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Atomic Force Microscopy of the Cell Nucleus

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In mammals and plants, the cell nucleus is organized in dynamic macromolecular domains involved in DNA and RNA metabolism. These domains can be visualized by light and electron microscopy and their composition analyzed by using several cytochemical approaches. They are composed of chromatin or ribonucleoprotein structures as interchromatin and perichromatin fibers and granules, coiled bodies, and nuclear bodies. In plants, DNA arrangement defines chromocentric and reticulated nuclei. We used atomic force microscopy to study the *in situ* structure of the plant cell nucleus. Samples of the plants *Lacandonia schismatica* and *Ginkgo biloba* were prepared as for electron microscopy and unstained semithin sections were mounted on glass slides. For comparison, we also examined entire normal rat kidney cells using the same approach. Samples were scanned with an atomic force microscope working in contact mode. Recognizable images of the nuclear envelope, pores, chromatin, and nucleolus were observed. Reticulated chromatin was observed in *L. schismatica.* Different textures in the nucleolus of *G. biloba* were also observed, suggesting the presence of nucleolar subcompartments. The observation of nuclear structure *in situ* with the atomic force microscope offers a new approach for the analysis of this organelle at high resolution. © 2000 Academic Press

Key Words: **atomic force microscopy; cell nucleus; chromatin; nucleolus; plant cell.**

INTRODUCTION

In the cell nucleus, DNA, RNA, and proteins are arranged in macromolecular domains involved in different steps of gene expression such as DNA replication, transcription, pre-mRNA splicing, polyadenylation, methylation, and RNA transport (see Spector, 1993; Singer and Green, 1997; Lamond and Earnshaw, 1998). The domains in mammalian and plant cell nuclei include chromatin, nuclear matrix, nuclear envelope and lamina, pore complexes, nucleolus, ribonucleoprotein particles, and nuclear bodies (see Spector, 1993; Testillano *et al.*, 1993; Wachtler and Stahl, 1993; Fakan, 1994; Vázquez-Nin and Echeverría, 1996; Simpson and Filipowicz, 1996; Shaw, 1996; Lamond and Earnshaw, 1998; Matera, 1999). These domains can be visualized by light and electron microscopy. A great deal has been achieved with regard to the structure, composition, and function of these domains by using microscopy and molecular approaches. While electron microscopy provides a way to study nuclear structure with nanometer resolution, no dynamic information can be obtained directly due to sample preparation. On the other hand, light microscopy offers a way to study more dynamic processes but in the range of micrometer resolution. Atomic force microscopy (AFM) potentially offers an opportunity to study nuclear structure with the resolution of electron microscopy in the range of nanometers, while offering the analysis of more dynamic processes because of the possibility of observing phenomena even in solution (Hansma *et al.*, 1997). As an initial attempt to test the real possibilities of AFM as mentioned, we were interested first in generating *in situ* images of this organelle by using AFM. Here, we have been able to show general nuclear structures that can be compared to those produced by electron microscopy. Further studies will be performed to try to visualize dynamic processes in the range of nanometer resolution within the cell nucleus.

MATERIALS AND METHODS

Samples. Normal rat kidney (NRK) epithelial cells (NRK-52E; ATCC CLR 1571, Rockville, MD) were grown on coverslips for 1 or 2 days in Dulbecco's modified Eagle's medium (Gibco) containing glutamine, pyruvate, and 10% fetal bovine serum (Gibco). *Lacandonia schismatica* E. Martínez & C. H. Ramos (Martínez and Ramos, 1989) bud flowers and young leaves of the tree *Ginkgo biloba* were processed for electron microscopy according to standard protocols (Spector *et al.*, 1998) with modifications. Briefly,

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samples were fixed with 6% glutaraldehyde for 24 h, postfixed in 1% osmium tetroxide for several hours, dehydrated with a series of graded concentrations of ethanol, and embedded in epoxy resin. A glass or diamond knife was used to obtain semithin sections that were mounted on glass slides. Some samples were stained with toluidine blue.

Atomic force microscopy. For entire cells, air-dried NRK cells were observed with an atomic force microscope (Digital Instruments) operating in contact mode. The scan size was 60 μm at a scan rate of 1.969 Hz. Images were generated by using the NanoScope IIIa control system. For plant material, unstained or stained samples were scanned with an atomic force microscope (Park Scientific) operating in contact mode. The microscope was equipped with a scanner of 100 μm and a 100-Å radius of a nitride silicone tip, mounted on a cantilever of 0.6 μm. A scan rate of 2–3 Hz, a force of 10 nN, and a gain of 0.5 arbitrary units were used. Images were generated with Proscan software (version 3.1, Park Scientific, 1997).

RESULTS

General Nuclear Structure

As a first step in characterizing the cell nucleus by AFM, we used NRK cells growing on coverslips and further air-dried. Figures 1A and 1B show the images generated by this procedure. The cytoplasm is recognizable as a flat and rough surface. The nucleus is an area where the three nucleoli of this cell type are evident as dense bodies rising above the plane of the slide. These images, however, do not show the details of the cell's interior since the cell membrane is still covering the cell body and the microscope detects a layer of membrane above it. We went on to use semithin sections to analyze structures within the cell nucleus. If we section the cell and visualize each surface of each section, then we may be able to see the internal nuclear structure. We chose plant material because it is more suitable since in many species, a reticulated pattern of DNA distribution is present (Lafontaine, 1974; Jordan *et al.*, 1980; Nagl, 1985), which allows an easy way to detect chromatin. In addition, cell walls are internal structures that would allow us to identify known structures. Figures 2A and 2B show low- and medium-magnification views of flower button sections of *L. schismatica*. Cell walls are rapidly recognized in cells from the teguments. Moreover, nuclei are also recognized when compared to published material of this species using light and electron microscopy (Jiménez-García *et al.*, 1992; Agredano-Moreno *et al.*, 1994). In several cells, even nucleoli are seen as clear spots within the nucleus. In Fig. 2B, a higher magnification of a single cell is shown. Cell walls are seen as bright lines and a nucleus inside is seen with chromatin forming a network, similar to what is observed in the nucleus of this plant when analyzed by light and electron microscopy (Jiménez-García *et al.*, 1992; Agredano-Moreno *et al.*, 1994). In addition, a nucleolus is observed.

Chromatin

Once we identified the nucleus, we analyzed in more detail known nuclear structures. Reticulated chromatin is shown in Figs. 2C and 2D. A clear network occupies the interior of the cell nucleus, while interchromatin spaces appear as black areas. Therefore, chromatin is a massive structure that adds texture to the section. At higher magnification, among the chromatin it is possible to identify some other structures that contribute to the roughness of the section. Many particles were observed in the nucleoplasm; these particles could be components of chromatin or more likely ribonucleoprotein particles.

Nucleolus and Nuclear Pores

Other *in situ* structures that we recognized were the nuclear pores and the nucleolus. Figures 2E and 2F show nuclear pores as discontinuities along the nuclear periphery, interrupting chromatin clumps and connecting the nucleoplasm with the cytoplasm, which appears as a thin layer surrounding the nucleus. The nucleolus in Fig. 2F is present among clumps of compact chromatin and displays a texture where two different regions can be seen. A nucleolar organizer region is also observed, penetrating the nucleolus from a clump of chromatin associated with the nuclear envelope (arrow in Fig. 2F).

DISCUSSION

We have been able to generate images of the *in situ* structure of the plant cell nucleus by atomic force microscopy. Recognizable images of nuclear pores, compact chromatin, nucleolus, and nucleoplasm are presented. In addition, nucleolar subcompartments were distinguished.

While much work has been done on isolated nuclear structures using AFM (see Hansma *et al.*, 1997; Stoffler *et al.*, 1999), no efforts were made previously to analyze the nuclear structure *in situ*. We have taken sections of cells or tissue and analyzed the surface of those sections in order to generate images of the interior of the cell, such as images of the cell nucleus. In fact, in addition to having a very flat section generated by a glass or diamond knife, we obtained sections that have a texture that can be detected by the AFM and indeed this texture correlates to well-known nuclear structures. Using this approach it may be possible to develop three-dimensional reconstructions using the texture of a

series of adjacent sections to generate high-resolution images of a complete nucleus. In addition, the possibility of performing experiments on the surface of sections opens up a new way to approach nuclear structure with high resolution while allowing reactions to take place at that moment. Analysis of thawed cryosections in solution might also be important for further AFM studies of the cell nucleus.

In our observation using entire NRK cells in culture, we were not able to distinguish nuclear structures since the plasma membrane covered the cell. However, different textures were visualized in the nucleus, such as the nucleolus and nucleoplasm. In the nucleoplasmic region, the observed texture may be related to underlying structures that are covered by the cell membrane and, therefore, may also offer additional information. On the other hand, our approach allows visualization of the nuclear interior. The recognizable structures are those that are very well known for the species used in this work. Chromatin in *L. schismatica* has been extensively studied using light and electron microscopy (Jiménez-García *et al.,* 1992). In fact, we have previously used the Feulgen reaction and phosphatungstic acid (PTA) contrast for chromatin and those methods produced the reticulated pattern for DNA known also for other species such as onion and corn (Lafontaine, 1974; Jordan *et al.,* 1980; Nagl, 1985). The ribonucleoproteins associated with the periphery of these strands of chromatin have also been described at the electron microscopy level (Jiménez-García *et al.,* 1992; Agredano-Moreno *et al.,* 1994). These particles measure 32 nm in diameter, are very abundant, and may be related to intranuclear RNA metabolism (Jiménez-García *et al.,* 1992; Agredano-Moreno *et al.,* 1994). We are currently focusing on the structure of these particles with the use of AFM.

The nuclear pores are nuclear structures that have been very well studied by AFM (see Stoffler *et al.,* 1999). We have recognized their presence as discontinuities along the nuclear periphery interrupting clumps of compact chromatin. The nucleolus was also readily observable by AFM. The nucleolus of *G. biloba* offers a good system to distinguish granular and fibrillar components. In this species, both components are well defined and the dense fibrillar component is abundant, as revealed by electron microscopy (data not shown). The dense fibrillar component is the place where synthesis and initial steps of pre-rRNA processing take place (see Wachtler and Stahl, 1993). In addition, the nucleolar organizer was identified as a region of chromatin penetrating the nucleolus from a clump of chromatin present in the periphery of the cell nucleus in this species. New studies are in progress using AFM to better understand nucleolar compartmentalization and its role in ribosome biogenesis.

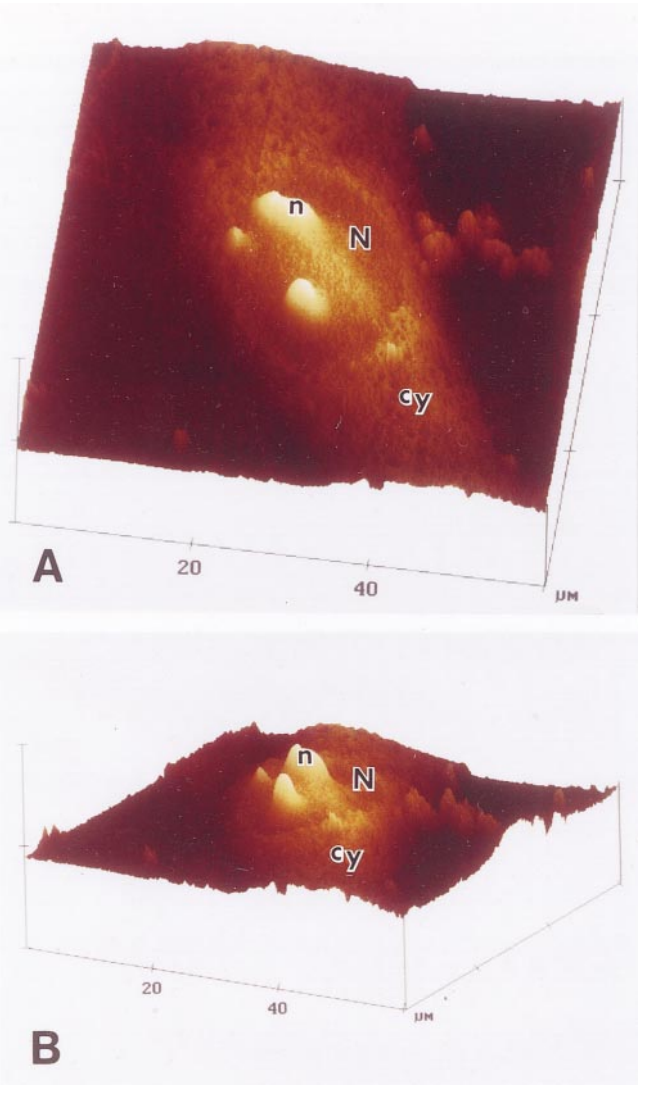

FIG. 1. Atomic force microscopy of entire NRK cells. (A) Bidimensional display of the cell where the cytoplasm (cy), nucleus (N), and nucleoli (n) are observed. (B) Three-dimensional projection of the same cell. Nucleoli (n) are structures contributing largely to the rough texture of the dried cell.

In conclusion, we have been able to generate images of the internal nuclear structure *in situ,* by using AFM and unstained semithin sections of cells or tissue processed for electron microscopy and mounted on glass slides. The texture of the section correlates to well-known nuclear structures such as chromatin, the nuclear envelope, and the nucleolus. In addition, surface imaging obtained with AFM in comparison to transmission electron microscopy has revealed a very rough texture of epon semithin sections that are cut with glass or diamond knives. New approaches to study more dynamic processes within the cell nucleus at high resolution are now in progress.

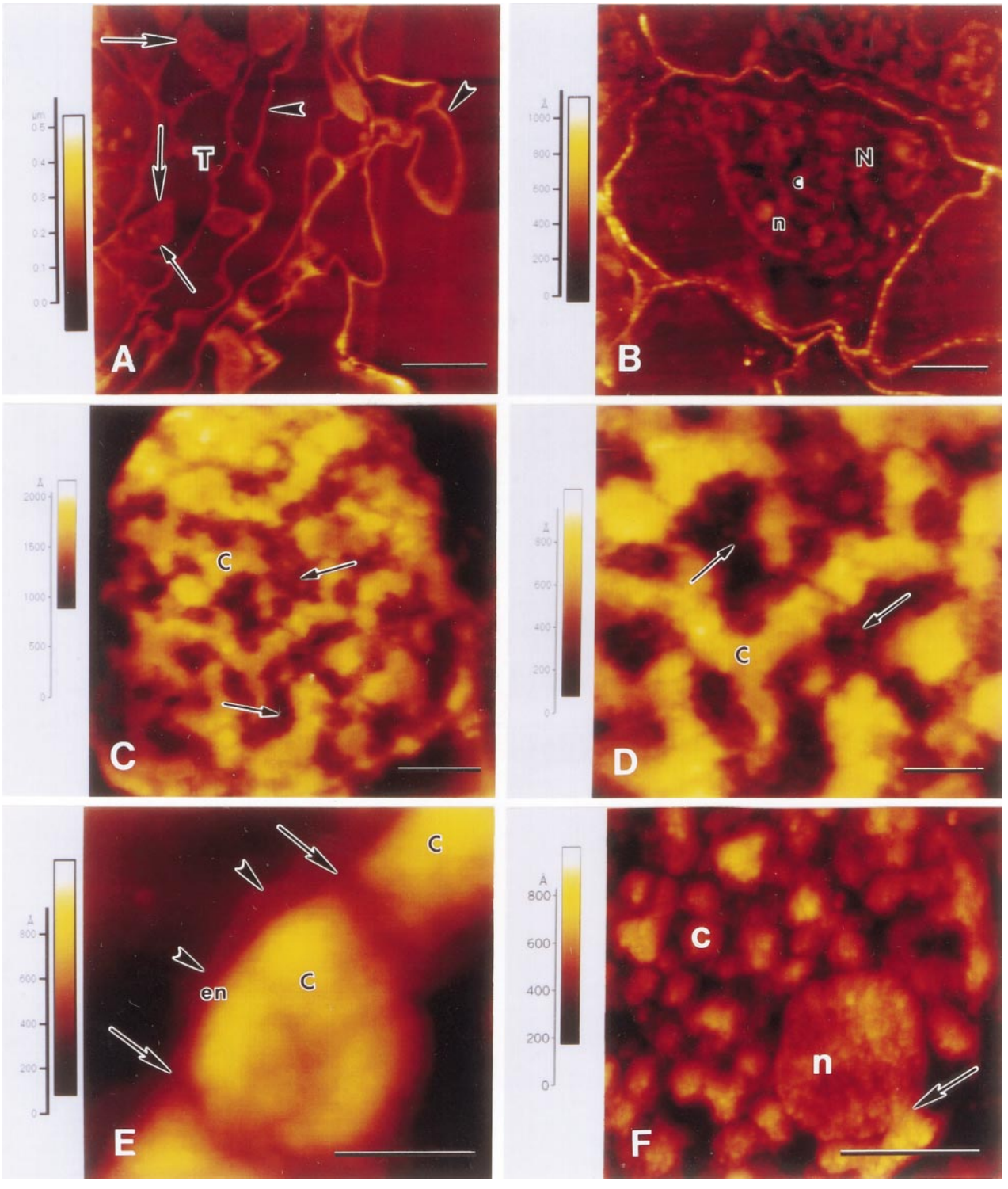

FIG. 2. Atomic force microscopy of unstained semithin sections of ovaries of *L. schismatica* (A–D) and leaves of *G. biloba* (E and F). (A) Low-magnification view where many cells are seen in the teguments (T). Cell walls (arrowheads) and nuclei (large arrows) are observed. Nucleoli are also observed as bright spots (small arrows). (B) Higher magnification view of a well-defined nucleus (N), showing chromatin strands (c) and the nucleolus (n). (C) The nucleus is composed of compact chromatin (c) forming a network and spaces corresponding to interchromatin areas (arrows). (D) Higher magnification view shows several particles (arrows) in the nucleoplasm associated with the periphery of compact chromatin (c). (E) Along the nuclear envelope (en), discontinuities are observed (arrows) interrupting clumps of compact chromatin (c). A thin layer (arrowheads) of cytoplasm is also observed. (F) The nucleolus (n) is observed among clumps of compact chromatin (c). In this nucleolus, a texture is observed that correlates to the presence of subdomains. A nucleolar organizer region from a clump of compact chromatin is associated with the nucleolus (arrow). Bars are (A) 20; (B) 5; (C) 2; (D and E) 1; and (F) 4 µm.

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