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SHORT COMMUNICATION

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Disruption of actin filaments delays accumulation of cell plate membranes after chromosome separation

Keisho Maeda[a] and Takumi Higaki[b]

[a]Graduate School of Science and Technology, Kumamoto University, Kumamoto, Japan; [b]International Research Organization for Advanced Science and Technology, Kumamoto University, Kumamoto, Japan

ABSTRACT

Phragmoplasts, which comprise microtubules, actin filaments, and membrane vesicles, are responsible for cell plate formation and expansion during plant cytokinesis. Our previous research using the actin polymerization inhibitor latrunculin B (LatB) to investigate the role of actin filaments suggested the existence of two types of microtubules: 1) initial microtubules sensitive to LatB but unassociated with NACK1 kinesin and 2) later LatB-insensitive, NACK1-associated microtubules. The organization of initial phragmoplast microtubules might have been disrupted by the LatB treatment; this hypothesis remained unverified, however, as the exact timing of cell plate membrane accumulation could not be determined. In the present study, we further investigated the timing of cell plate formation during LatB treatment. We monitored chromosome separation during anaphase as well as accumulation of FM4-64-stained cell plate membranes in dividing transgenic tobacco BY-2 cells expressing RFP-tagged histone H2B. We observed that LatB treatment prolonged the time between the slowdown of daughter chromosome migration and the accumulation of cell plate membranes. This result suggests that disruption of actin filaments resulted in delayed cell plate formation possibly by perturbation of initial phragmoplast microtubules or cell plate assembly.

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Plant cytokinesis is achieved by centrifugal expansion of the cell plate from the cell center to the parental cell wall. Cell plate formation and expansion are driven by the phragmoplast, a cell division-specific intracellular structure composed of microtubules, actin filaments, and membrane vesicles.[1,2] Phragmoplast microtubules, which are oriented with their plus-ends toward the cell plate,[3,4] are the main contributors to cell plate formation and expansion, being used for directional transport of Golgi-derived vesicles containing cell plate materials toward the equatorial planes. In contrast, the role of phragmoplast actin filaments has been unclear. We recently observed that disruption of actin filaments with an actin polymerization inhibitor, latrunculin B (LatB), perturbed midzone constriction of phragmoplast microtubules and caused dispersion of cell plate vesicles at the early phase of cytokinesis in tobacco BY-2 cells.[5] Interestingly, we also determined that LatB treatment shortened the lag time in equatorial planes between the accumulation of cell plate membranes and that of green fluorescent protein-tagged NACK1 kinesin, which plays a crucial role in cell plate expansion.[5] From these observations, we hypothesized the existence of two distinct phragmoplast microtubules: LatB-sensitive initial phragmoplast microtubules not associated with NACK1 and later LatB-insensitive, NACK1-associated microtubules.[5] In other words, LatB treatment might perturb the organization of the initial phragmoplast microtubules, thus causing the normally later-generated phragmoplast microtubules to appear first. We were unable, however, to determine the exact timing of accumulation of LatB-induced dispersed cell plate membranes, which might be driven by the later phragmoplast microtubules.

In this study, we focused on chromosome separation as a benchmark for the start of anaphase. To visualize chromosomes, transgenic tobacco BY-2 cells stably expressing red fluorescent protein-tagged histone H2B (Histone-RFP) were established as previously reported.[6] The cell cycle of the transgenic BY-2 cells was synchronized with aphidicolin, an inhibitor of DNA polymerase alpha,[7] and the cell plate membranes were vitally stained with the fluorescent dye FM4-64, an endocytic marker.[8] Using a spinning disk confocal microscope, we captured fluorescence signals from Histone-RFP and FM4-64 in dividing BY-2 cells either untreated or subjected to LatB treatment (2.5 μM for 60–90 min before observations) (Figure 1a). Because some of the Histone-RFP fluorescence leaked into the FM4-64 channel in our optical setting, the FM4-64-specific signals were identified by comparison of both channel images (Figure 1a,b). We thought it possible that LatB treatment might alter FM4-64 uptake and cell plate labeling, therefore we first checked the FM4-64 fluorescence intensity in the cell plate of cells treated with DMSO or LatB (Figure 1c). No significant differences in FM4-64 fluorescence intensity were observed in the cell plate 5 min after cell plate formation, suggesting that FM4-64 can be useful to monitor the cell plate membranes even in LatB-treated cells (Figure 1c). Time-sequential observations at 30-s intervals and kymograph analysis revealed that LatB treatment significantly prolonged the time from chromosome separation to cell plate membrane accumulation (Figure 1b,d). This result suggested two possibilities: LatB treatment (1) decreased the speed of daughter chromosome migration and/or (2) delayed the post-migration accumulation of cell

CONTACT Takumi Higaki thigaki@kumamoto-u.ac.jp International Research Organization for Advanced Science and Technology, Kumamoto University, Kumamoto 860-8555, Japan.

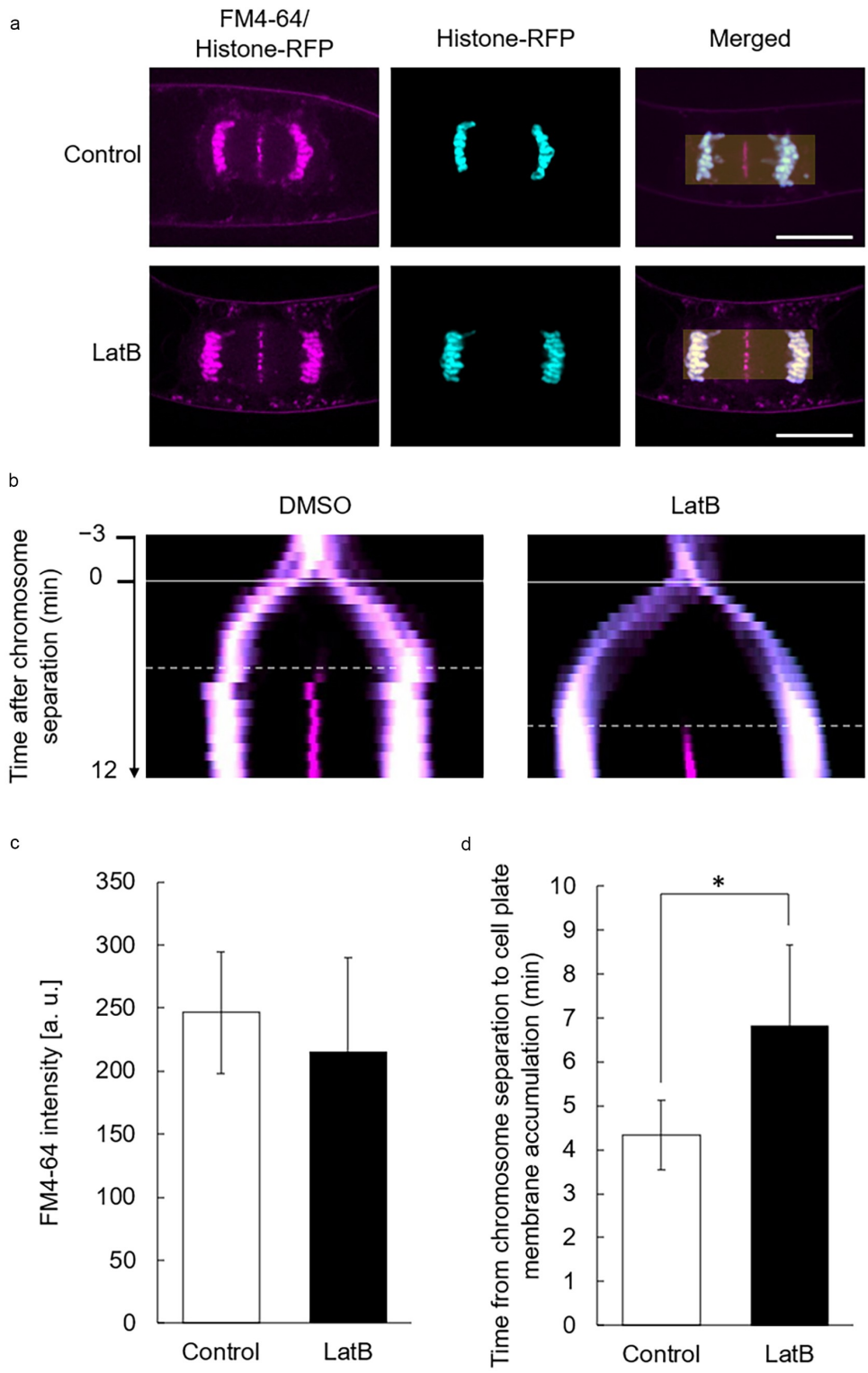

Figure 1. Time from chromosome separation to cell plate membrane accumulation. (a) Representative images of dividing tobacco BY-2 cells stably expressing RFP-tagged histone H2B (Histone-RFP) in cell plates stained with FM4-64. DMSO control (top) and LatB-treated cells are shown. Some of the Histone-RFP fluorescence leaked into the FM4-64 channel in our optical setting. Yellow lines (10 μm width) shown on the merged images were used for making kymographs. Scale bars = 10 μm. (b) Kymographs of (a). White solid and dashed lines indicate time points of chromosome separation and the onset of accumulation of FM4-64-labeled cell plate membranes, respectively. (c) FM4-64 fluorescence intensities in the cell plate. The intensities were calculated as the FM4-64 peak fluorescence intensities at 5 min after the onset of FM4-64 accumulation minus the background intensities in the kymographs. Note that significant differences were not detected. (d) Effects of LatB treatment on the time from chromosome separation to cell plate membrane accumulation. Data are presented as means ± SD (n = 10–12). Significance was determined using Mann–Whitney's U-test (*, $p < .01$).

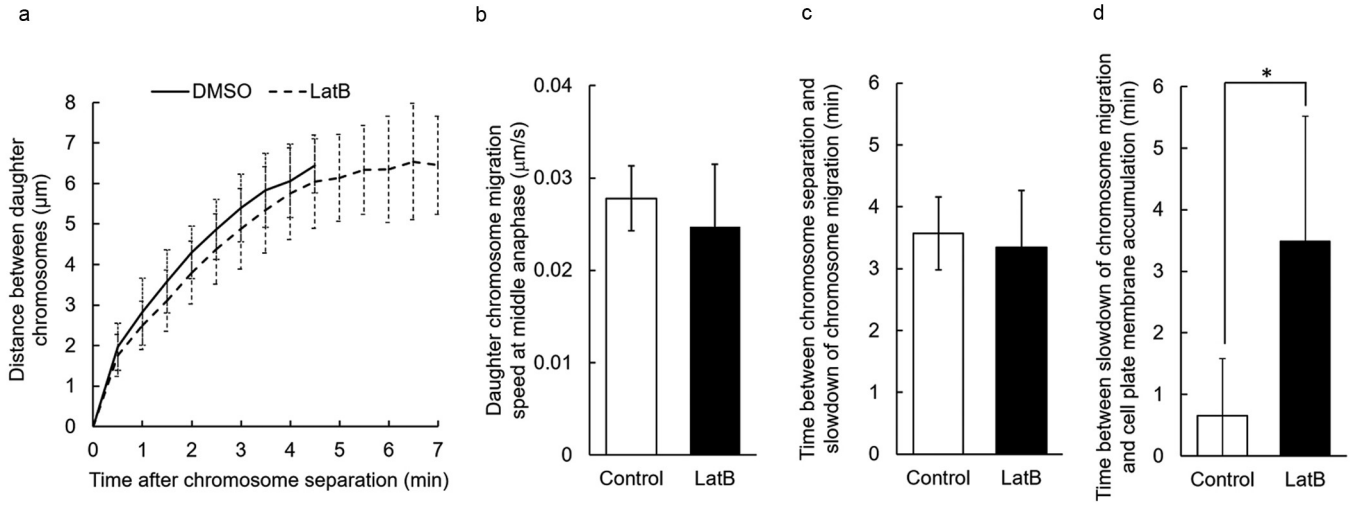

Figure 2. Time between the slowdown of daughter chromosome migration and the accumulation of cell plate membranes. (a) Changes in the distance between daughter chromosomes after chromosome separation in cells treated with the DMSO control (solid line) or LatB (dashed line). Data are presented as means ± SD ($n = 9$). (b) Daughter chromosome migration speed at middle anaphase. Data are presented as means ± SD ($n = 9$). (c) Effects of LatB treatment on the time between chromosome separation and the slowdown of chromosome migration. Data are presented as means ± SD ($n = 9$). (d) Effects of LatB treatment on the time between the slowdown of chromosome migration and cell plate membrane accumulation. Data are presented as means ± SD ($n = 9$). Significance was determined using Mann–Whitney's *U*-test (*, $p < .01$).

plate membranes. To clarify this issue, we attempted to define the ending point of daughter chromosome migration (i.e., the time when the speed of chromosome migration was reduced). We then plotted distances between daughter chromosomes using kymographs and analyzed the speed of daughter chromosome migration (Figure 2a). Although their migration slowed over time, daughter chromosomes did not completely cease migrating until FM4-64-labeled cell plate membranes accumulated (Figure 2a). We therefore defined the time point of daughter chromosome migration slowdown to be the moment when the daughter chromosome migration speed was half that observed at middle anaphase (30–60 s after chromosome separation). At middle anaphase, we observed no significant differences in the speed of daughter chromosome migration between cells treated with DMSO or LatB (Figure 2b). In addition, similar times were observed between chromosome separation and slowdown of daughter chromosome migration in control and LatB-treated cells (Figure 2c), which suggested that actin filament disruption did not affect the speed of daughter chromosome migration. This result is reasonable given that fewer actin filaments have been observed in mitotic spindles.[5,8,9] In contrast, the time between slowdown of daughter chromosome migration and accumulation of FM4-64-labeled cell plate membranes was significantly prolonged by LatB treatment (Figure 2d). This observation supports our hypothesis that actin filament disruption interrupts the formation of initial phragmoplast microtubules[5] and also suggests that the timing of the appearance of initial and later phragmoplast microtubules is strictly controlled by the cell cycle. Mechanisms of actin filament-mediated initial phragmoplast microtubule organization and cell plate membrane accumulation are still largely unknown. At present, we cannot deny the possibility that actin filament disruption may indirectly alter initial phragmoplast microtubule organization by inhibiting cell plate assembly. Future high temporal- and spatial-resolution multi-color imaging of actin filaments, microtubules, and cell plate membranes during the cell cycle transition from anaphase to telophase would clarify their relationships.

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Disclosure of potential conflicts of interests

The authors declare that they have no competing interests.

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ORCID

Takumi Higaki http://orcid.org/0000-0002-1379-3930

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