Journal of
APPLIED
BIOMEDICINE

J. Appl. Biomed.
5: 49–56, 2007
ISSN 1214-0287

ORIGINAL ARTICLE

Nucleolar proteins change in altered gravity

Margarita A. Sobol[1, 2], **Fernando González-Camacho**[2], **Elizabeth L. Kordyum**[1], **Francisco Javier Medina**[2]

[1]Institute of Botany (NASU), 01004 Kiev, Ukraine
[2]Centro de Investigaciones Biológicas (CSIC), E-28040 Madrid, Spain

Received 16th January 2007.
Revised 23rd January 2007.
Published online 12th February 2007.

Summary
Nucleolin is a major highly phosphorylated nucleolar protein involved in the regulation of r-chromatin condensation/expansion and rDNA transcription as well as in rRNA processing. The nucleolar protein homologous to the mammalian nucleolin and to the onion nucleolin-like protein NopA100, was detected in the nuclear soluble protein fraction, and in the nuclear matrix fraction from *Lepidium sativum* root meristematic cells, using the selective silver staining method and the cross-reaction with the anti-NopA100 antibody. In 2-DE Western blots of both nuclear fractions, the nucleolin-like protein was revealed as a smear on the level of 90 kDa extending through a certain range of pI. In both extracts obtained from seedlings germinated and grown under slow clinorotation, the extension of the pI range was shorter and the molecular weight range was thinner than in the 1 g control; moreover, in the nuclear matrix fraction, the spread of the pI range was separated into two clusters. The results obtained could indicate a lower phosphorylation of the protein, suggesting a decrease in the activity of *L. sativum* nucleolin-like protein under clinorotation.

Keywords: clinorotation – *Lepidium sativum* – nucleolus – nucleolin-like protein – two-dimensional immunoblotting

INTRODUCTION

Numerous spaceflight and ground experiments with plants modelling different aspects of real space flight have concluded that the major continuously acting factor is microgravity (Kordyum 1997). Microgravity or extended reduced gravity is available only aboard space vehicles. However, because of the high cost and limited access to experimentation in space, and the necessity to prepare space experiments, various devices have been developed to partially reproduce the conditions of the space environment. One of the most popular pieces of equipment for this purpose is the clinostat, currently used in many laboratories (e.g., Centis-Aubay et al. 2003, Hou et al. 2004, Kern et al. 2005). Since the clinostat rotates in the gravitational field, clinorotation clearly does not eliminate, alter, or scalarize gravity.

Rotation of a biological specimen on a horizontal clinostat at an appropriate rate only provides a continuous reorientation of objects with regard to a gravity vector that prevents them from perceiving a gravitational stimulus or realizing the subsequent response. It has become popular to use the terms "simulated microgravity" (clinorotation) or "altered gravity" (clinorotation, centrifugation,

✉ Margarita A. Sobol, Institute of Botany (NASU), 2 Tereschenkivska St., 01004 Kiev, Ukraine
margaret_sobol@yahoo.com
☎ +380 442723236
+380 442723236

or microgravity in spaceflight), as well as "real microgravity" (spaceflight) (Lorenzi and Perbal 1990, Albrecht-Buehler 1992, Kessler 1992, Dong et al. 1995). Essential re-arrangements of the structural and functional organization of cell organelles occur in microgravity indicating changes in cell metabolism under the conditions of altered gravity (Moore and Evans 1986, Halstead and Dutcher 1987, Claassen and Spooner 1994, Kordyum 1994).

The nucleolus, a highly dynamic cell compartment, responds to the influence of the environmental factors affecting cell metabolism generally (Sobol 2001). Actually, this organelle is a nuclear domain in which the major part of ribosome biogenesis takes place.

This is a basic process for cell vitality beginning with rDNA transcription followed by processing of newly synthesized pre-rRNA molecules (Dundr and Raska 1993, De Cárcer and Medina 1999, Scheer and Hock 1999, Medina et al. 2000, Yano and Sato 2002, Pendle et al. 2005, Shaw and Doonan 2005). A wide range of nucleolar proteins is involved in the regulation of different stages of ribosome biosynthesis (Saez-Vasquez et al. 2004, Shaw and Brown 2004, Sobol et al. 2005, Sobol et al. 2006).

Among them, nucleolin and nucleolin-like proteins play a key role; they are multifunctional proteins involved in the modulation of r-chromatin structure, the activation of ribosomal gene transcription, and the regulation of pre-rRNA processing, and they connect the process of ribosome biogenesis with other cellular processes (González-Camacho and Medina 2004, Saez-Vasquez et al. 2004, Sobol et al. 2005, Sobol et al. 2006). Up till now, the effects of microgravity on the nucleolus have been analyzed in a few publications focused on the description of changes in its structure (Dedolph et al. 1965, Shen-Miller and Gordon 1967, Shen-Miller and Hinchman 1968, Shen-Miller and Hinchman 1995).

Recently, we have undertaken a deeper characterization of these effects, using both real and simulated microgravity, studying ribosome biogenesis in the context of the cell activities and processes occurring in root meristematic cells, in particular cell proliferation and cell cycle, and analyzing how the microgravity environment affects these cellular processes (Matía et al. 2005, Sobol et al. 2005, 2006).

Moreover, the identification and characterization of functionally significant nucleolar proteins in plant cells, particularly under the influence of environmental factors, still remains an excitingly difficult problem, demanding profound and intense research. The purpose of our investigations was to reveal the peculiarities of nucleolin-like protein in root meristematic cells under clinorotation.

MATERIAL AND METHODS

We investigated cress (*Lepidium sativum* L.) root meristematic cells. Initially, cress seeds oriented with the embryonic root downwards were placed in 1% agar medium in tubes (d = 30 mm; h = 100 mm). Some of the tubes were placed in the dark, in stationary conditions, as controls; other tubes were attached with clamps to the horizontal axis of a horizontal clinostat, rotating at 2 rpm. Randomly distributed seeds and, consequently seedlings, experienced the total force of 0–6.7 x 10^{-5} g (Sobol 1999), which is considerably less than the threshold value of 10^{-3}–10^{-4} g (Shen-Miller and Hinchman 1968). Totally, two thousand control and two thousand clinorotated seedlings were harvested after two days of growth, and root meristems were dissected from them.

Polyclonal anti-NopA100 antibodies had been raised in rabbits against the complete protein from onion cells sharing immunological determinants with mammalian nucleolin (González-Camacho and Medina 2004, González-Camacho and Medina 2006). The dilution used was 1:100 in Western blotting experiments. Nuclei were purified as described previously (Cerdido and Medina 1995, González-Camacho and Medina 2004, Sobol et al. 2005, Sobol et al. 2006). Root meristems were homogenized, and the extract was repeatedly filtered and centrifuged.

The nuclear fraction purity was tested with methyl green-pyronin staining (bright field) or phase contrast microscopy. For this purpose, a drop of fresh nuclear fraction was deposited on a microscope slide, a drop of methyl green-pyronin solution was added, and a coverslip was placed for allowing microscopical observation. Then, nuclei were stored in nuclei stock buffer (NSB: 10 mM Tris-HCl, 10 mM HEPES, 10 mM KCl, 2 mM $MgCl_2$, 4 mM n-octanol, 0.1 mM $CaCl_2$, pH 7.4). All buffers contained a cocktail of protease inhibitors consisting of 10 µg of both leupeptin and aprotinin per ml and 1 mM phenylmethylsulfonyl fluoride (PMSF). All procedures were performed at 0–4 ^{0}C.

Nuclei were fractionated essentially as described (De Cárcer et al. 1997, González-Camacho and Medina 2004, Sobol et al. 2005, Sobol et al. 2006), firstly by extraction in a low ionic strength buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), then by DNase digestion, and finally, by solubilisation of the proteins of the nuclear matrix by incubation in 8M urea and sonication. All buffers and media used in nuclei fractionation contained the same cocktail of protease inhibitors as described for nuclei purification.

Proteins from each of these nuclear fractions were electrophoresed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

(Cerdido and Medina 1995, De Cárcer et al. 1997, Sobol et al. 2005, Sobol et al. 2006).

In addition the nuclear proteins of S2 fraction as well as the proteins of nuclear matrix fraction were subjected to the two-dimensional gel electrophoresis as described (González-Camacho and Medina 2004). For this purpose, isoelectric focusing (IEF) was carried out with 100 µg of protein from each fraction separately, using a Protean IEF cell (Bio-Rad, USA) and 11 cm gel strips forming an immobilized linear pH gradient from 3–10 (IPG Ready-Strips). The strips were rehydrated for 12 h at 18 ^{0}C at 50 V with a rehydration buffer, containing 100 µg proteins. The rehydration buffer contained 40 mM Tris, 2 M thiourea, 7 M urea, 4 % Triton X-100, 100 mM DTT, 2 % carrier ampholytes (pH 4–7 carrier ampholytes were mixed with pH 3–10 ampholytes at a 2:1 ratio) and 0.001% bromophenol blue. Focusing profiles for these strips included 15 min at 250 V followed by a rapid ramping to 8000 V and additional focusing at this level to accumulate 35 000 Vh. The current was limited to 50 mA per strip throughout the run and temperature was maintained at 18 ^{0}C. Upon completion of IEF, the IPG Ready-Strips were incubated first with an equilibration buffer (6 M urea, 2% SDS, 0.375 M Tris-HCl, pH 8.8, 20% glycerol) containing 130 mM DTT for 10 min and then with an equilibration buffer containing 135 mM iodoacetamide for 10 min. The strips were plated on top of vertical 12% SDS polyacrylamide gels, and the proteins were fractionated by electrophoresis in the second dimension.

In some experiments, the mono-dimensional gels with nuclear and nucleolar proteins were treated using the non-specific silver staining method (González-Camacho and Medina 2004, Sobol et al. 2005, Sobol et al. 2006). The stained gels were analyzed using the program "Image Quant" (Bio-Rad, USA). Other mono-dimensional gels as well as the two-dimensional gels were subjected to Western blotting. For mono-dimensional and two-dimensional Western blotting, the proteins from mono- and two-dimensional gels were electroblotted onto PVDF membranes. The membranes with nuclear and nucleolar proteins were washed in phosphate buffered saline-Tween 20 (PBST) for 10 min and blocked in this buffer supplemented with 5% dried nonfat milk for 1 h. The membranes were incubated with the anti-NopA100 antibodies overnight at 4 ^{0}C followed by incubation with the secondary antibodies for 1 h. The membranes were washed five times with PBST for 20 min between each step. The immune complexes were visualized with enhanced chemiluminescence using the ECL reagent kit (Amersham International, UK). The blots were scanned and analysed using the program "Image Quant" (BioRad, USA).

For each condition analyzed, mono- and two-dimensional gels were made in triplicate from six independent protein extractions. In all immunological experiments, negative controls consisting of the omission of the first antibody were performed. For detecting argentophile proteins of the nucleolar organizer (Ag-NOR proteins), the selective silver staining method was carried out on Western blots (Hozák et al. 1992, González-Camacho and Medina 2004, Sobol et al. 2005, Sobol et al. 2006). The selective silver staining solution consisted of a 2:1 v:v ratio mixture of solution A (50% $AgNO_3$ in deionized water) and solution B (2% gelatin, 1% formic acid in water). All solutions were prepared in freshly deionized water to avoid non-specific silver reactions. Samples were stained for 15 min in staining solution at room temperature under continuous stirring in the darkness. The reactions were stopped by washing in water. The membranes were scanned and analysed using the software "Image Quant" (BioRad, USA).

RESULTS

Nuclei isolated from the root apices were tested for purity and structural integrity with methyl green-pyronin staining or phase-contrast microscopy. With bright field microscopy, stained nuclei showed a rounded shape with conspicuous nucleoli occupying most of the nuclear area. In general, the aspect of purified nuclei appeared very similar to that showed in situ, showing signs of good structural preservation (Fig. 1).

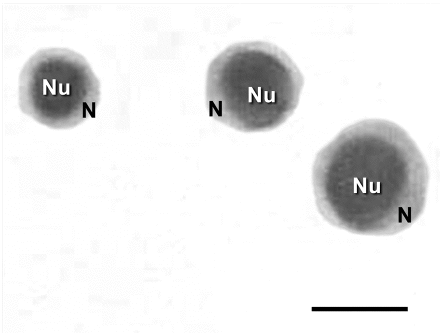

Fig. 1. **Extracted nuclei, stained with methyl green-pyronin, observed with bright field microscopy.** The nuclear fraction shows a good degree of purity and isolated nuclei (N) appear well preserved, with large and prominent nucleoli (Nu) in their interior. The original colors are pale lilac for the extranucleolar chromatin and dark pink for the nucleolus. Bar = 10 µm.

Fractionation and SDS-PAGE of total nuclear proteins followed by non-specific silver staining revealed 43 bands in 5 fractions; among them prominent bands in the soluble fraction and the

fraction of the nuclear matrix were detectable (Fig. 2).

Using the selective silver staining method (Ag-NOR method) for staining fractionated nuclear proteins on Western blots, argentophile nucleolar proteins were localized. According to previous reports on a variety of animal and plant biological models, nucleolin or nucleolin-like proteins are major targets of this staining procedure (Hozák et al. 1992, Martín et al. 1992, Roussel et al. 1992, González-Camacho and Medina 2004). The most soluble protein extract S2 was shown to be enriched in nucleolin-like proteins, since a prominent band, 90 kDa in molecular weight, appeared clearly stained by this specific silver staining procedure (Fig. 3). The protein with a molecular weight of 90 kDa localized in both the S2 fraction and the fraction of the nuclear matrix, took a minor position in the optical density among all nuclear and nucleolar proteins stained by the non-specific procedure.

Furthermore, we detected in *L. sativum* the putative homolog of nucleolin in the soluble fraction of nuclear proteins as well as in the insoluble protein fraction, i.e. the fraction of the nuclear matrix, on Western blots of these fractions probed with the antibody raised against the onion protein NopA100. The anti-NopA100 antibody showed a positive signal in both soluble and insoluble fractions of the nuclear proteins from cress. The protein of 90 kDa was recognized by the antibody, this molecular weight differing slightly from the corresponding homologous protein of *A. cepa* (Fig. 4).

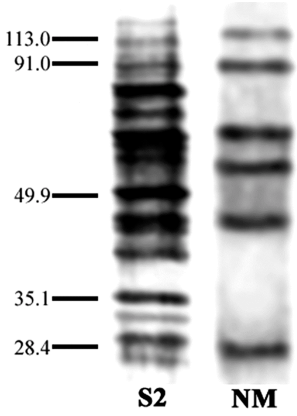

Fig. 2. ***Lepidium sativum*** **nuclear proteins revealed with the non-specific silver staining.** The most soluble fraction (S2), enriched in ribonucleoproteins, and the most insoluble fraction, identified as the nuclear matrix (NM) are shown. Molecular weight standards are at the left, in kDa.

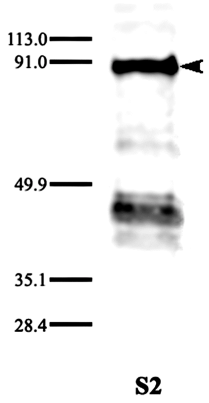

Fig. 3. **Western blot of the cress soluble nuclear ribonucleoprotein extract (S2),** stained with the specific silver method for the nucleolar organizer proteins (Ag-NOR staining). Arrowhead indicates the most stained protein band with molecular weight of 90 kDa. Molecular weight markers in kDa are given at the left.

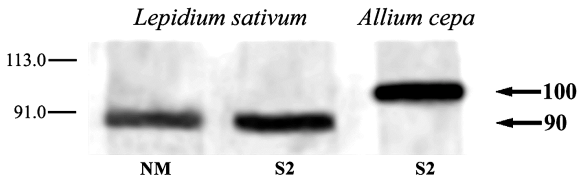

Fig. 4. **Western blots of *L. sativum* soluble (S2) and insoluble (NM) nuclear protein fractions** together with *A. cepa* soluble protein fraction (S2) probed with a polyclonal antibody raised against the onion nucleolin-like protein NopA100. The antibody recognizes a homologous cress protein of 90 kDa, which is present in the two functionally significant nuclear fractions. Arrows indicate the molecular weights of the stained bands in the two species. Molecular weight markers are at the left, in kDa.

The cress nucleolin-like protein was also revealed in two-dimensional (2-DE) Western blots of the same soluble and insoluble nuclear protein fractions. For this purpose, proteins were electrophoretically separated in two dimensions, and then they were electrotransferred to the PVDF membranes, and probed with the anti-NopA100 antibody. The result was a smear at the level of 90 kDa in molecular weight, extending through a range of isoelectric points (pI) (Fig. 5A, B). The band appeared as a stronger signal in the range of pI 4.5–6.0 for the soluble nuclear protein fraction (Fig.5A) and 4.5–7.0 for the insoluble nuclear protein fraction (Fig. 5B), and a weaker signal shifted in both cases towards the basic region of the blot.

Under clinorotation, we showed the shortening of its molecular weight range (Fig. 6A, B). In the case of the nuclear matrix fraction, a single band was

separated into two clusters; one was concentrated at the pI range of the 90 kDa protein and the thinning of pI 4.5-5.5 and the second was observed around pI 7 (Fig.6B).

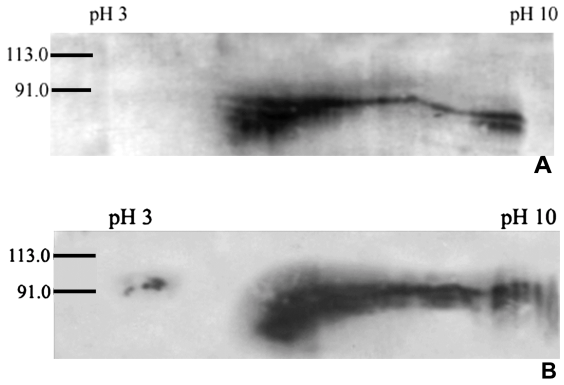

Fig. 5. **2-DE Western blots of nuclear proteins from *L. sativum*** S2 (A) and NM (B) fractions probed with the anti-NopA100 antibody in the stationary control.

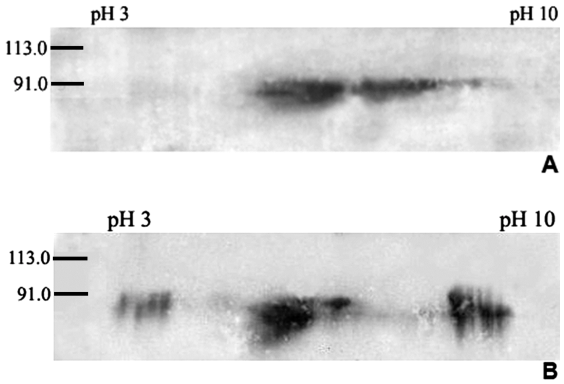

Fig. 6. **2-DE Western blots of nuclear proteins from *L. sativum*** S2 (A) and NM (B) fractions probed with the anti-NopA100 antibody under clinorotation.

DISCUSSION

In this work we continue the exploration of the effects of altered gravity on ribosome biogenesis, an essential process for cell vitality. For this purpose, we have chosen an approach involving SDS-PAGE and two-dimensional gel electrophoresis of the nuclear protein fractions, as well as one- and two-dimensional Western blotting, and used the slow-rotating clinostat. The slow rotating clinostat (1–5 rpm) is an appropriate tool for simulating reduced gravity (Merkys and Laurinavichius 1990, Albrecht-Buehler 1992, Kordyum 1994, Hilaire et al. 1995). Slow clinostats are widely used to investigate the biological effects of altered gravity because they make possible experiments with diverse analytical methods, without many of the limitations of spaceflight (safety, operator availability, storage, etc.).

With SDS-PAGE of total nuclear proteins and non-specific silver staining, we revealed a putative homolog of nucleolin in *L. sativum* with a molecular weight of 90 kDa. Owing to their domain structure, nucleolin and nucleolin-like proteins are well known to have a high affinity for silver staining under the particular conditions of the selective procedure (originally cytological) known as "Ag-NOR staining" (Hernandez-Verdun et al. 1984, Erard et al. 1990, Roussel et al. 1992, Ginisty et al. 1999). Therefore, we applied this sensitive method to the fractionated nuclear proteins and localized the major argentophile protein with a molecular weight of 90 kDa. Our results are in good agreement with the data obtained for onion, where silver-stained nucleolar proteins have been demonstrated to appear in S2 fraction (González-Camacho and Medina 2004). The major protein specifically stained with silver in *A. cepa* has been shown to be the plant homolog of nucleolin with a molecular weight of 100 kDa, called NopA100 (González-Camacho and Medina 2004, González-Camacho and Medina 2006). Nucleolin and nucleolin-like proteins from different types of organisms have been characterized; their molecular weights range from 52 kDa (Nopp52 of *Tetrahymena thermophilia*) (McGrath et al. 1997) to 110 kDa (rat nucleolin) (Bourbon and Amalric 1990). According to results reported elsewhere (Lee et al. 1992), the nucleolin-like protein of *Saccharomyces cerevisiae*, Nsr1p, have a molecular weight of 67 kDa. The radish nucleolin of 67 kDa capable of associating specifically with 5' ETS (External Transcribed Spacer) has been characterized (Echeverria and Lahmy 1995). In *Xenopus laevis*, two forms of nucleolin with molecular weights of 90 kDa (Caizergues-Ferrer et al. 1989) and 95 kDa (Rankin et al. 1993) have been found. A nucleolin homolog from alfalfa of 95 kDa, nucMs1 (Bogre et al. 1996), and a pea nucleolin of 90 kDa (Tong et al. 1997) have been revealed. Hence, we suggest that the major specific argentophile protein in *L. sativum* is a nucleolin homolog with molecular weight of 90 kDa.

Furthermore, the anti-NopA100 antibody recognized the nucleolin-like protein of 90 kDa in both soluble and insoluble fractions of nuclear proteins from *L. sativum.* The cross-reaction of the anti-onion NopA100 antibody with our biological material was clear and unequivocal, so we decided to use anti-NopA100 antibodies for comparing the behaviour of the cress nucleolin homolog in cells grown at 1 g and cells influenced by clinorotation.

The patterns of 2-DE Western blots, using the appropriate antibody on selected protein extracts can be useful in providing substantial information on protein peculiarities in relation to cell metabolic

activity (Bogre et al. 1996, Medina et al. 2000). We analyzed the 2-DE patterns of the soluble and insoluble nuclear protein fractions from cress cells germinated and grown under stationary control conditions probed with the anti-NopA100 antibody. Nucleolin-like protein was revealed in 2-DE Western blots of both nuclear fractions. We observed a band at the level of 90 kDa in molecular weight, spreading through a diapason of isoelectric points. The majority of the protein revealed among soluble nuclear proteins corresponded to the more acid conditions. The cluster of nucleolin homolog localized among insoluble nuclear proteins differed from it by a slight extension towards the basic region. The smear pattern of cress nucleolin in 2-DE Western blots is very similar to that shown by onion nucleolin, and the extension of the signal through a wide range of isoelectric points was shown in this species to be due to the existence of differently phosphorylated protein variants, as evidenced by an *in vitro* dephosphorylation assay (González-Camacho and Medina 2004). In fact, it has been shown that nucleolin and nucleolin-like proteins are highly phosphorylated and contain several phosphorylation sites (Bourbon et al. 1983, Caizergues-Ferrer et al. 1987, Peter et al. 1990, Roussel et al. 1992, Zhou et al. 1997, Ginisty 1999). So, the data of 2-DE seem to indicate the existence of phosphorylated variants of nucleolin homolog in *L. sativum*. The stronger signal in the acidic pI range is probably associated with the presence of major acidic isoform(s) of this protein.

In order to clearly establish if the nucleolin-like protein is related to the mechanisms of nucleolar response to the changed gravity environment, we compared the characteristics of nucleolin homolog, as they are detected in 2-DE Western blots, in extracts of proteins obtained from control samples with similar extracts obtained from clinorotated samples.

We showed that, under clinorotation, the range of putative phospho-isoforms of cress nucleolin-like protein spread through the pI range was reduced, as was the molecular weight extension of the signal revealed by the antibody. This could be related to a reduction in phosphorylation of the protein. It is known that phosphorylation of nucleolin and nucleolin-like proteins is intensely regulated by different kinases during various processes occurring in a cell (Caizergues-Ferrer et al. 1987, Peter et al. 1990, Medina et al. 1995, De Cárcer et al. 1997, Zhou et al. 1997), and this controlled phosphorylation modulates the activity of nucleolin and nucleolin-like proteins in rDNA transcription and rRNA processing. Actually, the variation observed in the 2-DE pattern of the nucleolin-like protein in *L. sativum* between control 1 g gravity and simulated microgravity was very similar to the variation described in the onion between meristematic (proliferating) and parenchymatic (non-proliferating) root cells (González-Camacho and Medina 2004). So, we could propose that a decrease in the quantity of the phosphorylated isoforms of *L. sativum* nucleolin-like protein under clinorotation indicates a lowering of the activity of the protein. Our results are consistent with the data recently reported (Sobol et al. 2004, Sobol et al. 2005, Sobol et al. 2006) on decrease in the activity of a nucleolus under clinorotation. Similar conclusions were reached after an experiment in real microgravity, on board the International Space Station (Matía et al. 2005). It is possible that the nucleolin-like protein is involved not only in the nucleolar response to the conditions of altered gravity, but also in the transmission of the signal of changed gravity environment into the nucleolus.

ACKNOWLEDGEMENT

This work was supported by a Grant of INTAS No. YSF 2001/2-144 and by Grants from the Spanish "Plan Nacional de Investigación Científica y Desarrollo Tecnológico" Nos. ESP2001-4522-PE, and ESP2003-09475-C02-02.

REFERENCES

Albrecht-Buehler G: The simulation of microgravity conditions on the ground. ASGSB Bull. 5:3V10, 1992.

Bogre L, Jonak C, Mink M, Meskiene I, Traas J, Ha DTC, Swoboda I, Plank C, Wagner E, HeberleBors E, Hirt H: Developmental and cell cycle regulation of alfalfa nucMs1, a plant homolog of the yeast Nsr1 and mammalian nucleolin. Plant Cell 8:417–428, 1996.

Bourbon HM, Amalric F: Nucleolin gene organization in rodents: highly conserved sequences within three of the 13 introns. Gene 88:187–196, 1990.

Bourbon H, Bugler B, Caizergues-Ferrer M, Amalric F: Role of phosphorylation on the maturation pathways of a 100 kDa nucleolar protein. FEBS Lett. 155:218–222, 1983.

Caizergues-Ferrer M, Belenguer P, Lapeyre B, Amalric F, Wallace MO, Olson MOJ: Phosphorylation of nucleolin by a nucleolar type–NII protein-kinase. Biochemistry 26:7876–7883, 1987.

Caizergues-Ferrer M, Mariottini P, Curie C, Lapeyre B, Gas N, Amalric F, Amaldi F: Nucleolin from *Xenopus laevis* – cDNA cloning and expression during development. Genes Dev. 3:324–333, 1989.

Centis-Aubay S, Gasset G, Mazars C, Ranjeva R, Graziana A: Changes in gravitational forces induce modifications of gene expression in *A. thaliana* seedlings. Planta 218:179–185, 2003.

Cerdido A, Medina FJ: Subnucleolar location of fibrillarin and variation in its levels during the cell cycle and during differentiation of plant cells. Chromosoma 103:625–634, 1995.

Claassen DE, Spooner BS: Impact of altered gravity on aspects of cell biology. Int. Rev. Cytol. 156:301–373, 1994.

De Cárcer G, Cerdido A, Medina FJ: NopA64, a novel nucleolar phosphoprotein from proliferating onion cells, sharing immunological determinants with mammalian nucleolin. Planta 201:487–495, 1997.

De Cárcer G, Medina FJ: Simultaneous localization of transcription and early processing markers allows dissection of functional domains in the plant cell nucleolus. J. Struct. Biol. 128:139–151, 1999.

Dedolph RR, Nagvi SM, Gordon SA: The effect of gravity compensation on the geotropic sensitivity of *Avena* seedlings. Plant Physiol. 40:961–965, 1965.

Dong LY, Liu CX, Shi JN: Enhancing effects of simulated microgravity on *Agrobacterium*-infected frequency of tobacco callus. Microgravity Sci. Technol. 7:336–338, 1995.

Dundr M, Raska I: Nonisotopic ultrastructural mapping of transcription sites within the nucleolus. Exp. Cell Res. 208:275–281, 1993.

Echeverria M, Lahmy S: Identification of a 67 kDa protein that binds specifically to the pre-rRNA primary processing site in a higher plant. Nucleic Acids Res. 23:4963–4970, 1995.

Erard MS, Lakhdar-Ghazal F, Amalric F: Repeat peptide motifs which contain beta-turns and modulate DNA condensation in chromatin. Eur. J. Biochem. 191:19–26, 1990.

Ginisty H, Sicard H, Roger B, Bouvet P: Structure and functions of nucleolin. J. Cell Sci. 112:761–772, 1999.

González-Camacho F, Medina FJ: Identification of specific plant nucleolar phosphoproteins in a functional proteomic analysis. Proteomics 4:407–417, 2004.

González-Camacho F, Medina FJ: The nucleolar structure and the activity of nucleolin-like protein NopA100 during the cell cycle in proliferating plant cells. Histochem. Cell Biol. 125:139–153, 2006.

Halstead TW, Dutcher FR: Plants in space. Annu. Rev. Plant Physiol. 38:317–345, 1987.

Hernandez-Verdun D, Derenzini M, Bouteille M: Relationship between the Ag-NOR proteins and ribosomal chromatin in situ during drug-induced RNA synthesis inhibition. J. Ultrastruct. Res. 88:55–65, 1984.

Hilaire E, Guikema JA, Brown CS: Clinorotation affects soybean seedling morphology. J. Gravit. Physiol. 2:149–150, 1995.

Hou G, Kramer VL, Wang YS, Chen RJ, Perbal G, Gilroy S, Blancaflor EB: The promotion of gravitropism in *Arabidopsis* roots upon actin disruption is coupled with the extended alkalinization of the columella cytoplasm and a persistent lateral auxin gradient. Plant J. 39:113–125, 2004.

Hozák P, Roussel P, Hernandez-Verdun D: Procedures for specific detection of silver-stained nucleolar proteins on Western blots. J. Histochem. Cytochem. 40:1089–1096, 1992.

Kern VD, Schwuchow JM, Reed DW, Nadeau JA, Lucas J, Skripnikov A, Sack FD: Gravitropic moss cells default to spiral growth on the clinostat and in microgravity during spaceflight. Planta 221:149–157, 2005.

Kessler JO: The internal dynamics of slowly rotating biological systems. ASGSB Bull. 5:11–22, 1992.

Kordyum EL: Biology of plant cells in microgravity and under clinostating. Int. Rev. Cytol. 171:1–78, 1997.

Kordyum EL: Effects of altered gravity on plant cell processes: Results of recent space and clinostatic experiments. Adv. Space Res. 14:77–85, 1994.

Lee WC, Zabetakis D, Melese T: NSR1 is required for pre-rRNA processing and for the proper maintenance of steady-state levels of ribosomal subunits. Mol. Cell Biol. 12:3865–3871, 1992.

Lorenzi G, Perbal G: Actin-filaments responsible for the location of the nucleus in the lentil statocyte are sensitive to gravity. Biol. Cell 68:259–263, 1990.

Matía I, González-Camacho F, Marco R Kiss JZ, Gasset G, Medina FJ: Nucleolar structure and proliferation activity of *Arabidopsis* root cells from seedlings germinated on the International Space Station. Adv. Space Res. 36:1244–1253, 2005.

Martín M, García-Fernández LF, Moreno Díaz de la Espina S, Noaillacdepeyre J, Gas N, Medina FJ: Identification and localization of a nucleolin homologue in onion nucleoli. Exp. Cell Res. 199:74–84, 1992.

McGrath KE, Smothers JF, Dadd CA: An abundant nucleolar phosphoprotein is associated with ribosomal DNA in *Tetrahymena* macronuclei. Mol. Biol. Cell. 8:97–108, 1997.

Medina FJ, Cerdido A, De Cárcer G: The functional organization of the nucleolus in proliferating plant cells. Eur. J. Histochem. 44:117–131, 2000.

Medina FJ, Cerdido A, Fernandez-Gomez ME: Components of the nucleolar processing complex (pre-rRNA, fibrillarin, and nucleolin)

colocalize during mitosis and are incorporated to daughter cell nucleoli. Exp. Cell Res. 221:111–125, 1995.

Merkys AI, Laurinavichius RS: Plant growth in space. In Asashima M, Malasinski GM (eds): Fundamentals of Space Biology, Springer-Verlag, Berlin 1990, pp. 64–89.

Moore R, Evans ML: How roots perceive and respond to gravity. Am. J. Bot. 73:574–587, 1986.

Pendle AF, Clark GP, Boon R, Lewandowska D, Lam YW, Andersen J, Mann M, Lamond AI, Brown JWS, Shaw PJ: Proteomic analysis of the *Arabidopsis* nucleolus suggests novel nucleolar functions. Mol. Biol. Cell. 16:260–269, 2005.

Peter M, Nakagawa J, Doree M, Labbe JC, Nigg EA: Identification of major nucleolar proteins as candidate mitotic substrates of cdc2 kinase. Cell 60:791–801, 1990.

Rankin ML, Heine MA, Xiao S, Leblanc MD, Nelson JW, Dimario PJ: A complete nucleolin cDNA sequence from *Xenopus laevis*. Nucleic Acids Res. 21:169, 1993.

Roussel P, Belenguer P, Amalric F: Nucleolin is an Ag-NOR protein; this property is determined by its amino-terminal domain independently of its phosphorylation state. Exp. Cell. Res. 203:256–269, 1992.

Saez-Vasquez J, Caparros-Ruiz D, Barneche F, Echeverria M: A plant snoRNP complex containing snoRNAs, fibrillarin, and nucleolin-like proteins is competent for both rRNA gene binding and pre-rRNA processing *in vitro*. Mol. Cell Biol. 24:7284–7297, 2004.

Scheer U, Hock R: Structure and function of the nucleolus. Curr. Opin. Cell Biol. 11:385–390, 1999.

Shaw PJ, Brown JWS: Plant nuclear bodies. Curr. Opin. Plant Biol. 7:614–620, 2004.

Shaw P, Doonan J: The nucleolus: playing by different rules? Cell Cycle. 4:102–105, 2005.

Shen-Miller J, Gordon SA: Gravitational compensation and the phototropic response of oat coleoptiles. Plant Physiol. 42:352–360, 1967.

Shen-Miller J, Hinchman RR: Nucleolar transformation in plants grown on clinostats. Protoplasma 185:194–204, 1995.

Shen-Miller J, Hinchman RR: Threshold for the georesponse to acceleration in gravity compensated Avena seedlings. Plant Physiol. 43:338–344, 1968.

Sobol MA: Structural-functional peculiarities of *Lepidium sativum* L. (Brassicaceae Burnett) root cell nucleoli under influence of altered gravity (In Russian). Ukr. Bot. J. 56:358–364, 1999.

Sobol MA: The role of the nucleolus in the reactions of plant cells on the influence of environmental physical factors (In Russian). Cytol. Genet. 35:72–84, 2001.

Sobol MA, González-Camacho F, Rodriguez-Vilariño V, Kordyum EL, Medina FJ: Clinorotation influences rDNA and NopA100 localization in nucleoli. Adv. Space Res. 36:1254–1262, 2005.

Sobol MA, González-Camacho F, Rodriguez-Vilariño V, Kordyum E, Medina FJ: Subnucleolar location of fibrillarin and NopA64 in *Lepidium sativum* root meristematic cells is changed in altered gravity. Protoplasma. 228:209–219, 2006.

Sobol MA, Kordyum EL, González-Camacho F, Rodriguez-Vilariño V, Medina FJ: Alterations of the intranucleolar DNA localization caused by simulated microgravity. J. Grav. Physiol. 11:189–190, 2004.

Tong CG, Reichler S, Blumenthal S et al.: Light regulation of the abundance of mRNA encoding a nucleolin-like protein localized in the nucleoli of pea nuclei. Plant Physiol. 114:643–652, 1997.

Yano H, Sato S: Combination of electron microscopic *in situ* hybridization and anti-DNA antibody labeling reveals a peculiar arrangement of ribosomal DNA in the fibrillar centres of the plant cell nucleolus. J. Electron Microsc. (Tokyo) 51:231–239, 2002.

Zhou G, Seibenhener ML, Wooten MW: Nucleolin is a protein kinase C-zeta substrate. Connection between cell surface signaling and nucleus in PC12 cells. J. Biol. Chem. 272:31130–31137, 1997.