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Research article

Endoplasmic reticulum targeted GFP reveals ER organization in tobacco NT-1 cells during cell division

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Abstract

The endoplasmic reticulum (ER) of plant cells undergoes a drastic reorganization during cell division. In tobacco NT-1 cells that stably express a GFP construct targeted to the ER, we have mapped the reorganization of ER that occurs during mitosis and cytokinesis with confocal laser scanning microscopy. During division, the ER and nuclear envelope do not vesiculate. Instead, tubules of ER accumulate around the chromosomes after the nuclear envelope breaks down, with these tubules aligning parallel to the microtubules of the mitotic spindle. In cytokinesis, the phragmoplast is particularly rich in ER, and the transnuclear channels and invaginations present in many interphase cells appear to develop from ER tubules trapped in the developing phragmoplast. Drug studies, using oryzalin and latrunculin to disrupt the microtubules and actin microfilaments, respectively, demonstrate that during division, the arrangement of ER is controlled by microtubules and not by actin, which is the reverse of the situation in interphase cells.

Keywords: Actin microfilaments; Cell division; Endoplasmic reticulum; Green fluorescent protein; Microtubules; Nuclear envelope; Nuclear invaginations

1. Introduction

In interphase cells of plants, the endoplasmic reticulum (ER) forms into numerous distinct arrays [16,39]. These arrays include a comparatively stable, reticulate, cortical network and highly dynamic subcortical ER tubules. These in turn are interconnected and continuous with the nuclear envelope. The presence of these different arrays in living cells was first demonstrated with the carbocyanine dye $DIOC_6(3)$ and DIC video microscopy [1,16,21,24,32,33], and more recently with green fluorescent protein (GFP) targeted to the lumen of the ER [5,6,13,19,29,34,38]. The movement of the motile ER tubules in interphase cells is a microfilament-based process, as actin bundles lie parallel to the ER [1,5,25], the motor protein, myosin colocalizes with the ER [25], disruption of the actin cytoskeleton with cytochalasin results in the cessation of ER movement [21]. Actin disruption also prevents the reorganization of ER that precedes cell division in cultured cells [38]. However, the factors responsible for organization of the cortical array, and whether they are cytoskeletal or not, have been more difficult to characterize.

In contrast to the numerous characterizations of ER organization in interphase plant cells, few detailed studies have been published of ER dynamics during plant cell division. This is important, however, for several reasons. First, the ER is continuous with the nuclear envelope during interphase so that proteins localized to the lumen of the ER are also found within the nuclear envelope, and knowing the fate of the ER during mitosis will suggest the localization of luminal proteins [6,18]. Second, electron micrographs of dividing plant cells show a close association between tubules of ER that run parallel to the microtubules in both the mitotic spindle [14,15,31] and

Abbreviations: DIC, differential interference contrast; ER, endoplasmic reticulum; GFP, green fluorescent protein.

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the phragmoplast, the plant specific structure responsible for the formation of the new cell wall in cytokinesis [2]. These results have been confirmed by observations of the accumulation of ER-targeted GFP within the spindle and phragmoplast [29]. It is unknown when in the cell cycle these ER tubules arrive at these regions. Third, phragmoplast formation in plants requires ER and Golgi apparatus-derived vesicles to run along microtubules to the equator of the cell [15,30] where they consolidate into a sheet that expands outwards as more vesicles fuse, eventually meeting with and fusing with the parent cell wall to complete cell division [35]. Understanding ER dynamics during cell division and their dependence on the cytoskeleton may suggest a mechanism of phragmoplast formation. And finally, some plant nuclei contain deep invaginations and trans-nuclear strands. As these structures are bounded by the nuclear envelope, they are continuous with the ER, but their formation, hypothesized to occur during cell division, remains uncertain [6].

In this study therefore, we have taken advantage of tobacco NT1 and BY-2 cell lines that stably expresses ER-targeted GFP to look at the behavior of ER during cell division, and how the ER interacts with the cytoskeleton. Our data confirm extensive accumulation of ER within the mitotic spindle and phragmoplast, and show that this reorganization of the ER during mitosis is maintained by microtubules, as opposed to the actin cytoskeleton.

2. Results

2.1. Endoplasmic reticulum organization in interphase cells

ER dynamics during interphase and cell division were characterized in tobacco NT-1 cells by visualizing GFP-ER with laser scanning and spinning disk confocal microscopy. In interphase cells, the ER formed three different arrangements, confirming earlier studies that used $DIOC_6(3)$ to stain membranes [1,33,24; our data, not shown], and ER-targeted GFP constructs in cultured tobacco [19,29,38] and other cell types [5,6,13,34]. First, cortical ER formed polygonal arrays similar to those found in animal cells and other plant cells (Fig. 1A, arrow). The cortical ER exhibited slow movement and intersections of cortical ER were generally three-way. Second, ER tubules extended from the cortical ER into the cytoplasm traversing between vacuoles (Fig. 1B, arrows). This ER was dynamic, and showed rapid cytoplasmic streaming relative to cortical ER, with streaming visible in successive images of a time course (Fig. 1D, arrow). Third, the ER surrounded and was continuous with the nuclear envelope (Fig. 1C, E, arrow). In many interphase cells, penetrations of the nuclear envelope extended into the nucleus forming invaginations and channels that crossed the entirety of the nucleus (Fig. 1E, F; supplemental file Video 1) [6]. These channels often showed extensive branching patterns, and could contain internal ER structures suggesting that they contain cytoplasm and ER rather than being a simple infolding of the inner nuclear membrane.

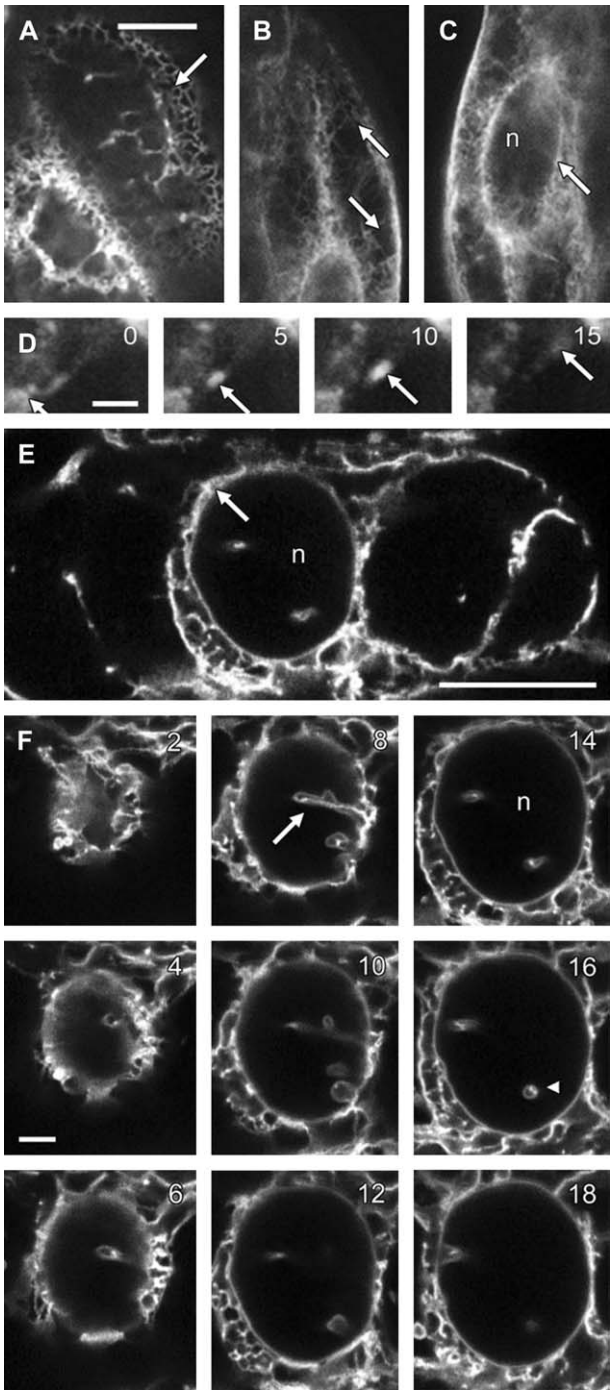

Fig. 1. The organization and dynamics of ER in interphase cells. (**A**–**D**) Spinning disk confocal optical sections showed polygonal arrays of ER in the outer cortex (**A**, arrow), transvacuolar ER strands that linked the nucleus (n) to the cortex (**B**, arrows), and links between the nuclear envelope and transvacuolar strands (**C**, arrow). **D** Sequential confocal images over 15 s showed ER movement in a transvacuolar strand (arrow). (**E**–**F**) Laser scanning confocal images showing nuclear morphology. **E** A low magnification confocal optical section through a centrally located nucleus (n) with a clear nuclear envelope (arrow). **F** Higher magnification optical sections through only the nucleus, shown at 2 μm intervals from the nuclear surface, reveal an invagination (arrowhead) and a channel that extends across the nucleus and which contains internal structure (arrow). Bar in **A** = 10 μm for **A**–**C**; bar in **D** = 5 μm; bar in **E** = 10 μm; bar in **F** = 5 μm.