AUTORADIOGRAPHIC AND ULTRASTRUCTURAL STUDIES OF THE EFFECT OF CHILLING ON SOYBEAN ROOT MERISTEM NUCLEOLI

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Root meristem nucleoli from soybean (Glycine max, cv. Aldana) seedlings germinated for 3 days at 25˚C and then for 4 days at 10˚C or still at 25˚C (control) were examined. Chill was observed to reduce root meristematic zone growth 15-fold. Nucleoli doubled their volume at 10˚C. Autoradiographic studies showed that after 20 min 3H-uridine incubation at 10˚C, incorporation of this precursor (postincubation time 0) into nucleoli of chilled seedlings was 4.7 times weaker than in the control. After 80 min postincubation in nonradioactive medium, the cytoplasm became the most intensely labelled cell area in the control material, while in chilled roots the nucleoli were still most intensely labelled and the cytoplasm was 11 times less labelled than in the control. The increase in nucleoli volume at 10˚C is suggested to result from greater cold-induced inhibition of the dynamics of maturation and transport of ribosome subunits than of rRNA synthesis dynamics. Ultrastructural studies of chilled seedling nucleoli showed a significant decrease in the fibrillar component and an increase in the granular component, forming characteristic clusters. They are supposed to correspond to shortened and condensed prerRNA transcription complexes (compacted “Christmas trees”).

Key words: 3H-uridine incorporation, ultrastructure, nucleolus, meristematic soybean root cells.

INTRODUCTION

Although there is much recent data to show the plurifunctionality of nucleoli, their best-confirmed function is as the site of synthesis and maturation of ribosome subunits (Pederson, 1998 a,b; Olson et al., 2000; Visintin and Amon, 2000; Ospina and Matera, 2002). Nucleoli are labile structures; their architecture depends on the functional status and developmental stage of a cell (Scheer and Hock, 1999; Olson et al., 2000). Generally their sizes correlate with cell metabolic activity (Flavell and O’Dell, 1979; Martini et al., 1982; Guerrero et al., 1989; Fischer et al., 1991). However, even if typical nucleoli are lacking, ribosome synthesis may take place in cells equipped with rDNA, suitable enzymes and regulatory factors (Nierras et al., 1997; Oakes et al., 1998; Scheer and Hock, 1999; Olson et al., 2000). Moreover, big nucleoli do not unequivocally mean dynamic ribosome synthesis. For example, in Chara vulgaris, small nucleoli poor in the granular component incorporate 3H-uridine as intensively as bigger ones abundant in the granular component present in the same nucleus. The former are more active in rRNA transport to the cytoplasm than the latter (Kwiatkowska and Maszewski, 1985). The size and structure of nucleoli result from the dynamics of two processes: (1) prerRNA synthesis and (2) its maturation and transport to the cytoplasm. These two systems can be modified independently in the cell (Olson et al., 2000; Verheggen et al., 2000; Yamamoto et al., 2000; Brown, 2001; Milkeriet al., 2001).

The present research uses 3H-uridine autoradiography to assess changes in soybean root meristem nucleoli functioning in chilling conditions, and examines changes in nucleoli ultrastructure caused...
by chilling of seedlings. Chilling at 10°C was employed, as this subtropical plant begins to degenerate at lower temperature. After 4–7 days of chilling, soybean seedlings are capable of fully regenerating metabolic activity and continuing growth (Stephiński, unpubl. data). There is a single nucleolus in a soybean meristematic interphase cell. NOR is localized only on chromosome 13 (Griffor et al., 1991).

MATERIALS AND METHODS

Seeds of soybean Glycine max (L.) Merr. cv. Aldana obtained from IHAR in Radzików were germinated for 3 days at 25°C in darkness in Petri dishes on filter paper wetted with distilled water. Some of these seedlings continued growth at 25°C (control) and some seedlings were transferred to cold (10°C) for 4 days. Meristematic root tip cells were examined.

MORPHOMETRIC MEASUREMENTS

Nucleolar morphometric measurements were made on squash slides stained by two independent methods: (1) apical parts of roots were fixed in 0.0125 M Sörensen's phosphate-buffered (pH 7.2) 2% glutaraldehyde and stained using the Ag-NOR impregnation method described by Howell and Black (1980); (2) root tips were fixed in absolute ethanol:glacial acetic acid (3:1), rinsed with ethanol and then water, the material was treated with pectinase at 45°C for 30 min and rinsed in McIlvain buffer (pH 5.0), squashed preparations were made and then stained with toluidine blue according to Smetana's method (Smetana et al., 1968).

It was assumed that the nucleoli were not flattened during squash slide preparation and that the nucleolar profiles are circular. Their areas were measured with the computer-aided Imal-512 system. After the radiuses of the nucleoli were established the nucleolar volumes were calculated mathematically.

INCUBATION IN ³H-URIDINE

Roots of seedlings growing 3 days at 25°C were incubated in water containing ³H-uridine (80 µCi/ml; 24.0 Ci/mM) for 20 min at 25°C (control) or at 10°C (3-day-old seedlings subjected to chill for 4 days). Half of the seedlings from each group were postincubated for 80 min in nonradioactive medium at 25°C or 10°C, respectively. Roots were fixed in absolute ethanol/glacial acetic acid (3:1) for 1 h at room temperature, rinsed with ethanol and kept in 70% ethanol for further procedures. The material was then treated with pectinase at 45°C for 30 min and rinsed in McIlvain buffer (pH 5.0). Meristematic parts of roots were placed on microscope slides in a water drop and squashed. After freezing and removal of coverslips the slides were dried, covered with emulsion for high-resolution microautoradiography (Amersham-Pharmacia-Biotech) and exposed in darkness at 8°C. After 14 days the autoradiograms were developed and stained with toluidine blue according to Smetana’s method (Smetana et al., 1968) and embedded in Canada balsam. Silver grains were counted over the nucleoli, extranucleolar 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% OsO4 in the same buffer. After dehydration in an ethanol series, the material was embedded in medium consisting of Epon 812 and Spurr’s resin. Ultrathin sections were double-stained with uranyl acetate and lead citrate according to Reynold (1963). The sections were examined and photographed in a JEOL JEM 1010 transmission electron microscope.

RESULTS

EFFECT OF CHILLING ON GROWTH DYNAMICS OF ROOT MERISTEMATIC ZONE

Examination of a 2 mm root meristematic zone (marks on root surface labelled with activated charcoal) showed that during 4-day development at 10°C the meristematic zone grew about 15 times slower than the control (Fig. 1). This means a decrease in cell elongation and/or a drastic decrease in cell division under chilling conditions but also that growth, though slowed, was not completely stopped.

EFFECT OF CHILLING ON SIZE OF NUCLEOLI IN ROOT MERISTEMATIC ZONE

Morphometric measurements of nucleoli from chilled seedlings showed that despite the significant decrease in growth activity in the root meristematic zone their volume was about twice that in the control (Fig. 2). However, the size of the nuclei in which the nucleoli were measured remained unchanged. This
regularity was observed in preparations after Howell and Black’s method (1980) as well as in those after Smetana’s method (1968) with the use of toluidine blue staining RNA.

3H-URIDINE INCORPORATION INTO MERISTEMATIC CELLS AFTER CHILLING AND IN CONTROL

In the control after 20 min 3H-uridine incorporation, the nucleolus was the most intensely labelled part of the cell (Fig. 3). After 80 min postincubation the cytoplasm was most intensely labelled (labelling was 4 times higher than at postincubation time 0), while the radioactivity of the nucleoli diminished.

In chill-treated plants after 20 min 3H-uridine incubation, labelling of meristematic zone nucleoli was about 4.7 times weaker than in the control (Fig. 3). Extranucleolar nucleoplasm and cytoplasm labelling was proportionally lower. After 80 min postincubation, unlike in the control, nucleoli remained the most intensely labelled area, while labelling of cytoplasm was still low. Comparing the dynamics of the labelling increase in chilled and control material during postincubation, it can be seen that chilling decreased the rate of maturation and transport of ribosome subunits into the cytoplasm about 11-fold. This effect is more than twice stronger than the effect of chilling on 3H-uridine incorporation into nucleoli.

ULTRASTRUCTURE OF NUCLEOLI IN CHILLED AND CONTROL MATERIAL

Central cross sections were analyzed, that is, the ones that were largest in serial sections of nucleoli of chilled and control seedlings.

In the control the structure of the nucleoli was dense. In the central part there was a distinct, abundant fibrillar component in which fibrillar centers (FC) could be seen, surrounded by a dense fibrillar component (DFC). The granular component (GC) was mainly peripherally situated (Figs. 4a, 5a).

After 4-day chilling, in the nucleoli, which were bigger than in the control, the fibrillar component significantly decreased while the granular component increased. Unlike in the control, the granules were not evenly spaced at the nucleolar periphery but formed characteristic clusters (Figs. 4b, 5b).

DISCUSSION

Autoradiographic studies showed that in the control the labelling of the nucleoli diminished while labelling of cytoplasm increased during postincubation. The increased radioactivity of the cytoplasm reflects mainly the activity of maturation and transport of ribosome subunits, since during mRNA splicing a significant part of the hnRNA becomes degraded in the nucleus (Moore, 2002).

In soybean plants, as in other species (Olszewska et al., 1985, 1987, 1988), chilling at 10°C causes a drastic decrease in the activity of 3H-uridine incorporation and transport of ribosome subunits to the cytoplasm. In chill-treated soybean seedlings the nucleoli enlarge because low temperature inhibits rRNA synthesis less than it inhibits ribosome subunit maturation and transport from the nucleolus to the cytoplasm. Uncoupling of these two processes may be part of soybean’s acclimation to chilling. The arrest of pre-ribosomes in the nucleolus may protect them against the destructive effect of ribonuclease,
as its activity increases in chill-treated seedlings (Stępiński, 2002). During regeneration at optimal temperature (25˚C), high activity of synthesis and transport of ribosome subunits into the cytoplasm is restored (Stępiński, unpubl. data), while ribonuclease activity drastically decreases (Stępiński, 2002).

Ultrastructural studies show that chilling distinctly changes the organization of the nucleolus. In chilled seedlings the nucleoli become loose. The amount of the fibrillar component decreases while that of the granular component increases. Possibly the granular component represents ribosome subunits “frozen” at different developmental stages and those ready to leave the nucleolus (Risueno et al., 1982; Brown and Shaw, 1998; Bassy et al., 2000). It is worth noting that these granules form specific structures resembling the condensed and shortened pre-rRNA transcription complexes that Scheer et al. (1997) called “Christmas trees” in Locusta migratoria oocytes in situ. These structures have axes 6 times shorter (with a positive terminal deoxynucleotidyl transferase reaction to DNA), with a radial arrangement of tightly packed lateral fibrils (con-

![Fig. 3. Mean number of silver grains above nucleolus (No), extranucleolar nucleoplasm (Nu) and cytoplasm (Cyt) of root meristematic cells of control and chilled seedlings after 20 min incubation in 3H–uridine and 80 min postincubation in nonradioactive medium. Bars represent standard errors.](image)

![Fig. 4. Nucleolus of seedling growing 3 days at 25˚C – control (a) and 3-day-old seedling subjected to chill for 4 days (b). FC – fibrillar centers; DFC – dense fibrillar component; GC – granular component. × 25 000.](image)
taining 28S RNA sequences observed in situ) terminated with granules (Scheer et al., 1997). Structures resembling "Christmas trees," thought to be transcription units, are also observed in nucleoli of onion root tip cells treated with cycloheximide. These structures are peripherally located on DFC and penetrate GC in nucleoli with a characteristic loose structure (Ghosh et al., 1993). Compacted "Christmas trees" in Pisum sativum plant nucleoli have been detected recently using a novel labelling technique followed by 3D electron microscopy with limited extraction of RNA and proteins (González-Melendi et al., 2001).

Localization of in situ ribosome synthesis on ultrathin sections is a very difficult, not entirely solved problem. Most scientists using precise molecular methods at ultrastructural level maintain that it takes place in the dense fibrillar component or between the fibrillar center and dense fibrillar component (Risueno et al., 1982; Raška et al., 1995; Brown and Shaw, 1998; Scheer and Hock, 1999; Bassy et al., 2000; Yano and Sato, 2000; Staněk et al., 2001; Koberna et al., 2002). However, Mais and Scheer (2001) and Cheutin et al. (2002) demonstrated recently that transcriptionally active rRNA genes are confined to the fibrillar centers of nucleoli. Morphological criteria alone are not sufficient to unequivocally determine whether the clusters of nucleolar granules we observed in soybean are really condensed transcription complexes. Moreover, the physiological status of chilled soybean roots significantly differs from that of Locusta migratoria: the former exhibit low and the latter very high dynamics of ribosome synthesis. However, the observed further slowing down of ribosome subunit maturation and transport in soybean makes it highly probable that these are indeed transcription complexes, forming a very slow-moving "ribosome production line" which in a fixed preparation may look like one actively producing ribosomes. These structures in the control probably are masked in an abundant and densely packed fibrillar component containing non-active rRNA genes as well. In plants under physiological conditions, 30–70% of rRNA genes are inhibited due to, among other things, rDNA cytosine methylation (Jacob and Ghosh, 1999). Stress, how-

**Fig. 5.** Fragment of nucleolus of a plant growing 3 days at 25°C (a) and 3-day-old plant subjected to chill for 4 days (b). GC – granular component; FbC – fibrillar component. Arrows show putative shortened and condensed transcription complexes (Christmas trees). × 75 000.
ever, including low temperature, may cause cytosine demethylation and activation of these genes (Demellemeyer et al., 1999). Thus it seems probable that in soybean an increase in the granular component (presumably shortened and condensed transcription complexes) accompanied by a decrease in the fibrillar component may result from activation of additional rRNA genes, which makes the nucleolar structure looser. Activation of new genes when ribosome production is too slow seems necessary for synthesis of proteins produced at low temperature, enabling cells to adjust to unfavorable conditions (Hughes and Dunn, 1996). This interpretation seems supported by the results of these experiments with soybean roots transferred after 4 days of chilling to a favorable temperature. They displayed enhanced activity of synthesis and maturation of pre-rRNA, much higher than in the control (Stepiński, unpubl. data). This may be the result of the functioning of additional rRNA genes activated during chilling, promoting the formation of transcription complexes resembling shortened, condensed “Christmas trees.”

REFERENCES


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