The Arubidopsis D-Type Cyclin CYCD4 Controls Cell Division in the Stomatal Lineage of the Hypocotyl Epidermis

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Cyclin D (CYCD) plays an important role in cell cycle progression and reentry in response to external signals. Here, we demonstrate that Arubidopsis thaliana CYCD4 is associated with specific cell divisions in the hypocotyl. We observed that cycd4 T-DNA insertion mutants had a reduced number of nonprotruding cells and stomata in the hypocotyl epidermis. Conversely, CYCD4 overexpression enhanced cell division in nonprotruding cell files in the upper region of the hypocotyl, where stomata are usually formed in wild-type plants. The overproliferative cells were of stomatal lineage, which is marked by the expression of the TOO MANY MOUTHS gene, but unlike the meristemoids, most of them were not triangular. Although the phytohormone gibberellin promoted stomatal differentiation in the hypocotyl, inhibition of gibberellin biosynthesis did not prevent CYCD4 from inducing cell division. These results suggested that CYCD4 has a specialized function in the proliferation of stomatal lineage progenitors rather than in stomatal differentiation. We propose that CYCD4 controls cell division in the initial step of stomata formation in the hypocotyl.

INTRODUCTION

During postembryonic development in plants, organs are formed not only from undifferentiated cells in the meristem but also from differentiated cells; for example, lateral roots are derived from root pericycle cells (Charlton, 1996). The onset of lateral root formation coincides with a series of anticlinal asymmetric divisions in the xylem pole pericycle (Malamy and Benfey, 1997a). Another example is stomata that are generated from differentiated protodermal cells of aerial organs. In Arubidopsis thaliana, stomatal development requires three different precursor cells, namely, the meristemoid mother cell (MMC), the meristemoid, and the guard mother cell (GMC) (Nadeau and Sack, 2003). The MMC divides asymmetrically to produce a small triangular meristemoid and a neighbor cell. Some meristemoids directly differentiate into GMCs, while others further divide one to three times before they are converted into GMCs (Geisler et al., 2000). GMCs always divide asymmetrically to produce two guard cells (Gs) that surround the pore.

For continuous functioning of the meristematic organization and the formation of new organs, cell division must be stringently controlled by machinery that regulates the cell cycle. Signaling pathways that regulate cell cycle progression ultimately converge to control the activity of cyclin-dependent protein kinases (CDKs). The activity and substrate specificity of CDKs depend on their binding to cyclins (Morgan, 1997). In plants, A-, B-, and D-type cyclins are assumed to play a major role in cell cycle control (de Jager et al., 2005). The A- and B-type cyclins are expressed from the S to the M phase, and they control DNA replication, the G2/M transition, and mitosis; the D-type cyclin is assumed to be a sensor of external signals and to play an essential role in cell cycle progression and in the reentry of quiescent cells into the cell cycle. In animals, cyclin D forms active kinase complexes with CDK4 and CDK6, which phosphorylate the retinoblastoma (Rb) protein and inactivate its suppressor function on the transcription factors E2F and DP; this leads to progression from the G1 to the S phase (Harbour and Dean, 2000). Recent studies have revealed that a similar Rb/E2F/DP pathway is also conserved in plants (Nakagami et al., 1999; Shen, 2002). Mitogen-induced signals stimulate cyclin D-CDK complexes at multiple levels, including those of gene transcription, translation, protein stability, and assembly and import of these complexes into the nucleus (Sherr and Roberts, 2004). Subsequently, active cyclin D-CDK complexes promote progression from the G1 to the S phase and thus enhance cell proliferation.

Plant cyclin D (CYCD) has been classified into the following six groups based on similarities in amino acid sequences: CYCD1, CYCD2/CYCD4, CYCD3, CYCD5, CYCD6, and CYCD7 (Wang et al., 2004). Recent studies have demonstrated the promotive effect of CYCD expression on cell division in plants; for example, overexpression of Antirrhinum majus CYCD1;1 in tobacco.
(Nicotiana tabacum) BY-2 cells accelerated cell entry into the S phase and into mitosis, and it was associated with CDK activity on histone H1 and Rb proteins (Koroleva et al., 2004). Overexpression of Arabidopsis CYCD2;1 in tobacco plants stimulated meristematic division by reducing the G1 phase (Cockcroft et al., 2000). Menges et al. (2006) reported that constitutive expression of CYCD3;1 in Arabidopsis cell suspension cultures reduced the proportion of G1-phase cells but extended the G2 phase, suggesting that CYCD3;1 dominantly drives the G1/S transition. In planta overexpression of CYCD3;1 caused hyperproliferation of leaf cells, inhibited cell differentiation, and reduced DNA ploidy, indicating that this gene may play an important role in the switch from cell proliferation to differentiation (Dewitte et al., 2003). However, the manner in which each CYCD is associated with the temporal and spatial control of cell division in the context of plant development is unclear. Recently, Masubelele et al. (2005) reported that during seed germination, transcription of Arabidopsis CYCD1;1 and CYCD4;1 was upregulated prior to the activation of cell division in the root meristem. In cycd1;1 and cycd4;1 mutants, the onset of cell proliferation was significantly delayed, while overexpression of CYCD1;1 resulted in a rapid increase in the number of cycling cells, which led to accelerated germination.

Arabidopsis has 10 CYCD genes; however, information regarding their molecular functions is quite limited. It was demonstrated that CYCD2;1 and CYCD3;1 were induced by the plant hormone cytokinin and/or sucrose at the mRNA level (Soni et al., 1995; Riou-Khamlichi et al., 1999, 2000). Furthermore, CYCD3;1 is a highly unstable protein that is degraded via the ubiquitin-proteasome pathway (Planchas et al., 2004). Healy et al. (2001) reported that CYCD2;1 and CYCD3;1 interact with CDKA;1, which is an ortholog of yeast Cdc2/Cdc28p. In Arabidopsis, CYCD4 includes two genes, namely, CYCD4;1 and CYCD4;2. We have recently demonstrated that both these CYCD4s form active kinase complexes with CDKA;1, whereas only CYCD4;1 can bind and activate CDKB2;1, which is a plant-specific CDK that is expressed from the G2 to the M phase (Kono et al., 2003, 2006). CYCD4;2 is unique in its amino acid sequence in that it lacks the Rb binding motif and the PEST sequence that are hallmarks of CYCDs. However, it was able to rescue G1 cyclin-deficient yeast, and its overexpression in hypocotyl explants caused faster callus induction than that in wild-type explants (Kono et al., 2006). This indicates that CYCD4;2 promotes cell division regardless of its unique amino acid sequence. Here, we observed knockout mutants and plants that overexpress CYCD4 genes and demonstrated that CYCD4 is involved in stomata formation in the hypocotyl. Our results suggest a specific requirement of CYCD control of cell divisions in populations outside of the meristems.

RESULTS

cycl4 Mutants Have Fewer Nonprotruding Cells in the Hypocotyl

To elucidate the in planta function of CYCD4, we isolated loss-of-function mutants of the CYCD4;1 and CYCD4;2 genes from T-DNA insertion collections (Figure 1A). Since a cycd4;1 mutant has been independently isolated by Masubelele et al. (2005), our cycd4;1 mutant was designated as cycd4;1-2. In this mutant, the T-DNA was inserted into the 2nd exon of CYCD4;1 that was 288 bp downstream of the start codon. We identified three cycd4;2 mutant lines; of these, we eliminated cycd4;2-1 from our analyses due to the complexity of T-DNA integration. In cycd4;2-2 and cycd4;2-3, the T-DNA was inserted into the 4th and 1st exons of CYCD4;2 that were 616 and 141 bp downstream of the start codon, respectively. Total RNA was isolated from the wild-type and mutant seedlings that were homozygous for T-DNA insertions; the RNA was subjected to RT-PCR (Figure 1B). The cDNAs that encompassed each T-DNA insertion site could be amplified from the wild-type seedlings but not from the mutant

![Figure 1. T-DNA Insertion Mutants of CYCD4.](image)
seedlings; this indicated that the CYCD4 genes were knocked out in the mutants. Note that the expression level of CYCD4;2 in cycd4;1-2 and vice versa did not change compared with the wild-type seedlings (data not shown).

All three mutants grew normally and did not exhibit any distinct macroscopic phenotype. However, in the hypocotyl, a significant reduction was observed in the number of stomata, that is, 62, 72, and 67% of the number in the wild-type plants in cycd4;1-2, cycd4;2-2, and cycd4;2-3, respectively (Figure 1C). The double mutants of cycd4;1-2 and cycd4;2-3 also exhibited fewer stomata. Introducing genomic fragments containing the CYCD4 genes restored the number of stomata visible on one surface of the mutants. Note that the expression level of the cycd4;1-2 and cycd4;2-3 mutants was 67% of the number in the wild-type plants in cycd4;1-2, and vice versa did not change compared with the wild-type plants (Table 1). This suggests that CYCD4;1 and CYCD4;2 are not essential for mitotic division or stomata formation in leaves.

The hypocotyl epidermis comprises two types of alternating cell files along the apical-basal axis, namely, protruding and nonprotruding cell files. The number of cells in the nonprotruding cell files was significantly reduced in the single and double mutants of cycd4;1 and cycd4;2 compared with the wild-type plants (Table 2). By contrast, there was no change in the number of protruding cells, that is, 15 to 16 cells in both the wild-type plants and the cycd4 mutants. We observed that the number of nonprotruding cells adjacent to the six most apical protruding cells (hereafter termed “upper nonprotruding cells”) in the cycd4 hypocotyls decreased to 60 to 70%. By contrast, the number of nonprotruding cells adjacent to the remaining 9 to 10 protruding cells near the hypocotyl-root junction was almost identical in the wild-type plants and cycd4 mutants (Table 2). Stomata are known to develop only in the upper region of nonprotruding cell files (Berger et al., 1998). In fact, we observed that almost all stomata were produced in the upper nonprotruding cells and that the number of these stomata in the cycd4 mutants was half that in the wild-type plants (Table 2). These results indicate that the destruction of CYCD4 genes reduced cell division in the nonprotruding cell files, thus reducing the number of stomata. In the hypocotyl epidermis, postembryonic cell proliferation is mainly restricted to the region associated with stomata formation (Gendreau et al., 1997). Consistent with this report, we observed that in the cycd4 mutants, cell division was compromised only in the upper nonprotruding cells where stomata are usually formed.

### CYCD4 Expression in Seedlings

We examined the spatial expression patterns of CYCD4 during the early stage of seedling development using promoter-β-glucuronidase (GUS) fusion genes. The 1st exon and intron of each CYCD4 gene were included in the fusion constructs because these constructs produced stronger signals than those carrying only the promoter fragment that was upstream of the start codon. In fact, GUS expression driven by either one of the CYCD4 promoters was observed in the hypocotyls, although CYCD4;1 exhibited stronger expression than CYCD4;2 (Figures 2A and 2B). Furthermore, CYCD4;1 expression was observed in a broad range of tissues, including shoot and root apices, cotyledons, and vascular cylinders. Interestingly, CYCD4;2 promoter activity was not observed in the shoot and root meristems (Figures 2C and 2D).

### CYCD4 Overexpression Stimulates Cell Division in the Nonprotruding Cell File

We observed that CYCD4 overexpression in tobacco BY-2 cells did not alter cell cycle progression (A. Kono and M. Umeda, unpublished data). This is in contrast with other CYCD genes, whose overexpression accelerated cell cycle progression in cell suspension cultures (Koroleva et al., 2004; Menges et al., 2006).

To determine whether CYCD4 is involved in in planta cell division, we generated transgenic Arabidopsis plants that overexpress hemagglutinin (HA)-tagged CYCD4;1 and FLAG-tagged CYCD4;2 under the control of the cauliflower mosaic virus (CaMV) 35S promoter. Immunoblot analysis revealed several independent cell lines that overexpressed CYCD4;1-HA or CYCD4;2-FLAG (Figure 3A). Immunoprecipitation of protein extracts with the anti-HA or anti-FLAG antibody revealed that CDKA;1, the ortholog of yeast Cdc2/Cdc28p, was coprecipitated with either CYCD4;1-HA or CYCD4;2-FLAG (Figure 3B). The immunoprecipitates exhibited histone H1 kinase activity, indicating that both CYCD4;1-HA and CYCD4;2-FLAG were functional in activating CDKA;1 in planta. The CYCD4-overexpressing (CYCD4-OE) plants exhibited

### Table 1. Cell Size, Cell Number, and Number of Stomata in the First Leaves of Wild-Type, cycd4 Mutant, and CYCD4-OE Seedlings

<table>
<thead>
<tr>
<th>Line</th>
<th>Leaf Blade Area (mm²)</th>
<th>Cell Area (μm²)</th>
<th>Cell Number</th>
<th>Number of Stomata</th>
<th>Stomatal Index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Columbia wild type</td>
<td>40.02 ± 2.58</td>
<td>1878 ± 98</td>
<td>21487 ± 1104</td>
<td>4125 ± 314</td>
<td>19.12 ± 0.84</td>
</tr>
<tr>
<td>cycd4;1-2</td>
<td>35.41 ± 2.11</td>
<td>1865 ± 89</td>
<td>19224 ± 1240</td>
<td>3551 ± 241</td>
<td>18.38 ± 0.44</td>
</tr>
<tr>
<td>cycd4;2-2</td>
<td>36.95 ± 1.34</td>
<td>1959 ± 47</td>
<td>18918 ± 745</td>
<td>3380 ± 214</td>
<td>17.82 ± 0.77</td>
</tr>
<tr>
<td>cycd4;2-3</td>
<td>37.62 ± 1.61</td>
<td>2094 ± 116</td>
<td>18292 ± 795*</td>
<td>3424 ± 229</td>
<td>18.59 ± 0.75</td>
</tr>
<tr>
<td>cycd4;1-2 cycd4;2-2</td>
<td>37.44 ± 1.65</td>
<td>1789 ± 47</td>
<td>21244 ± 1381</td>
<td>3835 ± 264</td>
<td>18.09 ± 0.41</td>
</tr>
<tr>
<td>cycd4;1-2 cycd4;2-2</td>
<td>36.96 ± 2.48</td>
<td>1732 ± 58</td>
<td>21639 ± 1646</td>
<td>3981 ± 367</td>
<td>18.19 ± 0.41</td>
</tr>
<tr>
<td>3SS:CYCD4;1 (F12)</td>
<td>40.55 ± 2.86</td>
<td>1295 ± 63***</td>
<td>31662 ± 2223***</td>
<td>5206 ± 317*</td>
<td>16.64 ± 0.54*</td>
</tr>
<tr>
<td>3SS:CYCD4;2 (O3)</td>
<td>40.14 ± 2.72</td>
<td>1554 ± 48**</td>
<td>28785 ± 1838**</td>
<td>4276 ± 350</td>
<td>16.27 ± 0.38**</td>
</tr>
</tbody>
</table>

The cell size, cell number, and number of stomata were estimated for the abaxial epidermal cells. All measurements were performed using seedlings at 20 d after germination. Data are presented as mean ± se (n = 12). Significant differences between the wild-type and mutant or transgenic plants are as follows: *P < 0.05, **P < 0.01, and ***P < 0.001; the other values are not significant (P > 0.05).
no distinct macroscopic phenotype (data not shown), and their DNA ploidy was almost identical to that of the wild-type plants (see Supplemental Figure 1 online). This is in contrast with CYCD3;1 overexpression that caused hyperproliferation of leaf epidermal cells, altered leaf architecture, and reduced DNA ploidy (Dewitte et al., 2003).

We observed a drastic change in the hypocotyls of the transgenic seedlings. In the CYCD4-OE seedlings, many small cells were generated in the nonprotruding cell files a few days after germination (Figure 4A). Cell division was also enhanced in the protruding cell files (~130% of that in the wild-type plants); however, a considerably higher enhancement was observed in the nonprotruding cell files (~260%) (Table 2). Accordingly, the numbers of nonprotruding cells and stomata in the upper hypocotyl region were significantly elevated (Table 2, Figure 1C). The stomatal index (i.e., the fraction of stomata in the upper non-cotyl region) were significantly elevated (Table 2, Figure 1C). The data obtained for the transgenic plants (data not shown), and GFP fluorescence was retained in the MMCs and daughter meristemoids that had a characteristic MMC (Figure 5A, top panel). GFP expression in wild-type hypocotyls using the TMM promoter. To identify each cell type during the turnover (Geisler et al., 2000; Nadeau and Sack, 2002). In leaves, like protein that is assumed to function in stomatal spacing pat-

ting (Geisler et al., 2000; Nadeau and Sack, 2002). In leaves, TOO MANY MOUTHS (TMM) encodes a Leu-rich repeat receptor-like protein that is assumed to function in stomatal spacing pattern-ning (Geisler et al., 2000; Nadeau and Sack, 2002). In leaves, TMM is expressed in cells of stomatal lineage, and its expression begins in MMCs that undergo formative asymmetric division (Nadeau and Sack, 2002). Here, we first investigated TMM expression in wild-type hypocotyls using the GFP gene under the control of the TMM promoter. To identify each cell type during the development of stomatal cells, GFP fluorescence was monitored from 1 to 2 d after germination. We observed that the promoter was already active in the cells that were to divide perpendicular to the apical-basal axis to produce an MMC or its precursor (a cell that continues to divide symmetrically before generating an MMC) (Figure 5A, top panel). GFP fluorescence was retained in the MMCs and daughter meristemoids that had a characteristic triangular shape and were completely filled with cytoplasm.

**Table 2. Number of Epidermal Cells and Stomata in the Hypocotyls of Wild-Type, cycd4 Mutant, and CYCD4-OE Seedlings**

<table>
<thead>
<tr>
<th>Line</th>
<th>Protruding Cells</th>
<th>Nonprotruding Cells</th>
<th>Nonprotruding Cells (Lower)</th>
<th>Nonprotruding Cells (Upper)</th>
<th>Stomata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Columbia wild type</td>
<td>15.4 ± 0.38</td>
<td>29.8 ± 0.85</td>
<td>11.9 ± 0.85</td>
<td>17.9 ± 0.63</td>
<td>2.44 ± 0.18</td>
</tr>
<tr>
<td>cycd4;1-2</td>
<td>15.2 ± 0.22</td>
<td>23.6 ± 0.87***</td>
<td>11.8 ± 0.74</td>
<td>11.8 ± 0.78***</td>
<td>1.22 ± 0.15***</td>
</tr>
<tr>
<td>cycd4;2-2</td>
<td>16.1 ± 0.31</td>
<td>24.1 ± 1.1***</td>
<td>11.4 ± 0.73</td>
<td>12.7 ± 0.69***</td>
<td>1.33 ± 0.17***</td>
</tr>
<tr>
<td>cycd4;1-2 cycd4;2-2</td>
<td>15.7 ± 0.33</td>
<td>22.3 ± 0.69***</td>
<td>10.9 ± 0.48</td>
<td>11.4 ± 0.65***</td>
<td>1.11 ± 0.20***</td>
</tr>
<tr>
<td>35S:CYCD4;2</td>
<td>19.8 ± 0.43***</td>
<td>76.4 ± 2.3***</td>
<td>27.8 ± 1.5***</td>
<td>48.7 ± 2.4***</td>
<td>4.89 ± 0.59**</td>
</tr>
</tbody>
</table>

The epidermal cells were counted in each protruding and nonprotruding cell file in the hypocotyl. The nonprotruding cells that lay adjacent to the six most apical protruding cells (upper) or those adjacent to the remaining 9 to 10 protruding cells near the hypocotyl-root junction (lower) were also counted. The stomata in the nonprotruding cell files adjacent to the six most apical protruding cells were counted. All the counting was performed using seedlings at 10 d after germination. Data are expressed as mean ± se (n = 12). Significant differences between the wild-type and mutant or transgenic plants are as follows: ***P < 0.01 and ****P < 0.001; the other values are not significant (P > 0.05).

**Specific Function of CYCD4 in the Hypocotyl**

Furthermore, we also observed the hypocotyl of Pro35S:CYCD3;1 plants that had been reported previously (Dewitte et al., 2003).
It is noteworthy that TMM expression disappeared rapidly in cells that did not divide further and that deviated from the stomatal lineage to differentiate into epidermal cells (Figure 5A, middle panel). GFP expression persisted in GCs that were generated by symmetric GMC divisions (Figure 5A, bottom panel). These results indicate that TMM is expressed in the early stages when protodermal cells enter the stomatal lineage to develop into MMC precursors, and its expression continues until GC differentiation.

In leaves, satellite meristemoids are created by divisions of the larger daughter cell of the MMC division (Geisler et al., 2000). However, we studied the division patterns of ∼30 sister cells of the meristemoid and observed that they did not generate satellite meristemoids in the hypocotyl; instead, they developed into GMCs that divided symmetrically to produce GCs. These results indicate that in the hypocotyl, the division of MMC precursors and not the sister of the meristemoid influences the cell population of stomatal lineage and thus controls the number of stomata in the epidermis.

We then examined TMM expression in CYCD4-OE plants using the ProTMM-GUS construct. A patchy pattern of TMM expression could be observed in the hypocotyl of mature embryos from both the wild-type and CYCD4;2-OE lines (Figure 5B). However, 1 d after germination, the CYCD4;2-OE seedlings exhibited small TMM-expressing cells that were aligned in tandem, and this expression pattern persisted for >10 d after germination (Figures 5B and 5C). The TMM expression observed here might include a carryover of the GUS protein due to the rapid cell division induced by CYCD4;2 overexpression; however, it is evident that the overproliferative cells in the nonprotruding cell files were derived from the TMM-expressing cells of stomatal lineage. The triangular meristemoids and GCs exhibited a high level of TMM expression continues until GC differentiation. In leaves, satellite meristemoids are created by divisions of the larger daughter cell of the MMC division (Geisler et al., 2000). However, we studied the division patterns of ∼30 sister cells of the meristemoid and observed that they did not generate satellite meristemoids in the hypocotyl; instead, they developed into GMCs that divided symmetrically to produce GCs. These results indicate that in the hypocotyl, the division of MMC precursors and not the sister of the meristemoid influences the cell population of stomatal lineage and thus controls the number of stomata in the epidermis.

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expression, while most of the other TMM-expressing cells were rectangular in shape. This suggested that they were MMCs or their precursors. By contrast, 11 d after germination, the wild-type seedlings expressed TMM only in the stomata and surrounding cells that were dispersed in the epidermis (Figures 5B and 5C). Hence, the number of TMM-expressing cells in the CYCD4;2-OE hypocotyls increased to twofold to threefold of that in the wild-type plants (see Supplemental Figure 3A online). Conversely, the number of these cells was significantly reduced in the single and double mutants of CYCD4, as shown in Supplemental Figure 3B online. The TMM expression in leaves was almost the same between the wild-type and CYCD4;2-OE leaves (see Supplemental Figure 4 online); this fact supports the above-mentioned assumption that CYCD4 is involved in proliferation of stomatal precursors in hypocotyls but not in leaves.

**CYCD4 Overexpression Promotes Cell Division before Meristemoid Differentiation**

Gibberellin (GA) is known to promote stomata formation in the hypocotyl, and this effect is pronounced when it is combined with ethylene (Saibo et al., 2003). Conversely, stomata are eliminated from the hypocotyls of the GA-deficient mutant ga1-3 and wild-type plants treated with the GA biosynthesis inhibitor paclobutrazol (PAC) (Saibo et al., 2003). When Arabidopsis seedlings were grown in the presence of GA and the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC), the number of stomata in the hypocotyl increased more than twofold (Table 3). However, we noticed that the number of upper non-protruding cells also increased to 150% (Table 3). When TMM expression was observed in the absence of an exogenous phytohormone, 65% of the hypocotyl stomata were surrounded by two or three TMM-expressing cells (Figure 6A). However, GA and ACC treatment reduced the number of stomatal neighbor cells, such that 60% of the stomata were accompanied by one or no TMM-expressing cells (Figure 6A). These results indicate that GA not only enhanced cell division in the nonprotruding cells but also promoted stomatal differentiation, resulting in the rapid disappearance of TMM expression in the neighbor cells. As a consequence, GA treatment increased the stomatal index slightly to 150% (Table 3).

Next, we observed the hypocotyls of seedlings grown on medium containing PAC. As expected, the number of upper nonprotruding cells had decreased, and stomata formation was
severely inhibited, as reported by Saibo et al. (2003); this caused a drastic decrease in the stomatal index to 19% (Table 3). As shown in Figure 6C, MMCs (or their precursors) and meristemoids could be identified as TMM-expressing cells in the PAC-treated hypocotyls, but only one or two stomata were found on one surface of the entire hypocotyl. These results indicate that GA is required for stomatal differentiation after cells have acquired meristemoid characteristics.

Even in the CYCD4;2-OE seedlings, stomata formation was severely inhibited by PAC treatment, and almost no stomata developed on the hypocotyl. However, we observed that small cells accumulated in the nonprotruding cell files (Figure 6B), and in fact, the number of upper nonprotruding cells reached 18.8 ± 1.2 (mean ± se), which is more than twice that in the wild-type seedlings (8.92 ± 0.38; see Table 3). This suggests that CYCD4 overexpression enhanced cell division in the nonprotruding cell files regardless of the inhibitory effect of PAC on cell division and stomatal differentiation. Moreover, the overproliferative cells expressed TMM, as was the case in the nontreated hypocotyls (Figure 6C). These results support our assumption that CYCD4 functions in the division of MMC precursors but not in the later process of stomata formation.

**DISCUSSION**

Previous reports have demonstrated that CYCDs promote cell cycle progression (Koroleva et al., 2004; Menges et al., 2006). However, overexpression of the CYCD4 genes in tobacco BY-2 cells did not affect cell cycle progression. Moreover, CYCD4 overexpression in Arabidopsis plants did not alter the morphology or DNA ploidy. In leaves, the number of epidermal cells increased, while the cell area decreased; this suggests that CYCD4 overexpression stimulated cell division. However, neither cell division nor cell growth was significantly affected in the leaves of single or double mutants of CYCD4, indicating that CYCD4 is not essential for the mitotic division of proliferating cells.

**Table 3. Number of Nonprotruding Cells and Stomata in Wild-Type Hypocotyls Treated with GA or PAC**

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<th>Treatment</th>
<th>Number of Nonprotruding Cells</th>
<th>Number of Stomata</th>
<th>Stomatal Index (%)</th>
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<td></td>
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<td></td>
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<tr>
<td>−GA, ACC</td>
<td>14.1 ± 0.59</td>
<td>1.67 ± 0.17</td>
<td>11.7 ± 0.91</td>
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<tr>
<td>+GA, ACC</td>
<td>20.7 ± 0.69***</td>
<td>3.56 ± 0.32***</td>
<td>17.2 ± 1.4**</td>
</tr>
<tr>
<td>−PAC</td>
<td>12.3 ± 0.63</td>
<td>1.67 ± 0.19</td>
<td>13.7 ± 1.5</td>
</tr>
<tr>
<td>+PAC</td>
<td>8.92 ± 0.38***</td>
<td>0.250 ± 0.13***</td>
<td>2.57 ± 1.4***</td>
</tr>
</tbody>
</table>

The nonprotruding cells adjacent to the six most apical protruding cells and the stomata in the same region were counted; the stomatal index was calculated. Wild-type plants were grown either in the presence of 10 μM GA and 50 μM ACC for 9 d or in the presence of 0.5 μM PAC for 7 d. Data are presented as mean ± se (n = 12). Significant differences between the nontreated and treated plants are as follows: **P < 0.01 and ***P < 0.001; the other values are not significant (P > 0.05).
cells or that the other CYCDs may be redundant in function. In this regard, it is noteworthy that the CYCD4;2 promoter activity was eliminated from the shoot and root meristems. During seed germination in the cycd4;1-1 mutant, the onset of cell proliferation was significantly delayed in the root tips, and the overall number of dividing cells was reduced (Masubelele et al., 2005). This suggests that CYCD4;1 may play a distinct role in regulating the extent of cell division that occurs during germination.

Schnittger et al. (2002) have reported that CYCD3;1 induced cell division in trichomes when it was expressed under the control of the GLABRA2 (GL2) promoter, while CYCD4;1 had no effect on the trichomes when it was expressed under the control of the same promoter. This further supports the assumption that CYCD4 function is distinct from that of other CYCDs.

In this study, we concluded that CYCD4 is associated with stomatal precursor formation in hypocotyls based on the following observations. (1) The cycd4 mutants had a reduced number of upper nonprotruding cells. As a result, the numbers of TMM-expressing cells and stomata were reduced. (2) CYCD4 overexpression enhanced cell division in the hypocotyl, particularly in the upper region of the nonprotruding cell file. This occurred after germination when stomata formation was initiated. (3) The overproliferative cells accumulated the GUS protein that was expressed under the control of the TMM promoter, suggesting that these cells were generated by reiterative symmetric divisions in the cells of stomatal lineage. Several reports have demonstrated that stomata formation in leaves was affected by the up- or down-regulation of cell cycle–related genes. Overexpression of CDC6 or CDT1, both of which are required for DNA replication, elevated the density of stomata on Arabidopsis leaves (Castellano et al., 2004). Expression of a dominant-negative type of CDKB1;1 disturbed cell division and reduced the stomatal density (Boudolf et al., 2004). However, we demonstrated that in leaves, CYCD4 overexpression reduced the stomatal density and that the TMM expression pattern did not change at all. These results are not surprising because some organ-specific rules may operate in stomata formation. For instance, tmm mutants have stomatal clusters on leaves but no stomata in the hypocotyl and inflorescence stem, suggesting the indispensability of TMM in stomata formation in the hypocotyl (Yang and Sack, 1995; Geisler et al., 1998). Moreover, it is known that GA is essential for stomatal development in hypocotyls but not in leaves (Sun et al., 1992; Saibo et al., 2003). Our results revealed that up- or down-regulation of CYCD4 had pronounced effects in the upper cells or that the other CYCDs may be redundant in function. In this regard, it is noteworthy that the CYCD4;2 promoter activity was eliminated from the shoot and root meristems. During seed germination in the cycd4;1-1 mutant, the onset of cell proliferation was significantly delayed in the root tips, and the overall number of dividing cells was reduced (Masubelele et al., 2005). This suggests that CYCD4;1 may play a distinct role in regulating the extent of cell division that occurs during germination. Schnitiger et al. (2002) have reported that CYCD3;1 induced cell division in trichomes when it was expressed under the control of the GLABRA2 (GL2) promoter, while CYCD4;1 had no effect on the trichomes when it was expressed under the control of the same promoter. This further supports the assumption that CYCD4 function is distinct from that of other CYCDs.

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**Figure 6.** Cell Division and TMM Expression in Response to GA.

(A) TMM expression in the GA-treated hypocotyls. Transgenic plants expressing GUS under the control of the TMM promoter were grown in the absence of phytohormones (w/o hormone) or in the presence of 10 μM GA and 50 μM ACC (GA + ACC) for 9 d. Bar = 20 μm. In the graph, the stomata that lay adjacent to the indicated number of TMM-expressing cells are expressed in terms of percentage: w/o hormone, n = 154; GA + ACC, n = 84.

(B) and (C) Effect of the GA biosynthesis inhibitor PAC. Wild-type or CYCD4;2-OE seedlings were grown on medium supplemented with 0.5 μM PAC for 4 d, and the upper hypocotyl region was observed (B). GUS expression driven by the TMM promoter was also observed (C). Bars = 20 μm.

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**Figure 7.** The Stomatal Pathway in Hypocotyls.

Some protodermal cells enter the stomatal pathway to develop into precursors of MMCs and begin to express TMM; this is indicated by gray color. The precursors reiterate cell divisions before they generate MMCs, which then divide asymmetrically to produce triangular meristemoids (M). Meristemoids are converted into GMCs, which then divide symmetrically to generate GCs. The other cells produced by the division of MMC precursors differentiate into epidermal cells.
nonprotruding cells that produce stomata. GL2, which encodes a homeodomain transcription factor, is only expressed in the protruding cell files of the hypocotyl, and in gl2 mutants, stomata formation, but not ectopic cell division, is observed in the protruding cell files (Berger et al., 1998; Hung et al., 1998). This indicates that GL2 may play an inhibitory role in GC differentiation in the protruding cell files. CYCD4 overexpression in gl2 mutants enhanced cell division only in the nonprotruding cell files, as observed in the wild-type background (data not shown), suggesting that some factor(s) other than GL2 may modify the CYCD4 function that is specific to the nonprotruding cell files. Therefore, differential mechanisms may operate to drive extra cell divisions in stomatal precursors and to produce the final differentiated GCs.

Using the GFP marker under the control of the TMM promoter, we noticed that typically, secondary meristemoids were not generated from existing meristemoids in hypocotyls; instead, the MMC precursors reiterated cell divisions before they acquired MMC characteristics. This indicates that the divisions of the MMC precursors increased the cell population and the number of stomata in the nonprotruding cell files. The number of nonprotruding cells in the cycld4 mutant was significantly reduced, while that in mature embryos was almost identical to that in the wild-type plants (data not shown). Therefore, we assume that the loss of CYCD4 function might cause a defect in the postembryonic divisions of the nonprotruding cells. This assumption was supported by the fact that CYCD4 overexpression caused the accumulation of many rectangular cells in tandem; this also occurred in the presence of PAC, which is a GA biosynthesis inhibitor that inhibits stomatal differentiation. Based on these results, we propose that CYCD4 plays a principal role in the divisions of MMC precursors but not in the asymmetric divisions of MMCs or in GC differentiation (Figure 7). In cycld4;1 and cycld4;2 double mutants, stomata were not completely eliminated from the hypocotyls (Figure 1C), which indicates that stomata formation is ensured even in the absence of CYCD4 function. CYCD4 is likely to increase the number of stomata by amplifying the MMC population.

In addition to TMM, a single loss-of-function mutation in the Arabidopsis STOMATAL DENSITY AND DISTRIBUTION1 (SDD1) or YODA (YDA) gene also induces increased stomatal density and clusters in leaves (Berger and Altmann, 2000; Von Groll et al., 2002; Bergmann et al., 2004). SDD1 and YDA encode a putative subtilisin-like extracytoplasmic protease and a mitogen-activated protein kinase kinase kinase, respectively (Berger and Altmann, 2000; Lukowitz et al., 2004). A model has been proposed in which SDD1 modifies a ligand for TMM, and the activated receptor signals downstream mitogen-activated protein kinase cascades via YDA to repress stomata formation (Bergmann et al., 2004). In contrast with TMM, loss of SDD1 or YDA increased the stomatal density not only in leaves but also in stems and hypocotyls (Berger and Altmann, 2000; Bergmann et al., 2004). Recently, Shpak et al. (2005) reported that ERECTA (ER) family Leu-rich repeat receptor-like kinases negatively control stomatal development in leaves and stems. In particular, ERECTA LIKE1 (ERL1) is important for maintaining stem cell activity and for preventing the terminal differentiation of meristemoids into GMCs. Genetic studies have suggested that TMM may inhibit ERL1 activity in the stem and promote stomatal differentiation; however, the mechanism by which TMM regulates the ER family is unknown. When CYCD4 was overexpressed in tmm mutants, overproliferation of the nonprotruding cells was observed as in the wild-type plants, but stomata were not formed (data not shown). This indicates that TMM is not a prerequisite for the divisions of the MMC precursors but is required for stomatal differentiation. Nevertheless, it is possible that the TMM/ER/YDA pathway controls CYCD4 function. Further genetic and molecular studies will clarify the mechanism by which the number of stomata in the hypocotyl epidermis is maintained.

CYCD4;2 has a unique amino acid sequence in that it lacks the Rb binding motif (LXCXE) and the PEST sequence that is the hallmark of unstable proteins (Kono et al., 2006). However, in this study, CYCD4;2 formed active kinase complexes with CDK4;1 in plant cells, indicating its functionality as a cyclin. The absence of the Rb binding motif suggests that the divisions of MMC precursors in the hypocotyl can be stimulated independent of the Rb/E2F/DP pathway; for example, CYCD4;CDKA1 may phosphorylate a substrate(s) other than Rb, which positively regulates cell division. Another possibility is that CYCD4 may interact with a gene-specific transcription factor(s) as reported in mammals; D-type cyclins control the transcription factors DMP1, C/EBPβ/ Nf-1, and AML1 via CDK-independent mechanisms (Inoue and Sherr, 1998; Lamb et al., 2003; Peterson et al., 2005). It is also known that CYCD1 is directly associated with the estrogen or androgen receptor and up- and downregulates their transcriptional transactivation abilities, respectively (Zwijsen et al., 1997; Knudsen et al., 1999). In future studies, these possibilities will be examined for Arabidopsis CYCD4s. It has been reported that in Drosophila, the division of germ line stem cells and their precursors (primordial germ cells) requires a specific function of cyclin B (Wang and Lin, 2005). Our study demonstrated a more specific requirement of CYCD in cell divisions associated with stomata formation. Recently, a gene named SPEECHLESS has been shown to direct the divisions of MCMs (MacAlister et al., 2007; Pillitteri et al., 2007), suggesting that it may control a specific set of cell cycle genes to induce the asymmetric divisions. The Arabidopsis genome includes >50 cyclin genes. Therefore, it is interesting to investigate the distinct functions of other cyclins that may be involved in the temporal and/or spatial control of cell division during postembryonic development in plants.

METHODS

Plant Materials and Treatments

Arabidopsis thaliana ecotype Col-0 was used for transformation. For in vitro cultivation, plants were grown on a medium containing 2.15 g/L of Murashige and Skoog basal salt mixture (Sigma-Aldrich) supplemented with 1% sucrose, 3 mg/L thiamine-HCl, 5 mg/L nicotinic acid, and 0.5 mg/L pyridoxine-HCl. GUS staining was conducted as described previously (Umeda et al., 2000). The DNA ploidy was measured using a flow cytometer (Ploidy Analyzer; Partec) according to the manufacturer’s protocol.

Identification of T-DNA Insertion Mutants

We isolated cycld4;1 and cycld4;2 mutants from the collections obtained from the Max-Planck Institute for Plant Breeding Research (Ríos et al.,
The open reading frame of CYCD4;1 that lacked the stop codon was PCR amplified with a SaII site at the N terminus and an NcoI site at the C terminus, and it was subcloned into the Smal site of pBluescript II SK- (Stratagene). Next, the plasmid was digested with HindIII and NcoI, and the resultant fragment was subcloned into pPlpy (Ferrando et al., 2000) that was digested with HindIII and Ncol to add a HA tag at the C terminus of CYCD4;1 under the control of the CaMV 35S promoter. The resultant plasmid was digested with KpnI, and the CYCD4;1-HA fragment was subcloned into the KpnI site of pSPTV20 (Becker et al., 1992). The open reading frame of CYCD4;2 that lacked the stop codon was PCR amplified and cloned into the pENTR-D/TOPO vector (Invitrogen). The resultant plasmid pENTR-CYCD4;2 was subjected to the LR reaction using the destination vector pGW11 (T. Nakagawa, unpublished data) to produce a binary vector containing the C-terminal FLAG-tagged CYCD4;2 under the control of the CaMV 35S promoter. Furthermore, pENTR-CYCD4;2 was also subjected to the LR reaction using the destination vector pGWB5 (T. Nakagawa, unpublished data) to produce a binary vector containing the C-terminal GFP-tagged CYCD4;2 under the control of the CaMV 35S promoter.

Plasmid Construction for Expression Analysis

The CYCD4;1 promoter region, which extends from 2491 bp upstream of the start codon, was PCR amplified with SalI sites at the 5’ and 3’ ends and subcloned into pCR-XL-TOPO (Invitrogen) to produce pCR-CYCD4;1pro. The genomic region containing the 1st exon and intron of CYCD4;1, which extends from 270 bp upstream to 585 bp downstream of the start codon, was PCR amplified with a BamHI site at the C terminus, and it was subcloned into the Smal site of pBluescript II SK- to produce SK’-CYCD4;1int. Furthermore, pCR-CYCD4;1pro was digested with SalI and HindIII that cuts it at 189 bp upstream of the start codon, and the SalI-HindIII fragment was subcloned into SK’-CYCD4;1int that was digested with SalI and HindIII. Next, the plasmid was digested with SalI and BamHI, and the resultant fragment was subcloned into the SalI-BamHI site of the binary vector pPCV812 (Koncz et al., 1994) to produce the GUS fusion gene.
Leaf Growth Analysis

The kinematic analysis of leaf growth was performed as described previously (De Veylder et al., 2001). Plants were grown in soil under continuous light conditions for 20 d. Healthy first/second leaves were harvested and fixed in a solution of 90% ethanol and 10% acetic acid at 4°C overnight, hydrated through a graded series of ethanol, and stored in water at 4°C. The samples were mounted on a slide glass and cleared with chloral hydrate solution during the overnight incubation. Data were collected by scanning images of the abaxial epidermis located at 50% the distance between the tip and the base of the leaf blade, halfway between the midrib and the leaf margin. The images, including at least 40 cells in focus, were excised using the photo-editing program Photoshop Elements 2.0 (Adobe). The epidermal cells, including GCs, in the excised image were counted, and the area of the excised image was measured using the image analysis program NIH image 1.63. The average cell area and stomatal index were determined based on these measurements. Next, the leaf blade area was measured, and the total number of epidermal cells on the abaxial side was estimated based on the average cell area. Finally, the total number of stomata was calculated based on the total cell number and stomatal index.

Immunoblotting and Kinase Assays

Total protein was extracted from the whole seedlings and subjected to immunoblotting using an ECL protein gel blotting detection system (Amersham Biosciences) with the anti-HA (Roche), anti-FLAG M2 (Sigma-Aldrich), and anti-CDKA;1 antibodies (Umeda et al., 2000). For the kinase assays, the protein extracts were immunoprecipitated using the anti-HA or anti-FLAG antibody, and the immunoprecipitates were subjected to a kinase reaction using 1 mM ATP and 1 mM [γ-32P]ATP. After an incubation of 1 h at 30°C, the reaction was stopped by boiling the samples. The proteins were separated on a 12% SDS-PAGE gel and then transferred to a nitrocellulose filter. The blot was incubated with the appropriate antibodies and then probed with an ECL detection reagent (Amersham). Biodetection was observed using an image analyzer (Fuji BAS-2000).

Accession Numbers

Arabidopsis Genome Initiative locus identifiers for the genes mentioned in this article are as follows: At5g65420 (CYCD4;1), At5g10440 (CYCD4;2), At3g48750 (CDKA;1), At4g34160 (CYCD3;1), and At1g80080 (TMM).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure 1. DNA Ploidy Distribution in Wild-Type and CYCD4- OE Seedlings.

Supplemental Figure 2. Epidermal Cells of Wild-Type and CYCD4-OE Leaves.

Supplemental Figure 3. Number of TMM-Expressing Cells in the Hypocotyl of Wild-Type, CYCD4-OE, and cycd4 Mutant Seedlings.

Supplemental Figure 4. TMM Expression in Wild-Type and CYCD4;2-OE Leaves.

ACKNOWLEDGMENTS

We thank James A.H. Murray for providing the CYCD3;1-OE seeds. We also thank the ABRC at Ohio State University and the Max-Planck Institute for Plant Breeding Research for providing the seeds of the T-DNA insertion mutants. Furthermore, we appreciate the assistance provided by Nobuhiro Tsutsumi and Shin-ichi Arimura with regard to the confocal laser scanning microscope system. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (Grants 17027007 and 18056006) and a Grant-in-Aid for Scientific Research (B) (Grant 16370019) from the Ministry of Education, Sports, Culture, Science, and Technology of Japan and by the Program for Promotion of Basic Research Activities for Innovative Biosciences.

Received August 19, 2006; revised March 22, 2007; accepted April 6, 2007; published April 20, 2007.

REFERENCES


The *Arabidopsis* D-Type Cyclin CYCD4 Controls Cell Division in the Stomatal Lineage of the Hypocotyl Epidermis

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*Plant Cell* 2007;19:1265-1277; originally published online April 20, 2007;
DOI 10.1105/tpc.106.046763

This information is current as of June 20, 2017

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