Microtubule and actin filament organization during acentral divisions in potato suspension culture cells

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Summary. Microtubule and filamentous (F)-actin organization in the potato suspension culture line HH260 was studied by fluorescence microscopy in double-labelled cells. During interphase, microtubules and F-actin were randomly arrayed in isodiametric cells but were aligned transversely to the direction of growth in elongated cells. Microtubules and F-actin co-aligned in preprophase bands which were, however, comparatively rare and diffuse. Interestingly, more than half of the cells in telophase contained phragmoplasts that were either horseshoe-shaped or straight, instead of being round. We traced the cause of this difference to preprophase, where misplaced nuclear localization away from the central axis of cells may give rise to acenetrally placed spindles and, subsequently, to acenetrally placed phragmoplasts and cell plates. Further, we hypothesize that it is the uneven fusion of the expanding cell plates with the parent plasma membrane, and the accompanying depolymerization of those parts of the phragmoplasts, that gives the incomplete phragmoplasts observed.

Keywords: Filamentous actin; Cytoskeleton; Microtubules; Phragmoplast; Phragmosome; Solanum tuberosum; Suspension culture.

Abbreviations: DAPI 4',6-diamidino-2-phenylindole; MBS 3-maleimidobenzoyl-N-hydroxysuccinimideester; PMSF phenylmethylsulfonyl fluoride; SB stabilization buffer.

Introduction

Plant development requires the precise control of cell division and cell expansion. Prior to cell division, the nucleus often lies in the cortical cytoplasm but, in G2 phase, it moves to the interior of the cell where its location determines the intersection of the future spindle–phragmoplast axis with the plane of cell division (Gunning 1982, Baskin and Cande 1990). The processes that determine the plane of cell division and the location of the nucleus within that plane remain unknown but may involve participation of the cytoskeleton. Nuclear movement to the cell centre accompanies the formation of the phragmosome, a disc of cytoplasm formed from the fusion of cytoplasmic strands (Sinnott and Bloch 1940) that is best seen in vacuolated cells entering mitosis (Venverloo et al. 1980). The phragmosome contains filamentous (F) actin and microtubules radiating from the nucleus, both of which are required for nuclear movement (Katsuta and Shibaoka 1988, Katsuta et al. 1990). The preprophase band, containing microtubules and F-actin, appears concurrently with the phragmosome, and as it forms adjacent to the plasma membrane where the phragmosome cytoplasm connects to the cortical cytoplasm, the two structures lie within the same plane (Venverloo et al. 1980). This plane predicts the eventual division plane of the cell (Gunning 1982, Staiger and Lloyd 1991). Thus, the processes that control cytoskeleton organization and nuclear positioning prior to the onset of cell division are critical in the determination of the correct axis and plane of cell division, and hence plant histogenesis. The nature of these processes is, however, unresolved.

Plant cells in suspension culture grow under unusual conditions with high auxin levels present and, partially because of these conditions, form irregular clusters and files in which the precise control of the cell division plane and direction of cell elongation has been lost. Even so, the cytoskeleton of suspension culture
cells is generally similar to that of cells in whole plants. Microtubules are present in the cortical array, preprophase band and radial phragmosome arrays, mitotic spindle and the phragmoplast (Lloyd et al. 1980, Simmonds et al. 1983). F-actin is also found in normal arrays; during interphase these are fine transverse filaments in the cortical cytoplasm, thicker cortical and transvacuolar bundles of filaments, and a basket of bundles around the nucleus while, during cell division, an extensive cortical actin array is retained along with the division specific arrays of the preprophase band and phragmoplast (Traas et al. 1987; Kakimoto and Shibaoka 1987a, b).

However, some differences between the cytoskeleton of suspension culture cells and cells in whole plants have emerged. Early reports of microtubule localizations in suspension cultures noted an apparent absence of microtubule preprophase bands, with this being attributed to the general lack of organized growth within these cells (Fowke and Gamborg 1980). While subsequent observations have shown that microtubule preprophase bands are present in suspension culture cells of some species (Simmonds et al. 1983, Simmonds 1986, Gorst et al. 1986, Traas et al. 1987, Sonobe and Shibaoka 1989, Tautorus et al. 1992), these often appear less organized in suspension culture cells than in cells in whole plants (Simmonds et al. 1983, Gorst et al. 1986). Furthermore, the PPB index, defined as the ratio of cells with preprophase bands of microtubules to cells with phragmoplasts, is significantly lower in nonembryogenic suspension cultures compared to root tip cells or embryogenic suspension cultures that are capable of organized growth and cell differentiation (Gorst et al. 1986, Tautorus et al. 1992). Given the irregular cell patterning in suspension cells, the early stages of division, including the centring of the nucleus through the formation of the phragmosome and preprophase band, may indicate crucial steps where suspension culture cells divert from the organized growth of cells in plants.

In this study, we ask the questions (i) at what stage(s) of cell development do the suspension cells divert from cells that give rise to patterned development in planta, and (ii) what changes occur in the cytoskeleton associated with this diversion of growth. Using an actin-microtubule double-labeling system, we confirmed that all configurations of the cytoskeleton occur in nonembryogenic potato suspension culture cells. However, the PPB index was low, typical of nonembryogenic suspension cultures and a high percentage of telophase cells contained incomplete phragmoplasts shaped like horseshoes or straight bands. We speculate that this anomaly results from acentral divisions due to acentral localization of the nucleus within the division plane at the onset of division.

Material and methods

Chemicals and antibodies

Specific chemicals and antibodies were obtained as follows: 4',6-diamidino-2-phenylindole (DAPI), 3-maleimidobenzoyl-N-hydroxy-succinimideester (MBS), phenylmethylsulfonyl fluoride (PMSF), leupeptin, pectolyase, anti-chicken brain α-tubulin, and fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Sigma, St. Louis, Mo., U.S.A.), rhodamine-conjugated phallolidin (Molecular Probes, Eugene, Oreg., U.S.A.), acetylated bovine serum albumin (BSA) (Aurion, Wageningen, The Netherlands) and cellulase onozuka R10 (Serva, Heidelberg, Federal Republic of Germany).

Plant material

A nonembryogenic potato suspension culture line (Solanum tuberosum L. genotype HH260; Binding et al. 1978, Gilissen et al. 1991) was subcultured weekly in Murashige-Skoog medium (Murashige and Skoog 1962) containing 3 % saccharose, 0.1 μg of 6-benzyladenine and 5.0 μg of 1-naphthylacetic acid per ml, and maintained at 25 °C in the dark while being shaken at 120 rotations per minute. For experiments, material was filtered through 250 μm nylon gauze to remove larger cell clusters.

Rhodamine-phalloidin labelling, fixation, and immunolabelling

For immunolabelling, small samples of cells were stabilized for 30 min prior to fixation in a 1 : 5 ratio with 400 μM MBS in stabilization buffer [SB; 100 mM piperezine-N,N'-bis(2-ethanesulfonic acid) (PIPES), pH 6.9, K⁺ 10 mM MgSO4, 10 mM ethylene glycol-bis(β-aminoethyl ether)-N,N',N'-tetraacetic acid, 0.2% Triton X-100, 1.0% DMSO] that also contained 0.3 mM PMSF and 10 μg of leupeptin per ml. Subsequently, the cells were fixed in 4% formaldehyde (15 min) and then 4% formaldehyde with 0.05% glutaraldehyde (15 min) in SB that contained 6 mM dithiothreitol. After washing in SB buffer (5 min) and then phosphate-buffered saline (PBS; 145 mM NaCl, 8.4 mM Na2HPO4, 1.6 mM NaH2PO4, pH 7.4) (5 min), cell walls were digested for 10 min at 30 °C in 1% cellulase onozuka R10 and 0.1% pectolyase in 10 mM MES (pH 6.1, K⁺) containing 10 μg of leupeptin per ml and 0.3 mM PMSF. After washing in PBS (2 x 5 min), cells were treated with 200 mM NH4OHCl in PBS (10 min) to reduce background and washed in PBS (5 min). Material was blocked in incubation buffer (PBS containing 0.1% acetylated BSA and 0.05% Tween-20) (10 min), incubated in the antitubulin antibody diluted 1/400 in incubation buffer (2 h), washed (3 x 10 min) and incubated in goat anti-mouse fluorescein isothiocyanate-conjugate (1/400 in incubation buffer, 2 h). Cells were then washed (3 x 10 min), stained with DAPI (5 min, 10 μg/ml) and mounted in incubation buffer containing p-phenylenediamine (0.1%) and rhodamine-phalloidin (0.083 μM, diluted from a 6.6 μM stock solution in methanol). F-actin was also labelled with rhodamine-phalloidin at intermediate stages of the immunolabelling procedure, from prior to MBS stabilization to after fixation,