The Plant Cell, Vol. 21: 2688–2699, September 2009, www.plantcell.org

The STRUCTURAL MAINTENANCE OF CHROMOSOMES 5/6 Complex Promotes Sister Chromatid Alignment and Homologous Recombination after DNA Damage in *Arabidopsis thaliana* [C][W]

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Sister chromatids are often arranged as incompletely aligned entities in interphase nuclei of *Arabidopsis thaliana*. The STRUCTURAL MAINTENANCE OF CHROMOSOMES (SMC) 5/6 complex, together with cohesin, is involved in double-strand break (DSB) repair by sister chromatid recombination in yeasts and mammals. Here, we analyzed the function of genes in *Arabidopsis*. The wild-type allele of *SMC5* is essential for seed development. Each of the two *SMC6* homologs of *Arabidopsis* is required for efficient repair of DNA breakage via intermolecular homologous recombination in somatic cells. Alignment of sister chromatids is enhanced transiently after X-irradiation (and mitomycin C treatment) in wild-type nuclei. In the *smc5/6* mutants, the x-ray–mediated increase in sister chromatid alignment is much lower and delayed. The reduced S phase–established cohesion caused by a knockout mutation in one of the α-kleisin genes, *SYN1*, also perturbed enhancement of sister chromatid alignment after irradiation, suggesting that the S phase–established cohesion is a prerequisite for correct DSB-dependent cohesion. The *radiation-sensitive51* mutant, deficient in heteroduplex formation during DSB repair, showed wild-type frequencies of sister chromatid alignment after X-irradiation, implying that the irradiation-mediated increase in sister chromatid alignment is a prerequisite for, rather than a consequence of, DNA strand exchange between sister chromatids. Our results suggest that the SMC5/6 complex promotes sister chromatid cohesion after DNA breakage and facilitates homologous recombination between sister chromatids.

INTRODUCTION

Double-strand breaks (DSBs), if not repaired, are lethal, at least for dividing cells, and, if misrepaired, may cause chromosome rearrangements, such as reciprocal translocation, insertions, inversions, duplications, and deletions (for review, see Schubert et al., 2004). DSBs are repaired either by homologous recombination (HR) or by nonhomologous end-joining (NHEJ). The gene products involved in these repair pathways are evolutionarily conserved. While NHEJ simply ligates free ends of double-stranded DNA, HR needs an intact homologous duplex to form a heteroduplex for repairing the damaged site by means of the undamaged homologous sequence (reviewed in Kanaar et al., 1998; Barzel and Kupiec, 2008). Since the physical proximity between the donor and acceptor strands is critical for strand exchange events during HR, closely aligned sister chromatids provide a preferred donor for DNA repair via HR (Kadyk and Hartwell, 1992).

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[W]Online version contains Web-only data.
www.plantcell.org/cgi/doi/10.1105/tpc.108.060525

STRUCTURAL MAINTENANCE OF CHROMOSOMES (SMC) complexes have multiple functions in sister chromatid cohesion and condensation and repair of eukaryotic chromosomes and are essential for faithful chromosome segregation (for review, see Lehmann, 2005; Nasmyth and Haering, 2005). Together with non-SMC proteins, including kleisin subunits, SMC proteins form multiprotein complexes, such as the cohesin, the condensin, and the SMC5/6 complex. Two large subunits of cohesin, SMC1 and SMC3, form together with an α-kleisin (SISTER CHROMATID COHESION1 [SCC1]/RADIATION-SENSITIVE21 [RAD21] in somatic cells and RECOMBINATION8 [REC8] in meiotic cells) a tripartite ring that establishes cohesion during DNA replication (S phase cohesion) and holds sister chromatids together. Cohesin, together with the SMC5/6 complex, is involved in DSB repair of G2 cells. Budding yeast mutants of cohesin and SMC5/6 complex components display errors in sister chromatid segregation (Uhlmann and Nasmyth, 1998; Torres-Rosell et al., 2005) and are deficient in DSB repair (Sjögren and Nasmyth, 2001; Ünal et al., 2004; De Piccoli et al., 2006). The Scc2/4 complex is needed to load cohesin and Smc5/6 complexes during the S phase onto chromosomes in budding yeast (*Saccharomyces cerevisiae*; Ciosk et al., 2000; Lindroos et al., 2006). For cohesion establishment in response to DSB formation, the Scc2/4 complex, the cohesion establishment factor (Ctf7/Eco1), Mre11, which acts as a sensor to DSBs in a complex with Rad50 and Xrs2 (Usui et al., 2001), the checkpoint kinases Mec1, Tel1, and Chk1, and Smc6 (probably in the form of the Smc5/6 complex) are required in

yeast (Ström et al., 2007; Ünal et al., 2007, Heidinger-Pauli et al., 2008). In human cultured cells, the SMC5/6 complex is involved in recruitment of cohesin to DSB sites (Potts et al., 2006). Mutations or reduced expression of cohesin or SMC5/6 complex components reduce the frequency of HR repair between sister chromatids (Cortes-Ledesma and Aguilera, 2006; De Piccoli et al., 2006; Potts et al., 2006) but has little impact on the frequency of DSB repair by NHEJ or intrachromatid-recombination in budding yeast (Cortes-Ledesma and Aguilera, 2006; De Piccoli et al., 2006). In human cultured cells, DSB repair by NHEJ was even increased after depletion of cohesion or SMC5/6 complex components (Potts et al., 2006). This suggests that cohesin and SMC5/6 complexes keep sister chromatids aligned and facilitate HR repair.

Arabidopsis thaliana homologs of repair factors involved in HR, NHEJ, and DSB signaling have been identified and characterized (Riha et al., 2002; Friesner and Britt, 2003; reviewed in Schuermann et al., 2005). Knowledge about the role of SMC proteins in DSB repair of plants is still limited. Sister chromatids of *Arabidopsis* are often aligned in a random manner along chromosome arms in interphase nuclei of meristematic and differentiated cells with a 4C or higher DNA content (Schubert et al., 2006, 2008). Accumulating evidence suggests that sister chromatid alignment in *Arabidopsis*, as in yeast and mammals, is mediated by cohesins (Cai et al., 2003; Schubert et al., 2009). Mutants of cohesin genes, *smc1* (*titan8*) and *smc3* (*titan7*), show aberrant seed development in *Arabidopsis* (Liu et al., 2002). One of the four α-kleisin paralogs in *Arabidopsis*, *SYN1*/*DETERMINATE*, *INFERTILE1*, is necessary for sister chromatid cohesion and correct chromosome segregation during meiosis but is expressed also in meristematic tissues (Peirson et al., 1997; Bai et al., 1999; Bhatt et al., 1999; Cai et al., 2003; da Costa-Nunes et al., 2006). The other three paralogs, *SYN2*/*RAD21.1*, *SYN3*/*RAD21.2*, and *SYN4*/*RAD21.3*, are expressed in all plant tissues and may participate in sister chromatid cohesion. γ-Irradiation induces expression of *SYN2*/*RAD21.1*, and the T-DNA insertion line for *SYN2*/*RAD21.1* shows higher sensitivity to ionizing radiation and bleomycin than do wild-type plants (da Costa-Nunes et al., 2006; Kozak et al., 2009). A T-DNA insertion line for one of the two *SMC6* genes (the *mim* [for *hypersensitive to MMS, irradiation, MMC*] mutant line; Mengiste et al., 1999) showed slow growth in early developmental stages, higher sensitivity to DNA damage than wild-ype plants, and a decreased frequency of HR in somatic cells.

To better understand the impact of DNA damage and of loss-of-function mutations of the SMC5/6 complex on interphase chromosome arrangement in plant cells, we analyzed HR and sister chromatid alignment in somatic cells with and without induced DNA damage in *Arabidopsis* wild-type, *smc5/6*, *syn1*, and *rad51* mutant plants.

RESULTS

All Homologs of *SMC5* and *SMC6* Genes Are Expressed in *Arabidopsis*

Arabidopsis carries one homolog for yeast *SMC5* (AT5G15920) and two homologs for *SMC6* (Losada and Hirano, 2005). We call one of the two *SMC6* homologs (AT5G07660) *SMC6A* and the other one *SMC6B* (AT5G61460, *MIM*; according to Mengiste et al., 1999). The expression of the three genes was examined in wild-type plants. RNA for RT-PCR was isolated from 2-week-old seedlings as well as from rosette leaves and from immature floral buds. All three transcripts were scarce in leaves but abundant in seedlings and floral buds (Figures 1A and 1B). The transcript level of *SMC6B* was 21-fold higher in seedlings and 6.4-fold higher in flower buds compared to that of *SMC6A*. However, the transcript level of *SMC6A* in floral buds was 6.9-fold higher than that in seedlings, while the increase in transcript level of *SMC6B* was 2.1-fold higher in flower buds than in seedlings (Figure 1C; see Supplemental Figure 1 online for the location of the PCR primers). Thus, SMC6B seems to be the major SMC6 subunit of the SMC5/6 complex in early developmental stages.

To analyze the role of the SMC5/6 complex in HR repair in a plant, we studied five T-insertion lines of *Arabidopsis* accession Columbia (Col): *smc5-1*, *smc5-2*, *smc6a-1*, *smc6b-1*, and *smc6b-2* (Figure 1A). The T-DNA insertions of *smc5-1* and *smc5-2* occurred within the second exon. In total, eight independent individuals, hemizygous for T-DNA insertion in the *SMC5* gene (*SMC5*/*smc5*-1 and *SMC5*/*smc5-2*), did not yield homozygous mutants among their progeny. Siliques of *SMC5*/*smc5-1* and *SMC5*/*smc5-2* contained ~25% shrunken seeds (Table 1), indicating that *SMC5* is essential for seed development. In *smc6a-1*, the T-DNA is inserted in the 11th exon and in *smc6b-1* in the 19th intron, while *smc6b-2* harbors a T-DNA within the 27th exon. RT-PCR revealed the absence of full-length transcripts in homozygous *smc6a-1* and *smc6b-2* mutants (Figure 1D). Amplified DNA fragments demonstrated that *SMC6A* transcripts from the *smc6a-1* mutant lack the central region around the T-DNA insertion site. PCR amplified two DNA fragments corresponding to the 3′ region of the *SMC6A* transcript (F3 fragment; Figures 1A and 1D) in the *smc6a* mutant as well as in the wild type. The larger fragment was amplified from a cDNA synthesized from a *SMC6A* transcript variant from which the 21st intron (from the position 5570 to 5666 of the genomic sequence of AT5G07660) was not spliced out (GenBank FJ869873), indicating an alternative splicing within the 3′ region of the *SMC6A* gene. A termination codon within the additional sequence of the longer transcript variant, having a poly(A) tail, would, if the transcript variant is translated, yield a protein that lacks the C-terminal 182 amino acids, which are required to interact with the δ-kleisin, NON-SMC ELEMENT4 (NSE4), in fission yeast (*Schizosaccharomyces pombe*) (Figure 2; Palecek et al., 2006). A very faint F2 fragment amplified from the *smc6a-1* mutant migrates faster than the F2 fragment from the wild-type plant, suggesting irregular splicing caused by the T-DNA insertion. The 3′ region of the *SMC6B* transcript downstream of the T-DNA insertion is absent in the *smc6b-2* mutant. The very faint F3 fragment obtained from the *smc6b-1* mutant is apparently due to rare splicing out of the intron that harbors the T-DNA insertion. These results indicate that *smc6a-1* and *smc6b-2* mutants do not express wild-type *SMC6* transcripts, while *smc6b-1* plants can generate wild-type transcripts via splicing out of the T-DNA insertion, albeit at a much lower level than wild-type plants.

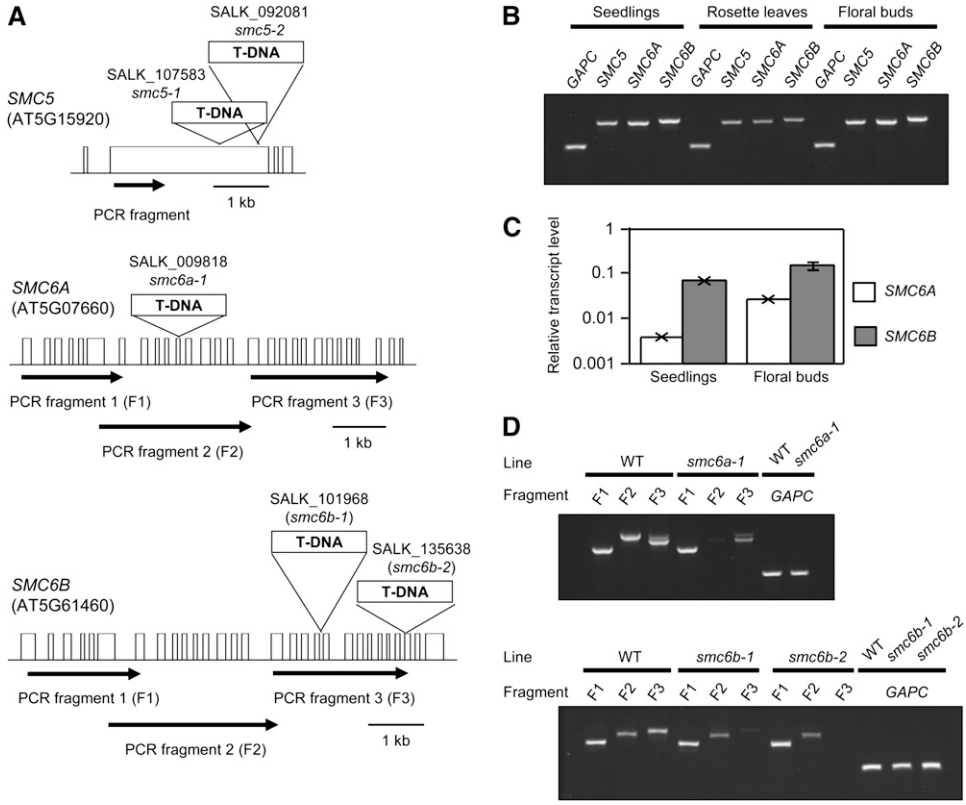

Figure 1. Structure and Expression of *SMC5* and *SMC6* Genes.

(A) Scheme of the genes with exons (open boxes) and T-DNA insertions of the corresponding SALK lines. The arrows below the boxes indicate the amplified PCR fragments of **(B)** and **(D)** and the direction of transcription. Primer sets used for PCR are shown in Supplemental Table 1 online.

(B) Expression analysis of *SMC5/6* genes by RT-PCR using RNA samples from seedlings, rosette leaves, and immature floral buds of wild-type plants. Glyceraldehyde-3-phosphate dehydrogenase C (*GAPC*) cDNA was amplified as a control.

(C) Differential expression of *SMC6* genes. Real-time RT-PCR was used to measure the amount of transcripts in the RNA samples from the indicated plant tissues. Transcript levels of *SMC6A* and *SMC6B* are shown in relation to that of *ACTIN2* in seedlings and floral buds of wild-type plants. White, *SMC6A*; gray, *SMC6B*. Vertical bar and crossed bars represent the SD of two biological replicates. Crossed bars are used to indicate SD not large enough to be depicted in a semilogarithmic graph.

(D) Top panel: DNA fragments amplified from *SMC6A* cDNA of wild-type and *smc6a-1* plants. Bottom panel: DNA fragments amplified from *SMC6B* cDNA of wild-type, *smc6b-1*, and, *smc6b-2* plants. RT-PCR was performed with RNA samples from rosette leaves. *GAPC* cDNA was amplified as a control.

Mutations on *SMC6A* and *6B* Genes Tend to Increase Sensitivity to X-Irradiation

Homozygous mutant plants harboring the *mim* allele of *SMC6B* are more sensitive to UV-C, x-rays, methyl methanesulfonate, and mitomycin C (MMC) than are wild-type plants (Mengiste et al., 1999). To find possible functional differences between *SMC6A* and *SMC6*B, the effect of X-irradiation (100 Gy) of seeds on root growth was analyzed in *smc6* mutant plants at various time points after irradiation (Figure 3). Compared with wild-type seedlings, *smc6a-1* and *smc6b-1* mutants revealed delayed root growth even in nonirradiated samples. All lines tested showed retarded root growth after irradiation. The difference between wild-type and mutant plants became apparent 10 d after irradiation. The *smc6a-1* and the *smc6b-1* mutant plants were equally sensitive to x-rays, suggesting that both *SMC6* genes are involved in DNA repair. MMC treatment results in retarded growth of the *smc6b-1* mutants in comparison to wild-type and *smc6a-1* mutant plants (see Supplemental Figure 2 online).

X-Rays Enhance Sister Chromatid Alignment in Wild-Type Plants

RAD51 homologs are involved in the repair of damaged DNA via HR (reviewed in Kanaar et al., 1998; Shibata et al., 2001). *Arabidopsis rad51* mutants show increased sensitivity to the DNA cross-linking agent MMC but not to DSB inducers, such as γ-rays or bleomycin (Bleuyard et al., 2005; Markmann-Mulisch et al., 2007), while mutants defective in NHEJ are hypersensitive to γ-irradiation (Friesner and Britt, 2003). Because MMC apparently induces DSBs only when cross-link repair interferes with DNA replication, HR might be involved mainly in postreplication repair and NHEJ in DSB repair during other phases of cell cycle.

Table 1. Shrunken Seeds in *smc5* Mutant Plants

Line	Normal Seeds (%)	Shrunken Seeds (%)	*n*
Wild type	96.7	3.3	514
SMC5/smc5-1	73.2	26.8**	935
SMC5/smc5-2	78.0	22.0**	943

n, total number of seeds examined. **, The observed segregation ratio is tested by the χ^2 test to determine whether it fits a ratio of 3:1 at the probability of >0.05.

Also, *smc6* mutants displayed a slightly increased sensitivity to x-rays compared to the wild type. To study interphase chromosome arrangement in response to X-irradiation in *Arabidopsis*, we applied fluorescent in situ hybridization (FISH) to nuclei of 4C DNA content (4C nuclei) prepared from the irradiated plants. Irradiation with 20 Gy of x-rays was previously found to induce chromosome bridges in 28% of anaphase nuclei in somatic pistil cells of DNA ligase IV–deficient (*lig4*) plants (a 25-fold increase compared to the 1.1% of anaphase nuclei of the nonirradiated *lig4* plants). The number of DSBs induced by 1 to 50 Gy follows a linear dose relationship, and 20 Gy should yield 9 to 26 DSBs in the *Arabidopsis* genome according a formula by Erixon and Cedervall (1995). In budding yeast, one DSB is sufficient to trigger a genome-wide cohesion establishment (Ström et al., 2007; Heidinger-Pauli et al., 2008). Therefore, we applied 20 Gy of x-rays to wild-type plants as well as to *smc5*/6, *syn1*, and *rad51* mutants. Because comet assay studies after bleomycin treatment had previously shown that nearly all single- and double-strand breaks are repaired within 60 min after treatment (Menke et al., 2001), we isolated nuclei 10 and 60 min after irradiation (mai).

Pecinka et al. (2004) have shown that somatic pairing of homologs in unchallenged 2C nuclei occurs mainly at random (in 0.8 to 13%, on average 4.9%, of nuclei) along chromosome arms. However, Abdel Halim et al. (2004) described, within human G1 cells, X-irradiated with 4 Gy, an increased homologous pairing frequency of heterochromatic loci, which are preferentially involved in chromosome rearrangements, but not of euchromatic loci. Therefore, we tested first whether X-irradiation enhances allelic pairing of homologs in 2C nuclei of the wild type. To analyze the frequency of positional pairing, we counted the number of hybridization signals after FISH with a single BAC or a BAC pair carrying inserts of adjacent genomic sequences from mid arm positions of chromosomes 1 and 3 on flow-sorted 2C nuclei of nonirradiated and irradiated 2-week-old wild-type seedlings. One FISH signal per BAC was regarded as allelic pairing, two signals were considered as separation of homologs at the corresponding locus (Figure 4A). Nonirradiated 2C nuclei showed 10.7 and 9.94% of positional pairing at the loci represented by BAC clones T7N9/T2P11 and F18C1 on chromosomes 1 and 3, respectively. The pairing frequency at these positions 10 and 60 mai did not differ significantly from that of nonirradiated samples (Table 2; $P > 0.05$, χ^2 test). This indicates that irradiation with 20 Gy of x-rays does not enforce allelic pairing between homologs and that donor sequences for HR repair are not regularly provided by allelic loci in 2C nuclei.

Next, we tested completeness of sister chromatid alignment for entire chromosome arms. With a FISH probe covering the top arm of chromosome 1, no completely separated sister chromatid arm territories were found in 264 4C nuclei of the irradiated 2-week-old wild-type seedlings at 10 mai. At 60 mai, 2.3% of 264 nuclei showed completely separated sister chromatid territories for at least one homolog of this chromosome arm. Nonirradiated nuclei display separated sister arm territories in 6.1% of rosette leaf nuclei (Schubert et al., 2006). The frequency of 6.1% is significantly higher than the frequency at 10 mai ($P < 0.001$, χ^2 test) and at 60 mai ($P < 0.05$, χ^2 test), indicating that X-irradiation promotes alignment rather than separation of sister arm territories.

Then we analyzed sister chromatid alignment at homologous loci in 4C nuclei prepared from nonirradiated and irradiated wild-type seedlings by FISH with the same BACs used to examine allelic pairing in 2C nuclei. In nuclei with one or two FISH signals per BAC, sister chromatids are aligned at the corresponding loci, while three or four signals indicate sister chromatid separation in one or both homologs. Ten minutes after irradiation, 4C nuclei showed between 17 and 21% higher frequencies of positional sister chromatid alignment at the two loci (T7N9/T2P11; F18/C1) than nonirradiated nuclei. These differences were highly significant according to the χ^2 test ($P < 0.001$). Sixty minutes after irradiation, the values decreased by 7 to 11% compared to those obtained at 10 mai (Table 3). These results imply that X-irradiation transiently increases cohesion between sister chromatids. Apparently, in *Arabidopsis*, the breakage-induced enforcement of sister chromatid cohesion relaxes again after completion of DNA repair.

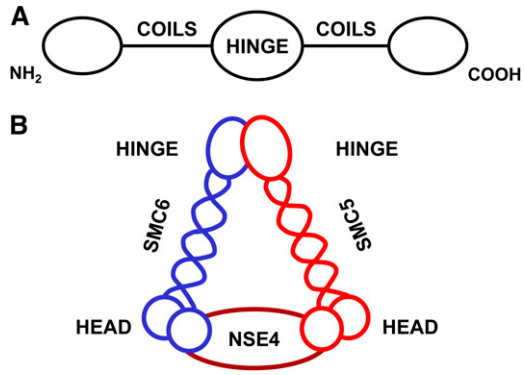

Figure 2. Architecture of SMC5 and SMC6 and Their Interaction with the δ-Kleisin NSE4.

(A) SMC5, SMC6A, and SMC6B have globular domains at both termini, each of which is connected to a hinge domain via a coiled-coil region. Each SMC protein is backfolded, creating a head domain composed of two globular domains at one end and a hinge domain at the other. NTP binding motifs (Walker A and B motifs) are identified in the N-terminal and the C-terminal globular domains of SMC5 and SMC6.

(B) SMC5 and SMC6 form heterodimers via interaction of their hinge domains, while the head domains of SMC5-SMC6 heterodimers associate with NSE4, according to Palecek et al. (2006).

[See online article for color version of this figure.]

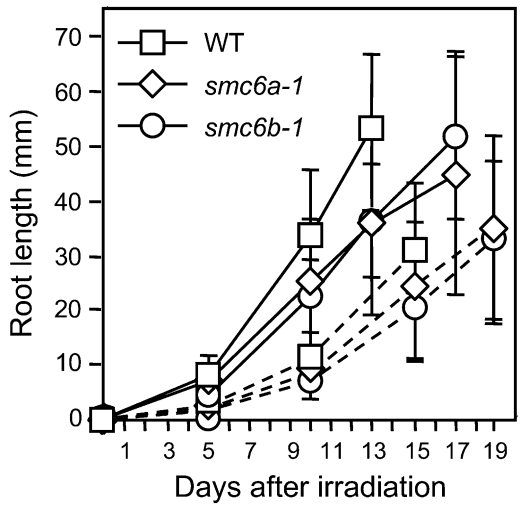

Figure 3. Root Extension in Control and Irradiated Plants.

Seeds on Murashige and Skoog-agar plates were exposed to 100 Gy of x-rays, and root length was measured at the indicated time. The mean values of root length are plotted. Error bars represent the SD for 20 plants per line. Open square, wild type; open diamond, *smc6a-1*; open circle, *smc6b-1*. Solid lines, mock-treated plants; dotted lines, irradiated plants.

Increased Sister Chromatid Alignment Needs the SMC5/6 Complex

To test the potential role of the *Arabidopsis* SMC5/6 complex in DSB repair, sister chromatid alignment was analyzed in nonirradiated and irradiated nuclei of heterozygous *smc5* and homozygous *smc6* mutant lines. First, we compared centromere cohesion in nonirradiated mutant and wild-type seedlings by FISH using the 178-bp centromere-specific probe. Full sister centromere cohesion in 4C nuclei should yield up to 10 FISH signals. More than 80% of nuclei of the wild type as well as of all mutant plants revealed 8 to 11 signals (Figure 4B), indicating no differences in sister centromere cohesion between wild-type and mutant plants and excluding the possibility that reduced sister chromatid alignment after X-irradiation of the mutants is due to precocious sister chromatid separation (before X-irradiation) in interphase nuclei with *smc5/6* mutant backgrounds.

To analyze sister chromatid alignment in nuclei from 2-week-old seedlings heterozygous for *SMC5*, we had to identify heterozygous plants. For genotyping, cotyledons were clipped from seedlings 2 d before preparation of sorted nuclei. Nonirradiated nuclei from the *smc5* mutants showed sister chromatid alignment at the tested positions in 60 to 67% of loci. Irradiation did not induce a significant increase in sister chromatid alignment in the *smc5* mutant nuclei at 10 mai (0.8 to 4.2% higher in irradiated nuclei than in nonirradiated nuclei), and at 60 mai the alignment frequency returned to the level observed in nonirradiated nuclei (Table 3). The higher alignment frequency in nonirradiated *smc5* mutants is apparently caused by cotyledon clipping, since wild-type seedlings showed a similar strong and significant increase of alignment ($P < 0.001$, χ^2 test) when exposed to cotyledon clipping (Table 3). Because of the high basic level of alignment caused by cotyledon clipping, it is difficult to decide whether enhancement of sister chromatid alignment is induced after DSB formation in heterozygous *smc5* nuclei or not.

The positional sister chromatid alignment in nonirradiated nuclei of *smc6a-1* and both *smc6b* mutant seedlings was as frequent as that of the wild type at both tested loci. After irradiation, the increase in sister chromatid alignment was delayed and clearly less pronounced in the nuclei of *smc6b* mutants than in wild-type nuclei. The 4C nuclei of the irradiated

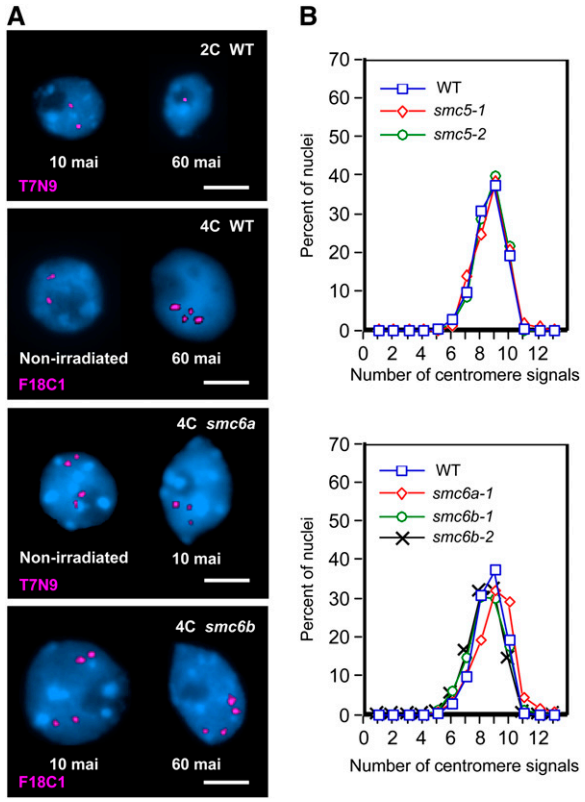

Figure 4. Positional Pairing and Sister Chromatid Alignment after FISH with Flow-Sorted *Arabidopsis* Nuclei.

(A) Top: FISH on wild-type 2C nuclei. Left: Separated loci in a nucleus 10 mai. Right: Paired loci in a nucleus, 60 mai. Second row: FISH on wild-type 4C nuclei. Left: Sister chromatid alignment at both homologs in a nonirradiated nucleus. Right: Positional sister chromatid separation at both homologs, 60 mai. Third row: FISH on 4C *smc6a-1* nuclei. Left: Positional sister chromatid separation at both homologs in a nonirradiated nucleus. Right: Sister chromatid alignment at one of the homologs, 10 mai. Bottom row: FISH on 4C *smc6b-2* nuclei. Left: Positional sister chromatid separation at both homologs, 10 mai. Right: 60 mai. Nuclei are counterstained with 4',6-diamidino-2-phenylindole (blue). Bars = 5 μm.
(B) Centromeric sister chromatid alignment in wild-type and in *smc5/6* mutants. Proportion of 4C nuclei with different numbers of FISH signals for centromeric repeats is demonstrated for wild-type plants and for *smc5/6* mutants. Top: Wild type versus heterozygotes for the *SMC5* gene. Bottom: Wild type versus homozygotes for *smc6* genes. The number of 4C nuclei analyzed in each line is as follows: wild type, 310; *smc5-1*, 209; *smc5-2*, 237; *smc6a-1*, 225; *smc6b-1*, 386; and *smc6b-2*, 386.

Table 2. Frequencies of Positional Homologous Pairing in Irradiated and Nonirradiated *Arabidopsis* 2C Wild-Type Nuclei

	Chromosome 1		Chromosome 3	
	T7N9/T2P11		F18C1	
mai	Frequency ± SD (%)[a]	*n*	Frequency ± SD (%)[a]	*n*
Nonirradiated	10.7	338	9.94	352
10	9.45	328	9.55	335
60	11.0	328	8.71	264

n, number of nuclei analyzed.
[a]Differences between nonirradiated and irradiated samples are not significant according to the χ^2 test ($P < 0.05$).

smc6 mutants showed at 60 mai 3.4 to 8.7% higher frequencies of sister chromatid alignment than nonirradiated nuclei, and the increase at 60 mai is statistically significant for position T7N9/T2P11 but not for position F18C1 in the *smc6b* mutants ($P < 0.05$, χ^2 test; Table 3). Although the relative transcript level of *SMC6A* is ~20 times less than that of *SMC6B* in wild-type seedlings, *SMC6A* seems to be necessary to enhance sister chromatid alignment after X-irradiation. These results suggest that both SMC6A and SMC6B are, likely in a complex together with SMC5, required for establishment of DSB-induced cohesion between sister chromatids.

Disturbed S Phase Cohesion in *syn1* Mutants Impairs Establishment of DSB-Mediated Cohesion

SYN1, a *REC8/SCC1* homolog, is required in *Arabidopsis* for sister chromatid cohesion in meiotic as well as in somatic cells (Schubert et al., 2009). Because SYN1 does not contain a Ser residue that becomes phosphorylated in response to DSB formation and is conserved among RAD21/SCC1 homologs (Heidinger-Pauli et al., 2008), SYN1 is likely to be involved in the establishment of S phase cohesion but not of DSB-induced cohesion. To test whether a reduced level of S phase cohesion impairs DNA breakage-mediated increase of sister chromatid alignment, we examined sister chromatid alignment in irradiated 4C nuclei from rosette leaves of a homozygous *syn1* mutant (Table 3). Unchallenged *syn1* nuclei showed significantly less ($P <$

Table 3. Frequencies of Positional Sister Chromatid Alignment in 4C *Arabidopsis* Nuclei of Nonirradiated and Irradiated (20 Gy) Wild-Type and Mutant Plants

			Chromosome 1		Chromosome 3	
			T7N9/T2P11		F18C1	
	Plant Material	mai	Frequency (%)	*n*	Frequency (%)	*n*
Wild type	Seedlings	Nonirradiated	54.8	704	51.4	704
		10	71.6***	880	72.7***	880
		60	61.1*	880	65.7***	880
Wild type[a]	Seedlings	Nonirradiated	67.2	640	65.5	640
SMC5/smc5-1 [a] (SALK_107583)	Seedlings	Nonirradiated	59.7	352	63.9	352
		10	63.9	352	67.6	326
		60	60.8	352	64.2	324
SMC5/smc5-2 [a] (SALK_092081)	Seedlings	Nonirradiated	63.4	352	67.3	352
		10	64.2	352	68.8	352
		60	60.5	352	64.8	352
smc6a-1/smc6a-1 (SALK_009818)	Seedlings	Nonirradiated	48.2	704	50.7	704
		10	52.0	704	50.4	702
		60	50.9	704	54.5	701
smc6b-1/ smc6b-1 (SALK_101968)	Seedlings	Nonirradiated	49.4	336	54.0	302
		10	54.3	352	53.5	318
		60	58.0*	350	60.1	336
smc6b-2/ smc6b-2 (SALK_135638)	Seedlings	Nonirradiated	49.7	348	55.7	352
		10	55.7	352	56.0	352
		60	57.7*	352	59.1	352
Wild type	Rosette leaves	Nonirradiated	60.7	704	62.1	704
syn1/syn1 (SALK_006687)	Rosette leaves	Nonirradiated	44.9	352	52.9	346
		10	52.3*	352	53.7	324
		60	46.6	352	44.9	316
rad51-1/rad51-1 (GABI_134A01)[b]	Rosette leaves	Nonirradiated	56.0	352	65.3	352
		10	66.2*	352	71.3	352
		60	59.7	352	67.9	352

n, number of homologous loci analyzed. *, Significance compared with the value for nonirradiated by the χ^2 test ($P < 0.05$). ***, Significance compared with the value for nonirradiated by the χ^2 test ($P < 0.001$).
[a]Cotyledons were clipped off 2 d before preparation of sorted nuclei.
[b]The line GABI_134A01 carries two T-DNA insertions and one of them was segregated out in *rad51-1* (Li et al., 2004).

0.005, χ^2 test) sister chromatid alignment than wild-type rosette leaf nuclei (60.7 and 62.1% in the wild type to 44.9 and 52.9% in *syn1*; Table 3), indicating that the absence of SYN1 impairs S phase–dependent cohesion. The generally higher frequency of sister chromatid alignment in rosette leaves than in seedlings might be due to changes in nuclear architecture along with development (Mathieu et al., 2003). Sister chromatid alignment was enhanced soon after irradiation in the *syn1* nuclei as well as in the wild-type nuclei and the *rad51* nuclei (Table 3), but the increase at 10 mai was small and less significant in the *syn1* mutant ($P < 0.05$ at position T7N9/T2P11 and $P > 0.05$ at F18C1; χ^2 test), suggesting that disturbed S phase cohesion impairs establishment of breakage-mediated cohesion in *syn1* mutants.

rad51 Mutants Show an Irradiation-Mediated Increase in Sister Chromatid Alignment

Heteroduplex formation between sister chromatids and subsequent DNA synthesis during HR require aligned sister chromatids. Rad51 and its homologs are involved in heteroduplex formation (Kanaar et al., 1998). To see whether mutants of repair components that act downstream of the SMC5/6 complex may influence chromatin dynamics after DNA damage, sister chromatid alignment was analyzed in 4C nuclei prepared from rosette leaves of *rad51-1* mutant plants (Table 3). Nonirradiated *rad51-1* nuclei showed sister chromatid alignment at a similar frequency as the nonirradiated wild-type nuclei from rosette leaves ($P > 0.1$, χ^2 test). In contrast with the results for *smc5/6* mutants, 20 Gy X-rays increased at 10 mai the sister chromatid alignment in *rad51-1* nuclei to values similar to those observed in nuclei from wild-type seedlings (71.6 and 72.7% in the wild type compared to 66.2 and 71.3% in *rad51*; $P > 0.05$, χ^2 test). The increase in sister chromatid alignment in irradiated *rad51-1* nuclei is reasonable because cohesin and SMC5/6 complexes are present in the *rad51* mutant. The increase indicates that sister chromatid alignment is a prerequisite for, rather than a consequence of, DNA strand exchange between sister chromatids.

Mutations in *SMC6A* and *6B* Genes Cause Reduced HR Frequencies in Somatic Cells

The efficiency of DSB repair via HR was analyzed with the recombination substrate, pDGU.US (Orel et al., 2003). This substrate contains, in direct orientation, two halves of a β-glucuronidase (GUS) gene with a 557-bp overlap separated by an unrelated sequence of 38 nucleotides. After treatment with bleomycin (causes DSBs directly) or with MMC (causes DSBs indirectly during repair of cross-links), a functional GUS gene can be restored by HR. Three different mechanisms can be envisaged (Figure 5): (1) intramolecular single-strand annealing after 5′-break end resection, (2) intermolecular synthesis-dependent strand-annealing, or (3) break-induced replication (BIR) with the sister chromatid or the homologous chromosome as a donor (Malkova et al., 1996; Puchta, 2005). In the case of synthesis-dependent strand-annealing, restoration of the GUS gene can be achieved by two intermolecular template switches: the first within the 557-bp overlap of the GUS sequences and the second behind this region, as both sister chromatids or homologs should be completely homologous distal to the overlap. Alternatively to the second switch, the chromatid might also be copied in toto, resulting in a BIR event (Figure 5). However, BIR would require rereplication licensing for all origins up to the chromosome arm

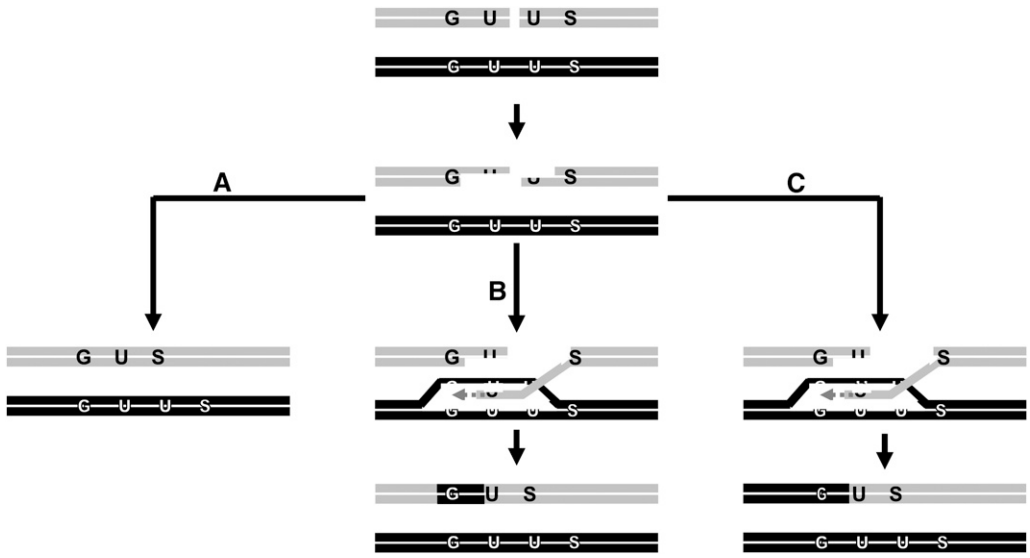

Figure 5. HR within the DGU.US Reporter Transgene.

Different mechanisms of DSB repair can be envisaged that result in the reconstitution of a functional GUS reporter gene.
(A) Single-strand annealing.
(B) Synthesis-dependent strand annealing via an intermolecular out-of-frame conversion.
(C) Break-induced replication.

end, which is unlikely to occur in large genomes. In two independent experimental series, the recombination events were monitored by counting GUS-stained blue sectors in seedlings of wild-type and *smc6a-1* or wild-type and *smc6b-1* mutant plants, both carrying pDGU.US in a homozygous state (Figure 6; see Supplemental Table 2 online). In comparison to the wild-type control, the number of recombination events is reduced to a third in the *smc6a-1* and to half in the *smc6b-1* mutant. After treatment with bleomycin and mitomycin, the *smc6a-1* mutant shows only a third of the recombination events of the wild type, and in the case of *smc6b-1*, the recombination events are reduced to a forth. Thus, up to three-quarters of the recombination events detected with our assay are due to intermolecular interactions. This might even be an underestimate, as both *smc6* mutants, compared to the wild type, still revealed some basic frequency of sister chromatid alignment and a small increase of sister chromatid alignment after irradiation, indicating that the two SCM6 homologs might be able to partially complement each other in HR.

DISCUSSION

Components of the SMC5/6 Complex Studied So Far Are Essential in *Arabidopsis*

The SMC5/6 complex is involved in DNA repair in yeasts and human. In yeasts, the SMC5/6 complex comprises two SMC subunits, SMC5 and SMC6, and six non-SMC elements, NSE1 to 6 (Losada and Hirano, 2005; Zhao and Blobel, 2005; Pebernard et al., 2006). Genes for SMC5, SMC6, and NSE1-4 are well conserved in budding and fission yeasts, *Drosophila melanogaster*, *Xenopus laevis*, and human. SMC5, 6, and NSE4, a δ-kleisin subunit, form a major tripartite complex interacting with the other NSE proteins (Losada and Hirano, 2005; Palecek et al., 2006). The *Arabidopsis* genome harbors homologs for these genes, and some of them are duplicated: two homologs for *SMC6* and two homologs for *NSE4* (*NSE4A* [AT1G51130] and *NSE4B* [AT3G20760]; Losada and Hirano, 2005). With RT-PCR, we detected transcripts of the *NSE4A* but not of the *NSE4B* gene in seedlings, rosette leaves, and immature floral buds (see Supplemental Figure 3 online), suggesting that *NSE4A* is a functional gene in somatic cells of *Arabidopsis*.

SMC5 and SMC6 are each composed of a globular head domain at both termini and a coiled-coil domain interrupted by a hinge domain (Figure 2A). In fission yeast, SMC5 and SMC6 interact with each other through their hinge domains and with NSE4 through their globular head domains (Figure 2B; Sergeant et al., 2005; Palecek et al., 2006). In *Arabidopsis*, the *smc6a-1* mutant expresses *SMC6A* transcripts lacking its central region around the T-DNA insertion site, which includes the sequence encoding a hinge domain. Therefore, SMC6A of the *smc6a-1* mutant might not be able to interact with SMC5. The *SMC6B* transcripts of *smc6b-1* and *-2* mutants lack the C-terminal head domain; therefore, the interaction of the mutated SMC6B with NSE4 might be disturbed in both mutants.

The *Arabidopsis* homolog for *NSE1* (AT5G21140; Losada and Hirano, 2005), which encodes a protein with a RING-like motif (Pebernard et al., 2008), is also essential for seed development and the terminal phenotype of the *nse1* line emerges at the preglobular embryonic stage (Tzafrir et al., 2004; SeedGenes Project at http://www.seedgenes.org/index.html). The *Arabidopsis* homologs for *NSE2* (AT3G15150), a SUMO ligase-encoding gene, and for *NSE3* (AT1G34770), a MAGE (melanoma antigen-encoding) gene, remain to be characterized. The primary structures of NSE5 and NSE6, identified in coprecipitates with the known SMC5/6 components, are quite different even between budding and fission yeasts (Zhao and Blobel, 2005; Pebernard et al., 2006). Functional homologs for these proteins are not yet identified in *Arabidopsis*.

The *Arabidopsis* SMC5/6 Complex Is Involved in DSB Repair

Homologous sequences in allelic or ectopic positions are potential donor sequences for DSB repair by HR. In human G1 cells, X-irradiation enforced homologous pairing of pericentromeric

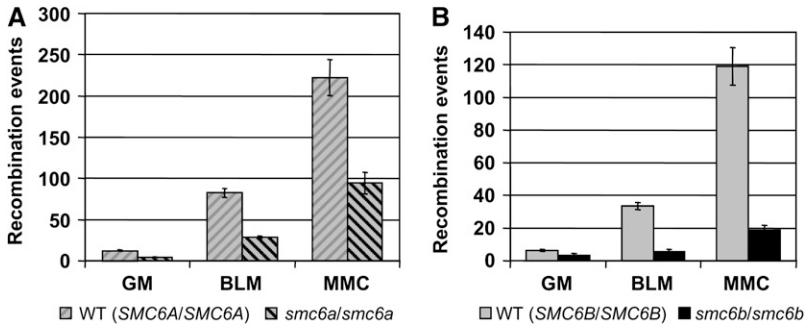

Figure 6. HR in *smc6a-1* and *smc6b-1* Mutants Compared to the Respective Wild Type.

(A) Analysis of HR events in the *smc6a-1* mutant compared to the corresponding wild type.

(B) Analysis of HR events in the *smc6b-1* mutant compared to the corresponding wild type.

The wild-type plants were obtained from a segregating population of a cross between a line carrying pDUGU.US and corresponding *smc6* mutant plants. Both mutants show a lower rate of HR compared to the wild type without and with DSB induction by application of bleomycin or MMC. HR events depicted for all conditions are mean values of three independent experiments. Vertical bars represent the SD. The data set and additional calculations are summarized in Supplemental Table 2 online.

heterochromatic loci, which are preferentially involved in chromosome rearrangements but not of euchromatic loci (Abdel Halim et al., 2004). Similarly, in *Arabidopsis* 2C wild-type nuclei, pairing of homologs at euchromatic regions was not enhanced by irradiation. The constrained movement of DSB ends in mammalian interphase nuclei indicates that search of broken chromosome ends for homology does not extend far beyond the chromosome territory (Soutoglou et al., 2007). Thus, HR in G1 seems to be restricted to homologous sequences, which are by chance in the spatial vicinity of a DSB. Since in *Arabidopsis* positional pairing between allelic loci occurs at random (i.e., in 0.84 to 13% of unchallenged 2C nuclei; Pecinka et al., 2004) and is not increased after irradiation, most DSBs seem to be repaired by NHEJ rather than by HR in 2C nuclei. Indeed, our previous work indicates that DSB repair using allelic or ectopic homology occurs very rarely, at least in tobacco (*Nicotiana tabacum*; Puchta, 1999; Gisler et al., 2002).

In budding yeast, the presence of sister chromatids immediately after DNA replication increases cell survival after DSB induction (Kadyk and Hartwell, 1992), and homologous alignment of sister chromatids by cohesin is important for DSB repair in S/G2 phase (Sjögren and Nasmyth, 2001; Ström et al., 2004). Cohesin loaded onto chromatin during S phase accumulates around centromeres (Megee et al., 1999; Bernard et al., 2001) and at defined loci every 5 to 10 kb along chromosome arms (Tanaka et al., 1999; Glynn et al., 2004; Lengronne et al., 2004). Thus, in yeast, sister chromatids are closely aligned during G2 phase. Additionally, cohesin is loaded onto sister chromatids in response to DSB formation and is maintained until the next M phase (Ström et al., 2004, 2007; Ünal et al., 2004, 2007). In contrast with the consistent sister chromatid cohesion along the chromosome arms in yeast, sister chromatid arms are incompletely aligned in *Arabidopsis* and other plant species (Schubert et al., 2006, 2007, 2008) and probably also in mammals (Volpi et al., 2001; Watrin and Peters, 2006). X-irradiation (and MMC treatment; see Supplemental Table 3 online) significantly increases sister chromatid alignment in 4C wild-type nuclei of *Arabidopsis* when functional *SMC6* genes are present. This suggests that in *Arabidopsis* as in yeast a tightened sister chromatid cohesion promotes (correct) DSB repair in 4C nuclei. The genotoxin-induced recombination frequency in somatic cells confirmed that the *SMC6* genes are required for nearly three-quarters of HR events in *Arabidopsis*. Thus, we conclude that the SMC5/6 complex enhances sister chromatid alignment after DNA damage and thereby facilitates correct DSB repair via HR between sister chromatids.

Rec8 artificially expressed in mitotic yeast cells binds to chromosome arms and contributes to establishment of S phase cohesion, but it is not deposited to DSB sites (Heidinger-Pauli et al., 2008). Because the *Arabidopsis* homolog *SYN1* is transcribed in somatic cells, and unchallenged *syn1* mutant nuclei show reduced sister chromatid alignment and occasional separation of sister centromeres (4.5 to 9.2% of 4C nuclei from different *syn1* mutants showed up to 18 centromere-specific FISH signals, while only 1.5% of wild-type 4C nuclei showed up to 12 centromere-specific signals.), SYN1 is thought to be involved in S phase cohesion in somatic cells (Schubert et al., 2009). The *syn2/rad21.1* mutant is deficient in DSB repair (da Costa-Nunes et al., 2006; Kozak et al., 2009), though it aligns sister chromatids in unchallenged conditions as frequently as the wild type does (Schubert et al., 2009), suggesting a role of SYN2 in DSB-responsive cohesion. We assume that the S phase cohesion is prerequisite to irradiation-enhanced cohesion establishment by SYN2-containing cohesins. The most prominent feature of multicellular organisms is cell differentiation. Most of the plant cells are differentiated and no longer proliferate. Some differentiated cells undergo endopolyploidization cycles. Expression of the *SCC1/RAD21/REC8* homolog *SYN1* and of *SMC5/6* is lower in mature leaves than in dividing tissues of *Arabidopsis* (Bhatt et al., 1999; Figure 1B). Similarly, the level of *RAD51* expression decreases in developing plants in correlation with a reduction of DSB repair by HR and with a complementary increase in expression of *KU70*, a component of NHEJ (Boyko et al., 2006). These data suggest that DSBs are preferentially repaired by NHEJ in differentiated cells and by HR between sister chromatids in meristematic cells. The predominance of the more accurate HR mechanism in dividing cells stabilizes the genome in meristematic tissues and ensures the correct transmission of genetic information to daughter cells and to subsequent generations.

METHODS

Plant Materials

The *smc5*, *smc6a*, *smc6b*, and *syn1* T-DNA insertion lines (in Col) (Figure 1A) were obtained from the SALK collection (Alonso et al., 2003). The *rad51-1* mutant (in Col) was described by Li et al. (2004) and provided by Bernd Reiss. The *lig4* mutant in accession Wassilewskija (Friesner and Britt, 2003) was provided by Ann Britt. The seedlings were cultured on germination media (GM)-agar (4.9 g/L Murashige and Skoog micro- and macro-elements, including vitamins [Duchefa], 10 g/L sucrose, pH 5.7, and 8 g/L micro-agar) under long-day conditions (16 h light/8 h dark) at 22°C for 2 weeks, followed by further cultivation in soil under short-day conditions (8 h light/16 h dark) at 21°C .

PCR-based genotype markers were used to identify the T-DNA insertion mutants. The PCR primers used for genotyping and the left border insertion junctions of these T-DNA insertion lines are listed in Supplemental Tables 4 and 5 online, respectively. PCR using the gene-specific primer sets yielded DNA fragments of ~1 kb representing the wild-type alleles of *SMC5*, *SMC6A*, and *SMC6B*. The PCR fragment specific for the *smc5-1*, *smc5-2*, *smc6a-1*, *smc6b-1*, *smc6b-2*, or *syn1* allele was amplified with the primer sets (107583RP, SALK_LB), (092081RP, SALK_LB), (009818RP, SALK_LB), (101968RP, SALK_LB), (135638RP, SALK_LB), or (006687RP, SALK_LB), and the amplification yielded products of ~0.5 kb. The allele of *rad51-1* was identified as described (Li et al., 2004).

X-Irradiation

Plant material was irradiated using the x-ray system YXLON MGC41 (YXLON International) at the Federal Research Center for Cultivated Plants (Quedlinburg, Germany). For analysis of sensitivity to ionizing radiation, seeds were sterilized, plated on Murashige and Skoog-agar media (4.4 g/L Murashige and Skoog micro- and macro-elements, including vitamins [Duchefa], pH 5.7, and 8 g/L micro-agar), and germinated at 4°C. Approximately 48 h later, plants were either mock-irradiated or exposed to 100 Gy (3 Gy/min) and cultured under long-day conditions. For analysis of sister chromatid alignment, plants were irradiated with 20 Gy (0.9 Gy/min) and fixed after the recovery time indicated in Tables 2 and 3. Nuclei were isolated as described below.

MMC Treatment

Seeds were sterilized, plated on GM-agar, and germinated under long-day conditions after 2 d of cold treatment at 4°C. For analysis of sensitivity to MMC, seedlings were transferred into 24-multiwell plastic plates (Falcon) 4 d after germination. Each well contained one seedling in 0.5 mL of liquid GM supplemented with MMC (Sigma-Aldrich) at concentrations of 2.5, 5, 10, 15, or 20 μg/mL. The plates were incubated for another 2 weeks under long-day conditions. For analysis of sister chromatid alignment, 10-d-old seedlings were moistened with water overnight, and the seedlings were mock-treated or exposed to 5 μg/mL of MMC in liquid GM for 30 min, followed by preparation of sorted nuclei.

Preparation of Nuclei, Probe Labeling, and FISH

Nuclei were isolated and flow-sorted according to their ploidy level from 2-week-old seedlings of wild-type, *smc5*, and *smc6* mutants or from leaves of wild-type, *syn1*, and *rad51* mutants as described (Jasencakova et al., 2003).

Isolation of BAC DNA, labeling by nick translation, and FISH were performed according to Jovtchev et al. (2008). BAC clones T2P11 (GeneBank accession number AC005508), T7N9 (AC000348), and F18C1 (AC011620) were labeled with biotin-dUTP, digoxigenin-dUTP, Alexa Fluor 488-5-dUTP, Cy3-dUTP, or Texas Red-12-dUTP. The 178-bp centromeric repeat probe was generated by PCR with specific primers from genomic DNA (Kawabe and Nasuda, 2005) and subsequently labeled with biotin-dUTP. For painting of the chromosome 1 top arm, 15 pools of in total 76 BACs were labeled with biotin-dUTP as described (Pecinka et al., 2004).

Microscopy Evaluation and Image Processing

Fluorescence signals in flow-sorted nuclei were analyzed using an Axioplan 2 (Zeiss) epifluorescence microscope with a ×100/1.4 Zeiss plan apochromat objective. In 4C nuclei, split FISH signals were considered to represent sister chromatid separation when their distance was larger than the signal diameter. Images were acquired separately for each fluorochrome using MetaVue (Molecular Devices) software, a cooled CCD camera (Spot 2e; Diagnostic Instruments), and appropriate excitation and emission filters. Monochromatic images were pseudocolored and merged using Adobe Photoshop 6.0 (Adobe Systems) software.

RNA Analysis

Total RNA was isolated from seedlings, rosette leaves, and floral buds using the RNeasy plant mini kit (Qiagen) according to the manufacturer's instructions. Reverse transcription was performed using a first-strand cDNA synthesis kit (Fermentas), oligo$(dT)_{18}$ primer (Fermentas), and 2 μg of total RNA as starting material. Primers used to amplify cDNA are listed in Supplemental Table 1 online. For conventional RT-PCR, PCR fragments were amplified using iCycler (Bio-Rad) and GoTaq Hot Start polymerase (Promega). All analyses were performed using three independent biological replicates. For quantitative RT-PCR, real-time RT-PCR was run using iCycler iQ (Bio-Rad) and iQ SYBR Green Supermix (Bio-Rad). Each transcript was quantified twice using two independent biological replicates. As a control, a fragment of *ACTIN2* cDNA was amplified for data normalization. The cDNA equivalent to 10 or 100 ng of total RNA was used in a 15-μL PCR reaction to amplify *ACTIN2* cDNA or *SMC6A* and *SMC6B* cDNAs, respectively. The positions of the primers used to amplify *SMC6A* and *SMC6B* cDNAs are depicted in Supplemental Figure 1 online. PCR amplification/cycle graphs are shown to indicate that amplification was in logarithmic phase for each DNA molecule being analyzed (see Supplemental Figure 4 online).

HR Assay

Arabidopsis thaliana seeds were sterilized in 6% sodium hypochlorite solution with a small amount of Tween 20 for 7 min and rinsed five times with sterile water before being stored in sterile agarose solution (1%, w/v) for 1 d at 4°C for stratification. Subsequently, seeds were sown with a pipette on 90-mm Petri dishes (40 seeds per plate) containing GM-agar and were grown in a growth chamber (Percival CU-36L; CLF Laborgeräte) under tightly controlled conditions (16 h light, 24°C/8 h dark, 20°C/100 μmol/m^2/s photosynthetic active radiation) for 7 d. Forty seedlings were then transferred with microceps into 90-mm Petri dishes filled with 18 mL of liquid GM and incubated for 24 h in the Percival growth chamber. Subsequently, 2 mL pure MMC (Duchefa) or Bleomycin (Duchefa) containing liquid GM was added to these plantlets, resulting in a final volume of 20 mL and a MMC or Bleomycin concentration of 5 μg/mL, respectively. The plants were then grown for another 5 d until histochemical staining.

Histochemical staining was performed as described (Schmidt-Puchta et al., 2004). Blue sectors were counted using a binocular microscope after the plants had been decolorized with 70% ethanol.

Accession Numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under the following accession numbers: *SMC5* (AT5G15920) mRNA, NM_121597; *SMC6A* (AT5G07660) mRNA, NM_125539; *SMC6A* mRNA (a transcript variant), FJ869873; *SMC6B* (AT5G61460) mRNA, NM_120848; *NSE4A* (AT1G51130) mRNA, NM_103992; and *NSE4B* (AT3G20760) mRNA, NM_112967.

Supplemental Data

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

Supplemental Figure 1. Position of the Primers Used in Quantitative RT-PCR.

Supplemental Figure 2. Sensitivity of *Arabidopsis* Lines to Mitomycin C Treatment.

Supplemental Figure 3. Structure and Expression of δ-Kleisin Genes, *NSE4A* and *NSE4B*.

Supplemental Figure 4. PCR Amplification/Cycle Graphs in Quantitative Real-Time PCR.

Supplemental Table 1. Oligonucleotide Primers Used for RNA Analysis.

Supplemental Table 2. Homologous Recombination Events in Wild-Type and *smc6* Mutants.

Supplemental Table 3. Frequencies of Positional Sister Chromatid Alignment in Mitomycin C–Treated 4C Nuclei.

Supplemental Table 4. Oligonucleotide Primers Used for Genotyping.

Supplemental Table 5. The Sequences of the Left Border Junctions of the T-DNA Insertion Lines.

ACKNOWLEDGMENTS

We thank Bernd Reiss (Max-Planck-Institut für Züchtungsforschung Köln) for providing the *Arabidopsis rad51-1* line, Anne Britt (University of California, Davis) for the *lig4* line, Jörg Fuchs (Leibniz-Institut für Pflanzengenetic und Kulturpflanzenforschung Gatersleben) for help

with flow-sorting, Ulrich Ryschka and Evelyn Klocke (Julius Kühne Institut, Quedlinburg) for X-irradiation, and Achim Bruder, Martina Kühne, Maren Nitze, and Rita Schubert for technical assistance.

Received May 7, 2008; revised July 13, 2009; accepted August 24, 2009; published September 8, 2009.

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