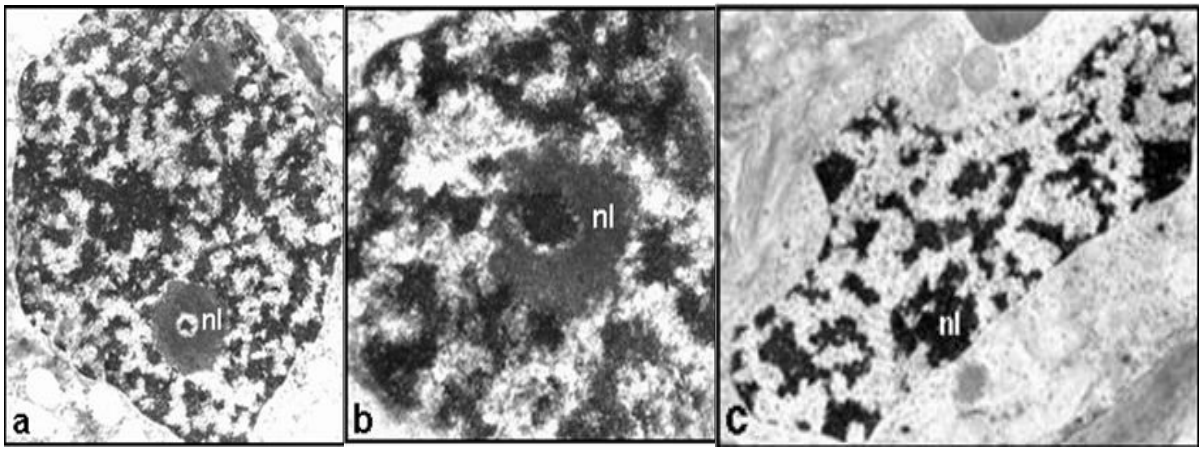

Fig. 5. Peculiar structural alterations arose in onion root tip meristem after exposure of plants at the dose of 5.0 Gy. Note massive blocks of condensed chromatin within the nucleoli (Fig. 5, a,b). Fig. 5,c demonstrates the extremely deformed nucleus with peculiar shape. Magnifications: a – 12.000; b – 18.500.

On the other hand, the nucleoli with clearly expressed and typical heterogeneous FCs are rare. All cellular as well as nuclear and nucleolar pathologies described in objects that were irradiated by high dose were more demonstrable when we utilized the option allowing the rotation of 3D models (Fig. 6).

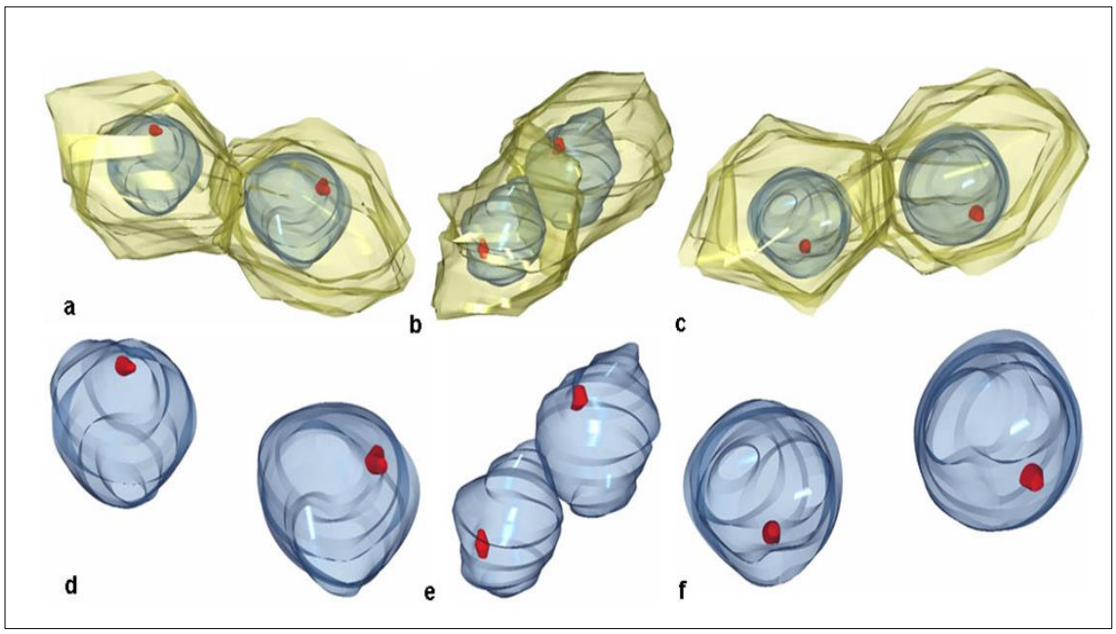

Fig. 6. Rotation of 3-D models makes cellular and nuclear damages clearly visible. Note the cellular and nuclear deformations at the angles presented in Fig. 6,b and Fig.6,e. Nucleoli are reduced in size and shifted to the nuclear periphery.

DISCUSSION

For many years, the nucleolus has appeared as a paradigm for the study of molecular and structural organization of active genes, of their transcription and of their processing. To understand how the different players of the transcriptional and processing nanomachineries are organized within NCs, many efforts have been devoted to the analysis of the nucleolus within living or fixed cells in animal and/or plant objects by using cellular imaging [20, 21, 27, 30-38]. Therefore, over the past 25 years the nucleolus has become recognised as the unique model to study the spatial organization of actively transcribing mammalian genes in functional and dynamic association with overall structure and metabolism of interphase cell.

The precondition for current investigation was the ability of FCs and DFC to move and fuse within a crowded nucleolar volume, revealed by classical EM studies on artificial inactivation of r-genes including the experiments where Actinomycin D (AmD) was first used as a chemical inhibitor of nucleolar processes [39, 40]. Since these experiments up to now AmD has been acknowledged the best studied, RNA polymerase I inhibitor inducing typical rearrangement of NCs, known as nucleolar segregation [30-33]. Corresponding ultrastructural changes of the nucleolus and linked inactivation dynamics under influence of chemical inhibitors were extensively documented in mammalian and human cells. However, same issues were less broadly studied in plant objects. By contrast, it is still unknown how physical factors, such as temperature, electromagnetic and γ-irradiation etc., considered as potential inhibitory agents disturbing the cellular metabolism by interfering with giant array of r-gene sequences will be reflected in 3D nuclear/nucleolar structural hierarchy. Meanwhile, it remains much more problematic to realize, which molecular as well as nano- and ultrastructural mechanism underlying territorial rearrangement of nuclear domains induced by physical factors. In respect to the influence upon any living systems γ-irradiation represents the most dangerous and quite realistic treat among all physical factors produced by the modern technological world.

Our hypothesis was based on presumption that during inhibitory action of γ-irradiation on r-genes level the dynamics of NCs rearrangement may develop via same mechanism as those caused by chemical inhibitors, thus yielding classical pattern of the nucleolar segregation. Correspondingly, we consider rapidly proliferating meristematic plant cells which nucleoli comprise high dose of r-genes, reflected in extremely big nucleolar size, as reliable experimental target. Accordingly, to address the γ-irradiation induced ultrastructural reorganization of the nucleus and nucleolus, we focused on postradiation displacement/spatial redistribution as well as ultrastructural appearance of FCs, DFC and ICC, being resorted to two complementary approaches. To identify the NCs and specify their rearrangement within the composition of normal and altered plant nucleoli we firstly applied conventional EM analysis. Secondly, to follow the changes in shape and sizes as well as spatial displacements of nuclei and nucleoli within cellular and nuclear volumes, respectively we resorted to 3D reconstruction using LM images obtained on serial semithin sections. These approaches allowed us to demonstrate precisely both the postradiation reshuffling of NCs in the form of non-canonic nucleolar segregation andintranuclear movement of the nucleolus inside nuclear volume. Undoubtedly, the results obtained are indicative to specific changes which are related to morphological signs of the radiation induced inhibition of entire cell metabolism. Hence, the responses of extremely sensitive nanostructural machineries involved in ribosome biogenesis are of particular importance. In fact, the nuleolar reaction fits perfectly all requests to be served as adequate criterion of plant cells and tissues functional state in prostradiation period. It is important to underline that intensity of developing postradiational nuclear/nucleolar damages correlates with the dose impact. Therefore, these alterations are certainly doze-dependent whereas general ultrastructural appearance after irradiation is indicative

of inhibited cell in which both nuclear processes and nuclear r-genes activity were artificially ceased or arrested.

Importantly, that current findings open exciting rout to analogous experiments in animal cells and tissues. Thus interpreting specific nucleolar reaction visualized in our plant object it's important to be reassured regarding specific character of the nucleolar reaction. Indeed, because of quite profound differences in ultrastructural appearance between the segregated nucleolus of irradiated plant cells and those in chemically inhibited animal objects, it's still hard to say whether peculliary altered ultrastructure observed in our experiments should be attributed to the specific postradiation reaction of the r-genes machineries. Definitely, in order to answer the question whether postradiation changes defined in plant meristematic cells are of universal character, next experimental series should be aimed on animal cells and tissues. Such an approach will help us to clarify if physical factors will produce in animal cell changes similar to the nucleolar reaction of the plant meristem or postradiation damage of r-genes will generate classical segregation of NCs.

As it was mentioned above one more specific expression of the nucleolar reaction was increase of chromatin condensation inside heterogeneous FCs. Arguably, heterogeneous FCs may occur as a result of condensation of ribosomal chromatin inside FCs (or on their periphery), that gives the rise to nucleosomal structure of inactivated part of rDNP-fibers**.** Most probably at high irradiation doses the further inactivation of r-chromatin takes place, resulting in massive condensation of rDNP fibrils. Nevertheless, all above described nucleolar changes are well known from literature and unambiguously indicate the inactivation of rRNA genes transcription and ribosome biogenesis.

In conclusion, we would emphasize: it's quite possible that cellular, nuclear and nucleolar changes visualized here, may be considered as specific morphological properties of postradiation death. Hence, functioning of r-genes and linked nucleolar modifications in postradiation conditions increasingly deserves further investigations.

REFERENCES

[1]. Jean-Pierre Pouget. Basics of radiobiology. Nuclear Medicine and Molecular Imaging, 2022, Volume 1 (Cover date: 2022), Pages 30-51

[2]. Long, E. O. & David I. B. (1980). Repeated genes in euckariotes. Ann Rev Biochem, 49, 727-764.

[3]. Hadjiolov, A. (1985) The Nucleolus and ribosome biogenesis, in Cell Biology Monographs (Alfert, M., Beerman, W. and Goldstein, L. ed.), Springer –Verlag, Wien, New York, pp. 1-263.

[4]. Risueno, M. C. & Medina, F. J. (1986). The nucleolar structure in plant cells. *Revis Biol Cell*, 7, 1-154.

[5]. Motte, P., Deltour, R., Mosen, H. &Bronchart R. (1988). Three-dimensional electron microscopy of the nucleolus and nucleolus-associated chromatin (NAC) during early germination of Zea mays L. *Biol Cell*, 62, 65-81.

[6]. Highett, M. J., Rawlins, D. J. & Shaw, P. J. (1993) Different patterns of DNA distribution in Pisum sativum nucleoli correlate with different levels of nucleolar activity. *J. Cell Sci*, 1993, 104, 843-852.

[7]. Shaw, P. J., Boven, A. F., Wells, B., Highett, M. J. & Jordan, E. G. (1996). The organization of nucleolar activity in plants. *J. Microsc*, 181, 178-185.

[8]. Rawlins, D. J. & Shaw, P. J. (1999). Three-dimensional organization of ribosomal DNA in interphase nuclei of Pisum sativum by in situ hybridization and optical tomography. *Chromosoma*. 99, 143-151.

[9]. Medina, F. J., Cerdido, A. & de Carcer G. (2000). The functional organization of the nucleolus in proliferating plant cells. *Eur J. Histochem*, 44, 117-131.

[10]. Saez-Vasques, J. & Medina, F. J. (2008). The plant nucleolus. *Adv Bot Res*, 47-1-46.

[11]. Shang, G, Wang, F, Hao, S. & Jiao, M. (2009). Dynamic changes of nucleolar DNA configuration and distribution during the cell cycle in Allium sativum cells. *Micron*, 449-454.

[12]. Kim S. H. (2009). Plant nucleolar dynamics. *J. Plant Biol*, 52, 193-201.

[13]. Stepinski, D. (2010). Organization of the nucleoli of soybean root meristematic cells at different states of their activity. *Micron*, 41, 283-288.

[14]. Stepinski, D. (2014). Functional ultrastructure of the plant nucleolus. *Protoplasm*, 251, 1285-1306.

[15]. Chelidze, P. & Zatsepina, O. (1988). Morphofunctional classification of the Nucleolus. *UspehiSovr. Biologii,* 105**,** 252-268 (in Russian).

[16]. Sheer, U. &Weisenberger, D. (1994). The nucleolus. *CurrOpin Cell Biol*, 6, 354-359.

[17]. Thiry, M. &Goessens, G. (1996). The nucleolus during the cell cycle, in *Molecular Biology Intelligence Unit* (Landes R.G ed.). Springer-Verlag, Heidelberg, 1-144

[18]. Heliot, L., Kaplan H., Lucas L., Klein C., Beorchia, A., Doco-Fenzy, M., Menager, M., Thiry, M., O'Donohue, M.F. &Ploton, D. (1997). Electron tomography of metaphase nucleolar organizers regions: Evidence for a twisted-loop organization. *Mol Biol Cell***,** 8, 2199-2216.

[19]. Chelidze, P., Dzidziguri, D. &Tumanishvili, G. (1998). Increased Functional Load on Mouse Kidney Proximal Tubule Epithelial Cells Causes Changes in Nucleolar 3-D Architecture. *J. Cell and Tissue Res,* 292,411-426.

[20]. Scheer, U. & Hock, R. (1999). Structure and function of the nucleolus. *CurrOpin Cell Biol*, 11, 385-390.

[21]. Cheutin, Th., O'Donohue, M.-F., Beorchia, A., Vandelaer, M., Kaplan, H., Defever, B., Ploton, D. &Thiry, M. (2002). Three-dimensional organization of active rRNA genes within the nucleolus. *J. Cell Sci*, 115, 3297-3307.

[22]. Cheutin, T., Misteli, T., and Dundr, M. (2003). Dynamics of nucleolar components, in *The Nucleolus* (Olson, M.O., ed.), Plenum Publesher, pp. 44-57.

[23]. Mosgoeller, W. (2003). Nucleolar ultrastructure in vertebrates, In: *Nucleolus* (Olson, M.O., ed.), Plenum Publesher, pp. 1-11.

[24]. Ploton, D.,O'Donohue, M.-F., Cheutin, T. &Thiry, M. (2004). Three-dimensional organization of rDNA and transcription. In: *Nucleolus* (Olson, M.O., ed.), Landes Bioscience, 154-169.

[25]. Misteli, T. (2005). Concepts in nucleolar architecture. *BioEssays*, 27, 477-487.

[26]. Thiry, M. and Lafontaine, D.L. (2005). Birth of a nucleolus: The evolution of Nnucleolar compartments. *Trends Cell Biol*, 54, 194-199.

[27]. Derenzini, M., Pasquinelli, G., O'Donohue, M.-F., Ploton, D. &Thiry, M. (2006). Structural and functional organization of ribosomal genes within mammalian cell nucleolus. *J. Histochem Cytochem*, 54, 131-145.

[28]. Tchelidze, P., Kaplan, H., Beorchia, A., O'Donohue, M.-F., Bobichon, H., Lalun, N., Wortham, L., Ploton, D. (2008). Three-Dimensional Reconstruction of Nuclear Components by Electron Microscope Tomography. *In: Methods in Cell Molecular Biology (R. Hanckock ed*.), 436, 137-158.

[29]. Tchelidze, P., Chatron-Colliet, A., Thiry, M., Lalun, N., Bobichon, H. &Ploton, D. (2009). Tomography of the cell nucleus using confocal microscopy and medium voltagwe electron microscopy. *Critical Reviews in Oncology/Hematology*,69,2, 127-143.

[30]. Tchelidze, P., Benaassarou, A., Kaplan, H., O'Donohue, M.-F., Lucas, L., Terryn, C., Rusishvili, L., Mosidze, G., Lalun, N. &Ploton D. (2017). Nucleolar sub-compartments in motion during rRNA synthesis inhibition: Contraction of nucleolar condensed chromatin and gathering of fibrillary centers are concomitant. PLoS ONE, 12, 1-37.

[31]. Michel, J., Nolin, F., Wortham, L., Lalun, N., Tchelidze, P., Banchet, V., Terryn, C. &Ploton, D. (2019). Various Nucleolar Stress Inducers Result in High Distinct Changes in Water, Dry Mass and Elemental Content in Cancerous Cell Compartments: Investigation Using Nano-Analytical Approach. Nanotheranostics, 2019, 3, 179-195.

[32]. Tchelidze, P., Kaplan, H., Terryn, C., Lalun N., Ploton, D. &Thiry, M. (2019). Electron Tomography Reveals Changes in Spatial Distribution of UBTF1 and UBTF2 Isoforms within Nucleolar Components during rRNA Synthesis Inhibition. Journal Struct Biol, 208, 1–23.

[33]. Shav-Tal, Y., Blechman, J., Darzacq, X., Montagna, C., Dye, BT. & Patton. J.G. (2005). Dynamic sorting of nuclearcomponents into distinct nucleolar caps during transcriptional inhibition. Mol Biol Cell, 2005, 16, 2395–2413.

[34]. Boisvert, F. M., van Koningsbruggen, S. N., Navascues, J. & Lamond A. I. (2007). The multifunctional nucleolus. Nat Rev Mol Cell Boil, 2007, 8, 574–585.

[35]. Sirri, V., Urcuqui-Inchima, S., Roussel, P. & Hernandez-Verdun, D. (2008). Nucleolus: the fascinating nuclear body. Histochem Cell Biol, 2008, 129, 13–31.

[36]. Pederson T. (2010). The nucleus introduced. Cold Spring HarbPerspect Biol, 2010, 10, 1–16.

[37]. Hernandez-Verdun, D, Roussel, P, Thiry, M, Sirri, V. & Lafontaine, D. (2010). The Nucleolus: structure/function rela-tionship in RNA metabolism. WIREs RNA, 2010, 1, 415–431.

[38]. Tafforeau, L, Zorbas, C, Langhendries, J. L., Mullineux, S.-T., Stamatopoulou, V. &Mullier, R. (2013). The complexity of human ribosome biogenesis revealed by systematic nucleolar screening of pre-rRNA processing factors. Molecular Cell, 2013, 51, 539–551.

[39]. Reynolds, R. C., Montgomery, P. O. & Hughes, B. (1964). Nucleolar "caps" produced by actinomycin D. Cancer Res, 1964, 24, 1269–1277.

[40]. Simard, R., Langelier, Y., Mandeville, R., Maestracci, N. & Royal, A. (1974). Inhibitors as tools in elucidating the struc-ture and function of the nucleus. In: Busch H, editor. The Cell Nucleus. New York: Academic Press, 1974, 447–487.