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Ultrastructural and autoradiographic studies of the role of nucleolar vacuoles in soybean root meristem

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Abstract: Ultrastructural and autoradiographic studies of nucleoli in soybean root meristematic cells in seedlings: (1) grown for 3 days at 25˚C (control), (2) grown for three days at 25˚C and for 4 days at 10˚C, and (3) grown as in (2) and recovered for 1 day at 25˚C were carried out. Control nucleoli had dense structure and a few small nucleolar vacuoles. Chilled plant nucleoli had less dense structure and no vacuoles. Nucleoli of plants recovered at 25˚C had big nucleolar vacuoles. In autoradiograms of squashed preparations, the labeling of nucleoli and cytoplasm after 20-min incubation in ^{3}H-uridine was 5- and 6-fold stronger, respectively, in control than in chilled roots. Following recovery, the labeling of nucleoli and cytoplasm was much stronger than after chilling or even than in control roots. After 80-min postincubation in non-radioactive medium, average labeling of particular areas of cells was the highest in recovered plants which indicated intensification of rRNA synthesis, maturation and transport into cytoplasm resulting from the resumption of optimal conditions which was correlated with the appearance of big nucleolar vacuoles. In autoradiograms of semi-thin sections from roots of seedlings chilled for 4 days then recovered and incubated for 20 min in ^{3}H-uridine, practically only extravacuolar parts of nucleoli were labeled. After 80-min postincubation, the labeling of nucleolar vacuoles was observed. Thus, during postincubation the labeled molecules were translocated from the nucleolar periphery into nucleolar vacuoles in cells where intensive transport of these molecules to the cytoplasm takes place. On the basis of these results, a hypothesis has been put forward that nucleolar vacuoles may be involved in the intensification of pre-ribosome transport outside nucleolus.

Key words: Nucleolus - Nucleolar vacuole - Soybean root meristem - Ultrastructure - Autoradiography

Introduction

Nucleoli are visible in nucleus of a living cell due to strong light refraction caused by their more densly packed structural components as compared to extranucleolar nucleoplasm. They are of very dynamic character - their structure is variable depending on the cell type and activity of production and maturation of ribosome subunits which is the major function of nucleoli [6].

The main elements of nucleoli visible in electron micrographs are fibrillar centers (FC), dense fibrillar component (DFC) and granular component (GC) [10]. Apart from those, in some types of plant and animal cells there are areas, which in the light microscope refract light to a lesser degree and in the electron microscope are electron transparent, containing a few granular and fibrillar structures. These areas are widely known as nucleolar vacuoles. Cytochemical studies revealed rRNA in them [6, 16].

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Observations of living cells revealed characteristic "pulsations" of nucleolar vacuoles. They grew gradually and then their content was transferred outside nucleolus into nucleoplasm [8, 13]. These studies suggest that nucleolar vacuoles are involved in the transport of nucleolar substance [13], most probably ribosomal subunits, to the cytoplasm.

Many authors believe that nucleolar vacuole formation results from ribosomal subunit migration from nucleolus to the cytoplasm [1, 3, 4, 11, 7]. However, Brown and Shaw [2] put forward a hypothesis that early processing of immature snoRNA could take place in nucleolar vacuoles, as they did not observe either rRNA or its precursors.

A lot of controversy has been also aroused by the fact that the appearance of nucleolar vacuoles is not strictly associated with any precise functional state of nucleoli and does not apply to all nucleoli in a given type of cells. Nucleolar vacuoles are present not only in nucleoli with high metabolic activity [1, 9, 3] but also in inactive ones, appearing at early germination stage of *Zea mays* seeds [3] and in primary root of *Sinapis alba* during initial six hours of germination [4, 5] as well as in cells inhibited

in G_{0-1} phase of *Pisum* cotyledon buds [7]. The appearance of nucleolar vacuoles can be also caused by some inhibitors of rRNA synthesis, such as cordicepin and FudR [9]. Thus it is hypothesized that nucleolar vacuoles appear as a result of the imbalance between rRNA synthesis and transport to the cytoplasm. Despite many experiments concerning nucleolar vacuole function, this problem is not unequivocally solved and needs further research.

Soybean, which is our material, originates from a warm climatic zone and is chill-sensitive. In chilled (10˚C) soybean seedlings, a 15-fold decrease in growth dynamics of meristematic root zone is observed. In soybean cell nucleus, only one nucleolus is present [15]. The aim of the present research was:

- to study ultrastructure of nucleoli and nucleolar vacuoles in seedlings grown in (1) control conditions (25˚C), (2) treated with 10˚C for 4 days and (3) recovered at optimal temperature for 1 day after 4-day chilling;
- to study the intensity of ^{3}H-uridine labeling of nucleoli and cytoplasm in autoradiograms of squashed preparations made from root meristems of seedlings grown as above, incubated in ^{3}H-uridine for 20 min and postincubated in the non-radioactive medium for 80 min;
- to study changes in labeling of nucleoli and nucleolar vacuoles after incubation in ^{3}H-uridine for 20 min and postincubation in the non-radioactive medium for 80 min in autoradiograms of semi-thin sections from roots of seedlings recovered at 25˚C.

Materials and methods

Material. Soybean seeds (*Glycine max* (L.) Merr.) cv. Aldana (from IHAR in Radzików) were germinated in darkness for 3 days at 25˚C (control) on distilled water moisted filter paper. Then some seedlings went on growing in the same conditions and some were transferred to 10˚C for 4 days. Some chilled seedlings were then recovered for 1 day at optimal temperature (25˚C).

^{3}H-uridine incubation. Seedling roots from control and recovered plants, and from chilled ones were incubated in ^{3}H-uridine solution (80 μCi/ml; 24.0 Ci/mM) for 20 min at 25˚C and 10˚C, respectively, then they all were postincubated in a non-radioactive medium for 80 min at the respective temperatures. Roots were fixed in absolute ethanol-glacial acetic acid mixture (3:1) for 1 h at room temperature and rinsed in ethanol. After hydration, the material was treated with pectinase solution (2.5 units mg^{-1} prot. ml^{-1}; Sigma) in Mc Illvain buffer (pH 5.0) for 30 min at 45˚C. Squashed preparations from apical parts of roots were made on dry ice. Dry preparations were covered with light-sensitive emulsion (EM 1; Amersham) and exposed at 8˚C. After 14 days autoradiograms were developed and stained with toluidine blue according to Smetana *et al.* [14]. Dry preparations were embedded in Canada balsam. Silver grains were counted over nucleoli, extra-nucleolar nucleoplasm and cytoplasm in 30 cells in preparations from 3 meristems from each variant.

Electron microscopy. Selected root tips were fixed in 2% glutaraldehyde in 1% cacodylate buffer (pH 7.2-7.4) for 3 h at 4˚C. Roots were postfixed in 1% OsO_4 in the same buffer. After dehydration in ethanol series, the material was embedded in a medium containing mixture of Epon 812 and Spurr's resin. Ultrathin sections were double stained with uranyl acetate and lead citrate according to Reynolds [12]. The sections were examined and photographed in a JEOL JEM 1010 transmission electron microscope.

Semi-thin section autoradiography. Roots of recovered seedlings were incubated in ^{3}H-uridine solution (100 μCi/ml; 28.0 Ci/mM) for 20 min at 25˚C and then postincubated in non-radioactive medium for 80 min. Fixation of the material and further procedure was as for EM except uranyl acetate/lead citrate treatment. Semi-thin sections were placed on microscopic slides, covered with light-sensitive emulsion and exposed for 35 days at 8˚C. After development and drying, the autoradiograms were stained with toluidine blue. Silver grains were counted over peripheral parts of nucleoli and nucleolar vacuoles of 60 cells in preparations from 3 meristems from each variant.

Results

Ultrastructure of nucleoli and nucleolar vacuoles in soybean root meristem

In meristematic cells of soybean roots growing in optimal conditions (25˚C), nucleoli were dense and contained FC and GC (Fig. 1) as well as a few small nucleolar vacuoles in some cells (not demonstrated).

Nucleoli in root cells treated with 4-day chilling (10˚C), contained FC surrounded with DFC but no nucleolar vacuoles. Their structure was less dense (Fig. 2). At their periphery, characteristic clusters of grains resembling shortened and condensed rRNA transcription complexes (Christmas trees) were abundant [15]. In roots of plants recovered for 24 h after 4-day chilling, huge nucleolar vacuoles were observed in big, dense nucleoli (Fig. 3). At their periphery composed of tightly packed fibrillar and granular components, small nucleolar vacuoles and FC were present. However, the big nucleolar vacuoles were not present in all nucleoli. Semi-thin section analysis revealed big nucleolar vacuoles in ca. 50% of soybean meristematic root cell nucleoli.

Labelling of nucleoli and cytoplasm in squashed preparations after 20-min ^{3}H-uridine incubation followed by postincubation in non-radioactive medium

Directly after 20-min ^{3}H-uridine incubation (postincubation 0 min), the labeling of nucleoli in control material was 4.7-fold higher than in cells chilled for 4 days (Tab. 1). The difference in cytoplasm labeling was even greater (5.8-fold weaker than in the control). In 24-h recovered seedlings the labeling directly after 20-min incubation was 8-fold higher than after 4-day chilling and higher by 44% than in the control. This was especially observed in the cytoplasm, where radioactivity after recovery was 2-fold higher than in the control, while in nucleoli a 60% increase in the number of silver grains in autoradiograms was observed. These data in-

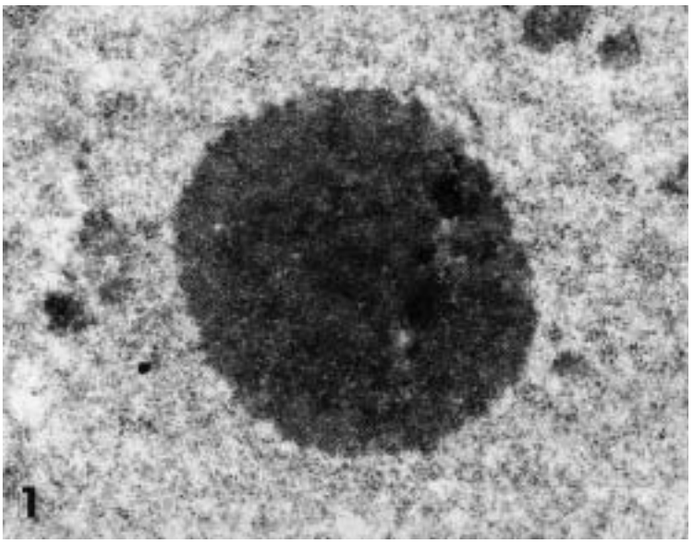

Fig. 1. Nucleolus of root tip meristematic cell of soybean seedling grown at 25˚C for 3 days (control). × 35 000.

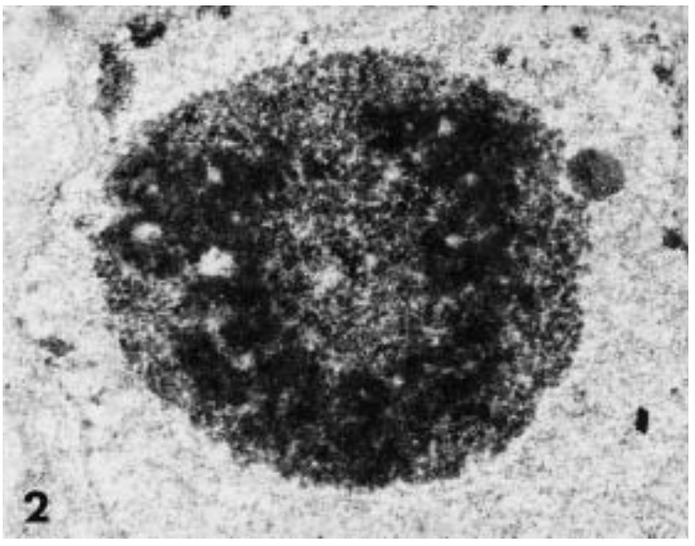

Fig. 2. Nucleolus of root tip meristematic cell of soybean seedling grown at 25˚C for 3 days then subjected to 4-day chilling (10˚C). × 35 000.

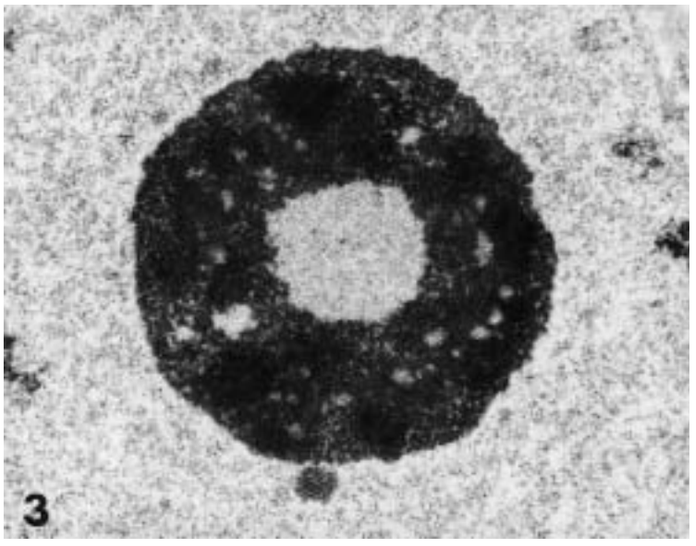

Fig. 3. Nucleolus of root tip meristematic cell of soybean seedling grown at 25˚C for 3 days, subjected to 4-day chilling (10˚C) and recovered at 25˚C for 1 day. × 35 000.

Table 1. ^{3}H-uridine labeling (number of silver grains) of nucleoli, extranucleolar nucleoplasm and cytoplasm in meristematic cells of soybean roots

Experimental variant	Labeling (number of silver grains)				
	Post-incubation	Nucleolus	Extranucleolar nucleoplasm	Cytoplasm	Whole cell
Control 3 d/25˚C	0	18.9 ± 4.7	10 ± 1.9	10.7 ± 1.7	40 ± 5.1
	80 min	13.4 ± 3.7	16 ± 2.7	43.6 ± 6.8	73 ± 8.8
Chilling 3d/25˚C + 4d/10˚C	0	4 ± 0.8	1.7 ± 0.3	1.8 ± 0.4	7.4 ± 0.8
	80 min	11.2 ± 2.3	2.9 ± 0.5	3.9 ± 0.8	18 ± 3.1
Recovery 3d/25˚C + 4d/10˚C + 1d/25˚C	0	31 ± 5.9	56 ± 7.9	21 ± 3.9	57.6 ± 8.7
	80 min	43.6 ± 7.2	36.7 ± 5.1	89.8 ± 11.4	170 ± 20.9

Squashed preparations. Means ± SD. Labeling of compared cellular areas is statistically significant, p=0.05, t_t=2.04.

dicate that the transport of ribosomal subunits from nucleolus to the cytoplasm was more dynamic during 20-min incubation in recovered seedlings than in the control.

Following 80-min postincubation in non-radioactive medium, the total labeling of cells from all experimental variants was stronger than after 0-min postincubation, which means that during postincubation the incorporation of ^{3}H-uridine taken up by roots during incubation into macromolecules continued. Radioactivity increase was more dynamic (3-fold) in recovered cells while in control and not-recovered ones it was ca. 2-fold higher than immediately after 20-min incubation. This indicates very dynamic synthesis, maturation and transport of rRNA to cytoplasm during 80-min postincubation following chilling and recovery.

Labeling of nucleoli and nucleolar vacuoles in autoradiograms from semi-thin sections

In semi-thin section autoradiograms from recovered seedling roots incubated in ^{3}H-uridine (postincubation 0 min), the number of grains over the peripheral part of nucleoli was much higher (average 5.4 grains) than over nucleolar vacuoles (0.33 grain). After 80-min postincubation, the overall radioactivity of the whole nucleolus increased, however, labeling of nucleolar vacuoles was stronger than that of the peripheral part (11-fold and 4.5-fold increase, respectively). These data seem to indicate that during postincubation a large number of labeled macromolecules was translocated from the peripheral part to the nucleolar vacuole (Tab. 2, Fig. 4).

Discussion

Many authors support the belief that the appearance of nucleolar vacuoles is connected with rRNA transport from nucleolus to cytoplasm [1, 3, 4, 11, 7]. Results of our autoradiographic experiments were consistent with this view. The data obtained from soybean root meristems of seedlings (1) grown in optimal conditions, (2) chilled (10˚C) as well as (3) chilled and recovered at 25˚C for 24 h indicated that big nucleolar vacuoles are accompanied by dynamic rRNA synthesis and its transport to the cytoplasm. It was more intensive in the roots of recovered seedlings in which the biggest vacuoles were observed. In roots grown in optimal conditions (25˚C) in which rRNA synthesis was 60% lower and ribosome transport dynamics was only half of that after recovery, the nucleolar vacuoles were scarce and small. In roots chilled (10˚C) for 4 days where transcriptional activity was very low while ribosome transport dynamics even lower, no nucleolar vacuoles were observed. The inhibition of nucleolar vacuole formation caused by chilling (6˚C) suppressing rRNA synthesis was also observed by de Barsy *et al.* [3] in *Zea mays* shoots and by Olszewska *et al.* [11] in *Helianthus annuus* roots chilled (10˚C) for 12 hours.

Our experiments on semi-thin section autoradiograms from plants after 20-min incubation in ^{3}H-uridine and 80-min postincubation in non-radioactive medium fully confirm the suggestion that nucleolar vacuoles are involved in rRNA transport from nucleolus to nucleoplasm and then to the cytoplasm. We observed that in recovered soybean seedlings after 20-min incubation in ^{3}H-uridine, only extra-vacuolar areas of nucleoli were labeled, while nucleolar vacuoles practically were not (Tab. 2). However, after 80-min postincubation, the nucleolar vacuoles were labeled 11 times stronger than just after incubation. It indicates that during that period radioactive macromolecules moved from the site of their synthesis into nucleolar vacuoles. It must be stressed, however, that nucleolar vacuoles are present only in 50% of nucleoli. One of the reasons could be the fact that nucleolar vacuoles appear only at the particular stage of a cell cycle, while soybean root meristematic cells were in different phases of the cycle. Such a hypothesis, however, is not supported by the experiments on *Helliantus annuus* roots showing that at G_1, S and G_2 phases there were similar numbers of ring nucleoli with

Table 2. ^{3}H-uridine labeling (number of silver grains) of the peripheral parts of nucleoli and nucleolar vacuoles in meristematic cells of soybean roots of recovered seedlings

Experimental variant	Postincubation	Labeling (number of silver grains)	
		Peripheral part of nucleolus	Nucleolar vacuole
Recovery 3d/25˚C + 4d/10˚C + 1d/25˚C	0	5.37 ± 0.9	0.33 ± 0.04
	80 min	23.9 ± 4.8	3.6 ± 0.7

Semi-thin preparations. Means ± SD.

big vacuoles [11]. On the basis of previous experiments of other authors performed on living cells [8, 13] and the present results we put forward a hypothesis that translocation of ribosomal subunits from nucleolar vacuoles to the cytoplasm may be involved in pulsation of nucleolar vacuoles. After accumulation of ribosomal subunits, the vacuoles may transiently disappear as a result of an extrusion of their content outside nucleolus as suggested by observations of living cells in tobacco [8] and potato tubers [13].

The fact that big nucleolar vacuoles coincide with an increase in the dynamics of labeling translocation from nucleolus to extranucleolar area may suggest that vacuoles may be involved in the mechanism which facilitates the movement of ribosomal subunits into the cytoplasm.

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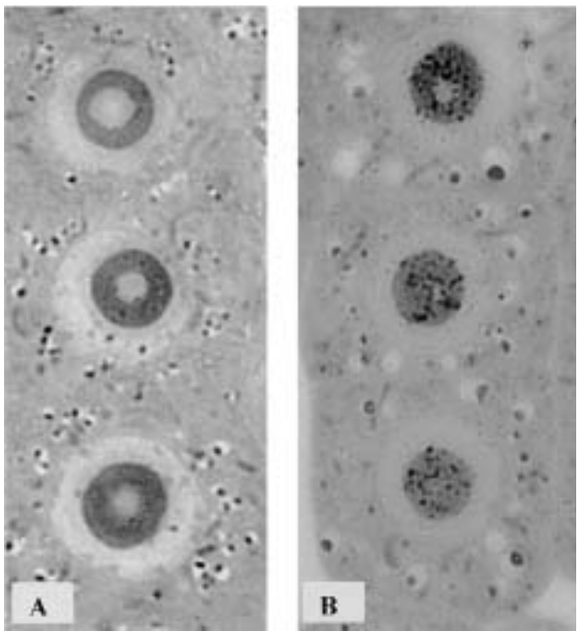

Fig. 4. ^{3}H-uridine labeling of nucleoli and nucleolar vacuoles in root tip meristematic cells of recovered soybean seedling after 20-min incubation (A) and 80-min postincubation (B). Semi-thin sections. × 3 000.

References

[1] Barlow P (1970) Vacuoles in the nucleoli of *Zea mays* root apices and their possible significance in nucleolar physiology. Caryologia 23: 61-70

[2] Brown JWS, Shaw PJ (1998) Small nucleolar RNAs and pre-rRNA processing in plants. Plant Cell 10: 649-657

[3] de Barsy TH, Deltour R, Bronchart R (1974) Study of nucleolar vacuolation and RNA synthesis in embryonic root cells of *Zea mays.* J Cell Sci 16: 95-112

[4] Deltour R, de Barsy T (1985) Nucleolar activation and vacuolation in embryo radicle cells during early germination. J Cell Sci 76: 67-83

[5] Deltour R, Mosen H, Bronchart R (1986) Three-dimensional electron microscopy of the internal nucleolus-associated chromatin and of the nucleolar vacuoles during early germination of *Sinapsis alba.* J Cell Sci 82: 53-71

[6] Goessens G (1984) Nucleolar structure. Int Rev Cytol 87: 107-158

[7] Jennane A, Thiry M, Diouri M, Goessens G (2000) Fate of the nucleolar vacuole during resumption of cell cycle in pea cotyledonary buds. Protoplasma 210: 172-178

[8] Johnson JM, Jones LE (1967) Behavior of nucleoli and contracting nucleolar vacuoles in tobacco cells growing in microculture. Annu J Bot 54: 189-198

[9] Moreno-Diaz de la Espina S, Medina FJ, Risueno MC (1980) Correlation of nucleolar activity and nucleolar vacuolation in plant cells. Eur J Cell Biol 22: 724-729

[10] Olson MOJ, Dundr M, Szebeni A (2000) The nucleolus: an old factory with unexpected capabilities. Trends Cell Biol 10: 189-196

[11] Olszewska MJ, Kuran H, Damsz B, Marciniak K (1985) Transport of rRNA into cytoplasm required for initiation of S phase in root meristem cells in *Helianthus annuus* L. Protoplasma 127: 110-118

[12] Reynolds SS (1963) The use of lead citrate of high pH as an electron-opaque stain in electron microscopy. J Cell Biol 17: 208-212

[13] Rose RJ, Setterfield G, Fowke LC (1972) Activation of nucleoli in tuber slices and the function of nucleolar vacuoles. Exp Cell Res 71: 1-16

[14] Smetana K, Lejner J, Potmesil M (1968) A further contribution to the demonstration of RNA and nucleoli of blood cells in smear preparations. Folia Hematol 91: 381- 393

[15] Stępiński D, Kwiatkowska M (2003) Autoradiographic and ultrastructural studies of the influence of chilling on soybean root meristem nucleoli. Acta Biol Cracov Ser Bot (in press)

[16] Williams LM, Charest PM, Lafonteine CF (1985) Nuclease-gold and protease-gold labeling over the nucleolar vacuoles of pea root tip cells. Biol Cell 55: 21-26

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