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Short communication

The structure of cortical cytoplasm in cold-treated tobacco cells: the role of the cytoskeleton and the endomembrane system

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1. Introduction

The eukaryotic cytoskeleton is a highly dynamic and complex structure supporting many basic metabolic processes. Generally, the organisation of cytoskeleton carries the spatial information important for various cellular compartments and moreover secures the mobility of protein-transporting membrane vesicles. One of the important features of cytoskeleton polymers is their high sensitivity to low (non-freezing) temperatures leading to reversible depolymerisation into subunits. However, cold sensitivity of cytoskeletal polymers differs in various plants and plant tissues depending on many interfering factors (Baluška et al., 1993; Egierszdorff and Kacperska, 2001; Kerr and Carter, 1990). In addition to that, spatial interconnection of cytoskeleton with endomembrane system plays an important role (for review see Lichtscheidl and Baluška, 2000). Cold-induced depolymerisation of both microtubules (MTs) and actin filaments (AFs) provided us with a tool to investigate the role of the cytoskeleton in the spatial organisation of the plant cell cytoplasm. The tobacco BY-2 suspension culture (Nagata et al., 1992) has been used as a model, physiologically homogenous plant cell line. We have focused on the cortical cytoplasm of BY-2 tobacco cells, where structural changes can be easily observed by conventional light and fluorescence microscopy (reviewed in Kumagai and Hasezawa, 2001). Together with this, the organisation of the endoplasmic reticulum (ER) was studied in vivo using BY-2 cells expressing a GFP-fusion protein targeted to the lumen of the ER. Protein analysis of cytoskeleton proteins was carried out to understand some cold-induced cytoskeletal changes on the protein level.

2. Results and discussion

In interphase, transverse parallel arrays of MTs (Fig. 1a) were observed in the cortical layer of cytoplasm and this typical organisation did not significantly change during the life cycle. There were no MTs in the cortical layer of cytoplasm during mitosis and cytokinesis. In contrast to MTs, AFs formed at least two different arrays in the cortical cytoplasm of interphase cells: transverse parallel arrays, and filaments without apparent orientation (Fig. 1b). AFs that formed transverse parallel arrays were thin in exponentially growing cells and became more prominent in cells in the stationary phase of growth. During mitosis and cytokinesis, AFs formed a very dense, non-oriented net in the cortical region. In vivo observations using ER-targeted GFP showed a lace-like network of tubules, lamellar sheets and mobile particles in the cortical cytoplasm of interphase cells (Fig. 1c). The net of ER did not show any noticeable structural changes during life cycle of tobacco cells, although it was denser during the exponential phase of growth. During mitosis, the cortical ER re-formed into a lace-like net in which lamellar sheets were absent.

The first effect of cold in the cortical region observed after 20 min of cultivation at 0 °C was the partial disassembly of both MTs and AFs. Progressive depolymerisation occurred during prolonged cultivation (12 h) at 0 °C. The rate of depolymerisation differed in cells found in different stages of life cycle. For example, exponentially growing and actively dividing cells maintained more AFs in the polymerised state for a longer time than elongating cells. After 12 h of cold treatment,

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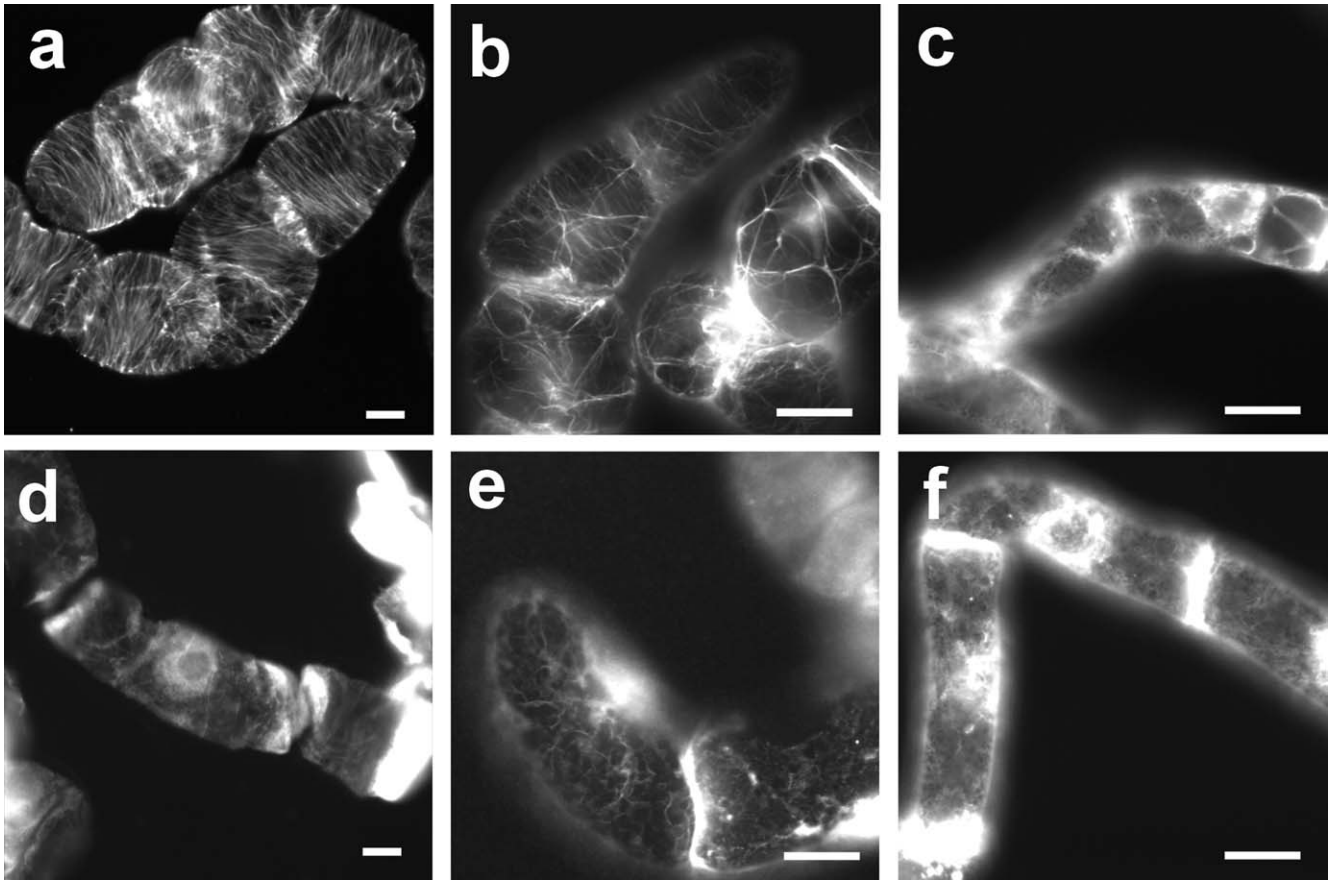

Fig. 1. The effect of cold (0 °C) on the organisation of MTs, AFs and ER in the cortical cytoplasm of interphase tobacco BY-2 cells. (a,d) Transversal arrays of MTs in the cortical cytoplasm of control cells (a) and in the cortical cytoplasm of cells incubated 12 h at 0 °C (d). Immunofluorescence staining with anti-α-tubulin antibody and FITC conjugated secondary antibody. Note degradation of MTs in cells cultivated 12 h at 0 °C (d). (b,e) AFs in the cortical cytoplasm of control cells (b) and in the cortical cytoplasm of cells incubated 12 h at 0 °C (e). Fluorescence visualisation with phalloidin conjugated with TRITC. Note partial degradation of AFs in cells cultivated at 0 °C for 12 h (e). Actin is detected as diffuse signal in the cortical region resembling the pattern of the ER. (c,f) BY-2 cells stably transformed with a gene construct coding for mGFP5-ER (Haseloff et al., 1997) containing a C-terminal ER retention signal sequence (HDEL). (c) GFP fluorescence in exponentially growing cells. (f) GFP fluorescence in cells cultivated at 0 °C for 12 h. Bars represent 20 μm.

the majority of cells did not contain any polymerised cytoskeleton or kept very short fragments of MTs (Fig. 1d) and AFs (Fig. 1e). In contrast to the cytoskeleton, no pronounced damage of ER structure was detected in the cortical layer of cold-treated cells and only subtle extension of lamellar sheets of ER was observed in cells cultivated for 12 h at 0 °C (Fig. 1f).

Although this ultra-low but non-freezing temperature (0 °C) was found to be sufficient to depolymerise the majority of both AFs and MTs, recovery experiments showed that polymerisation competence of subunits of both cytoskeletal structures was preserved during the cold treatment. Immediately after transfer of cells to optimal temperature conditions (25 °C), recovery of both AFs and MTs in all surviving cells was observed. Recovery of cytoskeletal polymers was preceded by the formation of transient structures.

Biochemical analysis revealed that the levels of actin and tubulin in cytosolic and sedimentable protein fractions did not change during cold treatment. Furthermore, although protein synthesis in vivo was strongly inhibited under cold stress conditions, in vitro translation assay indicated that no degradation of particular mRNAs occurred.

The stability of ER structure in the absence of cytoskeletal structures manifests a certain organisational independence of this membranous organelle. It can also be concluded that depolymerisation of the cytoskeleton is not the primary cause of death of cells cultivated at 0 °C. Furthermore, our data suggest that membranes of ER might provide the cytoskeleton with some kind of structural support. For example, actin in cells cultivated at 0 °C was often detected as diffuse signal in the cortical region whose pattern resembled the structure of the ER. When cells cultivated at 0 °C were transferred to a standard temperature (25 °C), first re-polymerisation of AFs occurred only in those places where the diffuse signal

of actin was assembled. After that, polymerised AFs reached other cortical areas to form a uniform net. Taken together, the data presented here suggest that cytoskeletal subunits released from polymers at 0 °C existed in a form immediately available for recovery of polymers. Further, our data indicate that membranes (particularly ER) might provide cytoskeletal monomers with the information important for their spatial organisation during cold treatment and also during subsequent recovery of AFs and MTs at optimal temperature.

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