Protoplasma (2002) 220: 105–109
DOI 10.1007/s00709-002-0029-4

PROTOPLASMA
Printed in Austria

Preprophase band loses its function as a cytokinetic apparatus in mitosis of neck canal mother cell

Short communication

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Received January 22, 2002; accepted April 26, 2002; published online October 31, 2001
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Summary. Preprophase bands in the neck canal mother cell and the central cell of the archegonium of the fern *Dryopteris crassirhizoma* are observed with immunofluorescence microscopy. No phragmoplast is found during mitosis of the neck canal mother cell; however, the phragmoplast develops very well in the central cell. The neck canal mother cell undergoes karyokinesis but not cytokinesis and finally produces only one binucleate neck canal cell. However, the central cell undergoes cytokinesis and produces an egg cell and a ventral canal cell. These observations suggest that the preprophase band in the neck canal mother cell loses its function as a cytokinetic apparatus and becomes an evolutionary vestige in the development of the archegonium.

Keywords: *Dryopteris crassirhizoma*; Mitosis; Neck canal mother cell; Preprophase band.

Introduction

The preprophase band (PPB) of microtubules was discovered by Pickett-Heaps and Northcote (1966a, b) as a band of cortical microtubules that girdled the nucleus during the G_2 phase in premitotic cells. Although the PPB disappears when the mitotic spindle is established, the memory of its location seems to be retained, and at subsequent cytokinesis a new cell wall is inserted into parent cell walls along the line previously delineated by the PPB (Gunning et al. 1978, Gunning 1982, Gunning and Hardham 1982, Gunning and Wick 1985, Gunning and Sammut 1990, Wick 1991, Mineyuki 1999, Pickett-Heaps et al. 1999). The PPB is the characteristic of dividing cells in land plants; however, it is not found in every cell type. The PPBs are absent in protonemata of the mosses (Schmiedel et al. 1981, Doonan et al. 1987, Doonan and Duckett 1988, Doonan 1991), but they are present in some moss cells once the gametophyte starts three-dimensional growth (Schmiedel et al. 1981, Apostolakos and Galatis 1985, Doonan et al. 1987). On the other hand, PPBs reported from fern protonemata and prothalli are usually well ordered (Wada et al. 1980, Murata and Wada 1989, Jenni et al. 1990) like those described in angiosperm plants (Pickett-Heaps 1969a–c; Wick and Duniec 1983, 1984; Gunning and Wick 1985; Mineyuki and Gunning 1990). Thus, the appearance of the PPB as cytokinetic apparatus in the cell division of plants is of evolutionary significance (Gunning 1982, Brown and Lemmon 1990, Wick 1991, Pickett-Heaps et al. 1999). Presently, our work shows that in the developmental process of the archegonium in the fern *Dryopteris crassirhizoma*, though a PPB appears in the neck canal mother cell, the mother cell only undergoes karyokinesis but not cytokinesis and only one binucleate neck canal cell is formed. Moreover, it is found that this PPB has lost its function as a cytokinetic apparatus which predicts the position of the new cell wall, and the PPB has become a vestige

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in the neck canal mother cell during the development of the archegonium in *D. crassirhizoma*.

Material and methods

Plant material and culture

Spores of *Dryopteris crassirhizoma* Nakai were gathered in Heilongjiang Province, China. They were grown in pure culture as described by Dyer (1979) and modified by the present authors as follows: Sterilized spores were sown directly onto Knop medium (0.5 g of $Ca(NO_3)_2$, 0.125 g of KNO_3, 0.125 g of $MgSO_4$, 0.125 g of KH_2PO_4, 0.001 g of $FeCl_3 \cdot 6H_2O$, 15 g of agar per liter of solution prepared with distilled water) in petri plates (60 mm in diameter) and kept in the dark for 12–48 h. Gametophytes were grown in petri plates under continuous fluorescent light at 25 °C. Each cordate gametophyte containing a sequence of archegonia from the initial stages to maturity, about 3–6 mm in length, was grown in pure culture and used throughout the study.

Fixation and embedding

Gametophytes were fixed with 8% paraformaldehyde and 0.2% picric acid in 0.1 M sodium phosphate buffer (containing 5 mM EGTA, 0.5 mM phenylmethylsulfonyl fluoride and 1% dimethyl sulfoxide, pH 7.0) for 30 min at room temperature (Murata and Wada 1989). After three rinses in phosphate buffer (10 min each) the fixed gametophytes were dehydrated in a graded ethanol series, 20 min in each change of 15, 30, 50, 70, and 90% and three changes of 100%. Then the specimens were gradually infiltrated (1 h for each step) at 37 °C with mixtures of absolute ethanol and Steedman wax (a mixture [9 : 1, w/w] of polyethylene glycol 400 distearate and 1-hexadecanol, from Aldrich) in the proportions 2 : 1, 1 : 1, and 1 : 2 (v/v) at each step separately. After three changes of pure Steedman wax, the specimens were embedded in the molds allowing the wax to be polymerized at room temperature (Brown and Lemmon 1995).

Immunofluorescence microscopy

Longitudinal sections of archegonia were cut 15 µm thick with a rotary microtome. Ribbons were mounted on coverslips coated with a thin film of Mayer albumen (Brown and Lemmon 1995). Before immunostaining, Steedman wax was removed from the section with absolute ethanol. Dewaxed sections were treated with 1% Triton X-100 in phosphate buffer about 15 min. Following a 5 min rinse in PBS (135 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2), the preparations were stained with mouse monoclonal anti-α-tubulin antibody (Sigma) for 4 h at 37 °C. After washing three times with PBS, the preparations were incubated at 37 °C with fluorescein isothiocyanate-labeled secondary antibody for 1.5 h. After the final washing, coverslips with processed sections are inverted in a drop of mounting medium on a clean slide. Mounting medium is prepared by combining 3 g of *n*-propyl gallate with 50 ml of glycerin and 50 ml of PBS (135 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, pH 8.5).

Control experiments were conducted in the same way, but with (1) omission of primary antibody; (2) omission of secondary antibody; and (3) omission of both antibodies.

Specimens were examined with a confocal laser scanning microscope (Bio-Rad MCR-1024).

Transmission electron microscopy

Archegoniate gametophytes of *D. crassirhizoma* were cut vertically and in a direction parallel to its longitudinal axis, each about 0.5 mm thick. The anterior boundary of each slice was formed by the meristem of the gametophyte at the base of the apical notch, and the length was adjusted so that each contained a sequence of archegonia from the initial stages to maturity (Bell and Mühlethaler 1962). Archegoniate gametophytes were prepared for transmission electron microscopy utilizing the fixation and embedding protocols as described by You and Jensen (1985) and modified. Briefly, the slices were fixed at room temperature in 3% glutaraldehyde in 0.05 M cacodylate buffer (pH 7), followed by three buffer rinses, then postfixed overnight with 2% osmic acid, dehydrated in acetone, and embedded in Epon 812. Thin sections were cut on a LKB-NOVA ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Hitachi H-300 electron microscope.

Results

Overview of archegonium development in D. crassirhizoma

Archegonia are located on the lower surface of the gametophyte, usually just behind the apical notch. The whole archegonium and its contents are developed from a single superficial cell that can be distinguished from the other superficial cells by its larger nucleus (Fig. 1 A). The superficial initial cell divides periclinally to give rise to a smaller outer cell and an inner one with a larger nucleus (Fig. 1 B). The inner cell makes another periclinal division to produce a column of three cells: the outer cell, the primary cell, and the innermost cell (Fig. 1 C). Further divisions of the outer cell and the innermost cell produce a jacket of vegetative wall cells (Fig. 1 D). The primary cell in the middle of the column of the three cells undergoes an asymmetric transverse division and forms a smaller distal neck canal mother cell and a larger proximal central cell (Fig. 1 E). A period of growth ensues, during which the central cell dramatically enlarges and becomes many times larger than the neck canal

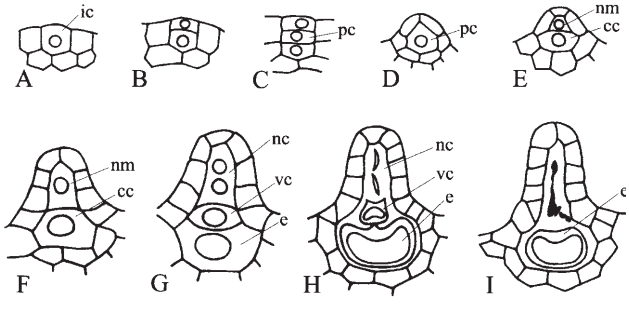

Fig. 1 A–I. Stages in the development of the archegonium of *D. crassirhizoma*. *ic* Initial cell of archegonium; *pc* primary cell; *nm* neck canal mother cell; *cc* central cell; *nc* neck canal cell; *vc* ventral canal cell; *e* egg

mother cell (Fig. 1F). Following an elongating stage, the neck canal mother cell undergoes the karyokinesis, ultimately resulting in a binucleate neck canal cell, but no new cell wall is formed between the two nuclei (Fig. 1G). At the same time, the central cell divides transversely and unequally into a larger proximal egg and a smaller distal ventral canal cell (Fig. 1G). As the egg increases in volume considerably, the archegonium is transformed from a cylindrical to a flask-shaped structure (Fig. 1H). When the egg reaches maturity, the neck canal cell and the ventral canal cell disintegrate and become a mucilaginous passage for sperms to reach the egg (Fig. 1I). Our observations in this study were concentrated on the role of the PPB during the last cell division in the neck canal mother cell and the central cell.

Development of preprophase bands in neck canal mother cell and central cell

When the neck canal mother cell and the central cell are produced from the primary cell, they enter the growth phase. The neck canal mother cell mainly grows along the longitudinal axis, cortical microtubules in this cylindrical cell run transverse to the long axis (Fig. 2A, B). While the neck canal mother cell reaches a certain size, the transverse cortical microtubules begin to aggregate and form the broad PPB. The broad PPB gradually narrows when the cell cycle progresses from interphase to prophase. Instead of girdling the nucleus at its equator, the typical narrow PPB is obvious at the basal part of the neck canal mother cell and still overlaps its nuclear region (Fig. 2C), while cortical microtubules disappear in the other part of this cell. Simultaneously, the central cell enlarges its volume, the cortical microtubules also transverse its long axis. Unlike the interphase meristematic cells, the central cell possesses abundantly both transverse cortical microtubules and random cytoplasmic microtubules (Fig. 2A–C), especially the perinuclear microtubules beneath the nucleus (Fig. 2A, C). Following the migration of the nucleus to the upper part of the central cell, a broad microtubule array is aligned parallel to the future division plane and then begins to form the broad PPB around the nuclear region. The PPB formation in the central cell is slightly prior to its counterpart in the neck canal mother cell. So in Fig. 2B the PPB of central cell appears well organized, while in the neck canal mother cell the PPB is in the early stages of formation. In

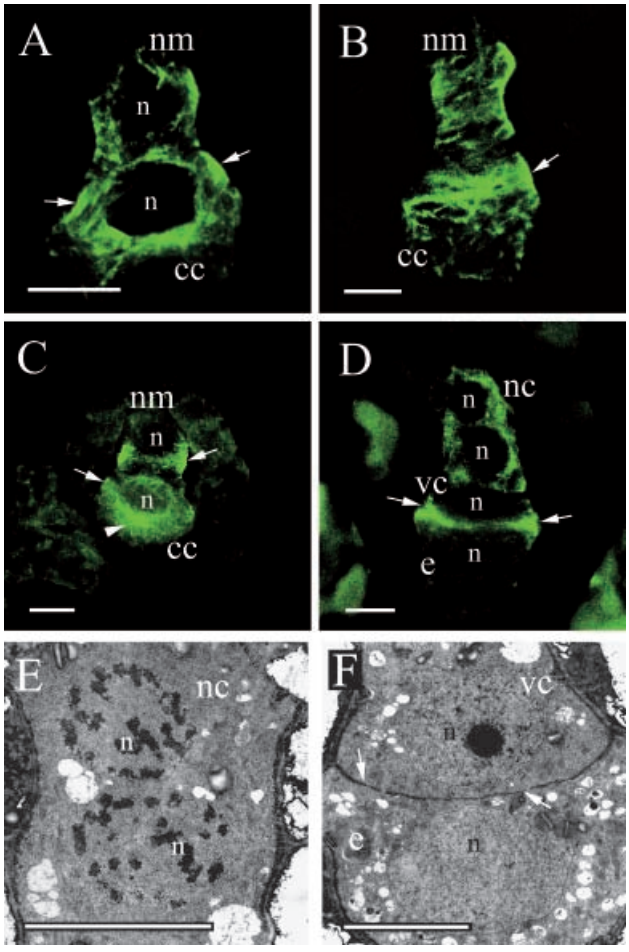

Fig. 2A–F. Longitudinal optical and thin sections of the archegonia. **A–D** Longitudinal optical sections of the archegonia (the archegonial jacket of vegetative wall cells were wiped out in **A** and **B**), showing the tubulin immunodetection of microtubular structures related to the last cell division (cortical microtubules, preprophase bands, and phragmoplast) of the neck canal mother cell (*nm*) and the central cell (*cc*). All figures were from stacks of 8 images taken 0.3 μm apart. **A** The transverse cortical microtubules found in the neck canal mother cell. The cortical microtubules began to gather in the upper part (pair of arrows) of the central cell (*cc*), random cytoplasmic microtubules were plentiful, especially the perinuclear ones. **B** The transverse cortical microtubules in the elongating neck canal mother cell and the broad PPB (arrow) in the central cell. **C** The well organized PPB (upper arrow) in the neck canal mother cell drifted away from the equator of the nucleus. The PPB in the central cell (lower arrow) began to break down and showed strong tubulin fluorescence at one pole of that cell (arrowhead). **D** No new cell wall formation in a newly formed binucleate neck canal cell (*nc*). The phragmoplast (arrows) still existed between the ventral canal cell (*vc*) and the egg (*e*). *n* Nucleus. Bar: 10 μm. **E** and **F** Electron photomicrographs of partial archegonia. **E** No cell wall observed between two nuclei in the longitudinal section of a newly formed neck canal cell (*nc*). Note the clear image of chromosomes in both newly formed nuclei. **F** A very delicate cell wall between the ventral canal cell (*vc*) and the egg (*e*) indicated with arrows. *n* Nucleus. Bar: 10 μm

Fig. 2C the PPB in the neck canal mother cell appears well organized, when the PPB in the central cell is beginning to break down. The mitotic spindles are not found in the mitoses of neck canal mother cells and the central cells, even though thousands of cells were examined in this study.

Phragmoplast and cell wall

In the neck canal mother cell no phragmoplast is formed at the site of the original PPB at telophase (Fig. 2D). If it existed during karyokinesis, the phragmoplast or its residual should be observed in any newly formed neck canal cells. The observation of thin sections by electron microscopy further confirms the absence of a cell wall between the two newly formed nuclei in the neck canal cell (Fig. 2E). In the central cell, however, the phragmoplast is well organized and the new cell plate appears in the region which was previously occupied by the PPB (Fig. 2D). At cytokinesis the central cell divides into an egg cell with a large nucleus and a ventral canal cell with a small nucleus. In the later stage, the two cells are separated by a very delicate cell wall (Fig. 2F) which inserts into the parental wall along the line delineated previously by the PPB.

Discussion

In our study we have found that some functional loss of PPB happens in the mitosis of the neck canal mother cell in comparison with descriptions of root tip cells (Gunning 1978; Wick and Duniec 1983, 1984; Gunning and Wick 1985), grass stomatal subsidiary cell mother cells (Galatis et al. 1984), tobacco BY-2 suspension cells (Granger and Cyr 2001), and fern protonemata (Murata and Wada 1989). In the neck canal mother cell of *D. crassirhizoma* the PPB loses its function as a cytokinetic apparatus which predicts the formation of a new cell wall at cytokinesis. The PPB becomes an evolutionary vestige in that cell, in which the karyokinesis is not followed by the cytokinesis and a neck canal cell with two nuclei is produced.

The possible functions of the PPB have been reviewed extensively by Mineyuki (1999): among them is its role as a source of tubulins and microtubules for mitotic spindles; in the establishment of the division site; and in the localization of cell wall deposition. In comparison with the above views, our findings are as follows: Firstly, no mitotic spindles are found in the mitosis of neck canal mother cells (and neither in the central cells), even though thousands of cells were observed with immuno-fluorescence microscopy. However, clearly discernible chromosomes emerge in the electron microphotograph of Fig. 2E. So it can be deduced that the spindles must exist but only in a very short time. Investigators have not caught it so far. Secondly, the observed PPB locates near the basal part of the neck canal mother cell. Though it does not affect the karyokinesis of the neck canal mother cell, it causes the failure in cytokinesis indeed. It seems to indicate that owing to the positional change of the PPB in that cell, its function in the establishment of the precise division site has been lost. Thirdly, no phragmoplast forms between two newly formed nuclei of the neck canal cell. The reason for this phenomenon could be ascribed to the fact that no precise division site is established.

In the evolutionary process of pteridophytes the regression has taken place in neck canal cells. The number of neck canal cells in the archegonia decreases from 16 in some species of *Lycopodium* to 1 in most leptosporangiate ferns (Foster and Gifford 1974). The fern *D. crassirhizoma* is a more evolutionary advanced species. Its mature archegonium contains a binucleated cell which results from an incomplete mitosis. This incomplete mitosis originates from the above-stated functional loss of the PPB. The deviation of the PPB from the precise division site, the equatorial plane, morphologically indicates the partial functional loss of the PPB. A similar situation has also been reported in *Marattia sambucina* (Stocky 1942) and *Psilotum nudum* (Zimmerly and Banks 1950, Bierhorst 1954). In those studies, however, it was occasionally found in some archegonia that a definite wall exists between the two nuclei and the neck canal mother cell forms two neck canal cells. It is thought that possibly the PPB incompletely or unstably loses its function as cytokinetic apparatus during mitosis. The assumption of the incomplete function of the PPB can also explain another case: the stoma of *Funaria hygrometrica* which developed from the guard cell parent cell is composed of a pair of incompletely separated guard cells with two nuclei (one on either side) as a result of cytokinesis that begins normally but is never completed (Sack and Paolillo 1985, Wick 1991).

After analyzing our results, we concluded that the PPB in the neck canal mother cell of *D. crassirhizoma* has become an evolutionary vestige because the PPB loses its function as a cytokinetic apparatus.

Acknowledgments

We thank Dr. J. Brush and Bai-Chen Wang for their careful reading of the manuscript. This work was supported by a grant from National Natural Science Foundation of China (nr. 39870073) to R.L.Y.

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Verleger: Springer-Verlag KG, Sachsenplatz 4–6, A-1201 Wien. — Herausgeber: Dr. D. Menzel, Botanisches Institut und Botanischer Garten, Rheinische Friedrich-Wilhelms-Universität Bonn, Kirschallee 1, D-53115 Bonn, Bundesrepublik Deutschland. — Redaktion: Sachsenplatz 4–6, A-1201 Wien. — Satz und Umbruch: SNP Best-set Typesetter Ltd., Hong Kong. — Druck: Adolf Holzhausens Nachfolger, Holzhausenplatz 1, A-1140 Wien. — Verlagsort: Wien. — Herstellungsort: Wien. — Printed in Austria