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Research

Methods

Establishment of a mitotic model system by transient expression of the D-type cyclin in differentiated leaf cells of tobacco (*Nicotiana benthamiana*)

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Summary

- Investigations of plant cell division would greatly benefit from a fast, inducible system. Therefore, we aimed to establish a mitotic model by transiently expressing D-type cyclins in tobacco leaf cells.
- Two different D-type cyclins, CYCD3;1 and CYCD4;2 from *Arabidopsis thaliana*, were expressed by agrobacterial infiltration in the cells of expanded leaves in tobacco (*Nicotiana benthamiana*). Leaf pavement cells were examined after cyclin expression while target and reference (histone or tubulin) proteins were marked by fluorescent protein-tagging.
- Ectopic expression of the D-type cyclin induced pavement cells to re-enter cell division by establishing mitotic microtubule arrays. The induced leaf cells expressed M phase-specific genes of *Arabidopsis* encoding the mitotic kinase AtAurora 1, the microtubule-associated proteins AtEDE1 and AtMAP65-4, and the vesicle fusion protein AtKNOLLE by recognizing their genomic elements. Their distinct localizations at spindle poles (AtAurora1), spindle microtubules (AtEDE1), phragmoplast microtubules (AtMAP65-4) and the cell plate (AtKNOLLE) were indistinguishable from those in their native *Arabidopsis* cells. The dividing cells also revealed two rice (*Oryza sativa*) microtubule-associated proteins in the phragmoplast and uncovered a novel spindle-associated microtubule motor protein.
- Hence, this cell division-enabled leaf system predicts hypothesized cell cycle-dependent functions of heterologous genes by reporting the dynamics of encoded proteins.

Introduction

Plant growth is brought about by sustained cell division and cell expansion in response to internal and external cues. Plants, like other eukaryotes, employ the evolutionarily conserved cyclin-dependent kinase (CDK) and cyclin family proteins to drive phase-to-phase progression during the cell division cycle (Polyn *et al.*, 2015). The D-type cyclin (CYCD) activates CDKA for the G1-to-S-phase transition, as demonstrated by CYCLIN D3;1 (CYCD3;1) in *Arabidopsis thaliana* (Menges *et al.*, 2006). Stable over-expression of CYCD3;1 converts unicellular leaf trichomes into multicellular ones in *A. thaliana* (Schnittger *et al.*, 2002). Conversely, mutants lacking multiple CYCD3 genes produce organs with decreased cell numbers due to reduced cell division (Dewitte *et al.*, 2007). A well-accepted principle is that CYCD3;1, like most D-type cyclins, contains a retinoblastoma (Rb)-binding site so that it targets CDKA to phosphorylate Rb and consequently the transcription factor E2F is liberated from the Rb inhibition in order to promote cell cycle progression (Polyn *et al.*, 2015). Plants also produce an atypical group of CYCD4 that lacks the Rb-binding site but still has a promotive function for cell division, as shown by the stable expression of CYCD4;2 in *A. thaliana* (Kono *et al.*, 2006). However, mitotic events in such CYCD-induced cell division have not been analyzed at the subcellular level other than their terminal phenotypes. On the other hand, it is unclear whether stable transformation is required for the induction of cell division by either CYCD3;1 or CYCD4;2.

To understand the molecular mechanisms that regulate plant cell division, we aimed to examine intracellular events that take place in specific locations at specific times after cell division is triggered. However, investigations of plant cell division often face the challenge of lacking a rapid, inducible experimental system. Because proteins that regulate the cell division cycle usually turnover rapidly and exhibit high dynamics, plant cell biologists have been thirsting for such a system that could allow live-cell imaging with relative ease. In young seedlings, cell division takes place primarily in root and shoot apical meristems. Because dividing cells are often enclosed inside tissues, they are not

conveniently accessible by popular fluorescence microscopic techniques. Alternatively, mitosis may be examined in meristematic cells after they are fixed. Ideally, experiments could greatly benefit from having plant cells that can be induced to divide at a high frequency in order to record protein dynamics during cell division by live-cell imaging. In the past, cultured tobacco BY-2 cells have been successfully synchronized by sequentially inhibiting DNA replication at S phase and depolymerizing microtubules (MTs) at M phase (Van Damme *et al.*, 2004; Kumagai-Sano *et al.*, 2006). However, it can be a challenging task for many laboratories to maintain synchronization-competent BY-2 cells and to prepare highly synchronized cells. Also, these experiments in BY-2 cells, like those in *A. thaliana*, require transformation and establishment of stable lines before microscopic observations, which would take months if not longer. Another concern is whether genetic abnormalities accumulated over time could cause problems in gene expression in systems such as BY-2.

In this study, we aimed to establish a mitotic system by testing the hypothesis that transient ectopic expression of D-type cyclin could induce mitosis in differentiated cells. As a result, we report a fast and simple but effective approach to induce differentiated leaf cells to undergo mitotic cell division in the robust tobacco (*Nicotiana benthamiana*) system that allowed us to perform live-cell imaging experiments to record protein dynamics during mitotic cell division.

Materials and Methods

Recombinant DNA techniques and plasmid construction

Amplification of DNA fragments was carried out by using the Phusion DNA polymerase (Thermo Fisher, Waltham, MA, USA). The PCR templates were an *A. thaliana* cDNA preparation for cloning of *AtCYCD3;1* and *AtCYCD4:2*, genomic DNA for histone H1.2 and other target genes described in the text, and a rice (*O. sativa* subsp. *japonica*) genomic DNA preparation for OsMAP65-3a and OsMAP65-3b (Guo *et al.*, 2009). The Gateway cloning technology (Thermo Fisher) was applied in plasmid construction. The primers used in DNA amplification are listed in Supporting Information Table S1. The resulting PCR products were first cloned into the vector pDONR221 by BP Clonase (Thermo Fisher), and the resulting pENTR plasmids were recombined with the pGWB series destination vectors (Nakagawa *et al.*, 2007a,b) by LR Clonase (Thermo Fisher). The plasmids used in this study are listed in Table S2. Before agrobacterial infiltration into leaves of *N. benthamiana*, plasmids were transformed into the *Agrobacterium tumefaciens* strain GV3101.

Agrobacterial infiltration to tobacco leaves

Tobacco (*N. benthamiana*) plants were grown in the growth chamber at 25°C with a 16-h : 8-h, light : dark photoperiod. Wild-type and green fluorescent protein (GFP)-α-tubulin (TUA6) (Gillespie *et al.*, 2002) plants were used in the experiments. The first to the third fully expanded true leaves of 4-wk-old plants were used for the infiltration experiment. Before infiltration, GV3101 agrobacteria transformed by plasmids were grown to stationary phase in liquid LB medium supplemented with respective antibiotics. They were then collected by centrifugation and resuspended in infiltration buffer of 10 mM MES (pH 5.6) supplemented with 10 mM $MgCl_2$ and 150 μM acetosyringone at an OD_{600} of 1.0. For co-infiltrations, agrobacterial cells containing different plasmids were mixed in equal volumes. Agrobacteria expressing the p19 viral protein, which functioned as a repressor of gene silencing (Silhavy *et al.*, 2002), was always included in infiltration experiments although not essential. After an agrobacterial mix was incubated for 3 h at room temperature, a small lesion on the abaxial side of a leaf was created by a needle, and the agrobacterial suspension was delivered into the extracellular space of the leaf through the lesion by using a blunt-tipped plastic syringe and applying gentle pressure. After infiltration, the plants were further grown in the 25°C growth chamber until observation by fluorescence microscopy.

Microscopic observation

Leaf samples were taken 36–48 h after agrobacterial infiltration when there was a copious number of cells undergoing mitosis. For examination of epidermal cells at the abaxial side of tobacco leaves, segments were sliced, mounted in double distilled H_2O, and observed under an Axio Observer inverted microscope equipped with the LSM710 laser scanning confocal module (Zeiss, White Plains, NY, USA). Cells were viewed by using a 40× C-Plan (water) objective, the GFP and red fluorescent protein (RFP)/TagRFP signals were excited by 488- and 561-nm lasers, respectively, and images were acquired with the ZEN software package (Zeiss) and processed in IMAGEJ (https://imagej.nih.gov/ij/).

Results and Discussion

Mitotic cell division in tobacco leaf cells following ectopic cyclin D expression

To test whether transient expression of the D-type cyclin could induce differentiated leaf cells of tobacco (*N. benthamiana*) to enter the cell division cycle, AtCYCD3;1 and AtCYCD4;2 from *A. thaliana*, chosen based on previous studies (Schnittger *et al.*, 2002; Kono *et al.*, 2006), were expressed under control of the viral 35S promoter in leaf cells by agrobacterial infiltration. The protein was localized in the nucleus when expressed in TagRFP fusions (data not shown). In later experiments, untagged cyclins were expressed so that target proteins could be tagged in red color. To monitor cell cycle progression, we used a line stably expressing a GFP-TUA6 fusion protein (Gillespie *et al.*, 2002) and had chromosomes labeled by AtHistone H1.2-TagRFP upon transient expression. When either AtCYCD3;1 or AtCYCD4;2 was expressed, the fully expanded pavement cells on the leaf epidermis, as marked by elaborate lobes outlined by green fluorescent signals, entered mitosis and exhibited the spindle and phragmoplast MT arrays coupled with respective chromosome configurations (Fig. 1a,b). This result indicated that the CDK protein(s) in differentiated leaf cells had been activated by either cyclin, regardless of its ability to target at the Rb protein. Upon

AtCYCD3;1 expression, pavement cells established the MT preprophase band (Fig. 1c). Mitotic progression led to rapid polymerization of MTs on the nuclear envelope at later stages of prophase (Fig. 1d). We were able to record MT reorganization, as evidenced by distinct MT arrays from establishing bipolar spindle arrays with chromosomes aligned at the metaphase plate and separated at anaphase to the expanding phragmoplast MT array coupled with the reformation of daughter nuclei (Fig. 1d). Hence, these cells actively entered the mitotic cell division cycle and completed all phases (Video S1). By contrast, the lobed pavement cells never entered the cell division cycle when AtHistone H1.2 or an MT marker was expressed alone (Fig. 1a).

We examined the frequency of epidermal cells with mitotic figures following agrobacterial infiltration. Compared to 24 h after infiltration when very few cells showed mitotic figures, at 36 h *c.* 8% and 4% of cells exhibited mitotic MT arrays upon AtCYCD3;1 or AtCYCD4;2 expression, respectively (Fig. 2a). The frequencies peaked at 48 h after infiltration and AtCYCD3;1 consistently rendered higher frequencies than AtCYCD4;2 (Fig. 2a). Therefore, our later experiments used AtCYCD3;1 expression and the results summarized hereafter were based on the ectopic expression of this protein. Seventy-two hours after agrobacterial infiltration, most if not all leaf pavement cells had already completed CYCD-induced cell division as marked by mature cell plates, compared to the control pavement cells that never re-entered cell division (Fig. 2b,c). The cell plate was formed mostly if not all at the neck of the pavement cells after entering division (Fig. 2c). Therefore, placement of the cell plate largely followed Errera's rule of minimizing the surface area of the cell plate, perhaps with modifications of a probability distribution as reported by Besson & Dumais (2011). We named this expression scheme CDELS for the Cell Division-Enabled Leaf System.

Expression of cell cycle-dependent genes in CDELS

The CDELS was first used to test whether it would induce the expression of mitotic genes from *A. thaliana.* In our experiments, we only selected genes that are known to exhibit cell cycle-dependent expression patterns. The mitotic Aurora kinase gene in *A. thaliana*, *AtAurora 1* (*AtAUR1*), exhibits a G2/M-specific expression pattern, and the AtAUR1 protein shows a spindle pole-biased association with MTs and plays an essential role in spindle formation and cell division plane orientation (Van Damme *et al.*, 2011). When a genomic construct of *AtAUR1* was tested along with AtCYCD3;1, we found that the GFP-AtAUR1 fusion protein was expressed and decorated spindle MTs, as

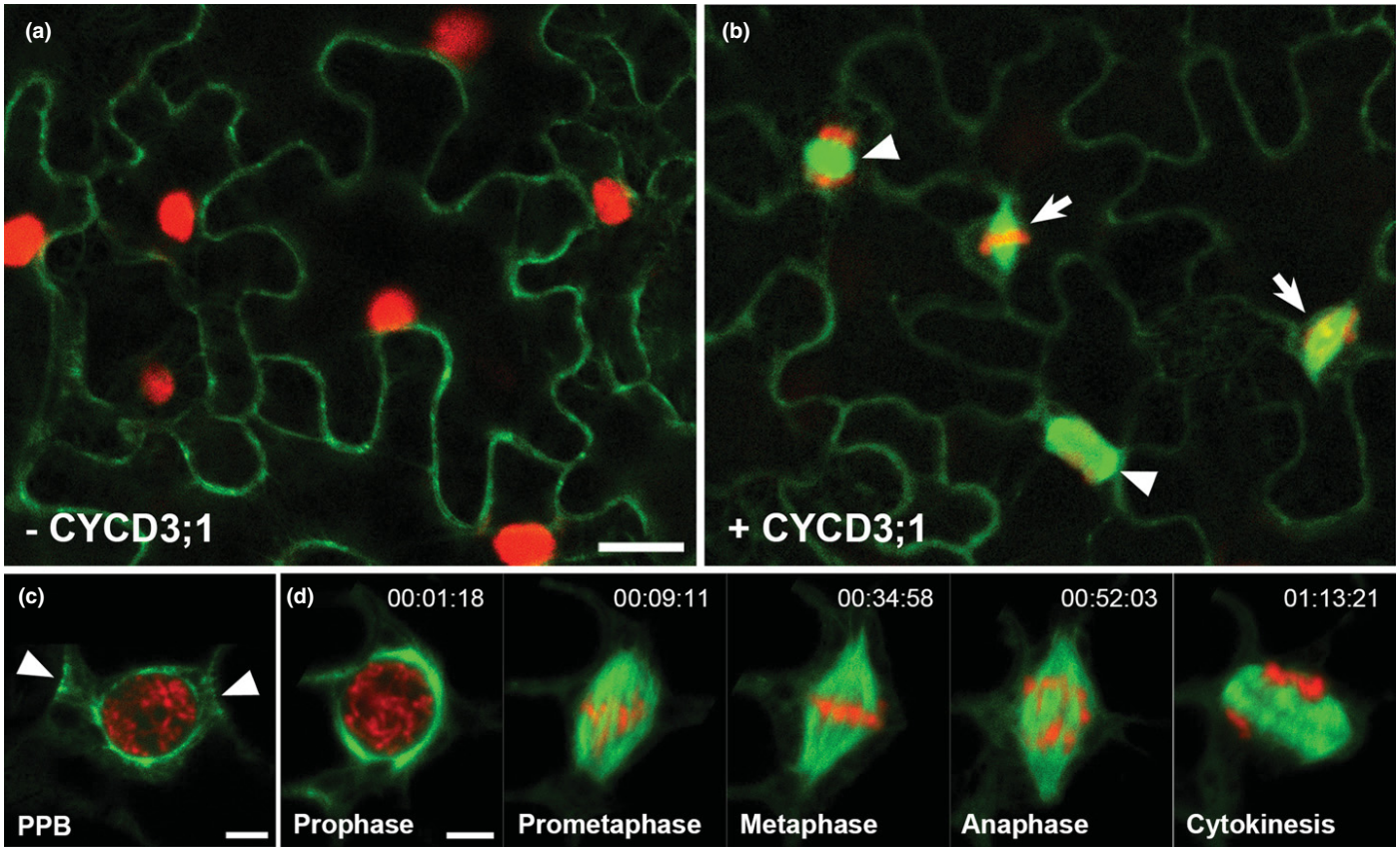

Fig. 1 Transient expression of CYCD triggers tobacco pavement cells to divide. Microtubules are marked by GFP-TUA6 (green) and nuclei highlighted by AtHistone H1.2-TagRFP (red). (a) Differentiated leaf pavement cells are outlined by microtubules at the cell cortex and contain interphase nuclei without ectopic CYCD expression (red). (b) Upon AtCYCD3;1 expression, leaf pavement cells enter mitotic cell division. Two spindles (arrows) and two phragmoplasts (arrowheads) have microtubules in green and chromatin in red. (c) An induced pavement cell forms a preprophase microtubule band (arrowheads). (d) Snapshots of Supporting Information Video S1 showing microtubule arrays (green) and chromosomes (red) from late prophase to cytokinesis. The time stamps are shown in h : min : s. Bars: (a,b) 20 μm; (c,d) 5 μm.

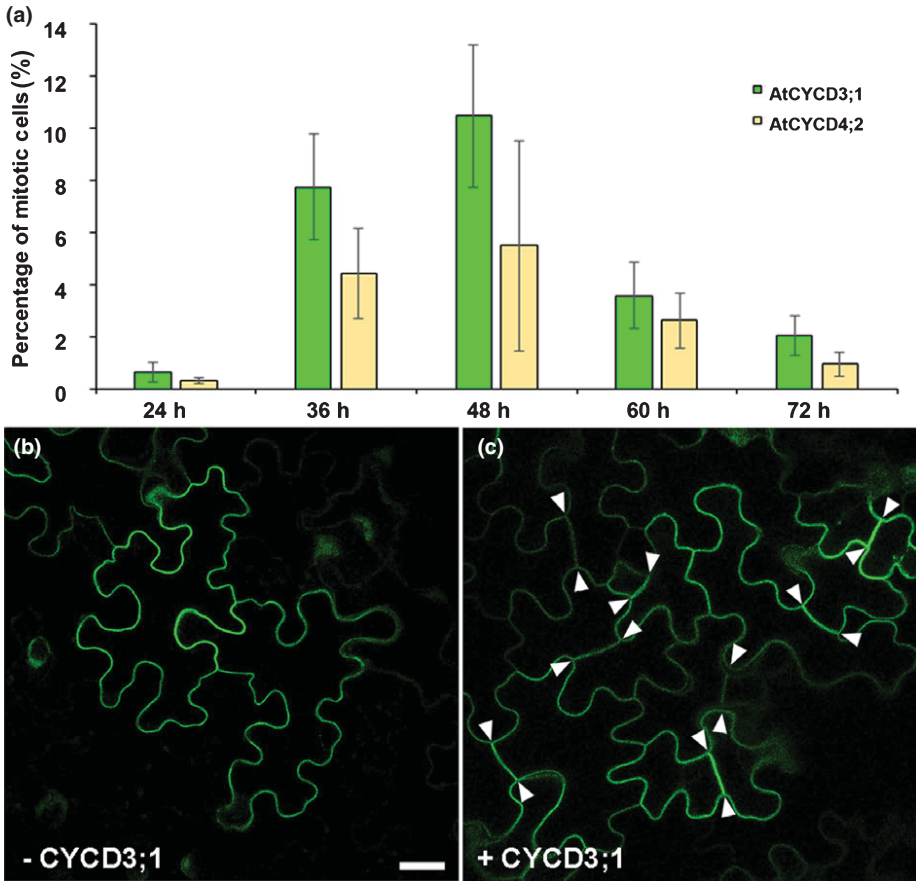

Fig. 2 Pavement cells complete cell division upon AtCYCD3;1 expression in *Nicotiana benthamiana*. (a) Frequencies of pavement cells at mitosis upon AtCYCD3;1 or AtCYCD4;2 expression. Samples were examined every 12 h starting at 24 h after agrobacterial infiltration. Twelve independent samples were examined at each time point and 275 cells were counted for each sample. At the five time points of 24, 36, 48, 60 and 72 h, cells expressing AtCYCD3;1 comprised 0.6 ± 0.3%, 7.8 ± 2.0%, 10.5 ± 2.7%, 3.6 ± 1.3% and 2.1 ± 0.7%, and those expressing AtCYCD4;2 comprised 0.3 ± 0.1%, 4.4 ± 1.7%, 5.5 ± 4.0%, 2.6 ± 1.1% and 1.0 ± 0.5% at mitosis, respectively. (b) Mature pavement cells establish sophisticated lobes, highlighted by cell wall autofluorescence, after differentiation and remain in the differentiated state without expression of CYCD3;1. (c) Seventy-two hours after infiltration of agrobacteria, most if not all pavement cells have already completed cell division as marked by cell plates (paired arrowheads) after CYCD3;1 expression. Bar, 20 μm.

viewed by the transiently expressed MT marker RFP-AtCKL6 CTD (Ben-Nissan *et al.*, 2008), by concentrating at spindle poles in a pattern indistinguishable from that in native cells (Fig. 3a–c). Because of the significantly larger spindles in tobacco cells than in *A. thaliana*, we were able to visualize the intracellular localization pattern of proteins such as AtAUR1 in greater detail by focusing on individual mitotic apparatus. We also examined the augmin complex, which acts along spindle MTs to generate new MTs for spindle MT reorganization during mitosis (Lee & Liu, 2019). It was reported that augmin's function in mitosis is defined by its mitotic-specific, MT-associated protein subunit AtEDE1 (Endosperm Defective1) (Pignocchi *et al.*, 2009; Lee *et al.*, 2017). When it was tested upon CYCD3;1 induction, its expression also was activated in tobacco cells and AtEDE1-GFP associated with spindle MTs (Fig. 3d–f), resembling its localization when expressed in *A. thaliana* (Lee *et al.*, 2017).

Furthermore, we tested two genes that act at later stages of mitotic division, encoding the MT-associated protein AtMAP65-4 and the vesicle fusion SNARE protein AtKNOLLE (SYP111) which functions in cell plate formation, using their respective genomic constructs (Enami *et al.*, 2009; Li *et al.*, 2017). While AtMAP65-4-GFP decorated MT bundles in the phragmoplast midzone, GFP-AtKNOLLE exclusively marked the developing cell plate (Fig. 3g–l), recapitulating their localizations in native cells. Among the genes tested above, *AtEDE1* and *AtKNOLLE* contain the mitosis-specific activator (MSA) element in their promoters, and their expression is regulated by the MYB3R class transcription factors (Haga *et al.*, 2011). Therefore, such an MSA-dependent regulatory mechanism was actively functioning when the genes of *Arabidopsis* were expressed in these heterologous tobacco cells.

Cell cycle-dependent expression of an artificial gene

We also tested whether we could artificially induce cell cycle-dependent gene expression by adopting a promoter of genes tested above. To do so, we used a construct having the *AtAUR1* promoter drive the expression of TagRFP-MBD (MT-binding domain), which has been shown to label mitotic but not interphase MT arrays after stable transformation in *A. thaliana* (Marhavy *et al.*, 2016). When this construct was delivered into leaf cells together with AtCYCD3;1 by agrobacteria, TagRFP-MBD was expressed only in dividing cells but not interphase cells and uniformly

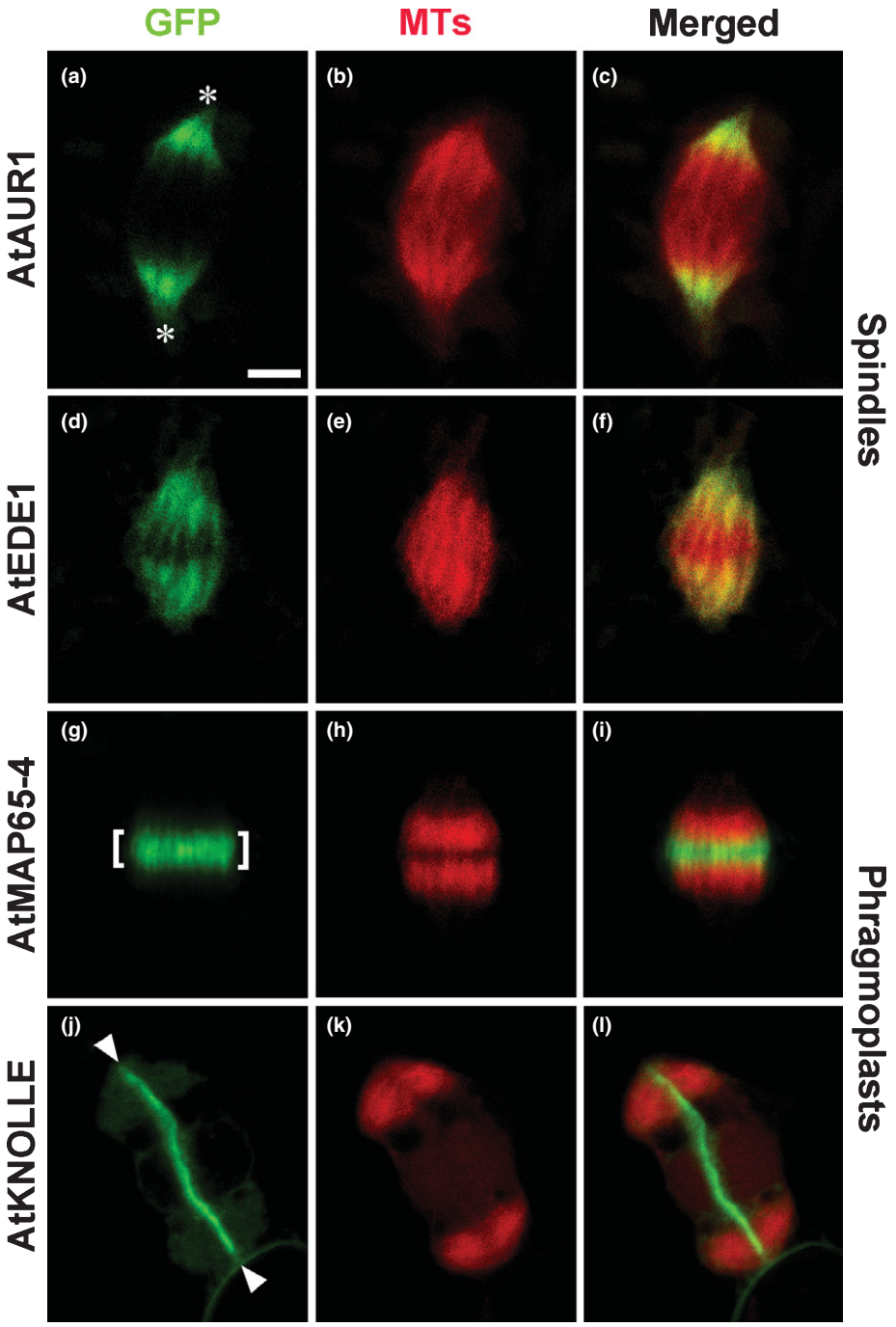

Fig. 3 Expression of mitotic genes from *Arabidopsis thaliana* in tobacco pavement cells. Four mitotic proteins were co-expressed in fusions of GFP (green) with *AtCYCD3;1* in leaf pavement cells. Microtubules (MTs) are marked by RFP-AtCKL6 CTD (red). They exhibit their distinct localization patterns during mitosis and cytokinesis. (a–c) GFP-AtAUR1 localizes to spindle microtubules by concentrating at spindle poles (asterisks). (d–f) AtEDE1-GFP associates with spindle microtubules with a bias towards the spindle poles as well but exhibits a broader distribution pattern than AtAUR1. (g–i) AtMAP65-4-GFP binds to microtubule bundles in the midzone of the developing phragmoplast (square brackets). (j–l) GFP-AtKNOLLE is concentrated at the developing cell plate during cytokinesis (arrowheads). Bar, 5 μm.

detected on the spindle MT array (Fig. S1). Therefore, we concluded that the promoters such as that of *AtAUR1* were sufficient to warrant cell cycle-dependent gene expression in the CDELS artificially. This finding paved the way for the delivery of factors that disturb MT dynamics, (e.g in the CDELS) to uncover how the cytoskeletal network reorganizes during the cell division cycle.

Cell cycle-dependent expression of monocot genes in tobacco leaf cells

We also assessed whether the CDELS could allow the ectopic expression of genes of the monocot rice (*Oryza sativa*) in a cell cycle-dependent manner. We used AtMAP65-3 as a reference as it is associated explicitly with MT plus ends in the phragmoplast (Ho *et al.*, 2011). The AtMAP65-3-TagRFP fusion protein exhibited a localization pattern indistinguishable from that in *A. thaliana* (Fig. 4a–c). Phylogenetic analysis of the MAP65 family proteins identifies two rice proteins that have a close homology to AtMAP65-3 (Guo *et al.*, 2009). To test whether these OsMAP65-3a and OsMAP65-3b behaved as AtMAP65-3, we made expression vectors based on their native genomic sequences of exons, introns and predicted promoters. We found that both OsMAP65-3a and OsMAP65-3b were expressed in M phase and their TagRFP fusion proteins decorated the phragmoplast MTs towards the plus ends in

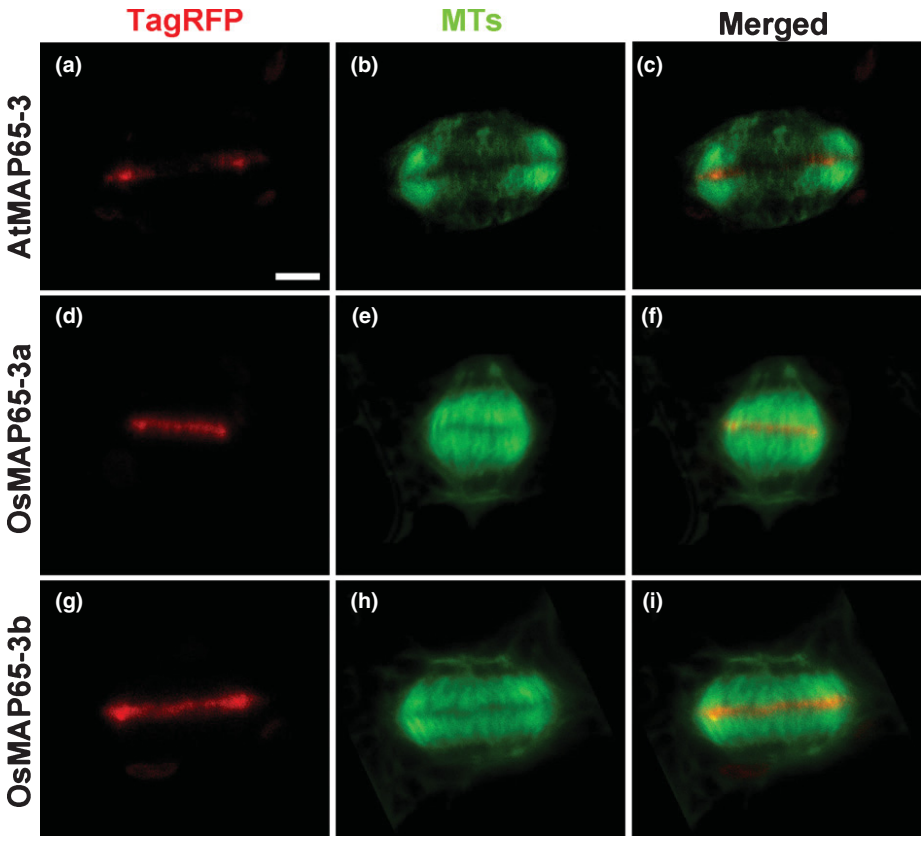

Fig. 4 Two MAP65-3 proteins from *Oryza sativa* exhibit identical localization patterns as AtMAP65-3 in tobacco cells. MAP65 proteins are expressed in TagRFP fusions (red) while microtubules (MTs) are labeled by GFP-TUA6 (green). (a–c) As a control, AtMAP65-3-TagRFP decorates the phragmoplast midline at or near the plus ends of phragmoplast microtubules during cytokinesis. (d–i) Both the OsMAP65-3a-TagRFP and the OsMAP65-3b-TagRFP fusion proteins exhibit identical localization patterns on phragmoplast microtubules as AtMAP65-3-TagRFP in induced leaf pavement cells expressing AtCYCD3;1. Bar, 5 μm.

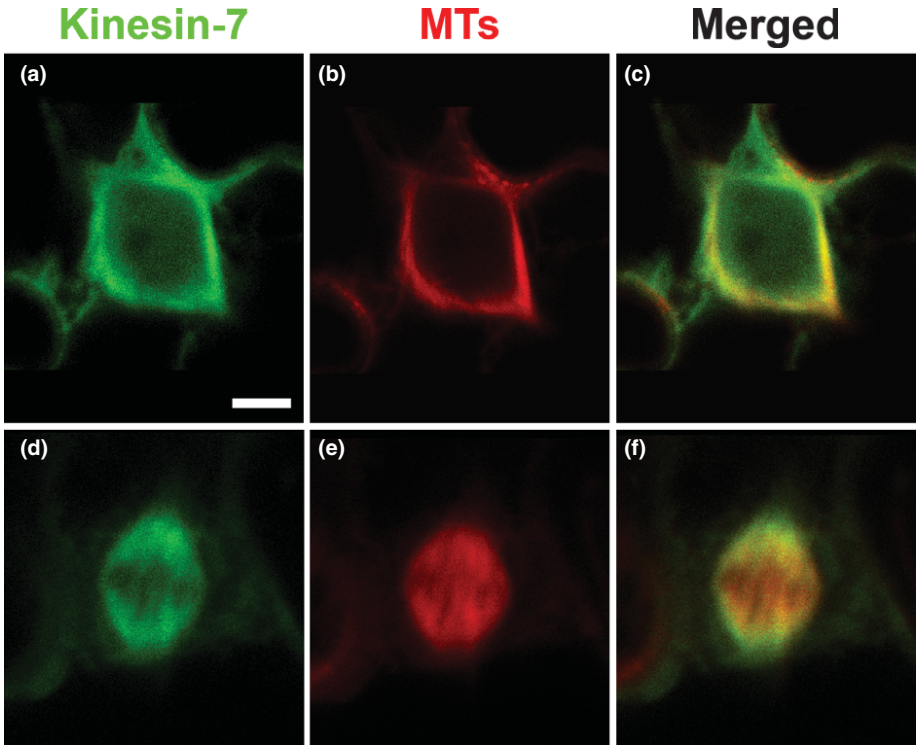

Fig. 5 Association of AtKinesin-7 with spindle microtubules. (a–c) AtKinesin-7-GFP (green) localizes to microtubules (MTs) marked by RFP-AtCKL6 CTD (red) formed on the nuclear envelope in a prophase cell. (d–f) AtKinesin-7-GFP is associated with spindle MTs with stronger signals towards poles than in the spindle midzone. Bar, 5 μm.

patterns indistinguishable from that of AtMAP65-3 (Fig. 4d–i). These results support the idea that the two homologous proteins may function redundantly in the organization of phragmoplast MTs in rice for cytokinesis as its *Arabidopsis* counterpart. Because typically it is more challenging to generate transgenic lines and to carry out live-cell imaging in monocot species such as rice when compared to *A. thaliana*, this finding could facilitate the investigation of cell division-related proteins from monocots.

Detection of a novel motor protein in mitotic spindles

In plant cells, directional transport along the MT tracks takes place in both interphase and dividing cells and is powered by MT-based motors collectively named kinesins (Lee *et al.*, 2015). An earlier gene expression analysis predicted that some are mitotically activated (Vanstraelen *et al.*, 2006). However, their localization is often unknown, so to date, functions of most mitotic kinesins remain unclear. We applied the CDELS to test a putative mitotic kinesin corresponding to the At1g59540 locus in the *A. thaliana* genome, which contains the MSA element in its promoter and exhibits an elevated expression pattern during mitosis (Vanstraelen *et al.*, 2006). This kinesin belongs to the Kinesin-7 subfamily, also known as Centromere protein-E (CENP-E). Kinesin-7/CENP-E motors in animals act at the kinetochores for chromosome congression by mediating the interaction between the kinetochore and spindle MTs (Maiato *et al.*, 2017). To date, only one Kinesin-7 motor has been detected at the kinetochores in the moss *Physcomitrella patens* (Miki *et al.*, 2014). However, whether Kinesin-7 also functions at the kinetochores during cell division in flowering plants remains unknown. We therefore tested whether this Kinesin-7 behaved like its animal or moss counterparts. Upon mitotic induction by CYCD3;1, this Kinesin-7 protein was expressed as a GFP fusion in actively dividing cells under control of its native promoter (Fig5), in line with the transcriptional evidence. We found that the AtKinesin-7-GFP fusion protein colocalized with MTs in the prophase spindle before the nuclear envelope broke down (Fig. 5a–c). When a typical metaphase spindle was established, the protein still associated with spindle MTs with its signal being stronger towards spindle poles (Fig. 5d–f). Therefore, we concluded that AtKinesin-7 did not behave like CENP-E during mitosis based on its localization pattern. Nevertheless, the intracellular localization of AtKinesin-7 further prompted us to investigate its mitotic function. This result also serves as an example of proving mitotic functions of genes that are suggested by transcriptional analysis.

Several transient expression methods have been successfully developed in cultured cells, leaves, hairy roots and young seedlings for the investigation of cytoskeletal dynamics and/or cell division with their respective advantages (Li & Nebenfuhr, 2010; Buschmann *et al.*, 2011; Ron *et al.*, 2014; Buschmann, 2016; Jimenez-Gongora *et al.*, 2019). For example, transient transformation of BY-2 cells makes the system attractive for examination of cytoskeletal proteins (Buschmann *et al.*, 2011; Buschmann, 2016), and may be used in combination with manipulation of synchronization. The challenge lies in both the maintenance of healthy cell cultures that are competent for synchronization and its transformation requirement. Both the hairy root and seedling-based transient expression systems are convenient in sample preparation but may not be suitable for routine examination of cytoskeletal dynamics during cell division (Li & Nebenfuhr, 2010; Ron *et al.*, 2014). Transient expression of the transcriptional regulator E2Fb in tobacco leaf cells triggered cells to divide (Jimenez-Gongora *et al.*, 2019). Therefore, E2Fb may be used in place of CYCD for similar studies as described here.

In conclusion, the transient expression of CYCD sequentially turned on all machineries for the mitotic cell cycle in order to drive the leaf cells to re-enter the cell cycle. As a result, this transient expression system enables us to examine the intracellular dynamics of proteins that exhibit cell cycle-dependent expression patterns so that their functions may be predicted according to their dynamic localization. Based on our results, we emphasize the following features or advantages associated with the CDELS.

(**1**) The system employs materials and reagents already available in most plant research laboratories that conduct transient gene expression experiments, and the procedure is relatively rapid and cost-effective.

(**2**) Epidermal cells are among the most conveniently accessible plant cells for live-cell imaging because they can be easily mounted on glass slides with aqueous solutions or agar media before being placed under either upright or inverted microscopes. The cells are rather robust for relatively long-term observation of a few hours.

(**3**) Compared to *Arabidopsis* cells that form metaphase spindles <10 μm in length, spindles formed in these tobacco cells often can be as long as 15–20 μm. Therefore, more details can be learned inside subcellular structures such as spindles and phragmoplasts in these tobacco cells than in those of *Arabidopsis*.

(**4**) The CDELS can be used to test the expression of genes that are predicted to function in a cell cycle-dependent manner. Their encoded proteins can be examined in living cells when they are tagged with fluorescent proteins.

(**5**) Multiple proteins can be co-expressed in the same leaf cells for examination of their relationship (e.g. their localization, interaction and association).

(**6**) Cell biology experiments in most plants such as cereals are particularly challenging. The CDELS can be used for heterologous expression of genes of both monocot and dicot plant species by using their original genomic elements.

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Author contributions

Y-RJL and BL planned and designed the research. JX and Y-RJL performed the experiments, and collected and analyzed the data. Y-RJL and BL wrote the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Expression of an artificial gene in a cell cycle-dependent manner.

Table S1 Primers used in this study.

Table S2 Plasmids used for transient expression in *Nicotiana benthamiana*.

Video S1 Time-lapse movie of mitosis induced by expression of AtCYCD3;1 in a leaf pavement cell.

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