Structure of plant cell nucleus viewed by scanning electron microscopy

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Abstract

The three-dimensional structure of the nucleus in meristematic and differentiated phloem ray parenchyma cells of shortleaf pine (*Pinus echinata* Mill.) is viewed by scanning electron microscopy. The fixed and dehydrated plant tissues were freeze-fractured in absolute ethanol and dried by the critical point method. Some of the features observed in the fractured nuclei are aggregates of chromatin some of which possess a spiral orientation, the attachment of chromatin to the nuclear envelope, and small canals in the fractured envelope probably representing nuclear pores.

INTRODUCTION

Methods for studying the three-dimensional organization of the protoplast in plant cells have recently been described by Panessa and Genarro (1972), Humphreys and Wodzicki (1972) and Wodzicki and Humphreys (1972). In any attempt to study fixed plant material the cells are subjected to varying degrees of distortion from fixing, dehydrating, freezing, freeze-drying, exposure to high vacuum, and other procedures commonly used for transmission or scanning electron microscopy. To prevent major displacement of protoplasmic components within the cell interior, especially within large, highly vacuolated plant cells, one may embed the plant specimen in plastic as is commonly done for obtaining ultrathin sections, then fracture the plastic block exposing surfaces along the planes of fracture for viewing with the scanning electron microscope (Wodzicki and Humphreys, 1973; Humphreys, Wodzicki and Paulin, 1973).

Another alternative is freeze fracturing the plant tissue frozen in absolute ethanol followed by Anderson's (1969) critical point method. Thus, distortion of protoplasmic structures by the formation of ice crystals in hydrated cells during freezing in liquid nitrogen or by subsequent damage during dehydration under high vacuum are believed to be prevented. One limitation of this method, however, is the possible loss of some cellular components from fractured, exposed cells when the solvents are replaced during thawing of the tissue. This paper shows some of three-dimensional features of the plant nucleus one may observe by using these procedures.

MATERIALS AND METHODS

Small cubes of tissue, 1 mm³, were cut from the cambial region of shortleaf pine (*Pinus echinata* Mill.) stems, and immediately fixed in cold (4° C). 2% glutaraldehyde buffered with 0.1 M sodium cacodylate to pH 7.2 for 2 hours, and post-fixed for 2 hours in cold 2% osmium tetroxide. The fixed cubes were dehydrated in a standard ethanol series, positioned in a shallow well in an aluminum specimen holder with a drop of absolute ethanol with the radial plane exposed, plunged into liquid nitrogen and fractured with a pre-cooled razor blade. The fractured tissue was thawed in absolute ethanol at room temperature and the alcohol carefully replaced with amyl acetate. The tissue was then transferred to a pressure chamber for drying by the critical point method using liquid carbon dioxide. Finally, the specimens were coated with a layer of gold about 200 Å in thickness before being viewed with a Cambridge Stereoscan II scanning electron microscope.

OBSERVATIONS AND DISCUSSION

Two rows of ray parenchyma cells are exposed in radial view in the cambial zone showing a cambial ray initial cell near the center with its fractured nucleus (Fig. 1). An enlarged view of the same nucleus is shown in Fig. 2. Another fractured nucleus from a slightly older phloem ray parenchyma cell is shown in Fig. 3. The granular and fibrillar structure of the nuclear chromatin appears to have a spiral configuration along some of the exposed fractured surfaces (small black arrows). Chromatin in the nucleus of the cell in Fig. 2 is more aggregated and the spiral arrangement more pronounced than in the ontogenetically older and more differentiated nucleus in Fig. 3. According to Lafontaine and Lord (1969) certain segments of the twisted filaments of chromosomal material at the prophase stage are 0.1 μ in diameter,

Fig. 1. Two horizontally aligned rows of cells are ray parenchyma. The shortest, central cell with fractured nucleus is the cambial initial. $\times 1300$.

Fig. 2. The fractured nucleus from ray initial seen in Fig. 1. $\times 9000$.

Fig. 3. Fractured nucleus from the phloem ray parenchyma cell advanced in the differentiation. $\times 6700$.

which is in the range of the aggregate sizes ($0.1 - 0.3 \mu$) seen in Figure 2. Thus, the observed structures in the nucleus of this meristematic ray initial may represent spiral aggregates of supercoiled nucleoprotein fibrils recognized by Ris (1969) and Kaye (1969).

One may recognize similar spiral aggregates of chromatin, although less distinct, in the fractured nucleus of the differentiating phloem parenchyma cell viewed in Fig. 3, although nuclei in these cells do not normally undergo division. In many places, the chromatin is visibly attached to the inner surface of the nuclear envelope (see large black arrows Fig. 3) which, agrees with the observations of Hearst and Botchan (1970). The outer surface of the nuclear envelope is coated with residual cytoplasm, as well as by a thin layer of gold, which makes it difficult to trace the pores of the nuclear envelope, however, the pores may be identified as small canals or inlets, less than $1000 \text{ \AA}$ in diameter, at a few places along the fractured edge of the nuclear envelope (see white arrows, Fig. 3).

The large cavity in the nuclear envelope at the upper end of the ellipsoidal nucleus seen in Fig. 3 may be associated with a type of nuclear invagination which occurs to varying degrees in the nuclei of differentiating secondary xylem cells of pine (Humphreys and Wodzicki, 1972; and Wodzicki and Brown, 1973). In Figures 1 and 3, one may also observe some of the residual cytoplasmic content of the fractured cells, although it is likely that much of the cytoplasm with its included organelles was washed out during replacements of solvents. The larger structures are of the size and appearance of plastids in pine cells.

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REFERENCES

- Anderson, T. F., 1969. Electron Microscopy of Microorganisms. [in:] Physical Techniques in Biological Research, Vol. 3 (ed. G. Oster and A. Pollister), pp. 177—240. Academic Press, New York.
- Hearst, J. E. and Botchan M., 1970. The eukariotic chromosome. *Ann. Rev. Biochem.* 39: 151—182.
- Humphreys, W. J. and Wodzicki, T. J., 1972. Methods for viewing by scanning electron microscopy the interior organization of protoplasts of plant

- cells. [in:] 30th Ann. Proc. Electron Microscopy Soc. Amer. (ed. C. J. Arceneau), pp. 238—239. Los Angeles, California.
- Humphreys, W. J., Wodzicki, T. J. and Paulin, J. J., 1973. Fractographic studies of plastic embedded cells by scanning electron microscopy. *J. Cell Biology*. 56: 876—880.
- Kaye, J. S., 1969. The ultrastructure of chromatin in nuclei of interphase cells and in spermatids. [in:] *Handbook of Molecular Cytology* (ed. A. Lima-de-Faria), pp. 361—380. Amsterdam — London: North — Holland Publishing Co.
- Lafontaine, J. G. and Lord A., 1969. Organization of nuclear structures in mitotic cells. [in:] *Handbook of Molecular Cytology* (ed. A. Lima-de-Faria), pp. 381—411. Amsterdam — London: North-Holland Publishing Co.
- Panessa, B. J. and Genarro, J. E., 1972. Preparation of fragile botanical tissues and examination of intracellular contents by S.E.M. [in:] *Proc. 5th Scanning E. M. Symposium* (ed. O. M. Johari and I. Corvin), pp. 328—334. Chicago, Illinois.
- Ris, H., 1969. The molecular organization of chromosomes. [in:] *Handbook of Molecular Cytology* (ed. A. Lima-de-Faria), pp. 221—250. Amsterdam — London: North-Holland Publishing Co.
- Wodzicki, T. J. and Brown, C. L., 1973. Organization and breakdown of protoplast in maturing pine tracheids. *Amer. J. Bot.* 60: 631—640.
- Wodzicki, T. J. and Humphreys, W. J., 1972. Cyto differentiation of maturing pine tracheids: The final stage. *Tissue and Cell* 4: 527—530.
- Wodzicki, T. J. and Humphreys, W. J., 1973. Fracturing plastic embedded plant material for scanning electron microscopy. *Micron* 4: 1—9.

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DESCRIPTION OF FIGURES

Plate 1 and 2. *Pinus echinata* (Mill.) stem cambial region fractured and exposed in radial plane. Arrows in all Figures indicate structural features as follows: Spiral configuration of chromatin (small black); attachment of chromatin to nuclear envelope (large black); probable sites of nuclear pores (white).

*Struktura jądra komórki roślinnej
obserwowana w skaningowym mikroskopie elektronowym*

Streszczenie

Przedstawiono mikrofotografie struktury jądra komórki merystematycznej i parenchymy promieniowej łyka. *Pinus echinata* Mill. uzyskane przy pomocy skaningowego mikroskopu elektronowego. Utrwalone, odwodnione tkanki roślinne przełupane zostały podczas zamrożenia w alkoholu absolutnym chłodzonym płynnym azotem i wysuszone metodą krytycznego punktu Andersona. W niektórych agregatach chromatyny obserwowanych w przełupanych jądrach dopatrzone się organizacji spiralnej. Widoczne jest także przytwierdzenie chromatyny do błony jądrowej. Drobne kanały w przełupanej błonie przedstawiają prawdopodobnie pory.