Arch. Biol. Sci., Belgrade, 67(2), 405-410, 2015 DOI:10.2298/ABS140810007W

NUCLEOLUS DISASSEMBLY AND DISTRIBUTION OF SEGREGATED NUCLEOLAR MATERIAL IN PROPHASE OF ROOT-TIP MERISTEMATIC CELLS IN *TRITICUM AESTIVUM* L.

Jianyue Wang[1] and Feixiong Zhang[1,*]

[1]*Capital Normal University, College of Life Sciences,* Beijing 100048, China

***Corresponding author:** fxzhang@hotmail.com

Abstract: This paper presents details of the process of nucleolar disassembly, studied by conventional transmission electron microscopy (TEM) in wheat root cells. In early prophase, chromatin condensation and irregular nucleolar morphology are observed, with many small particles appearing around the nucleolus. In middle prophase, the nucleolus radiates outwards; in late prophase, the fine structure of the nucleolus disappears and nucleolar material diffuses away. Using "en bloc" silver-staining to distinguish between nucleoli and chromatin, we observed that the dispersed nucleolar material aggregates around the chromatin, forming a sheath-like perichromosomal structure that coats the chromosomes in late prophase.

Key words: Nucleolus disassembly; distribution of segregated nucleolar materials; prophase; root-tip meristematic cells; *Triticum aestivum* L.

Received August 10, 2014; **Revised** January 16, 2015; **Accepted** January 26, 2015

INTRODUCTION

The nucleolus is a large nuclear domain involved in ribosomal biosynthesis, a complex process that involves the transcription of ribosomal genes (rDNA), the processing of rRNA, and assembly with ribosomal proteins to form the large and small ribosome subunits (Soldani et al., 2006). In ultrathin EM sections prepared by standard procedures, most nucleoli display a concentric arrangement of three types of components, defining a tripartite organization with fibrillar centers (FC), dense fibrillar components (DFC) and granular components (GC). Besides these three principal components, perinucleolar condensed chromatin can often be seen (Raška et al., 2006).

The nucleolus is a dynamic organelle involved in the cell cycle. It disintegrates in the early prophase, disappears at the end of late prophase and reassembles in telophase (Olson, 2011). A question that has been put forward concerns the segregation of nucleolar materials. A series of research papers have described the whereabouts

of nucleolar components by conventional TEM, immuno- and cytochemical staining for TEM observation, immunofluorescence staining, etc. (Moreno-Diaz de la Espina et al., 1976; Paweletz and Risueño, 1982; Shi et al., 1987, van Hooser et al., 2005), and it is generally accepted that nucleolar components move to the edges of chromosomes, and then gradually reconnect to form a perichromosomal compartment (Hernandez-Verdun et al., 2010). A fuller understanding of the details of the process, i.e. how the nucleolar material gradually diffuses outwards from the nucleoli, the size, morphological changes, localization and movement of this material remain to be elucidated.

In this study, using TEM with conventional and "en bloc" silver-staining procedures, we examined the diffusion of disintegrated nucleolar material in root-tip meristematic cells of wheat. We observed that this is an orderly process that proceeds from early to middle and late prophase. We also undertook a systematic analysis of the size, morphological changes and distribution of the nucleolar material.

MATERIALS AND METHODS

Plant material and reagents

Wheat (*Triticum aestivum* L., CB037-A) seeds were from Capital Normal University, China. Glutaraldehyde and osmium tetroxide were obtained from Sigma (Shanghai, China). Epon 812 epoxy resin was purchased from Zhongjingkeyi Technology Co., Ltd. (Beijing, China).

Observation of conventional ultrathin sections

Wheat seeds (*Triticum aestivum* L.) were pre-imbibed for 30 min and then placed in 10 cm Petri dishes on moistened filter paper for germination at 25°C. When the length of the root was ~1 cm, the root-tips were carefully excised and immediately fixed in 3% glutaraldehyde in 0.2 mol/L phosphate buffer (PBS, pH 7.4) for 12 h at room temperature. After rinsing in the PBS, the specimens were postfixed in 2% osmium tetroxide in the same buffer for 2 h. After a thorough wash with distilled water, the specimens were dehydrated in an ethanol-acetone series and embedded in Epon 812 epoxy resin. Ultrathin sections were cut on a Leica UC6 ultramicrotome at a thickness of 60-70 nm and stained with uranyl acetate and lead citrate. The sections were observed under a Hitachi H-7500 TEM.

Observation of "en bloc" silver-stained ultrathin sections

The wheat root-tips were carefully excised and fixed immediately in 3% glutaraldehyde (0.2 mol/L PBS, pH 7.4) at 4°C for 30 min, washed 3 times with the PBS, postfixed in methanol/glacial acetic acid (3:1 v/v) at 25°C for 30 min and dehydrated in an ethanol-water gradient. Samples were stained with a mixture of 50% $AgNO_3$/2% gelatin (in 1% formaldehyde) (2:1 v/v) at 65°C for 30 min, reduced in 5% NaS_2O_3 for 5 min, rehydrated with ethanol-acetone gradient and embedded in Epon 812 epoxy resin. Sections (60-70 nm thick) were cut, observed and photographed as described above.

RESULTS AND DISCUSSION

In higher eukaryotes, when cells enter mitosis, rDNA transcription declines sharply in early prophase and is completely repressed before nuclear envelope breakdown (Raška et al., 2006; Hernandez-Verdun et al., 2010), while the nucleolus undergoes disassembly. Fig. 1A shows an early

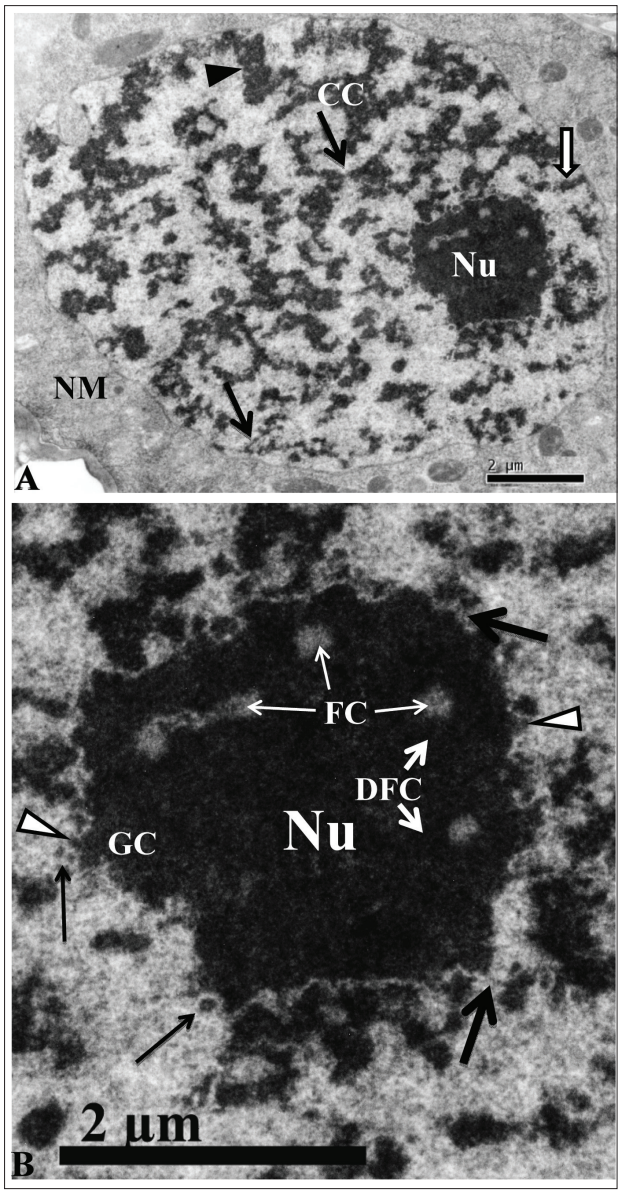

Fig. 1. The diffusion and distribution of segregated nucleolar materials in early prophase of root-tip meristematic cells in wheat. A – An early prophase nucleus. The bold, fine, hollow and triangle arrows indicate the protruded area of the nucleolus; 25 nm, 160 nm and 600 nm chromatin fibers. Bar: 2 μm. B – Detail of Fig. 1A. Particles of 0.05-0.20 μm around the nucleolus. The particles appear as protrusions (white triangle arrows), detached but still connected to the nucleolus (bold arrows), removed from the nucleolus (fine arrows). CC: condensed chromatin; DFC: dense fibrillar components; FC: fibrillar center; GC: granular component; NM: nuclear membrane; Nu: nucleolus; Bar: 2 μm.

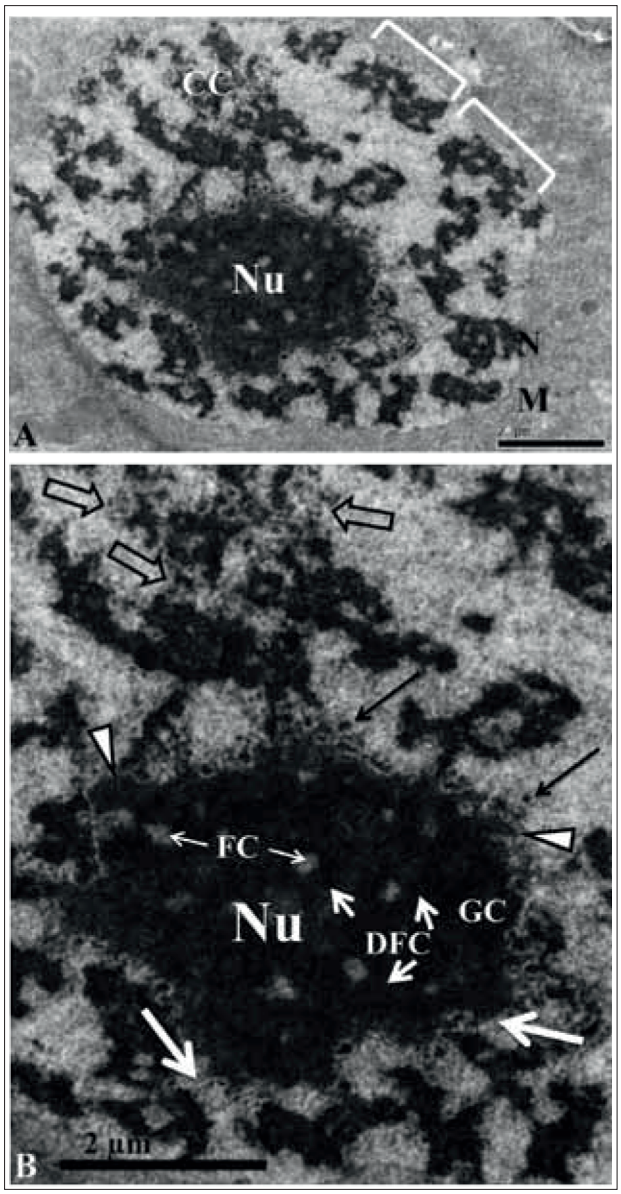

Fig. 2. The diffusion and distribution of segregated nucleolar materials in middle prophase of root-tip meristematic cells in wheat. A – middle prophase nucleus. Note chromatin condensation. The white bars indicate that the basic chromosome outlines. Bar: 2 μm. B – Detail of Fig. 2A. Ultrastructure of the nucleolus during middle prophase. The white triangle, bold, fine and hollow arrows indicate particles that are protruding from the nucleolus, particles detached but still connected to the nucleolus, particles that have moved out of the nucleolus and particles distributed around/between the chromatin, respectively. CC: condensed chromatin; DFC: dense fibrillar components; FC: fibrillar center; GC: granular component; NM: nuclear membrane; Nu: nucleolus; Bar: 2 μm.

prophase nucleus of root meristematic cell of *T. aestivum*. At this stage, the chromatin started to condense. Though some fine 25 nm and 160 nm chromatin fibers could be discerned, the proportion of thicker fibers was much higher, the thickest fibers being about 600 nm in diameter. The nucleolus was prominent; however, its morphology appeared irregular. Nevertheless, the fine structures of the nucleolus, such as the fibrillar center (FC), dense fibrillar component (DFC) and granular component (GC), could be easily distinguished (Fig.1B). We also observed many particles 0.05-0.2 μm in size distributed around the entire nucleolus. Some particles barely protruded out from the nucleolus, some appeared detached but were still connected by fine fibers, while a smaller proportion of fibers were observed outside the nucleolus. Nucleoli began to segregate in early prophase and many particles 0.05-0.20 μm in size diffused from the GC.

Fig. 2A shows a middle prophase nucleus. The degree of chromatin condensation at this stage was increased. The chromatin fibers appeared thicker while fine fibers were harder to detect. The basic chromosome outlines were visible in some places. While nucleolar morphology was severely irregular, the nucleolar fine structure were preserved (Fig. 2B). The whole nucleolus appeared to radiate to different regions, observed as numerous small particles of varying size.

In the late prophase nucleus, the chromatin was more condensed (Fig. 3A). The fine structures of the nucleolus disappeared, and diffusion of segregated nucleolar material was much more obvious. Though there were still particles that protruded outwards from the nucleolus, most remained localized around the nucleolus and connected with each other, forming short fibers.

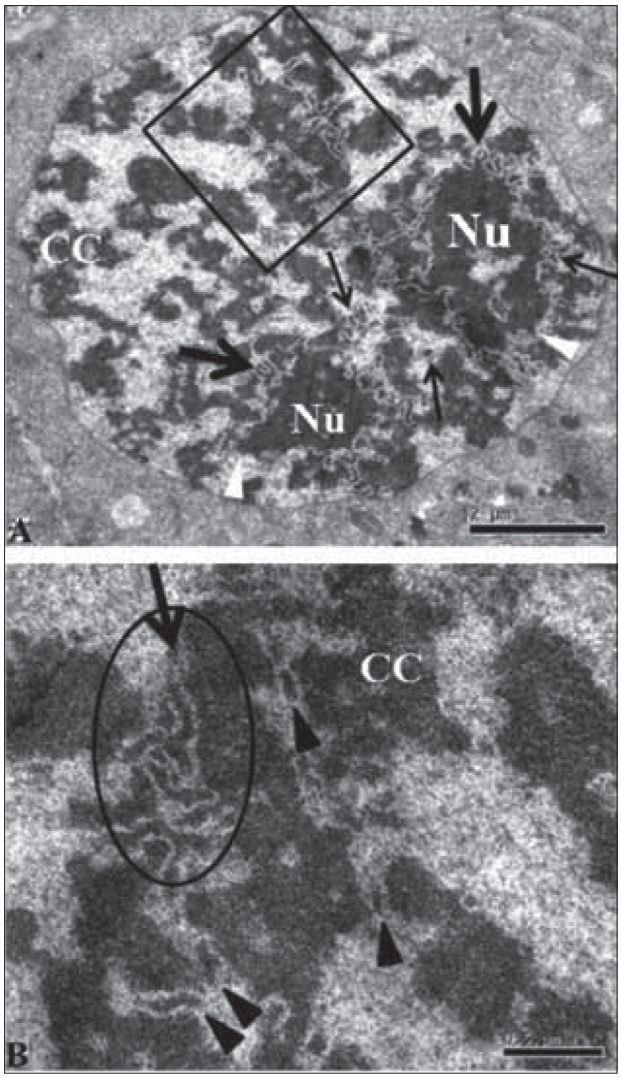

Fig. 3. The diffusion and distribution of segregated nucleolar materials in late prophase of root-tip meristematic cells in wheat. A – late prophase nucleus. Segregated nucleolar materials (square), the protruded granules (white triangle arrows), detached granules (fine arrows), the short fibers connected by the granules (bold arrows). Bar: 2 μm. B – Detail of Fig. 3A. Fine fibers with a 0.05 μm diameter. The triangle and bold arrows indicate fibers lying between and near the chromatin. CC: condensed chromatin; Nu: nucleolus. Bar: 0.5 μm.

Although these were of different lengths, their thickness was uniform, about 0.05 μm. While some fibers remained in close proximity to the nucleolus, most were distributed along the chromatin (Fig. 3B). With cell cycle progression,

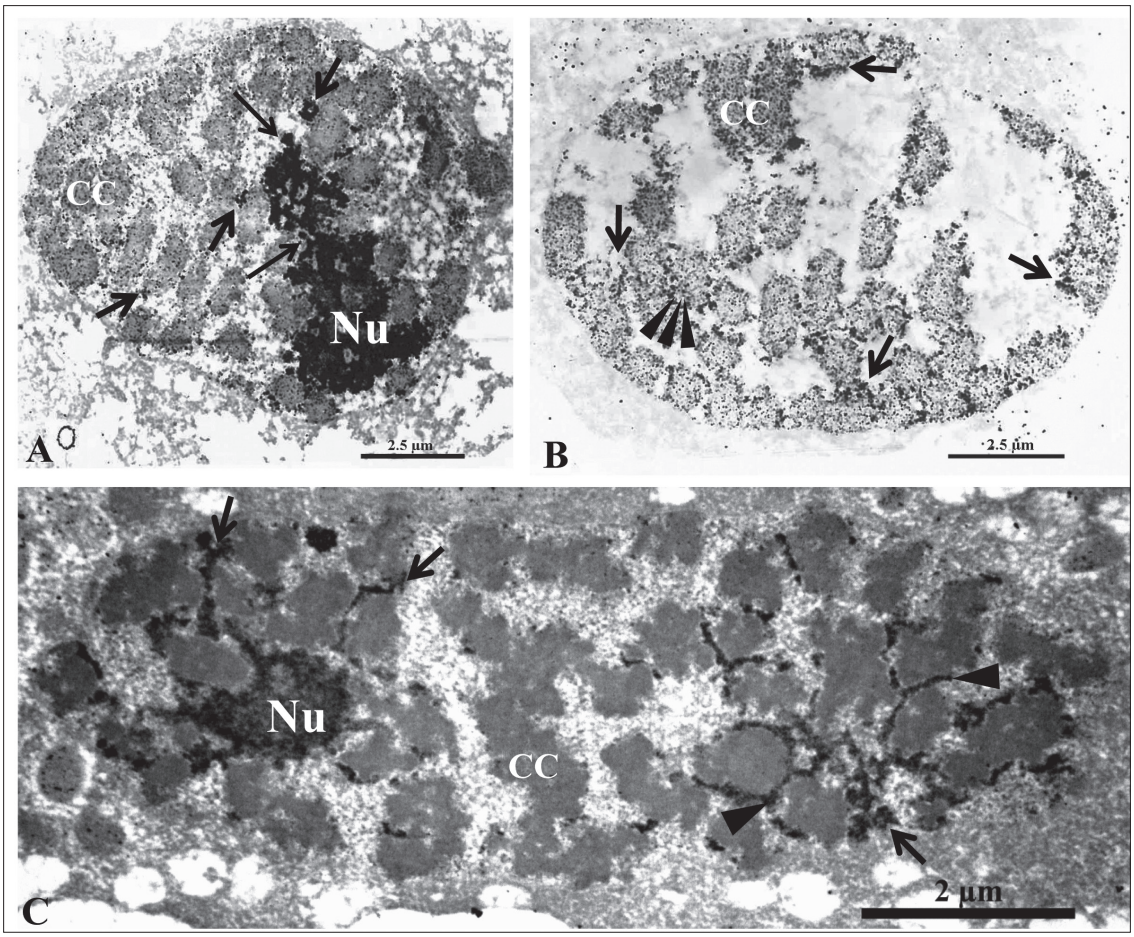

Fig. 4. The diffusion and distribution of segregated nucleolar materials in late prophases of root-tip meristematic cells. A – Segregated nucleolus. The fine and bold arrows indicate the segregated nucleolar material attached to the nucleolus, or diffused to the edges and between chromatin, respectively. Bar: 2.5 μm. B – Segregated nucleolar material. The arrows indicate segregated nucleolar material aggregated around the chromosomes; the triangle arrows show that the particles segregated from the nucleolus are interconnected, forming short fibers. Bar: 2.5 μm. C – Segregated nucleolar material (long arrows). The sheath-like perichromosomal structure is indicated by black triangle arrows. CC: condensed chromatin; Nu: nucleolus; Bar: 2 μm.

the particles segregated from the nucleolus approached the chromatin and linked up with each other to form short fibers with a diameter of 0.05 μm, which constituted a sheath-like perichromosomal structure wrapped around the chromosomes.

Since nucleolar proteins have a high affinity to silver nitrate (Trere, 2000), "en bloc" silver-staining TEM was performed to distinguish between nucleolar material and chromatin according to their staining properties (Fig. 4A-C). Silver staining revealed that dispersed nucleolar material (Fig. 4A) aggregated around the chromosomes (Fig. 4B), forming a sheath-like perichromosomal structure coating the chromosome (Fig. 4C). Moreno-Diaz de la Espina et al. (1976) showed that a chromosome "sheath"-like structure is formed in anaphase, while Paweletz and Risueño (1982), Shi et al. (1987), van Hooser et al. (2005) and Hernandez-Verdun et al. (2010) argued that it emerged in metaphase.

In contrast to these reports, we observed that the chromosome periphery was formed in late prophase, therefore being visible before metaphase.

Acknowledgments: This work was supported by the National Natural Science Foundation of China (NSFC, grant No. 30971453). We are grateful to Prof. Yueming Yan at Capital Normal University, China, for kindly providing wheat (*Triticum aestivum* L., CB037-A) seeds. The authors also appreciate the reviewers' comments to this paper and thank Dr. Kishore Pasumarthi (Dalhousie University, Halifax, Canada) for his critical reading of the manuscript and language improvement.

Author's contributions: F.Z. provided the conceptual framework, design experiments and wrote the manuscript. J.W. designed and performed the experiments and collected data. The data was analyzed by J.W and F.Z.

Conflict of interest disclosure: The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

Hernandez-Verdun, D., Roussel, P., Thiry, M., Sirri, V. and *D.L. Lafontaine* (2010). The nucleolus: structure/function relationship in RNA metabolism. *Wiley Interdiscip. Rev. RNA.* **1**, 415-431.

Moreno-Diaz de la Espina, S., Risueño, M.C., Fernandez-Gomez, M.E. and *C.J. Tandler* (1976). Ultrastructural study of the nucleolar cycle in meristematic cells of Allium cepa. *J. Microsc. Biol. Cell.* **25**, 265-278.

Olson, M.O. (2011). The nucleolus. Springer Press, New York.

Paweletz, N. and *M.C. Risueño* (1982). Transmission electron microscopic studies on the mitotic cycle of nucleolar proteins impregnated with silver. *Chromosoma.* **85**: 261-273.

Raska, I., Shaw, P.J. and *D. Cmarko* (2006). New insights into nucleolar architecture and activity. *Int. Rev. Cytol.* **255**: 177-235.

Shi, L.J., Ni, Z.M., Zhao, S., Wang, G. and *Y. Yang* (1987). Involvement of a nucleolar component, perichromonucleolin, in the condensation and decondensation of chromosomes. *Proc. Natl. Acad. Sci. U S A.* **84**: 7953–7956.

Soldani, C., Bottone, M.G., Pellicciari, C. and *A.I. Scovassi* (2006). Nucleolus disassembly in mitosis and apoptosis: dynamic redistribution of phosphorylated-c-Myc, fibrillarin and Ki-67. *Eur. J. Histochem.* **50**: 273-280.

Trerè, D. (2000). AgNOR staining and quantification. *Micron.* **31**:127-131

van Hooser, A.A., Yuh, P. and *R. Heald* (2005). The perichromosomal layer. *Chromosoma.* **114**: 377-388.