A DNA2 Homolog Is Required for DNA Damage Repair, Cell Cycle Regulation, and Meristem Maintenance in Plants

Ning Jia, Xiaomin Liu, and Hongbo Gao*

College of Biological Sciences and Biotechnology, Beijing Forestry University, Beijing, 100083, China (N.J., X.L., H.G.)

Plant meristem cells divide and differentiate in a spatially and temporally regulated manner, ultimately giving rise to organs. In this study, we isolated the Arabidopsis "jing he sheng 1" (jhs1) mutant, which exhibited retarded growth, an abnormal pattern of meristem cell division and differentiation, and morphological defects such as fasciation, an irregular arrangement of siliques, and short roots. We identified JHS1 as a homolog of human and yeast DNA Replication Helicase/Nuclease2, which is known to be involved in DNA replication and damage repair. JHS1 is strongly expressed in the meristem of Arabidopsis. The jhs1 mutant was sensitive to DNA damage stress and had an increased DNA damage response, including increased expression of genes involved in DNA damage repair and cell cycle regulation, and a higher frequency of homologous recombination. In the meristem of the mutant plants, cell cycle progression was delayed at the G2 or late S phase and genes essential for meristem maintenance were misregulated. These results suggest that JHS1 plays an important role in DNA replication and damage repair, meristem maintenance, and development in plants.

In higher plants, organ morphogenesis depends on the persistent activity of apical meristems during development (Donnelly et al., 1999; Fleming, 2006). Apical meristem development is sustained and driven by cell division in meristematic regions (Traas and Bohn-Courseau, 2005; Zhou et al., 2011). Plant meristems, which contain stem cells and have a powerful regenerative ability, enable sustained plant growth and give rise to new organs such as roots, stems, leaves, and flowers. Plant architecture and the final form of organs depend on spatially and temporally regulated cell proliferation in meristems.

Cells in the shoot apical meristem (SAM) function coordinately and are distributed in distinct regions, namely the central zone, which includes stem cells and the organizing center, the peripheral zone, and the rib zone (Aichinger et al., 2012). Meristem-specific genes, such as SHOOT MERISTEMLESS (STM), WUSCHEL (WUS), and CLAVATA3 (CLV3), are key regulators of SAM formation (Aichinger et al., 2012). STM encodes a transcription factor that promotes cytokinin biosynthesis and inhibits gibberellin biosynthesis, thus preventing cell differentiation in the SAM (Yanai et al., 2005). WUS, which also encodes a transcription factor, defines the organizing center (OC) of SAMs (Mayer et al., 1998; Lenhard and Laux, 2003). CLV3 encodes a small protein that can be processed into a small peptide, which interacts with and probably activates a putative receptor kinase, CLAVATA1 (CLV1), to regulate SAM size (Clark et al., 1997; Fletcher et al., 1999; Shinohara and Matsubayashi, 2015). The SAMs of csl1 and csl3 mutants are significantly enlarged relative to the wild type and produce more floral meristems than wild-type plants (Clark et al., 1993, 1995). The wus mutant lacks a SAM and overexpression of WUS results in enlarged meristems, suggesting that WUS prevents the differentiation of stem cells and is essential for maintaining the structural and functional integrity of the SAM (Mayer et al., 1998; Lenhard and Laux, 2003). In the SAM, a feedback regulation loop exists between WUS and CLV3; WUS maintains CLV3 expression in the OC and CLV3 functions as an intercellular signal that represses WUS transcription (Schoof et al., 2000). The controlled expression of WUS in the distal region of the OC is important for maintaining the boundaries between stem cells and differentiating cells.

Quiescent center (QC) cells are the mitotically inactive cells in the center of the root apical meristem (RAM) that inhibit differentiation of the surrounding stem cells (van den Berg et al., 1997). Together with their adjacent cells, QC cells establish intercellular signal communication and regulate the balance between division and differentiation in tissues such as the steel, ground tissue, epidermis, lateral root caps, and starch-containing...
and gravity-sensing columella (Lenhard and Laux, 2003). WUSCHEL-RELATED HOMEBOX5 (WOX5) is a homolog of WUS that is specifically expressed in QC cells (Haeckner et al., 2004). WOX5 maintains stem cells in the RAM and represses the differentiation of columella stem cells (Sarkar et al., 2007). Several CLV3-related peptides, such as CLE40, antagonize WOX5 function and promote differentiation of stem cells (Stahl et al., 2009).

Several fasciated mutants display similar developmental defects as clv3 and clv1. However, the genes affected in most of those mutants seem not to be directly related to CLV3 and CLV1 function. For example, FAS1 and FAS2, which encode proteins identified as counterparts of human Chromatin assembly factor1, have also been shown to be involved in maintaining the cellular and functional organization of the SAM and RAM, and mutation of either FAS1 or FAS2 causes fasciation, abnormal phyllotaxy, and defective root growth (Kaya et al., 2001). Further analyses suggested that fas1 is sensitive to DNA damage agents and exhibits enhanced endoreplication (Endo et al., 2006; Kirik et al., 2006). In addition, fasciated mutants with defects in BRU1/MGO3/TSK, which is thought to be involved in chromatid separation during mitosis (Takeda et al., 2004; Suzuki et al., 2005), are hypersensitive to DNA damage agents, show a release of gene silencing, and are delayed in cell cycle progression. Furthermore, MGO1, which encodes a type-IB DNA topoisomerase, was shown to be involved in stem cell regulation (Takahashi et al., 2002; Graf et al., 2010). DNA polymerase ε was shown to influence the cell cycle, embryonic patterning, epigenetic regulation, somatic homologous recombination, and ABA signaling (Jenik et al., 2005; Yin et al., 2009). teb, a mutant of DNA polymerase θ, exhibited a constitutively activated DNA damage response and defects in cell cycle progression (Inagaki et al., 2006). Mutants of MRE11 and BRCA2, which function in DNA damage repair, were also shown to have a fasciated phenotype (Bundock and Hooykaas, 2002; Abe et al., 2009). These data together suggest that chromatin assembly, DNA replication, DNA damage repair, cell cycle regulation, and related events are important for meristem organization and plant development.

DNA2 is a conserved essential protein with a helicase and a nuclease domain, and is thought to be involved in Okazaki fragment synthesis and DNA damage repair (Budd et al., 2006). During maturation of the Okazaki fragment, the short RNA/DNA primer flap is removed by FEN1 (Zheng and Shen, 2011). However, long flaps may also occur, and these are removed by the sequential action of DNA2 and FEN1 (Kang et al., 2010). During DNA break repair, DNA2 mediates 5'–end resection of DNA by cleaving the 5'–single-stranded DNA (ssDNA), with the aid of Sgs1 and RPA (Zhu et al., 2008; Mimitou and Symington, 2009; Čejka et al., 2010). Sgs1 is a helicase, whose activity is stimulated by RPA, and which can unwind DNA and produce an ssDNA substrate for DNA2. Homologs of Sgs1 and RPA are also conserved in plants with important roles in DNA repair and other processes (Aklilu et al., 2014; Schröpfer et al., 2014). Yeast DNA2 has Pat4, Pat7, and bipartite nuclear localization signal (NLS) sequences and is localized to the nucleus (Zheng et al., 2008). Yeast dna2 mutants exhibit increased sensitivity to DNA-damaging agents, such as UV, MMS (methyl methane sulfonate), and x-ray irradiation, and yeast DNA2 participates in DNA repair by facilitating homologous recombination (Budd and Campbell, 2000). In mammals, DNA2 plays a role in DNA replication and repair in both mitochondria and nuclei (Zheng et al., 2008; Duxin et al., 2009). In humans, depletion of hDNA2 results in cell cycle delay and aberrant cell division (Duxin et al., 2009). DNA2 is widely believed to have important roles in DNA replication and repair and in mitochondrial and nuclear genomic DNA maintenance in metazoans and fungi (Kang et al., 2010). However, the role of DNA2 in plants has yet to be investigated.

In this study, we isolated an Arabidopsis mutant, jing he sheng (jhs1), which exhibits fasciation, abnormal phyllotaxy, and defective root growth, as observed in fas1, fas2, and numerous other mutants as mentioned above. Map-based cloning indicated that JHS1 encodes a DNA2 homolog. Our analysis suggests that this DNA2-like gene acts in the response to stalled DNA replication and is also involved in double-stranded DNA break repair. Further results indicated that a mutation in this gene causes an increased sensitivity to DNA damage stress, accumulation of DNA damage, and a delay of cell cycle progression in plants, which may interfere with the expression of key genes involved in meristem maintenance and normal SAM and RAM development.

RESULTS

Mutation of JHS1 Affects SAM and RAM Development and Organ Morphogenesis

We isolated jhs1 in a screen for EMS (ethyl methane sulfonate)-induced Arabidopsis mutants with abnormal shoot growth. The mutant had a fasciated stem, which is called “jing he sheng” in Chinese. Therefore, we named this mutant jhs1.

Compared with the wild type, the growth of true leaves was retarded in young jhs1 seedlings (Fig. 1, A and B). Bolted jhs1 plants had a fasciated stem (Fig. 1, C–E) and abnormalities in silique arrangement (Supplemental Fig. S1) and phyllotaxy (Fig. 1C). These phenotypes suggest that SAM development is abnormal in jhs1. Sectioning of the SAM of young jhs1 seedlings indicated that the SAM was enlarged and consisted of enlarged cells with an abnormal arrangement, especially in the L1 and L2 layers (Fig. 1B). In cross section, the stem of the jhs1 mutant was oval-shaped, whereas it was cylindrical in the wild type (Fig. 1E). We analyzed the size of pavement cells of the first true leaves of 15-d-old plants. The cell size of the jhs1 mutant was significantly larger than that of the wild type (Fig. 1F).
Figure 1. Shoot phenotypes of the jhs1 mutant. A, The phenotype of 8-d-old wild-type and jhs1 seedlings and jhs1 seedlings complemented with JHS1 (Com) grown on MS medium. Bars = 2 mm. B, Toluidine blue-stained SAM sections of 8-d-old wild-type, jhs1, and Com seedlings. The L1 and L2 layers are outlined in wild type (left) and Com (right). Individual cells in the L1 and L2 layers are indicated in jhs1 (middle). Bars = 20 μm. C, Floral organs of 40-d-old wild-type, jhs1, and Com plants. Bars = 1 cm. D, Floral organs of 50-d-old wild-type, jhs1, and Com plants. Bars = 1 cm. E, Toluidine blue-stained stem cross sections of 40-d-old wild-type, jhs1, and Com plants. Bars = 100 μm. F, Comparison of the cell size of abaxial epidermal cells in the true leaves of 15-d-old wild-type, jhs1, and Com plants. The border of a cell is marked in white. Bars = 75 μm. G, Analysis of the cell area of abaxial epidermal cells in the true leaves of 15-d-old wild-type, jhs1, and Com plants. The average area of 50 cells is shown. Error bars represent sd. Student’s t-test; for **, P < 0.01. WT, wild type.
The average cell size was 3101 μm² in the wild type, but 6502 μm² in the mutant (Fig. 1G). Thus, *jhs1* has defects in cell division in the SAM and in SAM-derived organs, such as stems, leaves, and siliques.

In 10-d-old plate-grown plants, the root length of *jhs1* mutants was approximately one-fifth that of the wild type (Fig. 2, A and B), suggesting that root growth is severely retarded in the mutant. Observation with a stereomicroscope revealed that the first root hair was much closer to the root tip in *jhs* than in the wild type, indicating that the RAM and elongation zone are much shorter in the mutant (Fig. 2C). Confocal microscopy analysis of roots stained with FM4-64 (a plasma membrane dye) further indicated that the cellular arrangement (Supplemental Fig. S2). The RAM was also shorter in *jhs* than in the wild type, with fewer cells (Fig. 2D). To determine the location of the QC, we transformed *jhs* and wild-type plants with *ProWOX5:GFP*, which was previously shown to label the QC (González-García et al., 2011). In the *jhs* mutant, the QC was not clear and *ProWOX5:GFP* expression was altered in comparison with the wild type (Supplemental Fig. S2). We then stained the roots with Lugol’s solution (a starch dye) to analyze the columella root cap. Whereas wild-type roots had four tiers of columella root cap cells with a regular arrangement (Fig. 2E, left panel), root cap columella cells were severely disordered in *jhs1* (Fig. 2E, middle panel).

As shown in Figure 2A, the development of roots, including lateral roots, was severely affected in *jhs1*. We generatedtransgenic plants expressing *DR5:GUS*, a marker of auxin (Ulmasov et al., 1997), to further study the development of lateral roots in *jhs1*. *β-glucuronidase* (GUS) staining indicated sites of lateral root emergence (Supplemental Fig. S3A). In 9-d-old wild-type plants, lateral roots initiated and elongated normally (Supplemental Fig. S3A). However, in *jhs1*, the number of lateral roots was reduced at least 4-fold (Supplemental Fig. S3, A and B).

**Identification of JHS1**

We crossed the *jhs1* mutant with wild-type plants of the Landsberg erecta (*Ler*) ecotype for map-based cloning of *JHS1*. All F1 plants had a wild-type phenotype. Among 692 F2 progeny, 182 exhibited a mutant phenotype and 510 had a wild-type phenotype, suggesting that *jhs1* is a single recessive mutation (χ² = 0.62 < χ² 0.05(1) = 3.84). Rough mapping indicated that *JHS1* was located on chromosome 1, between the molecular markers LUG887 and CH1-4.3. Fine mapping indicated that the *JHS1* locus was in a region of approximately 270 kb between molecular markers CH1-2.62 and CH1-2.89 (Supplemental Fig. S4). We sequenced this region and identified a G-to-A transition mutation in *At1g08840* in the *jhs1* mutant plant (Fig. 3A). This mutation is at the splicing site of the 11th intron, and cDNA sequencing indicated that the splicing site in the mutant was shifted 63 bp upstream, to include a piece of intron sequence, and that this caused premature termination of the protein encoded by *At1g08840* (Fig. 3A).

A 10.259-kb DNA fragment containing the wild-type *At1g08840* gene and the 1.323-kb upstream sequence spanning the promoter and 5’ UTR was introduced into *jhs1* plants by Agrobacterium-mediated transformation. The transgenic plants were verified by genotyping, DNA sequencing, and reverse transcription (RT)-PCR analysis (Supplemental Fig. S5). All of the mutant phenotypes described above, such as retarded and abnormal development of the shoot and root, were complemented in the transgenic plants (Figs. 1 and 2). This finding confirmed that *At1g08840* is *JHS1* and that the mutation in *jhs1* is responsible for the mutant phenotype.

**JHS1 Encodes a DNA2-Like Protein That Is Localized to the Nucleus and Expressed Mainly in the Meristem**

*JHS1* consists of 33 exons and 32 introns (Fig. 3A) and encodes a protein of 1296 amino acids. Sequence analysis of the protein indicated that *JHS1* contains two conserved domains: an N-terminal DNA2 domain of nuclease/ATPase, and a C-terminal DNA helicase domain (Fig. 3B).

We analyzed the amino-acid sequence of JHS1 by PSORT II Prediction (http://psort.hgc.jp/form2.html) and found that *JHS1* contained an NLS sequence (Fig. 3B). We studied the localization of *JHS1-YFP* in the leaves of Nicotiana benthamiana and Arabidopsis plants transgenically expressing *Pro-35S:JHS1-YFP*. In both cases, the YFP signal colocalized with the DAPI (4’,6-diamidino-2-phenylindole) signal (Supplemental Figs. S6 and S7), suggesting that *JHS1* was localized to the nucleus. In control plants expressing YFP alone, YFP was localized to both the nucleus and cytoplasm (Supplemental Figs. S6 and S7).

To determine the expression pattern of *JHS1*, a fusion construct in which GUS expression is driven by the *JHS1* promoter (*ProJHS1:GUS*) was transformed into wild-type plants. GUS staining indicated that *JHS1* was strongly expressed in the RAM and SAM (Fig. 4, A and B). Moreover, GUS activity was detected in the vasculature at different developmental stages (Fig. 4, C and D) and in young floral tissues (Fig. 4E).

**jhs1 Is Sensitive to DNA Damage Stress**

In yeast, dna2 mutants are sensitive to DNA damage stress induced by MMS and x-ray irradiation and DNA2 was shown to have an important role in DNA damage repair (Formosa and Nittis, 1999; Budd and Campbell, 2000). Therefore, it is likely that the *jhs1* mutant is also sensitive to DNA-damaging agents. To test this hypothesis, we examined the effects of two
types of DNA-damaging agents, HU (hydroxyurea) and zeocin, on jhs1 plants. HU, a DNA replication stress-inducing agent that functions as a ribonucleotide reductase inhibitor, reduces the dNTP pool and results in stalked replication forks with ssDNA, whereas zeocin, a radiomimetic drug of the bleomycin family, induces a double-strand break (DSB).

We evaluated the effect of HU on the growth of 15-d-old plate-grown plants. Treatment with 1.0 mM and 2.0 mM HU severely limited the growth of the mutant, but not of the wild type. In the mutant, the fresh weight of treated plants was only 78% and 38% that of untreated plants, respectively. By contrast, the fresh weight of treated plants was 92% and 78% that of untreated plants in the wild type, respectively (Fig. 5, A and E). We also studied the effect of HU on root growth in the mutant. The root length of jhs1 plants treated with 1.0 mM HU was 28% that of the untreated jhs1 plants. However, on plates containing 2.0 mM HU, root growth was severely inhibited in both the wild type and jhs1, and no difference was observed between these lines (Fig. 5, C and F).

We then evaluated the effect of zeocin on the growth of 15-d-old plate-grown plants. Treatment with 5 μM and 10 μM zeocin severely limited the growth of the mutant, but not of the wild type. In the mutant, the fresh weight of treated plants was only 70% and 55% that of untreated plants, respectively. By contrast, the fresh weight of treated plants was 89% and 72% that of untreated plants in the wild type, respectively (Fig. 5, B and G). We also studied the effect of 5 μM and 10 μM zeocin on root growth in the mutant. The root length of the treated mutant was reduced to 40% and 32% that of untreated plants, respectively. However, the root length of treated wild-type plants was 81% and 60% that of untreated plants, respectively (Fig. 5, D and H).

JHS1 Is Localized to Nuclear Foci in Response to DNA Damage

The above results indicate that JHS1 has an important role in DNA damage repair in Arabidopsis. To further examine the role of DNA2 in the DNA damage response, we observed the subcellular localization of YFP-JHS1 in N. benthamiana and Arabidopsis plants treated or not with zeocin or HU (Fig. 6). JHS1-YFP was localized to subnuclear foci upon zeocin treatment in both plants. Because zeocin induces DSBs (Chankova et al., 2007), JHS1 may be localized to those foci to repair the breaks. In HU-treated plants, the localization of YFP-JHS1 was similar to that of the untreated control plants. This could be because no nuclear DNA replication and cell division occurred in these leaf cells after

Figure 2. Root phenotypes of the jhs1 mutant. A, Roots of 10-d-old wild-type (left), jhs1 (middle), and Com (right) seedlings. B, Analysis of the root length of seedlings shown in (A). Error bars represent SD. Student’s t-test; for **, *P < 0.01. C, Root tips of 6-d-old wild type (left), jhs1 (middle), and Com (right) seedlings. Arrows indicate the bottom of differentiated cells with root hairs. Bars = 1 mm. D, FM4-64-stained root tips of 6-d-old wild-type (left), jhs1 (middle), and Com (right) seedlings. White lines indicate the transition zone between the meristem and the elongation differentiation zone. The QC cells are outlined in white. Bars = 100 μm. E, Lugol-stained root caps of 6-d-old wild type (left), jhs1 (middle), and Com (right) seedlings. Starch granules were stained in columella root caps. Bars = 50 μm. WT, wild type.
the treatment. In the absence of severe DNA damage or stalled replication forks, no YFP-JHS1 foci formed.

**jhs1** Has Increased DNA Damage and Damage Responses

To examine DNA damage in the *jhs1* mutant, we used the terminal deoxynucleotide transferase-mediated dUTP nick-end labeling (TUNEL) method, which detects 3’-OH DNA breaks. Signals were barely detected in the leaves and root tips of the wild type (Fig. 7, A and B), whereas they were observed in numerous TUNEL-stained nuclei in the *jhs1* mutant (Fig. 7, A and B). These results suggest that more DNA damage occurred in the *jhs1* mutant than in the wild type.

We next analyzed whether the *jhs1* mutation affected the expression of several marker genes for the DNA damage response. *Rad51* is a homolog of the bacterial *RecA* gene and plays a crucial role in DSB repair as the central component of the DNA recombinational repair system (Doutriaux et al., 1998; Li et al., 2004). *BREAST CANCER SUSCEPTIBILITY1* (*BRCA1*) is a homolog of the human breast cancer susceptibility gene and has an important role in DNA damage repair as a key component of the BRCA1-associated genome surveillance complex (Wang et al., 2000; Lafarge and Montané, 2003). *GAMMA RESPONSE1* and *MRE11* are nucleases involved in DSB end-processing in the repair of cross-linked DNA (Bundock and Hooykaas, 2002; Uanschou et al., 2007; Roth et al., 2012). *KU70* plays an important role in the maintenance of the telomeric region and DSB repair by modulating the nonhomologous end-joining pathway (Tamura et al., 2002). *MSH2* and *MSH6* play important roles in the DNA mismatch repair system (Li et al., 2006; Lario et al., 2011). We examined the expression of these genes by real-time, quantitative reverse transcription-PCR (qRT-PCR). As shown in Figure 7C, the expression levels of *RAD51*, *BRCA1*, and *GR1* were all increased approximately 2- to 4-fold in *jhs1* in comparison to the wild type. The expression of *MRE11* and *KU70* was a little higher in the *jhs1* mutant than in the wild type. However, the expression of *MSH2* and *MSH6* was not changed in *jhs1* mutants.

It has been shown that plant meristematic cells are hypersensitive to DNA damage (Fulcher and Sablowski, 2009). We stained the roots of young seedlings with PI (propidium iodide) to observe dead cells in the RAM of the wild type and *jhs1* mutant (Fig. 7, D–G). The area of dead cells in PI-stained roots was significantly larger (50-fold) in the *jhs1* mutant than in the wild type (Fig. 7G). We also analyzed the PI-stained roots of wild-type,

---

**Figure 3.** Gene structure of *JHS1* and amino-acid sequences of the corresponding wild-type and mutant proteins. A, White boxes represent the 5’- and 3’-untranslated regions; black boxes represent protein coding sequences; and lines represent introns. Comparison of the cDNA and amino-acid sequences of the wild type and *jhs1*. Asterisk (*) represents the stop codon. B, *JHS1* (1–1296) contains an NLS (outlined in blue, 2–8), DNA2 (red, 336–544), and DNA helicase (green, 766–1282) domain. Numbers show the positions of specific amino acids.

**Figure 4.** Expression pattern of *ProJHS1:GUS* in Arabidopsis. A, Root tip. B, 10-d-old seedling. C, D, Rosette leaf of a 25-d-old plant (C) and a 25-d-old plant (D). E, Flowers. Bars = 500 μm (A and E), 1 mm (B and C), and 2 mm (D).
**Figure 5.** HU and zeocin treatment analysis. A, Comparison of wild-type, jhs1, and Com seedlings grown on MS medium containing 0, 1.0, and 2.0 mM HU. B, Comparison of wild-type, jhs1, and Com seedlings grown on MS medium containing 0, 5, and 10 mM zeocin. C, Root growth of wild-type, jhs1, and Com seedlings grown on MS medium containing 0, 1.0, and 1.0 mM HU. D, Root growth of wild-type, jhs1, and Com seedlings grown on MS medium containing 0, 5, and 10 mM zeocin. E, Relative fresh weight of the seedlings shown in (A). Each point represents the average result of 50 plants and error bars represent SD. Student’s t-test; for **, P < 0.01. F, Relative root length in (C). n = 50. Error bars represent SD. Student’s t-test; for **, P < 0.01. G, Relative fresh weight of the seedlings shown in (B). Each point represents the average result of 50 plants and error bars represent SD. Student’s t-test; for **, P < 0.01. H, Relative root length in (D). n = 50. Error bars represent SD. Student’s t-test; for **, P < 0.01. MS, Mirashige and Skoog.

**jhs1**, and Com seedlings that had been treated with 1.0 mM HU or 5 mM zeocin for 36 h. After the treatment, the area of dead cells in the jhs1 mutant was significantly increased (Fig. 7, D–G). However, treatment did not affect the area of dead cells in the wild type (Fig. 7, D–G).

**The Frequency of Homologous Recombination Is Increased in jhs1**

In response to DNA damage, DNA repair mechanisms such as homologous recombination (HR) or non-homologous end-joining are upregulated in the cell. To examine the frequency of HR in the jhs1 mutant, HR reporter lines 1415 and 1406, which contain two fragments of the GUS reporter gene in the direct and inverse orientation, respectively (Fig. 8A), were used. Line 1415 can be used to examine intramolecular HR, whereas line 1406 can be used to examine intermolecular HR. If HR events occurred between the homologous regions, i.e. the regions represented by “U” from “GU” and “US” in Figure 8A, DNA recombination between two U-regions of GUS would produce a complete and functional GUS gene. Cells that have undergone this kind of event appear blue upon GUS staining (Supplemental Fig. S8). We first crossed the jhs1 mutant with the 1406 and 1415 lines, respectively. We selected lines of the F3 generation that were homozygous for both jhs1 and the GUS reporter gene for our analysis. GUS staining resulted in more blue spots in the jhs1 mutant than in the wild type (Fig. 8, B and C). For line 1415, the mutant plants had an average of 4.52 spots per plant, while the wild-type plants had only 1.18 blue spots (Fig. 8, B and C). For line 1406, the mutant plants had 3.42 spots per plant on average, while the wild-type plants had only 0.57 blue spots (Fig. 8, B and C). These results indicate that the jhs1 mutant has an increased level of HR.

After HU treatment, the average number of blue spots was increased to 2.22 in the wild type in line 1415, while the jhs1 mutant had 4.81 blue spots. The average number of blue spots was increased to 0.98 in the wild type in line 1406, while the jhs1 mutant had 3.42 spots per plant on average, while the wild-type plants had only 0.57 blue spots (Fig. 8, B and C). These results indicate that the jhs1 mutant has an increased level of HR.

After HU treatment, the average number of blue spots was increased to 2.22 in the wild type in line 1415, while the jhs1 mutant had 4.81 blue spots. The average number of blue spots was increased to 0.98 in the wild type in line 1406, while the jhs1 mutant had 3.42 spots per plant on average, while the wild-type plants had only 0.57 blue spots (Fig. 8, B and C). After treatment with zeocin, the average number of blue spots increased to 7.34 in the wild type in line 1415, while the jhs1 mutant had 13.92 blue spots. The average number of blue spots was increased to 2.77 in the wild type in line 1406, while the jhs1 mutant had 6.26 blue spots (Fig. 8, B and C). Both HU and zeocin treatment significantly increased the HR events in the wild-type plants, while the latter had a much stronger effect. Furthermore, the HR events of both GUS reporters in the HU-treated jhs plants were
similar to those of the untreated plants. In all of these analyses, zeocin-treated \textit{jhs1} plants had the highest number of HR events.

\textbf{Cell Cycle Progression Is Delayed in the \textit{jhs1} Mutant}

DNA damage can affect cell cycle progression, at least partially through changes in the regulation of the expression of cell cycle-related genes. We thus compared the expression level of several cell cycle-related genes in the wild type and \textit{jhs1} mutant. Specifically, we evaluated the expression of \textit{CYCB1:1}, which is induced at the G2 phase and encodes a kinase protein with activity that peaks at the G2/M transition (Cools et al., 2010); \textit{CYCD4:1}, which encodes a protein that is expressed from the G2 to M phase and binds to and activates CDKB2;1 (Kono et al., 2003); CDKA;1, which is required for cell cycle regulation during pollen, embryo, root, and leaf development in Arabidopsis (Dissmeyer et al., 2009); \textit{CYCD3:3}, which is expressed in the G1 phase (Menges et al., 2005); histone H3.1 and histone H4, which are specifically expressed in the S-phase (Menges et al., 2003; Yin et al., 2009); CYCA3;1, whose transcripts are highly accumulated at the G1/S phase (Juraniec et al., 2016); \textit{CYCB1:2} and \textit{MAD2}, which are markers for G2/M phase (Menges et al., 2005; Juraniec et al., 2016) and \textit{CYCA2;1}, which is induced during the S phase and peaks at the end of the G2 phase (Shaul et al., 1996), using qRT-PCR analysis (Fig. 9A). Besides \textit{HISTONE H2A PROTEIN9} (HTA9), \textit{PLASTID DIVISION2} was also used as a reference gene and similar results were observed (Supplemental Fig. S9). Expression of \textit{CYCD3:3}, \textit{CYCA3:1}, and \textit{MAD2} were the same in the mutant and the wild type. By contrast, expression of \textit{CYCB1:1}, \textit{CYCB1:2}, \textit{CDKB1:1}, \textit{CYCD4:1}, \textit{CDKA;1}, H3.1, histone H4, and \textit{CYCA2;1} was greater in the \textit{jhs1} mutant than in the wild type. Most strikingly, the expression of \textit{CYCB1:1} was upregulated 4.3-fold compared with the wild type (Fig. 9A). The up-regulation of these genes suggests that the DNA damage response is activated in \textit{jhs1}, which may affect cell cycle progression.
with 1.0 mM HU, which primarily induces G1 arrest, but in addition, we treated wild-type and mutant plants with or without 2.0 mM HU and 10 μM zeocin treatment, respectively. Error bars represent SD. “GU” and “US” are two GUS gene fragments.

To analyze the G2 checkpoint response in the jhs1 mutant, we crossed jhs1 mutant plants with a transgenic line carrying the CYCB1:1 promoter fused with the GUS reporter gene. CYCB1 and GUS are expressed upon entry into the G2 phase (Doerner et al., 1996). Only a few cells in the wild-type SAM and RAM accumulated GUS protein, as revealed by GUS staining (Fig. 9, C and D). However, more cells were stained in the SAM and RAM of jhs1, which suggests that the DNA damage response was greater in these cells and that more cells underwent G2 cell-cycle arrest in the jhs1 mutant. In addition, we treated wild-type and jhs1 mutant plants with 1.0 mM HU, which primarily induces G1 arrest, but also induces G2 arrest (Culligan et al., 2004); aphidicolin, which inhibits the replicative DNA polymerases δ and ε and induces G2 arrest (Culligan et al., 2004); and zeocin, which induces G2 arrest (Twentyman, 1983). HU treatment can induce the GUS level in the RAM of the wild type, but not in the RAM and SAM of jhs1.

Besides, aphidicolin and zeocin can induce GUS expression in the wild type and jhs1 mutant. The GUS level was higher in jhs1 plants subjected to these two treatments than in the wild-type plants. However, both the wild type and the mutant had similar responses to these two treatments.

To analyze cell cycle progression in jhs1 plants, we determined the endoreduplication level of 10-d-old seedlings by flow cytometry. As shown in Figure 9B and Supplemental Figure S10, the proportion of cells with a 4C nuclear content was much lower in jhs1 mutant plants than in wild-type plants, and the proportion of cells with a 8C, 16C, and 32C nuclear content was higher in jhs1 mutant plants. This result, in combination with the data shown above, suggests that the G2/M phase or late S/G2 phase of the cell cycle is delayed in the jhs1 mutant.

Genes Important for SAM Development Are Misregulated in the jhs1 Mutant

To decipher the mechanism that controls the phenotype of the jhs1 mutant, we analyzed the expression level of SAM-specific genes, such as WUS, CLV3, and STM using real-time qRT-PCR. As shown in Figure 10A, the expression of STM and WUS was 7-fold and 3-fold greater, respectively, in the jhs1 mutant than in the wild type. However, the expression of CLV3 was slightly reduced.

To investigate the expression pattern of these three SAM-specific genes, we introduced the ProWUS:GUS, ProCLV3:GUS, and ProSTM:GUS reporter constructs into jhs1 plants by crossing, and analyzed GUS activity in the SAMs of the wild-type and jhs1 mutant. In 9-d-old wild-type seedlings expressing ProWUS:GUS or ProSTM:GUS, GUS staining was localized to the organizing center of the SAM. However, in the jhs1 mutant of the same age, GUS staining extended to the outer cell layers (Fig. 10, B and D). ProWUS:GUS and ProSTM:GUS expression was also detected in the apex of the inflorescence (Supplemental Fig. S11). In the wild type, both of these constructs were expressed in the central region of the SAM. In the jhs1 mutant, GUS was detected in multiple regions arranged in a linear fashion. This arrangement is due to the multiple meristems present in the shoot apex of fasciated mutants. Similar to our findings in 9-d-old seedlings, these two GUS reporter genes were also expressed at a higher level and in a larger area in the mutant than in the wild type.

The expression level of CLV3, as determined by real-time qRT-PCR, was slightly reduced in the mutant (Fig. 10A). Our GUS staining results indicate that ProCLV3:GUS activity was low and limited to a small region of the SAM in young seedlings of the wild type (Fig. 10C). The expression area was smaller and the activity was lower in the jhs1 mutant (Fig. 10C). In the SAM that gives rise to flowers, ProCLV3:GUS expression was barely detectable (Supplemental Fig. S11).

Figure 8. Frequency of homologous recombination in wild-type and jhs1 plants. A, Schematic diagram of the GUS reporter gene in the 1415 (direct repeat) and 1406 (inverted repeat) vectors used in HR assays. Arrows represent the process of HR. HR between two overlapping sequences produces a functional GUS gene. B, C, The average number of GUS spots per plant in the wild type and jhs1 background with or without 2.0 mM HU and 10 μM zeocin treatment, respectively. Error bars represent SD. “GU” and “US” are two GUS gene fragments.
Some of the fasciated mutants, such as \textit{fas1}, \textit{bru1}, \textit{mre11}, and \textit{abo4}, also have a defect in epigenetic regulation and a release of silencing of transcriptionally silent information (TSI) (Takeda et al., 2004; Kirik et al., 2006; Yin et al., 2009; Amiard et al., 2010), which may be related to the misregulation of \textit{WUS} and \textit{STM} expression in these mutants. However, in other mutants, such as \textit{teb}, there is no release of TSI (Inagaki et al., 2006). To determine the TSI status in \textit{jhs1}, we analyzed the expression of TSI repeats by RT-PCR (Supplemental Fig. S12). We found that there was no difference between the mutant and wild type. This suggests that epigenetic regulation is not altered in \textit{jhs1}, at least regarding TSI repeats.

To further elucidate the relationship between DNA damage and the fasciation phenotype, we treated 9-d-old wild-type plants with HU and zeocin, and analyzed the expression level of \textit{WUS}, \textit{CLV3}, and \textit{STM} using RT-PCR. We found that \textit{WUS} and \textit{STM} expression were induced by these DNA damaging agents and that \textit{CLV3} expression was not significantly changed (Fig. 10D). GUS staining also indicated that \textit{ProWUS:GUS} and \textit{ProSTM:GUS} was expressed at higher levels and over a larger region in plants treated with HU and zeocin (Fig. 10, B and D). Overall, \textit{WUS} and \textit{STM} expression was induced to a greater extent by 5 mM zeocin than by 1 mM HU, but it still did not reach the levels observed in the \textit{jhs1} mutant. These results further suggest that the DNA damage affects meristem maintenance in plants.
damage observed in the jhs1 mutant promotes the expression of WUS and STM, which may affect SAM development.

DISCUSSION

DNA2 is similar to its homologs in fungi and metazoans, suggesting its function may be conserved. Studies of DNA2 to date have mainly been performed in fungi and mammals (Kang et al., 2010). This protein removes the RNA primer during lagging-strand DNA replication and Okazaki fragment maturation (Budd and Campbell, 1997; Bae et al., 2001; Ayyagari et al., 2003) and is also involved in DNA resection during double-stranded DNA break repair (Zhu et al., 2008; Mimitou and Symington, 2009). The dna2Δ mutation is lethal in yeast, suggesting that the wild-type protein is essential for cell viability (Budd et al., 2011). In this study, we showed that JHS1 is targeted to the nucleus in Arabidopsis (Supplemental Figs. S6 and S7), as reported for DNA2 in yeast (Choe et al., 2002). The jhs1 mutation occurred at the splicing site of the 11th intron (Fig. 3), which resulted in mis-splicing of the intron and introduced a premature stop codon. This defect might severely affect or even abolish the function of the gene, as reflected by the many severe developmental and physiological defects of the jhs1 mutant shown in this study.

The yeast dna2 mutants are sensitive to DNA-damaging agents, such as MMS and x-ray and γ-ray irradiation, suggesting that DNA2 is involved in DNA damage repair (Formosa and Nittis, 1999; Budd and Campbell, 2000). To test whether JHS1 is also involved in DNA damage repair, we evaluated the effect of two DNA-damaging agents, HU and zeocin. HU blocks DNA synthesis and arrests DNA replication by reducing dNTP levels and causing stalled replication forks. Zeocin is a DSB-inducing agent. The jhs1 mutant was more sensitive to all of these agents than was the wild type (Fig. 5). After Zeocin treatment, JHS1 was localized to foci in the nucleus (Fig. 6). Indeed, the TUNEL staining results indicated that the level of DNA damage was high in jhs1 (Fig. 7). Moreover, many dead cells were observed in the SAM of jhs1, which might be the result of severe DNA damage. All these data suggest that the DNA2 homolog in plants also has an important role in DNA damage repair.

To further examine the working mechanism of DNA2 homologs in plants, we analyzed the expression levels of key genes in DNA damage responses (Fig. 7C). The expression of BRCA1, which functions in genome surveillance and DNA damage repair (Wang et al., 2000; Lafarge and Montané, 2003), was increased more than 4-fold in jhs1, suggesting that much DNA damage occurs in this mutant. The GR1 and MRE11 nucleases, which are involved in DSB end processing (Bundock and Hooykaas, 2002; Uanschou et al., 2007; Roth et al., 2012), were also induced in the mutant. The expression level of RAD51, which mediates DNA repair directly by homologous recombination (Li et al., 2004), was increased more than 4-fold in jhs1. The increased expression of genes involved in DNA damage repair and HR likely reduces the amount of damaged DNA in jhs1 cells, but does not repair all damaged DNA.

We crossed two recombination lines, 1415 and 1406, with the jhs1 mutant to examine HR. We found that the frequency of HR events is several times higher in jhs1 than in the wild type (Fig. 8). Previous studies in Arabidopsis suggest that bru1 and abo4-1 plants have a greatly increased frequency of HR events (Takeda et al., 2004; Yin et al., 2009). In jhs1 and abo4, an increased amount of DNA damage was also observed. As stated above, genes involved in DNA recombination and damage repair, such as Rad51 and BRCA2, were also up-regulated, probably in response to the DNA damage. The up-regulation of these genes would increase the frequency of HR, which has been shown by the two GUS reporter genes, and help the cell to repair damaged DNA through HR.

Our real-time qRT-PCR and GUS staining results showed that many cell cycle-related genes, such as CYCB1;1, CDKB1;1, CYCD4;1, CDKA;1, H3.1, and CYCA2;1, were up-regulated in jhs1 (Fig. 9, A and C). In addition to being a response to DNA damage, this up-regulation would affect the progression of the cell cycle in jhs1. The accumulation of DNA damage can activate the check-point response in the cell cycle, which can delay cell cycle progression and allow time to repair DNA damage. Otherwise, apoptosis and cell death may be triggered in response to severe DNA damage. The reduced growth of the SAM and RAM and a significant amount cell death in the RAM of jhs1 all suggest that cell cycle delay and apoptosis were triggered. On the other hand, cells with much DNA damage tend to stop dividing and undergo endoreplication (Kirik et al., 2007). Our flow cytometry analysis indicated that in the mutant, the nuclear DNA content was increased and the proportion of 3C cells was significantly reduced (Fig. 9B). Moreover, the cells in the SAM, RAM, and leaf epidermis of the jhs1 mutant were significantly larger than those in the wild type (Figs. 1, B and F, and 2D), indicating that endoreplication occurred in these cells. Furthermore, the phenotypes of jhs1 plants were similar to those of other mutated mutants, such as fas1, fas2, abo4-1, and teb-1 (Endo et al., 2006; Inagaki et al., 2006; Kirik et al., 2006; Yin et al., 2009). It has also been suggested that plant stem cells are hypersensitive to DNA damage (Fulcher and Sablowski, 2009). Thus, all these data support the notion that the DNA damage response may delay cell cycle progression and cause endoreplication in jhs1.

The ataxia telangiectasia and rad3-related (ATR) and ataxia telangiectasia-mutated (ATM) protein kinases are conserved among eukaryotes and involved in DNA damage sensing and the regulation of cell cycle checkpoints. ATR senses the ssDNA of stalled replication forks, whereas ATM is activated by DSBs (Sancar et al.,
For example, strong alleles of the mutant of DNA polymerase 1β caused seedling lethality, while disruption of DNA topoisomerase 1α alone caused fasciation (Takahashi et al., 2002; Graf et al., 2010). Thus, the phenotype of a mutant may largely depend on the severity and type of DNA damage. A similar situation may exist in cell cycle mutants. For example, only double knockdown of CDKB2;1 and CDKB2;2 caused an abnormal SAM, whereas the single-knockdown plants appeared normal (Andersen et al., 2008). Moreover, it is possible that only the mutants that affect specific steps of the cell cycle cause misexpression of WUS and STM and produce an abnormal SAM.

DNA2 has been shown to function in DNA replication and DNA damage repair in humans and yeast (Budd and Campbell, 2000; Zheng et al., 2008). In this study, we showed that, in plants, DNA2 is important for DNA damage repair during DNA replication in the stem cells of the SAM and RAM, and that mutation of this gene causes DNA damage, a delay in cell cycle progression, endoreplication, and severe developmental defects.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The Arabidopsis plants used in this study were of the Col-0 ecotype unless otherwise stated. Seeds were surface-sterilized with 75% (v/v) ethanol for 5 min, washed five times with sterile water, and sown on Murashige and Skoog (MS) solid medium. The plates were placed at 4°C for 3 d and then transferred to a growth chamber under long-day conditions (16 h of light/8 h of darkness) at 22°C. Plants were grown in a perlite/vermiculite/peat soil (1:1:1, v/v/v) mixture under long-day conditions at 22°C and with a controlled humidity range of 40–60%.

Map-Base Cloning of jing he sheng1

The Arabidopsis jing he sheng 1 (jhs1) mutant plants were crossed with the Ler wild type to obtain F1 seeds. The F1 plants were self-crossed to obtain F2 seeds. Approximately 700 plants from the F2 population were used for the fine mapping of JHS1. PCR-based molecular markers were developed for the mapping and the primer sequences used are listed in Supplemental Table S1.

Plasmid Construction

For complementation analysis, a 10.259-kb DNA fragment of JHS1, including a 1.323-kb upstream sequence of the promoter and 5′ UTR, and a 145-bp downstream region, was amplified from the genomic DNA of the wild type with the primers 5′-CCT ACT AGT CGA AGT TTA TCA GCT CCA TC-3′ and 5′-CCT AGC CGT GCA AGT GAT TCA TCT GTC TCT G-3′. The PCR product was digested with MluI and inserted into a binary vector 3302Y3 digested with Smal and MluI. The constructed plasmid was transformed into Agrobacterium tumefaciens C58C1 and the gene was introduced into the jhs1 mutant by floral dipping (Clough and Bent, 1998).

To construct the ProJHS1:GUS fusion gene, a 1368-bp promoter of JHS1 with the 5′ UTR was amplified from the wild-type genomic DNA with the primers 5′-GTC AAT AAG GAT AGT ACC TTT GCT AC-3′ and 5′-CCT AGC GGA TCC TTC CAA TGG CTA ATA CTG GCC AC-3′. The PCR product was digested with BamHI and inserted into the 3302GUS binary vector digested with Smal and BglII to produce the fusion gene.

To construct the Pro35S-JHS1:YFP fusion gene, a 8.788-kb fragment of JHS1 with the 5′ UTR was amplified from wild-type genomic DNA with the primers 5′-GCA GAC ATG ATC CGA ACA AGG CC-3′ and 5′-CCT AGC GGT TGG TTT TAA ATC TCC CCG CAA CAG-3′. The PCR product was digested with AatII and MluI and inserted into the 3302Y3 binary vector digested with AatII and MluI to produce the fusion gene.
**Microscopy Analysis**

To study the cellular arrangement of shoot apical meristems (SAMs) and stems in the wild type, jhs1, and complementation lines, 8-d-old seedlings and the stems of 6-week-old plants were fixed in cold FAA solution (70% ethanol, formalin/acetic acid, 18:1:1, v/v/v), dehydrated in a series of ethanol concentrations (50, 70, 80, 90, and 100%, v/v), and infiltrated and embedded in LR White resin (Electron Microscopy Sciences, Hatfield, PA). The resin was polymerized at 60°C for 24 h. Sections (1-3 μm thick) were cut with an Ultra Microtome (Leica Microsystems, Wetzlar, Germany), stained with 1% w/v toluidine blue, and viewed with a model no. CX21 microscope (Olympus America, Melville, NY).

To study the cellular arrangement of roots, 5-d-old seedlings of the wild-type, jhs1, and complemented mutant plants were stained with 5 μM FM4-64, a plasma membrane dye, for 5-10 min. FM4-64 fluorescence was observed using a confocal laser scanning microscope (model no. SP8; Leica Microsystems). Starch granules in root caps were stained with Lugol’s solution as previously described in Takahashi et al. (2003) and viewed with a model no. CX21 microscope (Olympus America).

To analyze the root apical meristem, jhs1 mutant plants were crossed with transgenic plants carrying ProWOXS:GFP and ProDKS:GUS, which are specifically expressed in the quiescent center and roots, respectively. F1 plants homozygous for the reporter gene and jhs1 were selected. Five-day-old seedlings were used to analyze GFP fluorescence, which was observed with a confocal laser scanning microscope (model no. SP8; Leica Microsystems). The number of lateral roots was determined in 9-d-old β-glucuronidase (GUS)-stained seedlings using a dissecting microscope (Optec, Chongqing, China).

To study the subcellular localization of JHS1, the pro-35S:JHS1:YFP fusion gene was first transiently expressed in Nicotiana benthamiana leaf cells by Agrobacterium infiltration (Voinnet et al., 2003). Pro-35S:JHS1-YFP was also transformed into Arabidopsis to obtain stable transgenic lines. For colocalization studies, the nucleus was stained with a DNA dye, DAPI (4′,6-diamidino-2-phenylindole). Fluorescence images of YFP and DAPI were obtained with a confocal laser scanning microscope (model no. SP5; Leica Microsystems).

To study the subcellular localization of JHS1 after DNA damage agent treatment, agrobacteria containing the Pro-35S:JHS1-YFP construct were cocultured with mock, 10 μM zeocin, or 2 mM HU (hydroxyurea) and then infiltrated into N. benthamiana leaves. Five-day-old transgenic Arabidopsis seedlings harboring YFP-JHS1 were transferred to MS medium with mock, 10 μM zeocin, or 2 mM HU for analysis. The localization of JHS1-YFP in N. benthamiana and Arabidopsis was observed 48 h later with a confocal laser scanning microscope (model no. SP8; Leica Microsystems).

To detect DNA damage in jhs1 mutants, a terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) Kit (One Step TUNEL Kit, Roche, Indianapolis, IN) was used. The manufacturer’s instructions were modified. Briefly, seedlings were fixed in 4% (w/v) paraformaldehyde in PBS at 20°C for 1 h and washed three times with PBS buffer for 10 min. Then, samples were incubated in 0.1% (w/v) Triton X-100 with 0.1% (w/v) sodium citrate for 30 min (4°C). After three washes in PBS buffer, the roots were soaked in TUNEL reaction mixture and incubated at 37°C for 90 min in darkness. Finally, the roots were examined with a confocal laser scanning microscope (model no. SP8; Leica Microsystems).

**DNA Damage Sensitivity Assay**

For the images shown in Figure 5, A and B, the seeds were sown directly on MS solid medium containing HU or zeocin and the plants were grown for approximately 3 weeks before photographs were taken. For the images shown in Figure 5, C and D, the seeds were sown directly on MS solid medium containing different concentrations of HU or zeocin and the plants were grown on vertically oriented dishes for 8 d before photographs were taken and root lengths were measured.

**Quantitative RT-PCR Analysis**

Total RNA was extracted from 10-d-old Arabidopsis seedlings using an RNA Isolation Kit (Appygen, Beijing, China) and an equal amount of total RNA was reverse-transcribed into cDNA using the RevertAid Premium First Strand cDNA Synthesis Kit (Fermentas, Thermo Fisher Scientific, Rochester, NY). For real-time quantitative RT-PCR (qRT-PCR), the Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) and a model no. 7500 Fast RT-PCR system were used (Applied Biosystems). PCR reactions were run in quadruplicate for each sample and the gene expression level was normalized to that of the reference genes, HISTONE H2A PROTEIN or PLASTID DIVISION2. All analyses were repeated at least three times. The primer pairs used for qRT-PCR are listed in Supplemental Tables S2 and S3. Transcriptionally silent information analysis was done as before (Yin et al., 2009).

**Homologous Recombination Assay**

The jhs1 mutant plants were crossed with the homologous recombination (HR) reporter lines 1415 and 1406, which were in the Col background (Lucht et al., 2002). Plants homozygous for the GUS reporter gene in the jhs1 or wild-type background were selected. Fourteen-day-old seedlings of the F3 generation were subjected to GUS staining and used for HR frequency analysis. For HU and zeocin treatment, seedlings were grown on MS medium containing 1 mM HU or 5 mM zeocin for 16 d. Then, the seedlings were subjected to GUS-staining. The number of blue (GUS-positive) spots per root, each of which represents an HR event, was determined using a dissecting microscope (Optec, Chongqing, China). GUS staining was performed using an S102 GUS Staining Kit (Obloibar, Beijing, China). For each line, 100 individuals were analyzed.

**Cell Cycle Assay**

The jhs1 mutant was first crossed with Col wild-type plants transformed with a ProCYCB1;1:GUS reporter gene (Doerner et al., 1996). Plants homozygous for the ProCYCB1;1:GUS reporter gene and jhs1 in the F1 generation were selected. Five-day-old seedlings were used to analyze GUS activity. For the HU, zeocin, and aphidicolin treatment, 5-d-old seedlings were transferred to MS medium containing 1 mM HU, 2 mM zeocin, or 12 μg/mL aphidicolin, respectively, and cultured for 3 d before being subjected to GUS staining. GUS activity in the SAMs and root apical meristems was observed using a dissecting microscope (Optec).

**Flow Cytometry Analysis**

To study the ploidy level of the jhs1 mutant and the wild type, 9-d-old seedlings were dropped with a blade in 1 mL cold extraction buffer (Doelezel et al., 2007). Crude samples were filtered through a nylon filter (30 μm) to remove cell debris and stained with 25 μL of 0.1 mg/mL 4′,6-diamidino-2-phenylindole (DAPI). The nuclei were analyzed using a MoFlo XDP Flow Cytometer (Beckman Coulter, Brea, CA) with CellQuestPro software (BD Biosciences, San Jose, CA). Four independent replicates were performed for each sample.

**Expression Pattern Analysis of SAM-Specific Genes**

The jhs1 mutant plants were crossed with wild-type transgenic plants carrying ProWUS:GUS, ProCLV3:GUS, or ProSTM:GUS (Angela et al., 2002; Meng et al., 2012; Song et al., 2013), respectively. F1 plants homozygous for the reporter gene and jhs1 were selected. GUS expression was analyzed in 9-d-old seedlings treated without or with 1 mM HU or 5 mM zeocin and in the inflorescence of 45-d-old plants. After dehydration in 70% (v/v) ethanol, seedlings were cleared with HCG solution (30 mL H2O, 80 g chloral hydrate, and 10 mL 100% glycerol) at room temperature overnight and viewed with a model no. CX21 microscope (Olympus America).

**Supplemental Data**

The following supplemental materials are available.

**Supplemental Figure S1.** Silique arrangement in the wild-type, jhs1, and complemented lines.

**Supplemental Figure S2.** Expression of ProWOXS:GFP in the roots of transgenic wild-type and jhs1 seedlings.

**Supplemental Figure S3.** Analysis of lateral root generation in wild-type and jhs1 plants.
Supplemental Figure S4. Map-based cloning and sequence analysis of JHS1.

Supplemental Figure S5. Genotyping and gene expression analysis of the complemented jhs1 plant.

Supplemental Figure S6. Subcellular localization of JHS1 in N. benthamiana leaves.

Supplemental Figure S7. Subcellular localization of JHS1 in Arabidopsis leaves.

Supplemental Figure S8. HR in jhs1 mutant plants.

Supplemental Figure S9. qRT-PCR analysis of cell cycle-related gene expression.

Supplemental Figure S10. Analysis of ploidy level distributions of wild-type and jhs1 plants.

Supplemental Figure S11. Expression analysis of SAM-specific genes in the floral tissues of 45-d-old wild-type and jhs1 plants.

Supplemental Figure S12. qRT-PCR analysis of TSL expression.

Supplemental Table S1. Molecular markers and primers used for the fine mapping of JHS1.

Supplemental Table S2. Primers used for the genotyping and RT-PCR analysis in Supplemental Figure S5.

Supplemental Table S3. Primers used for the qRT-PCR analysis.

ACKNOWLEDGMENTS

We thank Dr. Zhizhong Gong, Dr. Tonglin Mao, Dr. Chunming Liu, Dr. Yuling Jiao, and Dr. Fengning Xiang for providing the materials; Dr. Meiqin Liu for technical assistance with the sections; Dr. Meiqin Liu for technical assistance with the microscopy; and Chuanjing An, Jingjing Wang, Yuhong Shi, and Xiaoxing Liu for laboratory support.

Received February 28, 2016; accepted March 4, 2016; published March 7, 2016.

LITERATURE CITED

Abe K, Osakabe K, Ishikawa Y, Tagiri A, Yamanouchi H, Takyuu T, Plants with Different Body Plans. Plans. Curr Bio

Aichinger E, Kornet N, Friedrich T, Laux T

Akilu BB, Soderquist RS, Culligan KM (2014) Genetic analysis of the Replication Protein A large subunit family in Arabidopsis reveals unique and overlapping roles in DNA repair, meiosis and DNA replication. Nucleic Acids Res 42: 3104–3118

Amiard S, Charbonnel C, Allain E, Pepeigne A, White CI, Gallego ME

Antoshechkin IA, Reis C, Wold BJ, Campbell JL (2011) Invi-

Bae SH, Bae KH, Kim JA, Seo YS (2001) RPA governs endonuclease switching during processing of Okazaki fragments in eukaryotes. Nature 412: 456–461

Budd ME, Antoshechkin IA, Reis C, Wold BJ, Campbell JL (2011) Invi-



DNA Damage Affects Meristem Maintenance in Plants

Budd ME, Campbell JL (1997) A yeast replicative helicase, DNA2 helicase, interacts with yeast FEN-1 nuclease in carrying out its essential function. Mol Cell Biol 21: 2136–2142

Budd ME, Campbell JL (2000) The pattern of sensitivity of yeast DNA2 mutants to DNA damaging agents suggests a role in DSB and post-

replication repair pathways. Mutat Res 459: 173–186

Budd ME, Reis CC, Smith S, Myung K, Campbell JL (2006) Evidence suggesting that Pif1 helicase functions in DNA replication with the DNA2 helicase/nuclease and DNA polymerase delta. Mol Cell Biol 26: 2490–2500

Bundock P, Hooykaas P (2002) Severe developmental defects, hypersen-

sitivity to DNA-damaging agents, and lengthened telomeres in Arabi-

dopsis MRE11 mutants. Plant Cell 14: 2451–2462

Cejka P, Cannavo E, Polacek P, Masuda-Sata T, Pokharel S, Campbell JL, Kowalczykowski SC (2010) DNA end resection by DNA2-Sgo1-RPA and its stimulation by Top3-Rmi1 and Mre11-Rad50-Xrs2. Nature 467: 112–116

Chankova SG, Dimova E, Dimitrova M, Bryant PE (2007) Induction of DNA double-strand breaks by zeocin in Chlamydomonas reinhardtii and the role of increased DNA double-strand breaks rejoining in the formation of an adaptive response. Radiat Environ Biophys 46: 409–416

Choe W, Budd M, Imamura O, Hoopes L, Campbell JL (2002) Dynamic localization of an Okazaki fragment processing protein suggests a novel role in telomere replication. Mol Cell Biol 22: 4202–4217

Clark SE, Running MP, Meyerowitz EM (1993) CLAVATA1, a regulator of meristem and flower development in Arabidopsis. Development 119: 397–418

Clark SE, Running MP, Meyerowitz EM (1995) CLAVATA3 is a specific regulator of shoot and floral meristem development affecting the same processes as CLAVATA1. Development 121: 2057–2067

Clark SE, Williams RW, Meyerowitz EM (1997) The CLAVATA1 gene encodes a putative receptor kinase that controls shoot and floral meri-

stem size in Arabidopsis. Cell 89: 575–585

Cough SJ, Bent AF (1998) Floral dpr: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J 16: 235–243

Cools T, Iantcheva A, Maes S, van den Daele D, De Veylder L (2010) A replication stress-induced synthesis mechanism for Arabidopsis thaliana root meristems. Plant J 64: 705–714

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

Dissmeyer N, Weimer AK, Busch S, De Schutter K, Alvim Kamei CL, Nowack MK, Novak B, Duan GL, Zha YG, De Veylder L, Schnittger A (2009) Control of cell proliferation, organ growth, and DNA damage response operate independently of dephosphorylation of the Arabi-
dopsis Cdk1 homolog CDKA1. Plant Cell 21: 3641–3654

Doerner P, Jørgensen JE, You R, Steppuhn J, Lamb C (1996) Control of root growth and development by cyclin expression. Nature 380: 520–523

Doležel J, Greilhuber J, Suda J (2006) Evidence for meristem integrity in Arabidopsis. Plant Physiol. Vol. 171, 2016 331

Doutriaux MP, Couteau F, Bergounioux C, White C

Doutriaux MP, Couteau F, Bergounioux C, White C (1999) Isolation and characterisation of the RAD51 and DMC1 homologs from Arabidopsis thaliana. Mol Gen Genet 257: 283–291

Duxin JP, Doo B, Martinsson P, Rajala N, Guittat I, Campbell JL, Spellbrink JN, Stewart SA (2009) Human DNA2 is a nuclear and mitochondrial DNA maintenance protein. Mol Cell Biol 29: 4274–4282

Endo M, Ishikawa Y, Osakabe K, Nakayama S, Kaya H, Araki T, Shibahara K, Abe K, Ishikawa H, Valentine L, Hohn B, Toki S (2006) Increased fre-

quency of homologous recombination and T-DNA integration in Arabidopsis CAF1 mutants. EMBO J 25: 5579–5590

Fleming AJ (2006) The co-ordination of cell division, differentiation and morphogenesis in the shoot apical meristem: a perspective. J Exp Bot 57: 25–32

Fletcher JC, Brand U, Running MP, Simon R, Meyerowitz EM (1999) Signaling of cell fate decisions by CLAVATA3 in Arabidopsis shoot meristems. Science 283: 1911–1914

Formosa T, Nittis T (1999) Dna2 mutants reveal interactions with DNA polymerase a and Ctf4, a Pol a-accessory factor, and show that full DNA2 helicase activity is not essential for growth. Genetics 151: 1459–1470
Mimitou EP, Symington LS (2009) DNA end resection: many nucleases make light work. DNA Repair (Amst) 8:983-995
Qiao M, Zhao ZJ, Song YG, Liu ZH, Cao LX, Yu YC, Li S, and Xiang FN (2012) Proper regeneration from in vitro cultured Arabidopsis thaliana requires the microRNA-directed action of an auxin response factor. Plant J 71:14-22
Reidt W, Wurz R, Wanieck K, Chu HH, Puchta H (2006) A homologue of the breast cancer-associated gene BARD1 is involved in DNA repair in plants. EMBO J 25: 4326-4337
Roncero A, Guillen-Morillo J, Lincker F, Gadea-Vacas J, Delorme V, Bechtold N, Pelletier G, Delseny M, Chabouéte ME, Devic M (2005) Genetic analysis of two Arabidopsis DNA polymerase epsilon subunits during early embryogenesis. Plant J 44: 223-236
Roth N, Klimesch J, Duchowie-Schulze S, Pacher M, Mannass A, Puchta H (2012) The requirement for recombination factors differs considerably between different pathways of homologous double-strand break repair in somatic plant cells. Plant J 72: 781-790
Sancar A, Lindsey-Boltz LA, Unsai-Kacmaz K, Sinn L (2004) Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu Rev Biochem 73: 39-85
Sarkar AK, Luijten M, Miyashima S, Lenhard M, Hashimoto T, Nakajima K, Scheres B, Heidstra R, Laxus T (2007) Conserved factors regulate signalling in Arabidopsis thaliana root and stem cell organizers. Nature 446: 811-814
Schoof H, Lenhard M, Haecker A, Mayer KF, Jürgens G, Laxus T (2000) The stem cell population of Arabidopsis shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. Cell 100: 635-644
Schröpfer S, Kobbe D, Hartung F, Knoll A, Puchta H (2014) Defining the roles of the N-terminal region and the helicase activity of RECQD4 in DNA repair and homologous recombination in Arabidopsis. Nucleic Acids Res 42: 1684-1697
Shaul O, Mironov V, Burssens S, van Montagu M, Inze D (1996) Two Arabidopsis cyclin promoters mediate distinctive transcriptional oscillation in synchronized tobacco BY-2 cells. Proc Natl Acad Sci USA 93: 4868-4872
Shinohara H, Matsuaysash Y (2009) Reevaluation of the CLV3-receptor interaction in the shoot apical meristem: dissection of the CLV3 signaling pathway from a direct ligand-binding point of view. Plant J 82: 328-336
Song XF, Guo P, Ren SC, Xu TT, Liu CM (2013) Antagonistic peptide technology for functional dissection of CLV3/ESR genes in Arabidopsis. Plant Physiol 161: 1076-1085
Stahl Y, Wink RH, Ingram GC, Simon R (2009) A signaling module controlling the stem cell niche in Arabidopsis root meristems. Curr Biol 19: 909-914
Suzuki T, Nakajima S, Morikami A, Nakamura K (2005) An Arabidopsis protein with a novel calcium-binding repeat sequence interacts with TONSO/K/MGN2/BRUSHY1 involved in meristem maintenance. Plant Cell Physiol 46: 1452-1461
Takahashi N, Yamazaki Y, Kobayashi A, Higashitani A, Takahashi H (2003) The Arabidopsis AtRAD51 gene is dispensable for vegetative development but required for meiosis. Proc Natl Acad Sci USA 100: 2937-2942
Takahashi N, Adachi Y, Chiba K, Oguchi K, Takahashi H (2002) Disruption of a DNA topoisomerase I gene affects morphogenesis in Arabidopsis. Curr Opin Plant Biol 5: 592-597
Twentyman PR (1983) Bleomycin—mode of action with particular reference to its cell cycle. Pharmacol Ther 23: 417-441
Uanschou C, Siwicic T, Pedrosa-Haran A, Kerzendorfer C, Sanchez-Moran E, Novatchkova M, Akimcheva S, Woglar A, Klein F, Schöglerhofer P (2007) An Arabidopsis plant gene essential for meiosis is related to the human CIP and the yeast COM1/SAE2 gene. EMBO J 26: 5061-5070
Ulmasov T, Murrett J, Hagen G, Guilfoyle TJ (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell 9: 1963-1971
van den Berg C, Willemsen V, Hendriks G, Weisbeek P, Scheres B (1997) Short-range control of cell differentiation in the Arabidopsis root meristem. Nature 390: 287–289

Voinnet O, Rivas S, Mestre P, Baulcombe D (2003) An enhanced transient expression system in plants based on suppression of gene silencing by the p19 protein of tomato bushy stunt virus. Plant J 33: 949–956

Wang Y, Cortez D, Yazdi P, Neef N, Elledge SJ, Qin J (2000) BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. Genes Dev 14: 927–939

Yanai O, Shani E, Dolezal K, Tarkowski P, Sablowski R, Sandberg G, Samach A, Ori N (2005) Arabidopsis KNOXI proteins activate cytokinin biosynthesis. Curr Biol 15: 1566–1571

Yin H, Zhang X, Liu J, Wang Y, He J, Yang T, Hong X, Yang Q, Gong Z (2009) Epigenetic regulation, somatic homologous recombination, and abscisic acid signaling are influenced by DNA polymerase epsilon mutation in Arabidopsis. Plant Cell 21: 386–402

Zheng L, Shen B (2011) Okazaki fragment maturation: nuclease take centre stage. J Mol Cell Biol 3: 23–30

Zheng L, Zhou M, Guo Z, Lu H, Qian L, Dai H, Qiu J, Yakubovskaya E, Bogenhagen DF, Demple B, Shen B (2008) Human DNA2 is a mitochondrial nuclease/helicase for efficient processing of DNA replication and repair intermediates. Mol Cell 32: 325–336

Zhou X, Li Q, Chen X, Liu J, Zhang Q, Liu Y, Liu K, Xu J (2011) The Arabidopsis RETARDED ROOT GROWTH gene encodes a mitochondria-localized protein that is required for cell division in the root meristem. Plant Physiol 157: 1793–1804

Zhu Z, Chung WH, Shim EY, Lee SE, Ira G (2008) Sgs1 helicase and two nucleases DNA2 and EXO1 resect DNA double-strand break ends. Cell 134: 981–994