Arabidopsis WEE1 Kinase Controls Cell Cycle Arrest in Response to Activation of the DNA Integrity Checkpoint

Kristof De Schutter,1 Jérôme Joubès,1,2 Toon Cools, Aurine Verkest, Florence Corellou,3 Elena Babiychuk, Els Van Der Schueren, Tom Beeckman, Sergeï Kushnir, Dirk Inzé, and Lieven De Veylder4

Department of Plant Systems Biology, Flanders Interuniversity Institute for Biotechnology, Ghent University, B-9052 Gent, Belgium

Upon the incidence of DNA stress, the ataxia telangiectasia–mutated (ATM) and Rad3-related (ATR) signaling kinases activate a transient cell cycle arrest that allows cells to repair DNA before proceeding into mitosis. Although the ATM-ATR pathway is highly conserved over species, the mechanisms by which plant cells stop their cell cycle in response to the loss of genome integrity are unclear. We demonstrate that the cell cycle regulatory WEE1 kinase gene of Arabidopsis thaliana is transcriptionally activated upon the cessation of DNA replication or DNA damage in an ATR- or ATM-dependent manner, respectively. In accordance with a role for WEE1 in DNA stress signaling, WEE1-deficient plants showed no obvious cell division or endoreduplication phenotype when grown under nonstress conditions but were hypersensitive to agents that impair DNA replication. Induced WEE1 expression inhibited plant growth by arresting dividing cells in the G2-phase of the cell cycle. We conclude that the plant WEE1 gene is not rate-limiting for cycle progression under normal growth conditions but is a critical target of the ATR-ATM signaling cascades that inhibit the cell cycle upon activation of the DNA integrity checkpoints, coupling mitosis to DNA repair in cells that suffer DNA damage.

INTRODUCTION

Genome integrity of cells is threatened by DNA damage that is the consequence of environmental stresses and endogenous causes. To cope with these stress conditions, cells have developed a set of surveillance mechanisms to monitor the status and structure of DNA during cell cycle progression. In Schizosaccharomyces pombe (fission yeast) and mammals, DNA damage activates the ataxia telangiectasia–mutated (ATM) and Rad3-related (ATR) signaling cascades that simultaneously turn on DNA repair complexes and arrest cell division; this mechanism allows cells to repair damaged DNA before proceeding into mitosis (Zhou and Elledge, 2000; Abraham, 2001; Bartek and Lukas, 2001; Kurz and Lees-Miller, 2004). ATM responds specifically to double-stranded breaks, whereas ATR primarily senses replication stress caused by a persistent block of replication fork progression. The ATM and ATR kinases transduce the DNA stress signal to the checkpoint kinases CHK1 and CHK2, which, in turn, arrest the cell cycle by directly modulating the activity of the effectors that control cell cycle progression (Chen and Sanchez, 2004; Sancar et al., 2004), the cyclin-dependent kinase (CDK) complexes.

CDK complexes consist of a catalytic kinase subunit and a regulatory cyclin. The sequential activation of different CDK/cyclin complexes drives the cell cycle through the phosphorylation of many different target substrates. CDK/cyclin activity is highly regulated at multiple levels. Control mechanisms include the regulated synthesis and destruction of the cyclin subunits (Peters, 1998; Murray, 2004), which are thought to target the CDKs to the substrates (Ohi and Gould, 1999), and the association of CDKs with inhibitory proteins and docking factors (Lees, 1995). Moreover, CDK activity is positively regulated by phosphorylation of a conserved residue (Thr-161 or equivalent) within the T loop and negatively regulated through phosphorylation of Tyr-15 and Thr-14 by WEE1 family kinases (Berry and Gould, 1996). Phosphorylation of Tyr-15 and Thr-14 residues of the CDK subunit inhibits ATP binding and blocks substrate recognition.

In fission yeast and mammals, rapid activation of the CDK/cyclin activity at the G2-M boundary is mediated by a dual-specificity phosphatase CDC25. Maintenance of the inhibition of CDK activity by Tyr-15 phosphorylation is the ultimate target of DNA damage checkpoint signaling. By activation of CHK1 and CHK2, CDC25 is phosphorylated and targeted for ubiquitin-dependent destruction or association with a 14-3-3 protein, resulting in nuclear export and exclusion of CDC25 from the nuclear pool of CDK/cyclin complexes (Boutros et al., 2006). Both WEE1 and the functionally related kinase MIK1 have been implicated as targets of the DNA damage and replication checkpoints as well. In Xenopus laevis (African frog) egg extracts,
activation of the DNA replication checkpoint stabilizes exogenously added WEE1 (Michael and Newport, 1998), whereas in fission yeast, MIK1 is a target for both the DNA damage and DNA replication checkpoints (Rhind and Russell, 2001). In response to the DNA replication checkpoint, MIK1 mRNA levels accumulate to high levels and, simultaneously, the MIK1 protein is stabilized, leading to dramatic increases in protein levels (Boddy et al., 1998; Baber-Furnari et al., 2000; Christensen et al., 2000).

The basic machinery that controls cell cycle progression in plants is similar to that of yeast and mammals (De Veylder et al., 2003; Dewitte and Murray, 2003; Inzé and De Veylder, 2006). Multiple CDKs and cyclins are encoded by the genomes of Arabidopsis thaliana and Oryza sativa (rice) (Vandepoele et al., 2002; Wang et al., 2004; La et al., 2006). In addition, a WEE1-related kinase has been described for maize (Zea mays), tomato (Solanum lycopersicum), and Arabidopsis (Sun et al., 1999; Sorrell et al., 2002; Gonzalez et al., 2004). Although the plant WEE1 gene is unable to complement mutations in its yeast homolog, its overexpression inhibits cell division in fission yeast. Additionally, recombinant purified WEE1 protein from maize is capable of inhibiting the kinase activity of biochemically purified CDKs (Sun et al., 1999). However, the in vivo role of WEE1 in plant cell cycle progression and growth is not well defined.

Our first insights into the role of DNA replication and damage checkpoints in plants came with the identification and characterization of Arabidopsis mutants in genes encoding orthologous ATM and ATR kinases (Garcia et al., 2003; Culligan et al., 2004). A defective DNA damage checkpoint is the reason why ATM-deficient plants are primarily hypersensitive to DNA-damaging agents, such as γ-irradiation, but rather insensitive to replication-blocking agents, such as hydroxyurea or aphidicolin (Garcia et al., 2003). In contrast, ATR mutants are hypersensitive to replication-blocking agents but also mildly sensitive toward γ-irradiation (Culligan et al., 2004). These results strongly indicate that the DNA checkpoint signaling pathways are conserved in plants. However, it is still unclear how activation of these signaling cascades leads to the arrest of the cell cycle in response to DNA damage. Here, we identify WEE1 as an important target of the DNA replication and DNA damage checkpoints. WEE1-deficient plants grow normally under optimal growth conditions but are hypersensitive to DNA-damaging agents. In accordance with a role for WEE1 in arresting the cell cycle in response to replication stress, WEE1 transcripts are found to be strongly upregulated by replication-inhibiting drugs in an ATR-dependent manner. Analogously, γ-irradiation and radiomimetic drugs induce WEE1 transcription in an ATM-dependent manner. The cell cycle arrest observed upon induction of WEE1 expression indicates that WEE1 is part of the mechanism that couples the onset of mitosis with the completion of DNA repair in cells that have suffered DNA damage.

Figure 1. CDKA;1 Target for Tyr Phosphorylation and Binding WEE1.

(A) CDK phosphorylation in response to checkpoint activation. Arabidopsis cell cultures were treated with 10 μg/mL aphidicolin (A), with 3 μM propyzamide (P), or mock-treated in controls (C). CDKs were purified from total protein extracts (300 μg/sample) with a p10CKS1At-Sepharose matrix, resolved by SDS-PAGE, and immunoblotted with the indicated antisera.

(B) Interaction of CDKA;1 with WEE1 in the yeast two-hybrid system. Yeast PJ69-4 cells containing a CDKA;1 or CDKB1;1 bait plasmid in combination with an empty control or WEE1 prey plasmid were spotted on plates with (+) or without (−) His. Only when the two proteins interact do cells grow on −His medium.
RESULTS

Tyr Phosphorylation of Arabidopsis CDKA;1 upon Activation of the DNA Replication Checkpoint

Tyr phosphorylation of CDKs has been shown to take place upon cytokinin deprivation in tobacco (Nicotiana tabacum) cells, application of water stress to wheat (Triticum aestivum) leaves, and stimulation of the DNA replication checkpoint in zygotes of the brown alga Fucus (Zhang et al., 1996; Schuppler et al., 1998; Corellou et al., 2000). To test whether Tyr phosphorylation occurs upon the activation of the DNA replication checkpoint in higher plants, cultured suspension cells of Arabidopsis were treated with aphidicolin, which inhibits all replicative DNA polymerases. Propyrazamide was used to block cell cycle progression into mitosis by depolymerizing the mitotic spindle (Planchais et al., 2000). The efficiency of the drugs to stop cell cycle progression was confirmed by flow cytometric analysis (data not shown). After the drugs had been applied for 24 h, the CDK complexes were purified and analyzed on protein gel blots with specific antibodies against CDKA;1 and CDKB1;1 (Hemerly et al., 1995; Porceddu et al., 2001). CDKA;1 belongs to the archetypical CDKs, characterized by the presence of a PSTAIRE amino acid sequence motif in the cyclin binding protein domain, and CDKB1;1 belongs to the group of plant-specific CDKs (De Veylder et al., 2003; Boudolf et al., 2004a, 2004b). Neither drug treatment had an effect on the abundance of CDKA;1 protein compared with that of control cells (Figure 1A). In contrast, CDKB1;1 levels increased slightly in the propyzamide-treated cells, probably because of the preferential expression of the CDKB1;1 gene during M-phase (Porceddu et al., 2001; Boudolf et al., 2004b). Next, the protein blots were probed with an anti-phosphotyrosine antibody. Whereas no antibody binding was detected in protein samples from control or propyzamide-treated cells (Figure 1A), a polypeptide band with the same electrophoretic mobility as that of CDKA;1 cross-reacted with the antibody in extracts prepared from the aphidicolin-treated cells. This analysis strongly indicates that CDKA;1 is the target for Tyr phosphorylation upon activation of the DNA replication checkpoint.

To analyze whether the Arabidopsis WEE1 kinase might be responsible for the observed Tyr phosphorylation of CDKA;1, both proteins were tested for their interaction using the yeast two-hybrid system. CDKA;1 and CDKB1;1 in fusion with the GAL4 DNA binding domain were cotransformed in an appropriate yeast reporter strain with an empty control vector or a vector encoding the GAL4 transactivation domain and WEE1. Transformants were streaked on medium with or without His. Cells expressing CDKA;1 and WEE1 grew in the absence of His, indicating that both gene products interacted. No association was observed between CDKB1;1 and WEE1 (Figure 1B).

WEE1 Gene Expression Is Induced in Response to Activation of the DNA Replication Checkpoint in Cultured Arabidopsis Cells and Seedlings

The observed phosphorylation of CDKA;1 upon DNA replication blockage and its association with WEE1 suggested an involvement of the WEE1 kinase in the checkpoint pathway. Therefore, we analyzed the transcriptional response of the WEE1 gene in cell suspensions treated with either the ribonucleotide reductase inhibitor hydroxyurea (HU) or aphidicolin (Figures 2A and 2B). Drugs were added to exponentially growing cells and samples were collected at different time points for the next 20 h, after which WEE1 and CDKA;1 transcript levels were analyzed by semiquantitative RT-PCR. The actin 2 (ACT2) gene was used as a loading control.

Figure 2. Transcriptional Response of the WEE1 Gene after Activation of the DNA Replication Checkpoint.

(A) and (B) Transcript levels of WEE1 and CDKA;1 in Arabidopsis cells treated with 40 mM HU (A) and 10 μL/mL aphidicolin (B). Samples were harvested at the indicated time points after addition of the drugs. Gene expression was analyzed by semiquantitative RT-PCR. The ACT2 gene was used as a loading control.

(C) and (D) Transgenic Arabidopsis roots harboring the WEE1 promoter fused to the GUS gene grown in the absence (C) or presence (D) of 10 mM HU. Plants were stained for GUS activity 20 h after drug application. Both images are at the same magnification. Bar = 50 μm.
By contrast, WEE1 transcript levels increased dramatically within 4 h after drug treatment, reaching maximum levels at 6 and 10 h after addition of HU and aphidicolin, respectively, indicating that replication inhibition transcriptionally activated the WEE1 gene.

To investigate the transcriptional induction of the WEE1 gene in response to DNA replication stress at the tissue level, transgenic Arabidopsis lines were generated that expressed the β-glucuronidase (GUS) reporter gene under the control of the WEE1 promoter. A reproducible expression pattern was found in three independent reporter lines. Under standard growth conditions, promoter activity was detected locally in the shoot apex of seedlings as well as in the vasculature of the cotyledons and roots (Figures 3A and 3B). Also in older seedlings, expression was confined to apex and vascular tissues of roots and leaves (Figure 3C). Surprisingly, only occasionally, a faint GUS signal was detected in the apex of both main and lateral roots (Figures 3D and 3E). By contrast, GUS staining was strong in developing flowers, particularly in the anthers and gynoecia (Figure 3F), but not in mature flowers (Figure 3G).

To characterize the transcriptional induction of the WEE1 gene in response to HU treatment, seeds of plants harboring the WEE1:GUS reporter were germinated on control medium. After 2 weeks, seedlings were transferred onto fresh control medium or a medium supplemented with HU. After 20 h, plants were harvested and assayed for GUS activity. No GUS staining was observed in the primary and lateral root meristems of plants transferred to the control medium (Figure 2C). In contrast, roots of the plants treated with HU for 20 h showed strong GUS staining in the root apical meristem, mostly confined to the cells of the central cylinder (Figure 2D). Similarly, WEE1::GUS reporter expression in the shoot apical meristem and vascular tissues was clearly induced by the HU treatment (data not shown). From these observations, we conclude that transcriptional control seems to play a major role in the regulation of WEE1 kinase activity during DNA replication stress.

WEE1 Activity Is Not Required for Cell Division or Endoreduplication

To study the role of WEE1 during normal plant development in more detail, plants were analyzed phenotypically that carried a mutation in the WEE1 gene as a result of the insertion of T-DNA. Three different T-DNA insertion lines were used (Figure 4A). The wee1-2 mutant allele harbored a T-DNA insertion in the first exon of the WEE1 gene, deleting most of the WEE1 protein, whereas the wee1-1 insert located between exons 7 and 8 resulted in a deletion of the last 197 (of 500) amino acids. Likewise, the wee1-3 T-DNA insertion was localized in the kinase domain, with a deletion of 112

Figure 3. Spatial Expression Pattern of WEE1.
Promoter activity was visualized through histochemical GUS staining.
(A) Young seedling with strong GUS staining in the shoot apical meristem and vascular tissues of the root and cotyledon.
(B) Transverse section of the root with GUS staining in the vascular bundle and pericycle.
(C) Older seedling with GUS staining in the shoot apical meristem and vascular tissues.
(D) and (E) Primary and lateral root apex without staining or with only weak GUS staining, respectively.
(F) and (G) Young developing flower bud and mature flower, showing strong and weak GUS staining, respectively.
Bars = 2 mm in (A) and (G), 50 μm in (B), and 200 μm (D) to (G).
amino acids as a consequence. Because the WEE1 kinase domain was located at the extreme C terminus of the WEE1 protein (amino acids 249 to 495), all mutants probably corresponded to null alleles. To test this hypothesis, both the full-length and the wee1 alleles with a truncated kinase domain were cloned under the control of the no message in thiamine (nmt1) promoter, which is repressed in the presence of thiamine and can be induced by growing cells in thiamine-free medium (Maundrell, 1990). The obtained constructs were used to transform fission yeast cells. No significant difference in cell size was observed for the different constructs under noninducing conditions. In agreement with previously published results, in the absence of thiamine, expression of the full-length WEE1 gene clearly interfered with the yeast cell cycle, resulting in an elongated cell phenotype (Figure 4B) (Sun et al., 1999; Sorrell et al., 2002). By contrast, expression of the truncated wee1 alleles did not arrest the yeast cell cycle, because no difference in cell size was observed between cells grown in the presence or absence of thiamine. These data show that a complete kinase domain is essential for WEE1 functioning.

In none of the insertion lines was any full-length WEE1 transcript detected (Figure 4C). When cultivated under standard growth conditions, the WEE1 T-DNA insertion plants had no obvious morphological deviation from wild-type plants. For all plants, the mature first leaves were of similar size and contained the same number of abaxial epidermal pavement cells (Table 1). Also, the primary root length and number of lateral roots per millimeter were similar for wild-type and mutant plants (see Supplemental Figure 1 online).

In both maize and tomato, WEE1 transcript levels have been found to peak in cell types entering the endoreduplication cycle, an alternative cell cycle during which DNA replication is not automatically followed by cytokinesis (Sun et al., 1999; Gonzalez et al., 2004). These data suggested a role for WEE1 as an important regulator of the mitosis-to-endocycle transition. However, no such role could be deduced from the Arabidopsis WEE1 knockout plants, because the DNA ploidy distribution profile of wild-type and mutant plants was found to be identical in all tissues tested (see Supplemental Figure 2 online).

**WEE1 Loss-of-Function Plants Are Hypersensitive to Replication-Inhibitory Drugs**

Because of the observed induction of WEE1 in response to HU and aphidicolin, the growth of wee1 mutant plants was tested in the presence of drugs that block DNA replication. Wild-type and WEE1-deficient plants were germinated and grown on control medium for 5 d and subsequently transferred to control medium or medium containing either HU or aphidicolin at a dose that had mild, but perceptible, effects on the growth of the wild-type root (Culligan et al., 2004). In the presence of 1 mM HU or 12 μg/mL aphidicolin, the length of wild-type roots was reduced by 32 and 72%, respectively, compared with that of untreated plants (Figures 5A, 5B, 5D, and 5F). In contrast, WEE1-deficient root growth was reduced by >70% in the presence of HU (Figures 5A and 5E) and root growth was totally arrested in the presence of aphidicolin (Figures 5A and 5G). The growth inhibition phenotype was even more severe for plants germinated directly on aphidicolin-containing medium. Aphidicolin treatment clearly inhibited the growth of both wild-type and wee1-1 mutant plants, although much more severely in the latter.

**Figure 4.** Molecular Analysis of WEE1-Deficient Plants.

(A) Intron-exon organization of the WEE1 gene. Black and gray boxes represent exons, and lines indicate introns. The coding regions corresponding to the kinase domain are indicated in gray. The triangles correspond to the insertion sites of the different mutant alleles.

(B) Yeast cells harboring the full-length (WEE1-FL) or truncated (WEE1Δ197 and WEE1Δ112) alleles of WEE1, grown in the presence (+thia) or absence (-thia) of thiamine. Bars = 10 μm.

(C) Two-step RT-PCR analysis performed on equal amounts of total RNA prepared from 8-d-old wild-type (Columbia [Col-0]) and mutant (wee1-1, wee1-2, and wee1-3) seedlings with primers that specifically amplify the WEE1-coding sequence flanking the T-DNA insertion site. The ACT2 gene was used as a loading control.
All three mutant lines had the same recessive phenotype that segregated with the respective insertions in WEE1. We reasoned that the root growth arrest observed for the WEE1-deficient plants was attributable to a failure to block their cell cycle in response to DNA stress. To test this hypothesis, we compared the number of dividing cells in the root tips of the wild-type and WEE1-deficient plants. Under control growth conditions, the number of cells in mitosis was similar in both genotypes (Figure 6A). When control plants were treated with HU, they experienced a dramatic decrease in the number of mitotic cells. This decrease correlated with the appearance of a Tyr-phosphorylated CDK that migrated with the same electrophoretic mobility as that of CDKA;1 (Figure 6B). Moreover, a decrease in CDK activity was observed within 5 h after transfer to HU-containing medium (Figure 6C). By contrast, in the WEE1-deficient plants, the number of mitotic cells decreased only slightly upon HU treatment (Figure 6A). In addition, neither CDK Tyr phosphorylation nor a decrease in CDK activity was seen (Figures 6B and 6C). These data suggest that the WEE1-deficient plants failed to activate a G2 arrest and progressed with a not fully replicated genome into mitosis.

### Table 1. Size and Number of Abaxial Pavement Cells in Leaves of WEE1-Deficient and Control Plants

| Line   | Leaf Size (mm²) | Abaxial Pavement Cell Size (µm²) | Estimated Number |
|--------|----------------|---------------------------------|-----------------|
| Columbia     | 22.4 ± 0.6     | 2129 ± 90                      | 10.746 ± 298    |
| wee1-1       | 22.1 ± 1.0     | 2183 ± 140                     | 10.334 ± 373    |
| wee1-2       | 23.0 ± 1.5     | 2090 ± 92                      | 10.981 ± 519    |
| wee1-3       | 20.0 ± 1.3     | 1946 ± 76                      | 10.250 ± 452    |

All measurements were performed on leaves harvested 21 d after sowing. The indicated values are means ± SE (n = 14 to 30).

Figure 5. Phenotypic Analysis of WEE1-Deficient Plants under Replication Stress.

(A) Root elongation rates of plants shown in (B) to (G). Error bars indicate SE (n = 14 to 20).

(B) to (G) Wild-type ([B], [D], and [F]) and wee1-1 ([C], [E], and [G]) plants grown for 5 d and then transferred for 5 d to control medium ([B] and [C]), medium supplemented with 1 mM HU ([D] and [E]), or medium supplemented with 12 µg/mL aphidicolin ([F] and [G]).

(H) and (I) Wild-type and wee1-1 seeds germinated on 12 µg/mL aphidicolin, respectively.
Transcriptional Upregulation of WEE1 upon Replication Stress Depends on the ATR Kinase

Wild-type and atr-2 plants were germinated and grown on control medium for 5 d and subsequently transferred to control medium or medium containing HU. Plants were grown for 24 h, after which WEE1 transcript level was analyzed by real-time quantitative PCR. Under control growth conditions, only a low basal WEE1 expression level was detected in both genotypes (Figure 7). When grown on HU-containing medium, the WEE1 expression levels increased by twofold in wild-type plants. By contrast, in the atr-2 plants, no induction of WEE1 upon HU treatment was observed. Therefore, we conclude that the transcriptional activation of WEE1 in response to replication-arresting drugs depends on ATR kinase function.

ATM Kinase Is Required for the Activation of the WEE1 Gene in Response to DNA Damage

To test whether the transcriptional induction of WEE1 is specifically linked to DNA replication stress or correlated with a general loss of DNA integrity, we analyzed whether the WEE1 gene is modulated in response to DNA damage. To induce the in vivo formation of double-stranded breaks in plant cell DNA, we used the radiomimetic drug zeocin, belonging to the family of bleomycin/phleomycin antibiotics that are known to bind and cleave DNA. As a positive control, we used plants that carried as transgene the poly(ADP-ribose)polymerase2 (PARP2) gene promoter fused to GUS (Babiychuk et al., 1998), as ionizing radiation and radiomimetic drugs are known to induce the Arabidopsis PARP2 gene (Doucet-Chabeaud et al., 2001; Chen et al., 2003). One-week-old WEE1:GUS reporter seedlings were transferred from standard germination medium to liquid medium supplemented with zeocin in serial dilutions, ranging from 1 to 100 μg/mL. Treatment of the PARP2:GUS reporter lines with zeocin induced a strong dose-dependent induction of GUS activity, demonstrating the efficiency of zeocin as a genotoxic drug (Figure 8).

Transcriptional Upregulation of WEE1 upon Replication Stress Depends on the ATR Kinase

The root phenotype of the WEE1-deficient plants seen upon replication stress mimicked that described for the atr-2 mutant (Culligan et al., 2004), showing densely clustered hairs at the root tip and outgrowth of lateral roots. These observations suggest that WEE1 and ATR operate in the same pathway. To test whether WEE1 activation upon replication stress depended on ATR, the WEE1 expression level in wild-type and atr-2 mutant plants was compared under control and HU stress conditions.
Similarly, WEE1 promoter activity was induced upon zeocin treatment in both root (Figure 8) and shoot (see Supplemental Figure 3 online).

We also tested the effects of ionizing radiation on expression of the WEE1 gene. Two-week-old plantlets exposed to 20 Gray of γ-rays were sampled for RNA preparation immediately after treatment (0 h) and after 30 min, 1, 3, 5, and 8 h. Only a low basal WEE1 transcript level was detected just after treatment. At 1 h after irradiation, the WEE1 mRNA abundance increased by fivefold (Figure 9A). The kinetics of WEE1 gene induction correlated with that of the RAD51 gene (Figure 9B), encoding an eukaryotic homolog of RecA, involved in double-stranded break repair, and demonstrated previously to be transcriptionally induced by γ-rays (Garcia et al., 2003). A very similar γ-irradiation-induced expression was observed for the PARP2 gene (see Supplemental Figure 4 online). This transcriptional activation of WEE1, RAD51, and PARP2 was only transient, although mRNA levels decreased with different kinetics.

Induction of RAD51 by γ-irradiation was shown to depend on ATM (Garcia et al., 2003). To test the ATM dependence of the WEE1 induction, atm-1 plants were treated with γ-rays as described above and transcript levels were measured. As can be observed in Figure 9A, WEE1 transcript levels did not increase in the mutant background, clearly illustrating that transcriptional activation of WEE1 in response to DNA damage is regulated through ATM. Also, the RAD51 and PARP2 genes were not induced in the atm-1 mutant (Figure 9B; see Supplemental Figure 3 online), confirming previous data (Garcia et al., 2003).

Induced Expression of WEE1 Induces a G2 Cell Cycle Arrest

To analyze the effects of induced WEE1 expression on plant growth, we attempted to generate transgenic Arabidopsis plants that constitutively overexpressed the WEE1 gene under the control of the cauliflower mosaic virus 35S promoter but failed to do so, indicating that high WEE1 levels severely impaired growth and interfered with the regeneration of transgenic plants. To overcome this difficulty, we decided to use a switch-on constitutive overexpression approach, which relies on CRE-mediated recombination at lox sites (Joubes et al., 2004) (see Supplemental Figure 5 online). In this design, CRE recombinase expression is controlled by the promoter of the heat-shock protein gene hsp90 and, hence, can be induced by heat treatment. CRE recombinase is able to switch on the expression of WEE1 by catalyzing the excision of the enhanced green fluorescent protein (EGFP) gene, which was flanked by two colinearly orientated lox recombination sites and separated the open reading frame encoding WEE1 from the CDKA;1 promoter. An additional advantage of the developed system is that the transcriptional activity of the transgene at a given chromosomal integration locus and the success of recombinational excision can be monitored visually by scoring the fluorescent tissues. All transgenic lines behaved identically upon WEE1 expression induced by heat-shock treatment. All analyses were performed on root tissues, because heat shock–inducible CRE-dependent recombination was found to be most efficient and homogeneous.

To induce WEE1 expression, transgenic plants were treated for 2 h at 37°C and subsequently returned to normal growth conditions. Two days after applying the heat shock, the WEE1 transcript level had clearly increased (Figure 10A). As expected, induction of WEE1 expression was accompanied by the loss of EGFP fluorescence in the root tissues, where the CDKA;1 promoter is normally active (Figure 10B, panels ii and iv), and by the outgrowth of root hairs close to the root tip (Figure 10B, panel iii), probably because of shrinkage of the root meristem (see below). Root growth was completely arrested by 3 d after...
increase in the expression level of the G2-to-M-phase–specific cyclin CYCB1;1 gene was seen upon WEE1 overexpression (Figure 11). By 4’,-6-diamidino-2-phenylindole and orcein staining of the genomic DNA, no increase in M-phase cells was observed, suggesting that the increase in CYCB1;1 was attributable to a block of the cell cycle during G2 rather than to a post-G2 mitotic arrest. No effect of the heat-shock treatment on the expression of the reporter genes was observed in a control line harboring an inducible GUS gene (Figure 11).

DISCUSSION

DNA can be damaged in a variety of manners. To maintain genome integrity, signaling cascades initiated by the phosphatidylinositol-3-OH kinase–like kinases ATM and ATR control the activity of DNA repair complexes, halt cell cycle progression, and, in some cases, initiate cell death programs, at least in mammals. In plants, the role of ATM/ATR-dependent signaling in the expression of several DNA repair genes, such as RAD51 and PARP1, has been demonstrated (Garcia et al., 2003). However, we know very little about molecular players in DNA damage response that modulate plant cell cycle progression. Here, we show that the Arabidopsis WEE1 gene is transcriptionally activated in response to treatments that induce either DNA damage or DNA replication stress, and this induction depends on the activity of the ATR and ATM kinases, marking WEE1 as a downstream target gene of the ATR-ATM signaling cascades. Because upregulated WEE1 transcription blocked cell cycle progression, we propose a model in which ATR and ATM sense genotoxic stress and enforce a G2 cell cycle phase arrest by activating WEE1 expression, allowing cells to complete the replication of their genome or to repair damaged DNA before proceeding into mitosis (Figure 12). In plants lacking WEE1, cells probably proceed into mitosis prematurely, resulting in loss of genome integrity, eventually triggering cell cycle and growth arrest. This model is corroborated by the observation that in WEE1-deficient lines the mitotic index in the root meristem did not decrease upon HU treatment, as can be observed in control plants (Figure 6A).

Our data indicate that CDKA;1 is a major WEE1 target. First, upon activation of the DNA replication checkpoint, a Tyr-phosphorylated CDK migrated with the same electrophoretic mobility as CDKA;1. Second, CDKA;1 associated directly with WEE1 in the yeast two-hybrid system. In addition, recently, CDKA;1 was shown to be directly phosphorylated by WEE1 in vitro in a Tyr-15–dependent manner (Shimotohno et al., 2006). In mammalian cells, the drug caffeine cancels the DNA replication checkpoint through inhibition of the ATM kinase (Schlegel and Pardee, 1986; Andreassen and Margolis, 1992; Blasina et al., 1999), but in plants, it overrides the replication checkpoint only in the presence of the mitotic cyclin CYCB2 (Weingartner et al., 2003). Therefore, we postulate that among many other partnerships, CDKA;1 in complex with the B2-type cyclin might be the major target for inhibition by the activated checkpoint control pathways.
The severe growth phenotype of WEE1-deficient plants upon activation of the replication checkpoint suggests that WEE1 is part of the prevailing pathway that blocks the cell cycle under DNA stress, because in the presence of a redundant mechanism, no mutant phenotype would have been observed. By contrast, in cells of animals and fission yeast, the CDC25 phosphatase rather than WEE1 is the main target of the DNA damage and replication checkpoint cascades. Through its phosphorylation by the CHK1 and CHK2 kinases at specific amino acids in the N-terminal regulatory domain, CDC25 is targeted for destruction or exported from the nucleus (Boutros et al., 2006). Recently, an Arabidopsis gene coding for a CDC25-like phosphatase was identified (Landrieu et al., 2004). However, although the plant CDC25-like phosphatase displays structural homology with the mammalian CDC25 proteins within its catalytic domain and can activate CDKs in vitro, it lacks the complete N-terminal regulatory domain. Moreover, a role for the Arabidopsis CDC25-like protein in cell cycle control is debated, because no clear effects on cell cycle progression can be seen upon either overexpression or knockout under normal growth conditions or under stress (Bleeker et al., 2006; Dhankher et al., 2006; our unpublished data). Therefore, the Arabidopsis CDC25 protein very likely is not a target of the DNA damage signaling cascades.

In the absence of DNA stress, WEE1 was expressed in the shoot apical meristem and the vascular tissues of roots and leaves, suggesting that it plays a role besides its involvement in checkpoint control. Because WEE1 is expressed during S-phase (Gonzalez et al., 2004; Menges et al., 2005) and CDK Tyr phosphorylation occurs predominantly during DNA replication (Mészáros et al., 2000), we hypothesize that WEE1 might prevent replicating cells from entering mitosis through inhibition of the complexes required for the G2-to-M transition. Upon the incidence of DNA stress, WEE1 expression might be maintained, arresting cells in the G2-phase until DNA synthesis or repair is completed. Such a regulation mechanism would establish a
cell cycle in response to loss of genome integrity. WEE1 activity might offer a more rapid mechanism to block the
pared with transcriptional activation, posttranslational control of
DNA damage or replication arrest (O’Connell et al., 1997; Boddy
protein becomes phosphorylated and stabilizes in response to either
African frog, WEE1 is activated posttranslationally and the pro-
are not rate-limiting for the onset of mitosis. In accordance, the
data suggest that a redundant control mechanism couples DNA
replication with mitosis. A putative candidate to coordinate this
situation in which cells in S-phase are intrinsically coupled to
mitosis. However, no obvious cell division phenotype has been
observed for WEE1-deficient plants under nonstressed growth
conditions, indicating that CDK Tyr phosphorylation and WEE1
are not rate-limiting for the onset of mitosis. In accordance, the
overexpression of a mutant cdka;1 allele in which the putative
phosphorylated Tyr residue is mutated also did not result in an
obvious cell division phenotype (Hemery et al., 1995). These
data suggest that a redundant control mechanism couples DNA
replication with mitosis. A putative candidate to coordinate this
event together with WEE1 is CDKB1;1, whose activity has been
demonstrated to limit the G2-to-M onset (Porceddu et al., 2001;
Boudolf et al., 2004b, 2006).

Alternatively, the discrepancy between the observed high
WEE1 expression in dividing tissues and the lack of a cell division
phenotype for WEE1 knockout plants might be explained by
posttranscriptional control of WEE1 levels. In fission yeast and
African frog, WEE1 is activated posttranslationally and the pro-	ein becomes phosphorylated and stabilizes in response to either
DNA damage or replication arrest (O’Connell et al., 1997; Boddy
et al., 1998; Michael and Newport, 1998; Lee et al., 2001). Com-
pared with transcriptional activation, posttranslational control of
WEE1 activity might offer a more rapid mechanism to block the
cell cycle in response to loss of genome integrity.

A strong expression of WEE1 was observed in flowers, par-
ticularly during gynoecium and anther development, indicating a
role for WEE1 during gametogenesis. Here again, WEE1 might be
induced in response to the double-stranded breaks that arise
during meiotic recombination events. Mutant atm plants are
partially sterile, because of aberrant meiosis accompanied by
chromosomal fragmentation (Garcia et al., 2003). By contrast,
WEE1-deficient mutants are normally fertile, illustrating that
WEE1 activity is not essential for a putative meiotic checkpoint
and suggesting that, if ATM controls a meiotic checkpoint, it
must do so through a mechanism that does not involve WEE1
activation.

Previously, WEE1 had been attributed a role in plant genome
endoreduplication, based on its expression in endoreduplicating
maize and tomato tissues (Sun et al., 1999; Gonzalez et al.,
2004). However, the Arabidopsis WEE1 T-DNA insertion lines
had a wild-type DNA ploidy distribution profile in all tissues
tested, suggesting that if WEE1 controlled the endocycle, it
would do so in a species-dependent manner. Nevertheless, at
this stage, we cannot exclude the possibility that WEE1 controls
the timing of differentiation events other than endoreduplication,
although no significant differences in the timing of leaf develop-
ment have been observed.

Our analysis of transgenic plants demonstrates that cis ele-
ments that are necessary and sufficient for WEE1 gene activation
are contained within the 591-bp DNA sequence upstream from
the translation start. It would be of interest to identify the
transcriptional cascade by which the WEE1 promoter is activ-
ated upon DNA stress. In mammals, the tumor suppressor gene
product p53 is an important transcriptional activator whose
protein levels increase in response to DNA damage. Activated
p53 induces genes coding for CDK inhibitor proteins, such as
p27Kip1, resulting in cell cycle arrest. However, WEE1 is not among
reported p53 gene targets. Moreover, no clear p53 homolog can
be found in the Arabidopsis genome, suggesting that other
pathways must regulate the activation of WEE1 transcription in
response to DNA stress. In fission yeast, transcriptional induc-
tion of WEE1 in response to stress was found to rely on the
mitogen-activated protein kinase (MAPK)–dependent pathway
(Suda et al., 2000) that is activated by a range of stress-inducing
stimuli, including DNA damage (Degols and Russell, 1997).
Interestingly, Arabidopsis MAPK orthologs are activated in response
to DNA damage, and the MAPK phosphatase mutant mkp1 is
hypersensitive to genotoxic stress treatments (Ulm et al., 2001).
Therefore, it would be worthwhile to test whether MKP1 operates
upstream of WEE1.

![Figure 11. Expression Levels of Cell Cycle Markers upon WEE1 Overexpression.](image)

Seven days after sowing, seedlings were mock-treated (−HS) or heat-
h shocked (+HS) for 2 h at 37°C and returned to standard growing
conditions; 24 h later, root tips (most distal 2 to 3 mm) were harvested
for RNA preparation. WEE1, histone H4, and CYCB1;1 transcript levels
were measured by real-time PCR. All values were normalized to the
expression level of the ACT2 housekeeping gene. The normalized value
of the untreated wild-type sample was arbitrarily set to 1.

![Figure 12. Model for WEE1 in the Control of the DNA Integrity Checkpoint.](image)

DNA stress induced by double-stranded DNA breaks (as induced by
γ-irradiation and zeocin) or by blockage of the replication fork (induced
by HU and aphidicolin) is sensed mainly by the ATM or ATR signaling
cascade, respectively (Garcia et al., 2003; Culligan et al., 2004). ATM and
ATR simultaneously induce the expression of DNA repair genes and
iWEE1. WEE1 arrests cells in the G2-phase of the cell cycle, allowing cells
to repair DNA before proceeding into mitosis.
METHODS

Plant Materials and Growth Conditions

Arabidopsis thaliana (ecotype Columbia) plants were grown under long-day conditions (16 h of light, 8 h of darkness) at 22°C on germination medium (Valvekens et al., 1988). The wee1-1 allele (GABI_270E05) was obtained from the GABI-Kat T-DNA mutant collection (http://www.mpiz-koeln.mpg.de/GABI-Kat/GABI-Kat_homepage.html) (Li et al., 2003), whereas the wee1-2 (SALK_147968) and wee1-3 (SALK_039890) alleles were found in the Salk Institute T-DNA Express database (http://signal.salk.edu). The seeds were acquired from the ABRC. To screen for homozygous insertion alleles, primers were used according to GABI-kat and SALK; to screen for the presence of the full-length WEE1 transcript, the following primer pairs were designed: 5′-TGCTGTCGAGCAATT-TTCATCGG-3′ and 5′-GGATATTTCTCCTGTTGGTTGAAAG-3′ for wee1-1, 5′-ATGGTCAGAAACGAAACAC-3′ and 5′-CTATG-ATGGAAGTGAAGCTTGG-3′ for wee1-2, and 5′-TGCTGTCGAGCAAT-TTTCATCGG-3′ and 5′-TGATGGATCTGATCTCCAAGCG-3′ for wee1-3. The WEE1-inducible gene construct was generated by cloning all required DNA fragments generated by PCR into pCRBlunt-TOPO (Invitrogen). Fragments corresponding to the promoter of the Arabidopsis HSP18.2 gene, the CRE recombinase-coding sequence, and the octopine synthase terminator (OCS3) sequence were assembled into the pZEROI-2 vector and subsequently inserted into the pCAMBIA1200 vector, resulting in the pJCRE vector. The Arabidopsis ecotype Landsberg (ecotype Columbia) plants were grown under long-day conditions (120 rpm) in a modified Murashige and Skoog medium (Menges et al., 2002). Suspension- and SALK T-DNA Express database (http://signal.salk.edu). The seeds were acquired from the ABRC. To screen for the presence of the full-length WEE1 transcript, the following primer pairs were designed: 5′-TGCTGTCGAGCAATT-TTCATCGG-3′ and 5′-GGATATTTCTCCTGTTGGTTGAAAG-3′ for wee1-1, 5′-ATGGTCAGAAACGAAACAC-3′ and 5′-CTATG-ATGGAAGTGAAGCTTGG-3′ for wee1-2, and 5′-TGCTGTCGAGCAATT-TTTCATCGG-3′ and 5′-TGATGGATCTGATCTCCAAGCG-3′ for wee1-3. The WEE1-inducible gene construct was generated by cloning all required DNA fragments generated by PCR into pCRBlunt-TOPO (Invitrogen). Fragments corresponding to the promoter of the Arabidopsis HSP18.2 gene, the CRE recombinase-coding sequence, and the octopine synthase terminator (OCS3) sequence were assembled into the pZEROI-2 vector and subsequently inserted into the pCAMBIA1200 vector, resulting in the pJCRE vector. The WEE1 gene was introduced into the pJLOX vector by the GATEWAY recombination site were assembled on a 1.2% agarose gel and transferred onto Hybond N+ membrane. Plant Treatments

Arabidopsis plants and cell cultures were treated with aphidicolin or HU as described by Culligan et al. (2004) and Nagata et al. (1992), respectively. For zeocin treatments, seeds were germinated under aseptic conditions. One-week-old seedlings were transferred onto 12-well plates containing 1 mL of water supplemented with different amounts of zeocin starting from a commercially available zeocin stock solution of 100 mg/mL (Invitrogen). After 24 h of treatment, plants were used for histochemical GUS staining. For γ-irradiation treatments, 2-week-old plantlets grown in vitro were irradiated with γ-rays at a dose of 20 Gray from a 137Cs source (Faculty of Agriculture, Ghent University). Plant material was harvested and immediately frozen in liquid nitrogen according to a time course as described.

DNA and RNA Manipulation

Genomic DNA was extracted from Arabidopsis leaves with the DNeasy Plant kit (Qiagen). RNA was extracted from Arabidopsis tissues and cultured cells with the TriZol reagent (Invitrogen). First-strand cDNA was prepared from 500 ng of total RNA with the Superscript RT II kit (Invitrogen) and oligo(dT)16 according to the manufacturer’s instructions. A 0.2-μL aliquot of the total RT reaction volume (20 μL) was used as a template in a semiquantitative RT-mediated PCR amplification, ensuring that the amount of amplified product remained in linear proportion to the initial template present in the reaction. Ten microliters from the PCR was separated on a 1.2% agarose gel and transferred onto Hybond N+ membranes (GE-Healthcare). The membranes were hybridized at 65°C with fluorescein-labeled probes (Gene Images random prime probe; GE-Healthcare), and the hybridized bands were detected with the CDP Star detection module (GE-Healthcare).

Yeast Two-Hybrid Interactions

Yeast two-hybrid bait and prey vectors were obtained through recombination-based GATEWAY cloning (Invitrogen). The CDKA;1, CDKB1;1, and WEE1 open reading frames were recombined into the pDEST22 and pDEST32 vectors (Invitrogen) by an LR reaction, resulting in translational fusions between the open reading frames and the GAL4 transcriptional activation and GAL4 DNA binding domains, respectively. Plasmids encoding the baits and preys were cotransformed into the yeast reporter strain PJ69-4a (MATa, trp1-901, leu2-3,112, ura3-52, his3-200, gal4D, gal80D, lys2::GAL1-HIS3, GAL2-ADE2, met2::GAL7-iacZ) by the lithium acetate method (Gietz et al., 1992) and plated on SD plates without Leu and Trp. After 2 d of growth at 30°C, yeast was transferred to SD plates without Leu and Trp (as a control) and to SD plates without Leu, Trp, and His. Plates were incubated at 30°C and scored for growth of yeast and, hence, protein–protein interaction after 2 d.
Antibodies and Protein Gel Blot Analysis

Protein extracts were prepared by grinding material in homogenization buffer (De Veylder et al., 1997). Protein concentrations were determined with the protein assay kit (Bio-Rad). CDKs were purified by affinity chromatography with either p105CREML or p95CREML-Sepharose beads as described (De Veylder et al., 1997). Protein gel blotting was performed according to standard procedures with primary anti-CDKA;1 and anti-CDKB1;1 antibodies (Porceddu et al., 2001) diluted 1:5000 and 1:2500, respectively, and a secondary horseradish peroxidase–conjugated sheep anti-rabbit antibody (GE-Healthcare) diluted 1:5000 or with the mouse monoclonal horseradish peroxidase–conjugated anti-phosphotyrosine p-Tyr antibody (PY99; Santa Cruz Biotechnology) diluted 1:5000. Proteins were detected by a chemiluminescence procedure (NEN Life Science Products). Kinase assays were performed as described previously (De Veylder et al., 1997).

Histochemical GUS Assays

Complete seedlings or tissue cuttings were stained on multiwell plates (Falcon 3043; Becton Dickinson). GUS assays were performed as described by Beeckman and Engler (1994). Samples mounted in lactic acid were observed and photographed with a stereomicroscope (Stemi SV11; Zeiss) or with a differential interference contrast microscope (Leica).

Microscopy and Flow Cytometric Analyses

Leaves were harvested at 21 d after sowing, cleared overnight in ethanol, stored in lactic acid for microscopy, and observed with a microscope fitted with differential interference contrast optics (Leica). The total (blade) area was determined from images digitized directly with a digital camera (Axiocam; Zeiss) mounted on a binocular (Stemi SV11; Zeiss). From scanned drawing-tube images of outlines of at least 30 cells of the abaxial epidermis located 25 and 75% from the distance between the tip and the base of the leaf, halfway between the midrib and the leaf margin, the following parameters were determined: total area of all cells in the drawing and total numbers of pavement and guard cells, from which the average cell area was calculated. The total number of cells per cotyledon was estimated by dividing the leaf area by the average cell area. To visualize mitotic cells, seeds were germinated and grown for 5 d as described above and then transferred to control plates or plates containing HU. Root tips were harvested 2 d after transfer and fixed in three parts ethanol and one part acetic acid, macerated in 0.1 N HCl, and squashed in orcein in 45% acetic acid. The number of mitotic cells was determined by observing mitotic figures through a Leica microscope with a 63× oil lens. For flow cytometric analysis, tissues were chopped with a razor blade in 300 μL of 45 mM MgCl₂, 30 mM sodium citrate, 20 mM MOPS, pH 7, and 0.1% Triton X-100 (Galbraith et al., 1991). One micro-liter of 4,6-diamidino-2-phenylindole from a stock of 1 mg/mL was added to the filtered supernatants. The nuclei were analyzed with the CyFlow (Partec) flow cytometer using FloMax (Partec) software. Sections of root tips for histological analysis were prepared according to Beeckman and Viane (1992). 2-Aminopurine overrides PARP2 in response to γ-irradiation. We thank Mirande Naudts for technical support and Martine De Cock for help in preparing the manuscript. This research was supported by grants from the Interuniversity Poles of Attraction Program–Belgian Science Policy (Grant P5/13), the Research Fund of Ghent University (Geconcerteerde Onderzoeksacties Grant 12051403), and the European Union Marie Curie Research Training Networks (Grant MRTN-CT-2004-005338). J.J. and F.C. are indebted to the European Molecular Biology Organization (Heidelberg, Germany) for postdoctoral fellowships. L.D.V. is a Postdoctoral Fellow of the Research Foundation–Flanders.

Received June 19, 2006; revised August 21, 2006; accepted November 14, 2006; published January 5, 2007.

REFERENCES

Abraham, R.T. (2001). Cell cycle checkpoint signaling through the ATM and ATR kinases. Genes Dev. 15: 2177–2196.

Andreassen, P.R., and Margolis, R.L. (1992). 2-Aminopurine overrides multiple cell cycle checkpoint in BHK cells. Proc. Natl. Acad. Sci. USA 89: 2272–2276.

Baber-Furnari, B.A., Rhind, N., Boddy, M.N., Shanahan, P., Lopez-Girona, A., and Russell, P. (2000). Regulation of mitotic inhibitor Mnk1 helps to enforce the DNA damage checkpoint. Mol. Biol. Cell 11: 1–11.

Babychuk, E., Cottrill, P.B., Storozhenko, S., Huangthong, M., Chen, Y., O’Farrell, M.K., Van Montagu, M., Inze, D., and Kushner, S. (1998). Higher plants possess two structurally different poly(ADP-ribose) polymerases. Plant J. 15: 635–645.

Bartek, J., and Lukas, J. (2001). Pathways governing G1/S transition and their response to DNA damage. FEBS Lett. 490: 117–122.
Beeckman, T., and Engler, G. (1994). An easy technique for the clearing of histochemically stained plant tissue. Plant Mol. Biol. Rep. 12: 37–42.

Beeckman, T., and Viana, R. (2000). Embedding thin plant specimens for oriented sectioning. Biotech. Histochem. 75: 23–26.

Berry, L.D., and Gould, K.L. (1996). Regulation of Cdc2 activity by phosphorylation at T14/Y15. In Progress in Cell Cycle Research, Vol. 2, L. Meijer, S. Guidet, and L. Vogel, eds (New York: Plenum Press), pp. 99–105.

Blasina, A., Van de Weyer, I., Laus, M.C., Luyten, W.H.M.L., Parker, A.E., and McGowan, C.H. (1999). A human homologue of the checkpoint kinase Cds1 directly inhibits Cdc25 phosphatase. Curr. Biol. 9: 1–10.

Bleeke, P.M., Hakvoort, H.W.J., Bliék, M., Souer, E., and Schat, H. (2006). Enhanced arsenate reduction by a CDC25-like tyrosine phosphatase explains increased phytochelatin accumulation in arsenate-tolerant Holcus lanatus. Plant J. 45: 917–929.

Boddy, M.N., Furnari, B., Mondesert, O., and Russell, P. (1998). Replication checkpoint enforced by kinases Cds1 and Chk1. Science 280: 909–912.

Boudoul, V., Barróco, R., de Almeida Engel, J., Verkest, A., Beeckman, T., Naudts, M., Inzé, D., and De Veylder, L. (2004a). B1-type cyclin-dependent kinases are essential for the formation of stomatal complexes in Arabidopsis thaliana. Plant Cell 16: 945–955.

Boudoul, V., Inzé, D., and De Veylder, L. (2006). What if higher plants lack a CDC25 phosphatase? Trends Plant Sci. 11: 474–479.

Boudoul, V., Vlieghe, K., Beemster, G.T.S., Magyar, Z., Torres Acosta, J.A., Maes, V., Van Der Schueren, E., Inzé, D., and De Veylder, L. (2004b). The plant-specific cyclin-dependent kinase CDKB1;1 and transcription factor E2Fa-DPa control the balance of mitotically dividing and endoreduplicating cells in Arabidopsis. Plant Cell 16: 2683–2692.

Boutros, R., Dozier, C., and Ducommun, B. (2006). The when and where of CDC25 phosphatases. Curr. Opin. Cell Biol. 18: 185–191.

Chen, I.-P., Haehnel, U., Altschmied, L., Schubert, I., and Puchta, H. (2003). The transcriptional response of Arabidopsis to genotoxic stress—A high-density colony array study (HDCA). Plant J. 35: 771–786.

Chen, Y., and Sanchez, Y. (2004). Chk1 in the DNA damage response: Conserved roles from yeasts to mammals. DNA Repair (Amst.) 3: 1025–1032.

Christensen, P.U., Bentley, N.J., Martinho, R.G., Nielsen, O., and Carr, A.M. (2000). Mik1 levels accumulate in S phase and may mediate an intrinsic link between S phase and mitosis. Proc. Natl. Acad. Sci. USA 97: 2579–2584.

Clough, S.J., and Bent, A.F. (1998). Floral dip: A simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. 16: 735–743.

Corellou, F., Biscgrove, S.R., Kropf, D.L., Meijer, L., Kloareg, B., and Bouget, F.-Y. (2000). A Sm DNA replication checkpoint prevents nuclear and cytoplasmic events of cell division including centrosomal axis alignment and inhibits activation of cyclin-dependent kinase-like proteins in fucoid zygotes. Development 127: 1651–1660.

Craven, R.A., Griffiths, D.J.F., Sheldrick, K.S., Randall, R.E., Hagan, I.M., and Carr, A.M. (1998). Vectors for the expression of tagged proteins in Schizosaccharomyces pombe. Gene 221: 59–68.

Culligan, K., Tissier, A., and Britton, A. (2004). ATR regulates a G2-phase cell-cycle checkpoint in Arabidopsis thaliana. Plant Cell 16: 1091–1104.

Degols, G., and Russell, P. (1997). Discrete roles of the Spc1 kinase and the Aft1 transcription factor in the UV response of Schizosaccharomyces pombe. Mol. Cell. Biol. 17: 3356–3363.

De Veylder, L., Joubès, J., and Inzé, D. (2003). Plant cell cycle transitions. Curr. Opin. Plant Biol. 6: 536–543.

De Veylder, L., Segers, G., Glab, N., Casteels, P., Van Montagu, M., and Inzé, D. (1997). The Arabidopsis Cks1At protein binds the cyclin-dependent kinases Cdc2aAt and Cdc2bAt. FEBS Lett. 412: 446–452.

Dewitte, W., and Murray, J.A.H. (2003). The plant cell cycle. Annu. Rev. Plant Biol. 54: 235–264.

Dhankher, O.P., Rosen, B.P., McKinney, E.C., and Meagher, R.B. (2006). Hyperaccumulation of arsenic in the shoots of Arabidopsis silenced for arsena reductase (ACR2). Proc. Natl. Acad. Sci. USA 103: 5413–5418.

Doucet-Chabaud, G., Godon, C., Brutesco, C., de Murcia, G., and Kazmaier, M. (2001). Ionising radiation induces the expression of PARP-1 and PARP-2 genes in Arabidopsis. Mol. Genet. Genomics 265: 954–963.

Gaillraith, D.W., Harkins, K.R., and Knapp, S. (1991). Systemic endopolyploidy in Arabidopsis thaliana. Plant Physiol. 96: 985–989.

Garcia, V., Bruchet, H., Camesasca, D., Granier, F., Bouchez, D., and Tissier, A. (2003). AtMik1 levels accumulate in S phase and may mediate the erasure of an endoreduplication checkpoint in Arabidopsis. Plant Cell 15: 119–132.

Gietz, D., St. Jean, A., Woods, R.A., and Schiestl, R.H. (1992). Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res. 20: 1425.

Gonzalez, N., Hernould, M., Delmas, F., Gévaudant, F., Dufpe, P., Caussé, M., Mouras, A., and Chevalier, C. (2004). Molecular characterization of a WEE1 gene homologue in tomato (Lycopersicon esculentum Mill.). Plant Mol. Biol. 56: 849–861.

Hemerly, A., de Almeida Engel, J., Bergouinioux, C., Van Montagu, M., Engler, G., Inzé, D., and Ferreira, P. (1995). Dominant negative mutants of the Cdc2 kinase uncouple cell division from iterative plant development. EMBO J. 14: 3925–3936.

Inzé, D., and De Veylder, L. (2006). Cell cycle regulation in plant development. Annu. Rev. Genet. 40: 77–105.

Joubès, J., De Schutter, K., Verkest, A., Inzé, D., and De Veylder, L. (2004). Conditional, recombinase-mediated, expression of genes in plant cell cultures. Plant J. 37: 889–896.

Karimi, M., Inzé, D., and Depicker, A. (2002). GATEWAY™ vectors for Agrobacterium-mediated plant transformation. Trends Plant Sci. 7: 193–195.

Kurz, E.U., and Lees-Miller, S.P. (2004). DNA damage-induced activation of ATM and ATM-dependent signaling pathways. DNA Repair (Amst.) 3: 889–900.

La, H., Li, J., Ji, Z., Cheng, Y., Li, X., Venkatesh, P.N., and Ramachandran, S. (2006). Genome-wide analysis of cyclin family in rice (Oryza sativa L.). Mol. Genet. Genomics 275: 374–386.

Landrieu, I., da Costa, M., De Veylder, L., Dewitte, F., Vandepoele, K., Hassan, S., Wieruszkes, J.-M., Corellou, F., Faure, J.-D., Van Montagu, M., Inzé, D., and Lippens, G. (2004). A small CDC25 dual-specificity tyrosine-phosphatase isoform in Arabidopsis thaliana. Proc. Natl. Acad. Sci. USA 101: 13380–13385. Erratum. Proc. Natl. Acad. Sci. USA 101: 16391.

Lee, J., Kumagai, A., and Dunphy, W.G. (2001). Positive regulation of Wee1 by Chk1 and 14-3-3 proteins. Mol. Biol. Cell 12: 551–563.

Lees, E. (1995). Cyclin dependent kinase regulation. Curr. Opin. Cell Biol. 7: 773–780.

Li, Y., Rosso, M.G., Strizhov, N., Viehoever, P., and Weisshaar, B. (2003). GABI-Kat SimpleSearch: A flanking sequence tag (FST) database for the identification of T-DNA insertion mutants in Arabidopsis thaliana. Bioinformatics 19: 1441–1442.

Maundrell, K. (1990). nmt1 of fission yeast. A highly transcribed gene completely repressed by thiamine. J. Biol. Chem. 265: 10857–10864.

Menges, M., de Jager, S.M., Gruissem, W., and Murray, J.A.H. (2005). Global analysis of the core cell cycle regulators of Arabidopsis identifies novel genes, reveals multiple and highly specific profiles of expression and provides a coherent model for plant cell cycle control. Plant J. 41: 546–566.
Menges, M., Hennig, L., Gruissem, W., and Murray, J.A.H. (2002). Cell cycle-regulated gene expression in Arabidopsis. J. Biol. Chem. 277: 41987–42002.

Mészáros, T., Miskolczi, P., Ayaydın, F., Pettkó-Szandtner, A., Peres, A., Magyar, Z., Horváth, G.V., Bakó, L., Fehér, A., and Dudits, D. (2000). Multiple cyclin-dependent kinase complexes and phosphatases control G2/M progression in alfalfa cells. Plant Mol. Biol. 43: 595–605.

Michael, W.M., and Newport, J. (1998). Coupling of mitosis to the completion of S phase through Cdc34-mediated degradation of Wee1. Science 282: 1886–1889.

Moreno, S., Klar, A., and Nurse, P. (1991). Molecular genetic analysis of fission yeast Schizosaccharomyces pombe. Methods Enzymol. 194: 795–826.

Murray, A.W. (2004). Recycling the cell cycle: Cyclins revisited. Cell 116: 221–234.

Nagata, T., Nemoto, Y., and Hasezawa, S. (1992). Tobacco BY-2 cell line as the “HeLa” cell in the cell biology of higher plants. Int. Rev. Cytol. 132: 1–30.

O’Connell, M.J., Raleigh, J.M., Verkade, H.M., and Nurse, P. (1997). Cdk1 is a wee1 kinase in the G2 DNA damage checkpoint inhibiting cdc2 by Y15 phosphorylation. EMBO J. 16: 545–554.

Ohl, R., and Gould, K.L. (1999). Regulating the onset of mitosis. Curr. Opin. Cell Biol. 11: 267–273.

Peters, J.-M. (1998). SCF and APC: The Yin and Yang of cell cycle regulated proteolysis. Curr. Opin. Cell Biol. 10: 759–768.

Planchais, S., Glab, N., Inzé, D., and Bergounioux, C. (2000). Chemical inhibitors: A tool for plant cell cycle studies. FEBS Lett. 476: 78–83.

Porceddu, A., Stals, H., Reichheld, J.-P., Segers, G., De Veylder, L., De Pinho Barroco, R., Casteels, P., Van Montagu, M., Inzé, D., and Mironov, V. (2001). A plant-specific cyclin-dependent kinase is involved in the control of G2/M progression in plants. J. Biol. Chem. 276: 36354–36360.

Rhind, N., and Russell, P. (2001). Roles of the mitotic inhibitors Wee1 and Mik1 in the G2 DNA damage and replication checkpoints. Mol. Cell. Biol. 21: 1499–1508.

Sancar, A., Lindsey-Boltz, L.A., Ünsal-Kaçmaz, K., and Linn, S. (2004). Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu. Rev. Biochem. 73: 39–85.

Schlegel, R., and Pardee, A.B. (1986). Caffeine-induced uncoupling of mitosis from the completion of DNA replication in mammalian cells. Science 232: 1264–1266.

Schupper, U., He, P.-H., John, P.C.L., and Munns, R. (1998). Effect of water stress on cell division and cell-division-cycle 2-like cell-cycle kinase activity in wheat leaves. Plant Physiol. 117: 667–678.

Shimotono, A., Ohno, R., Bisova, K., Sakaguchi, N., Huang, J., Koncz, C., Uchiyama, Y., and Umeda, M. (2006). Diverse phospho-regulatory mechanisms controlling cyclin-dependent kinase-activating kinases in Arabidopsis. Plant J. 47: 701–710.

Sorrell, D.A., Marchbank, A., McMahon, K., Dickinson, J.R., Rogers, H.J., and Francis, D. (2002). A WEE1 homologue from Arabidopsis thaliana. Planta 215: 518–522.

Suda, M., Yamada, S., Toda, T., Miyakawa, T., and Hirata, D. (2000). Regulation of WEE1 kinase in response to protein synthesis inhibition. FEBS Lett. 486: 305–309.

Sun, Y., Dilkes, B.P., Zhang, C., Dante, R.A., Carneiro, N.P., Lowe, K.S., Jung, R., Gordon-Kamm, W.J., and Larkins, B.A. (1999). Characterization of maize (Zea mays L.) WEE1 and its activity in developing endosperm. Proc. Natl. Acad. Sci. USA 96: 4180–4185.

Ulm, R., Revenkova, E., di Sansebastiano, G.-P., Bechtold, N., and Paszkowski, J. (2001). Mitogen-activated protein kinase phosphatase is required for genotoxic stress relief in Arabidopsis. Genes Dev. 15: 699–709.

Valvekens, D., Van Montagu, M., and Van Lijsebettens, M. (1988). Agrobacterium tumefaciens-mediated transformation of Arabidopsis thaliana root explants by using kanamycin selection. Proc. Natl. Acad. Sci. USA 85: 5536–5540.

Vandepoele, K., Raes, J., De Veylder, L., Rouzé, P., Rombouts, S., and Inzé, D. (2002). Genome-wide analysis of core cell cycle genes in Arabidopsis. Plant Cell 14: 903–916.

Wang, G., Kong, H., Sun, Y., Zhang, X., Zhang, W., Altman, N., dePamphilis, C.W., and Ma, H. (2004). Genome-wide analysis of the cyclin family in Arabidopsis and comparative phylogenetic analysis of plant cyclin-like proteins. Plant Physiol. 135: 1084–1099.

Weingartner, M., Pelayo, H.R., Binarova, P., Zwerer, K., Melikant, B., de la Torre, C., Heberle-Bors, E., and Bögler, L. (2003). A plant cyclin B2 is degraded early in mitosis and its ectopic expression shortens G2-phase and alleviates the DNA-damage checkpoint. J. Cell Sci. 116: 487–498.

Zhang, K., Letham, D.S., and John, P.C.L. (1996). Cytokinin controls the cell cycle at mitosis by stimulating the tyrosine dephosphorylation and activation of p34cdc2-like H1 histone kinase. Planta 200: 2–12.

Zhou, B.-B.S., and Elledge, S.J. (2000). The DNA damage response: Putting checkpoints in perspective. Nature 408: 433–439.
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Kristof De Schutter, Jérôme Joubès, Toon Cools, Aurine Verkest, Florence Corellou, Elena Babychuk, Els Van Der Schueren, Tom Beeckman, Sergeï Kushnir, Dirk Inzé and Lieven De Veylder

Plant Cell 2007;19:211-225; originally published online January 5, 2007;
DOI 10.1105/tpc.106.045047

This information is current as of April 27, 2019