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The role of microtubules in the maintenance of regular localization and arrangement of Golgi apparatus in root cells of *Triticum aestivum* L.

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ABSTRACT

In plant cells Golgi apparatus organization, maintenance and distribution differ from that in mammalian cells and the mechanisms for this are not clearly understood. Here we investigate the role of microtubules in the positioning and arrangement of Golgi apparatus in the root cells of *Triticum aestivum* L. by using dual immunofluorescent labeling and laser confocal microscopy to localize both throughout the cell cycle. We observed that Golgi stacks (i) in interphase cells predominantly occupied the perinuclear region, (ii) during mitosis they redistributed to the spindle periphery and/or areas above spindle poles, and (iii) in telophase accumulated around the phragmoplast and the chromosomes/nuclei of daughter cells. Inhibition of microtubule assembly by colchicine resulted in aggregation of Golgi in the cortical cytoplasm of interphase cells and accumulation around the chromosomes in C-mitotic cells, in stark contrast with the distribution in untreated cells. Electron microscopy revealed that in colchicine treated cells many Golgi units became disorganized, yet others were abnormally enlarged. Overall, our results indicate that in plant cells microtubules play a key role in restricting the position and maintaining the arrangement and structural integrity of the Golgi apparatus.

1. Introduction

The cytoplasm of eukaryotic cells is partitioned into specialized structural and functional domains/compartments, occupied by multiple molecular ensembles and organelles, which are specialized for different metabolic and biochemical processes. In the cell walled, immobile plant cells, the cellular growth, development and differentiation are accompanied by redistribution of organelles in the cytoplasm, leading to the formation of new domains and/or reorganization of the old ones. The anchoring and retention of organelles at the newly formed domains, which are set up for specific functional activity, occur after the rearrangement of the cytoplasm. The cytoskeleton contributes to the appropriate distribution of organelles within a cell, providing tracks for active intracellular transport. The Golgi apparatus in plant cells plays a key role in the synthesis of the cell wall material, modification and sorting of proteins destined for the cell surface and vacuoles [1]. The mode of Golgi organization, maintenance and distribution in plant cells differs from that in mammalian cells, reflecting both the different structural properties and functional requirements. In contrast to mammalian cells, plant Golgi apparatus consists of multiple individual stacks, dispersed over the cytoplasm. Each stack is a functionally independent unit, composed of flattened cisternae, and has distinctive structural and functional polarity [2].

Golgi units retain their structural integrity and remain functionally active during mitosis [3–5], presumably, because plant cells maintain active synthesis and secretion of complex carbohydrates, required for cell plate formation during cytokinesis [6]. The number of stacks per cell varies among different cells and tissues [7,8]. Another distinctive feature of plant Golgi is that in vacuolated cells stacks undergo myosin dependent movement through the cytoplasm along actin filaments [3,9,10], and Golgi membranes and sites of protein export on ER (ERES) behave as a single secretory complex [11].

Even though actin provides the tracks for organelle movement, microtubules also may be involved in the translocation of Golgi stacks and Golgi-derived secretory products. Cortical microtubules organize cellulose synthase complexes and guide their trajectories in the plasma membrane, thus regulating the formation of the cell wall [12,13]. The preprophase band (PPB) of microtubules has been indirectly implicated in the formation of the "Golgi belt" in the equatorial region under the plasma membrane. It has been suggested that the Golgi belt predicts the future site of cell division [4], yet Dixit and Cyr [14] showed that Golgi secretion is not required for marking the PPB site. During cytokinesis, Golgi-derived vesicles deliver membranes and polysaccharides along microtubules to the midzone of the phragmoplast, where they fuse and form the cell plate [15]. Apparently, endocytosis actively participates

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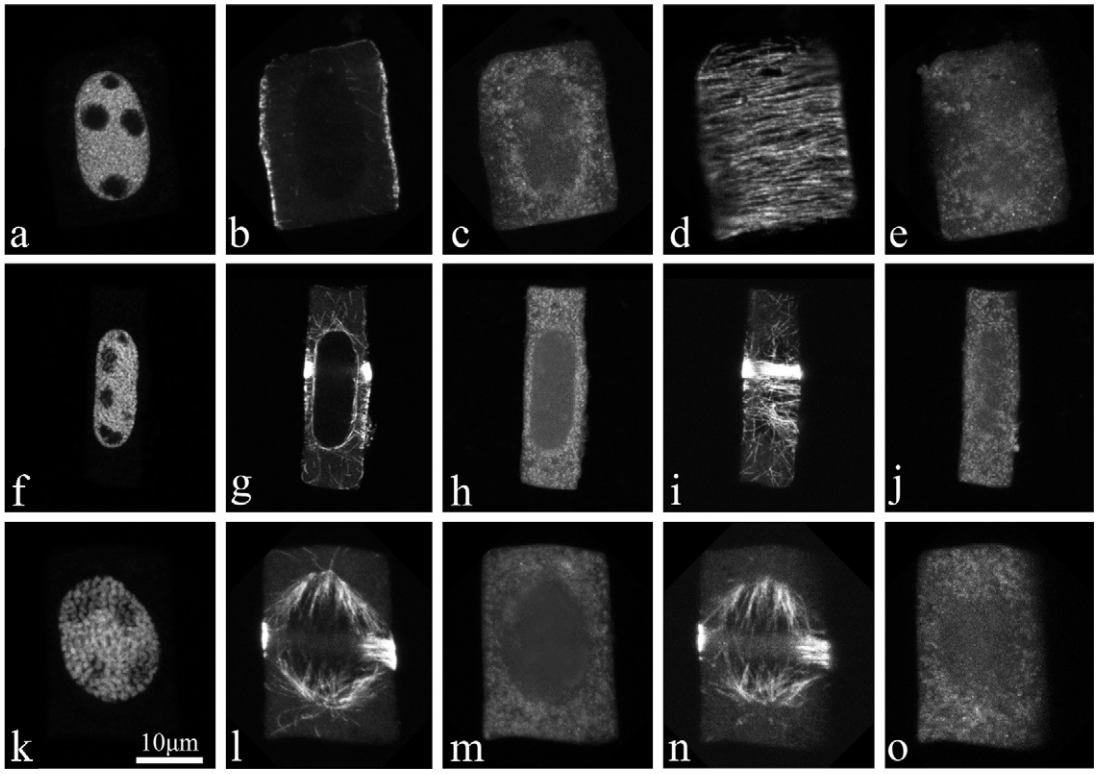

Fig. 1. Immunofluorescence of *T. aestivum* root tip cells labeled with DAPI (a, f, k), antibodies against tubulin (b, d, g, i, l, n) and Golgi marker 58K/FTDC (c, e, h, j, m, o). (a–e) – interphase; (f–j) – late G_2 phase; (k–o) – prophase; (b, c, g, h, l, m) – perinuclear region; (d, e, i, j, n, o) – cortical cytoplasm.

in cytokinesis, and the cell plate formation is considered a special type of secretion [6,16]. Cumulative evidence indicates that in plant cells Golgi stacks and Golgi derived vesicles translocate along actin filaments for long distances and along microtubules for shorter distances, which is in stark difference with mammalian cells [17]. Plant Golgi stacks, being highly mobile units, must retain subcellular localizations within particular domains or compartments during specific stages of the cell cycle. However, the evidence on the role of the cytoskeleton, in particular, microtubules, in the maintenance of intracellular architecture is incomplete. Thus, radial microtubule arrays were implicated in anchoring the nucleus and configuring the premitotic cytoplasm in vacuolated cells [16]. Microtubule end binding protein EB1 was found in association with endomembranes, and it has been suggested that the organization of the ER and their polarized distribution may require an intact and functional microtubule cytoskeleton [18]. Golgi stacks are also frequently associated with microtubules [13], but the role of microtubules in the maintenance of the proper organization of endomembranes, is ambiguous. The goal of this study was to investigate the role of microtubules in the maintenance of regular distribution and retention of Golgi at specific cytoplasmic domains in non-vacuolated and polarized cells of *Triticum aestivum* L. roots.

2. Materials and methods

2.1. Plant material

Seeds of wheat *T. aestivum* L. were sterilized and germinated on moist filter paper placed in Petri dishes, at 25 °C until the rootlets were 1 cm long.

2.2. Immunofluorescence

The root tips were cut and fixed for 1.5–2 h in 4% paraformaldehyde dissolved in PHEM buffer, pH 6.9 (60 mM PIPES, 25 mM HEPES, 10 mM EGTA, 2 mM $MgCl_2$), and incubated for 15–20 m in 0.4 M mannitol containing 1% cellulase (Sigma Aldrich) and 5 mM EGTA. The root tips were placed on glass coverslips and macerated with thin surgical needles [19]. The cell suspension was left to dry overnight at 4 °C. Coverslips were immersed in 0.2% gelatin, air-dried, and transferred into PHEM buffer solution. Specimens were placed for 30 m in 0.5% Triton X-100 prepared on PHEM and supplemented with 5% DMSO. The coverslips were rinsed in 10 mM Mg–Tris–HCl buffer (pH 7.6), and incubated with antibodies. For double labeling of microtubules and Golgi, (1) microtubule detection: rat monoclonal antibodies against α-tubulin YOL1/34 (Abcam), Texas Red conjugated donkey anti-rat IgG (Jackson), (2) Golgi detection: mouse monoclonal antibodies against Golgi 58K protein (Sigma Aldrich), Alexa 488 conjugated donkey anti-mouse IgG (Invitrogene), (3) Prolong Gold embedding solution (Invitrogene).

The preparations were examined with Axiovert 200 M light microscope (Zeiss), both equipped with epifluorescence illumination, standard filter sets and a Neofluar 100× objective. Images were recorded with a DC camera Kodak 260 and a High resolution Camera AxioCam HRm, and analyzed with Adobe Photoshop 6.0 and 7.0. The samples were also analyzed using a confocal laser-scanning microscope (TCS SP2, Leica Microsystems, Germany).

2.3. Electron microscopy

For transmission electron microscopy, the root tips were fixed with 2.5% glutaraldehyde in 0.1 M Na–K–phosphate buffer (pH 7.2) supplemented with sucrose (15 mg/ml). Then samples were post fixed in 1% OsO_4, left overnight at 4 °C in 70% ethanol containing 2% uranyl acetate, dehydrated in ethanol and acetone, and embedded in epon resin. Thin sections were placed on Formvar-coated slot grids, stained with lead citrate, imaged and photographed in JEM-1011 (JEOL) microscope equipped with a GATAN ES500W digital camera.

2.4. Incubation with colchicine

The seedlings were placed in water solution containing 0.2% colchicine (Sigma Aldrich) and grown at 25 °C. The root tips were cut after 1, 6, 12 and 24 h, fixed and processed for immunochemistry or electron microscopy.

3. Results and discussion

3.1. Localization of Golgi apparatus during the cell cycle of wheat root cells

Four microtubule arrays replace each other during the cell cycle of plant cells: cortical bundles in interphase, preprophase band in G2-prophase, spindle and phragmoplast in mitosis. We have cor-