Lacandonia granules are present in the cell nucleus of Welwitschia mirabilis

Lourdes Teresa Agredano-Moreno1,2, María de Lourdes Segura-Valdez1,2, Jaime Jiménez-Ramírez3, and Luis Felipe Jiménez-García1,2*

1Electron Microscopy Laboratory-Tlahuizcalpan, Faculty of Sciences. National Autonomous University of Mexico, Mexico.
2Cell Nanobiology Laboratory, Department of Cell Biology. Faculty of Sciences. National Autonomous University of Mexico, Mexico.
3Department of Comparative Biology. Faculty of Sciences. National Autonomous University of Mexico, Mexico.
*Corresponding author: luisfelipe_jimenez@ciencias.unam.mx

Abstract

Background: Lacandonia granules are extranucleolar ribonucleoprotein (RNPs) particles, 32 nanometers in diameter that were first described in the nucleus of Lacandonia schismatica. Cytochemical and immunocytochemical studies suggest that these particles are equivalent to perichromatin and Balbiani ring granules described in mammals and salivary glands cells of the insect Chironomus tentans, respectively. Lacandonia granules are also present in the related Triuris brevystilis, and they were later described in the gymnosperm Ginkgo biloba. These findings suggest that Lacandonia granules have a wider distribution in the plant kingdom.

Species study: Welwitschia mirabilis, a gymnosperm of the order Gnetales.

Hypothesis: Lacandonia granules are present in the cell nucleus of W. mirabilis.

Methods: Plants were cultivated in a germination chamber and samples of leaves were processed for transmission electron microscope. Thin sections were stained with the EDTA technique preferential for ribonucleoproteins and osmium amine specific for DNA and observed with an electron microscope.

Results: Light, electronic and atomic force microscopy revealed that cell nuclei of W. mirabilis display a reticulated arrangement of chromatin. Moreover, granules of 32.17 ± 1.7 nm in diameter were observed among strands of reticulated chromatin.

Conclusions: Our results indicate that Lacandonia granules are present in the nuclei of the gnetal W. mirabilis.

Keywords: Atomic force microscopy, gymnosperms, Lacandonia granules, plant cell ultrastructure, Welwitschia.

Resumen

Antecedentes: los gránulos de Lacandonia son partículas ribonucleoproteicas extranucleolares (RNPs), de 32 nanómetros de diámetro, que se describieron por primera vez en el núcleo de Lacandonia schismatica. Los estudios citoquímicos e inmunocitoquímicos sugieren que estas partículas son equivalentes a los gránulos pericromatinianos y de los anillos de Balbiani descritos en mamíferos y células de glándulas salivales del insecto Chironomus tentans, respectivamente. Los gránulos de Lacandonia también están presentes en Triuris brevystilis, y posteriormente se describieron en la gimnosperma Ginkgo biloba. Estos hallazgos sugieren que los gránulos de Lacandonia tienen una distribución más amplia en el reino vegetal.

Especie de estudio: Welwitschia mirabilis, una gimnosperma del orden Gnetales.

Hipothesis: Los gránulos de Lacandonia están presentes en el núcleo celular de W. mirabilis.

Métodos: Las plantas se cultivaron en una cámara de germinación y se procesaron muestras de hojas para microscopía electrónica de transmisión. Los cortes delgados se tían con la técnica de EDTA preferencial para ribonucleoproteínas y amina de osmio específicas para ADN y se observaron al microscopio electrónico.

Resultados: La microscopía de luz, electrónica y de fuerza atómica revelaron que los núcleos celulares de W. mirabilis muestran una disposición reticulada de la cromatina. Además, se observaron gránulos de 32.17 ± 1.7 nm de diámetro entre las hebras de cromatina reticulada.

Conclusiones: Nuestros resultados indican que los gránulos de Lacandonia están presentes en los núcleos del gnetal W. mirabilis.

Palabras clave: gimnospermas, gránulos de Lacandonia, microscopía de fuerza atómica, ultraestructura de plantas, Welwitschia.
Lacandonia granules in *Welwitschia mirabilis*

*Lacandonia schismatica* E. Martínez & C. H. Ramos (Triuridaceae) is a plant from the Lacandona forest in Chiapas, Mexico. It is a plant displaying distribution of gynoecium surrounding the androecium (Márquez-Guzmán et al. 1989, Martínez & Ramos 1989). The cell nucleus of this species is characterized by the presence of granules of 32 nm in diameter called *Lacandonia* granules (Jiménez-García et al. 1992). These particles are ribonucleoproteins (RNPs), intermediate in size and distribution between interchromatin and perichromatin granules present in mammalian cell nucleus. *Lacandonia* granules contain SR proteins and poly-A RNA tail as perichromatin granules (Agredano-Moreno & Jiménez-García 2000). In addition, the morphology of these particles was previously analyzed using atomic force microscopy (Fragoso-Soriano et al. 2009).

*Lacandonia* granules are also present in the related *Triuris brevistylis* Donn.Sm., also a member of the order Pandanales as *L. schismatica* (Stevens 2017). The observation of *Lacandonia* granules in the nuclei of the gymnosperm tree *Ginkgo biloba* L., (Jiménez-Ramírez et al. 2002), suggests that granules are present in other groups and may display a general function. In the present work we report the presence of *Lacandonia* granules in the nuclei of *Welwitschia mirabilis* Hook.f., a member of Welwitschiaceae (order Gnetales; Stevens 2017).

**Materials and methods**

**Plants.** *W. mirabilis* seeds were disinfected with 1 % captain for 1 hour and washed with deionized water. Seeds were cultivated in tepojal (small volcanic grain covered with clay), substrate in a germination chamber Lab-Line at 26 °C, 60 % relative humidity and photoperiod (light/dark): 16/8.

**Transmission Electron Microscopy.** 1 mm³ fragments of young leaves were processed following the standard protocol for electron microscopy (Jiménez-García & Segura-Valdez 2004). Briefly, fragments were fixed overnight at room temperature in a mixture of 6 % glutaraldehyde and 4 % paraformaldehyde, in PBS buffer (pH 7.2). Post-fixation was performed with 2 % osmic acid overnight. Samples were subsequently dehydrated in a graded series of ethanol and embedded in an epoxy resin at 60 °C for 48 h following the standard protocol for electron microscopy. Thin sections were placed on copper grids covered with formvar. Contrast was conducted with 5 % uranyl acetate and 0.5 % lead citrate. Grids were observed with a transmission electron microscope (JEOL, JEM 1010, Peabody, MA) working at 80 kV. Images were obtained with a charge-coupled device camera coupled to the microscope. The diameter of the nuclear granules was determined on 100,000x electron micrographs of thin sections stained with Bernhard’s EDTA technique (Bernhard 1969).

**Light microscopy.** Thin sections were stained with toluidine blue and 100x pictures were taken in brightfield illumination with an optical microscope (Nikon, Eclipse E800).

**Atomic force microscopy.** Atomic force microscopy was conducted as previously described (Jiménez-García & Segura-Valdez 2004, Segura-Valdez et al. 2010). Briefly, semithin sections (about 250 nm thickness) were mounted on glass slides and observed with an atomic force microscope (model BioScope, Digital Instruments, Santa Barbara CA, USA) working in contact mode. The scan size was from 30 to 100 µm at a scan rate of 2.1 Hz. Images were produced with the NanoScope IIIa control system. The AFM tips were silicon nitride tips with a curvature radius of 20-60 nm (model NP).

**EDTA staining for RNPs.** Thin sections of samples fixed with 6 % glutaraldehyde and 4 % paraformaldehyde without osmium tetroxide were used for Bernhard’s EDTA technique, for preferential staining of ribonucleoproteins (RNPs) (Bernhard 1969). Basically, 5 % uranyl acetate was used for 3 min, followed by treatment with EDTA for 13 min and 0.5 % lead citrate for 3 min.

**Osmium amine.** Specific staining of DNA in the cell nucleus of *W. mirabilis* was performed according to Vázquez-Nin et al. (1995) with modifications. Briefly, 60-90 nm sections mounted in gold grids without formvar were floated on a drop of 5N HCl for 1 hour at room tempera-
ture (acid hydrolysis). Grids were rinsed with deionized water and incubated in a wet chamber containing a drop of osmium amine solution. Finally, grids were washed and observed with an electron microscope without additional staining.

Results

The cell nuclei of *Welwitschia mirabilis* were observed with light, atomic force, and transmission electron microscopy.

*Compact chromatin.* In the cell nuclei of *Welwitschia mirabilis* dense strands are observed between areas of low density (Figure 1A, B and C). The three dimensional arrangement of the strands is evident when they are observed with light (Figure 1A) and atomic force microscopy (Figure 1B). Conventional staining for transmission electron microscopy shows the strands heavily stained with uranyl acetate and lead citrate (Figure 1C). To determine whether the dense strands observed in the nuclei of *W. mirabilis* correspond to DNA, we used the osmium ammine technique specific for DNA. The strands were densely stained indicating that they correspond to DNA. Little or no DNA is present in the nucleoplasm and nucleolus and no staining is observed in the cytoplasm (Figure 2).

*Nuclear particles.* The nucleoplasm of *W. mirabilis* is composed of a fibrogranular environment among the strands of chromatin, with electrondense particles about 32 ± 1.7 nm in diameter. Granules are associated or interconnected to electron dense fibers (Figures 3-4), both, in interchromatin and perichromatin areas (Figure 4). The EDTA regressive technique for ribonucleoproteins bleached the compact chromatin strands and granules and fibers are heavily stained (Figure 5). The association of *W. mirabilis* extranucleolar granules to reticulated chromatin, their location, abundance and ribonucleoproteic nature indicate that these particles correspond to *Lacandonia* granules reported in the species *L. schismatica*, *T. brevystilis* (Jiménez-García *et al.* 1992) and *Ginkgo biloba* (Jiménez-Ramírez *et al.* 2002).

Discussion

*Compact chromatin.* Optical microscopy showed strands that stain with toluidine blue distributed in the nuclei of cells of *Welwitschia mirabilis* (Figure 1A). Thin sections of samples prepared for transmission electron microscopy and observed with the electron microscope corroborated the reticulated pattern of the strands (Figure 1B) and atomic force microscopy showed the three dimensional arrangement of the strands in the nucleoplasm. The strands are also positive for the osmium ammine technique specific for DNA. Therefore, these strands correspond to the reticulated pattern reported previously using also results from atomic force microscopy (Jiménez-Ramírez *et al.* 2002).

*Nuclear particles.* In previous studies, the presence of ribonucleoprotein granules has been described in nuclei that have a reticulated pattern such as *Lacandonia schismatica* and *G. biloba* (Agredano-Moreno *et al.* 1994, Agredano-Moreno *et al.* 2000, Fragos-Soriano *et al.* 2009, Jiménez-García *et al.* 1992, Jiménez-García & Fragos-Soriano 2000, Jiménez-Ramírez *et al.* 2002). Therefore, we searched for *Lacandonia* granules in cell nuclei of *W. mirabilis*. Using standard transmission electron microscopy, we observed granules in the nucleoplasm, about 32 nm in diameter, associated to fibers in the periphery of chromatin. These granules were stained after the EDTA regressive staining preferential for ribonucleoproteins, while compact chromatin is observed with low contrast. These particles also may correspond to similar although scarce, particles observed in some members of bryophytes (Alonso-Murillo & Jiménez-García 2015).

**Figure 1.** *Welwitschia mirabilis* cell nuclei show a reticulated pattern of compact chromatin. (A) Bright field of a cell nucleus stained with toluidine blue. (B) Atomic force microscopy of this organelle showing three dimensional arrangement of intranuclear strands. (C) Conventional staining for transmission electron microscopy showing strands of compact chromatin (c). Nucleus (N), cytoplasm (cyt), cell wall (cw), Nucleolar Organizer Region (NOR), nucleolus (nu).
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