

The Arabidopsis homologue of Xrcc3 plays an essential role in meiosis

Jean-Yves Bleuyard and Charles I White*

CNRS UMR6547, Université Blaise Pascal, Aubière, France

The eukaryotic RecA homologue Rad51 is a key factor in homologous recombination and recombinational repair. Rad51-like proteins have been identified from yeast (Rad55, Rad57 and Dmc1) to vertebrates (Rad51B, Rad51C, Rad51D, Xrcc2, Xrcc3 and Dmc1). These Rad51-like proteins are all members of the genetic recombination and DNA damage repair pathways. The sequenced genome of *Arabidopsis thaliana* encodes putative homologues of all six vertebrate Rad51-like proteins. We have identified and characterized an Arabidopsis mutant defective for one of these, *AtXRCC3*, the homologue of *XRCC3*. *atxrcc3* plants are sterile, while they have normal vegetative development. Cytological observation shows that the *atxrcc3* mutation does not affect homologous chromosome synapsis, but leads to chromosome fragmentation after pachytene, thus disrupting both male and female gametogenesis. This study shows an essential role for *AtXrcc3* in meiosis in plants and possibly in other higher eukaryotes. Furthermore, *atxrcc3* cells and plants are hypersensitive to DNA-damaging treatments, supporting the involvement of this Arabidopsis Rad51-like protein in recombinational repair.

The EMBO Journal (2004) 23, 439–449. doi:10.1038/sj.emboj.7600055; Published online 15 January 2004

Subject Categories: genome stability & dynamics; plant biology

Keywords: *Arabidopsis thaliana*; meiosis; Rad51; recombination; Xrcc3

Introduction

Meiosis is the specialized cell division used in sexually reproducing organisms to produce haploid gametes from diploid cells (reviewed by Petronczki *et al*, 2003). During meiosis, a single round of DNA replication is followed by two successive divisions: the first (reductional) division segregates homologous chromosomes, and the second (equational) division separates sister chromatids from each other. The first division requires pairing and synapsis of homologous chromosomes to ensure accurate segregation, while the second releases sister chromatid cohesion. Homologous chromosome recognition (pairing), synaptonemal complex assembly (synapsis) and homologous recombination are the three events that promote and ensure bivalent formation and

stability before the first anaphase division (reviewed by Yamamoto and Hiraoka, 2001).

In *Saccharomyces cerevisiae* and most likely in all eukaryotes, meiotic recombination is initiated by the Spo11 protein, a member of the type II topoisomerase family (Bergerat *et al*, 1997; Keeney *et al*, 1997). Spo11 creates DNA double-strand breaks (DSBs) along meiotic chromosomes (reviewed by Keeney, 2001); the resulting DNA ends are then processed to produce 3' single-stranded tails (Sun *et al*, 1989, 1991; reviewed by Connelly and Leach, 2002), which can invade a homologous duplex. This meiotic recombination step is promoted by Rad51 and Dmc1, a Rad51-like protein expressed only in meiotic cells (Bishop *et al*, 1992; Shinohara *et al*, 1992, 1997; reviewed by Masson and West, 2001). These two proteins are members of the *Escherichia coli* RecA-like recombinase family (Aboussekhra *et al*, 1992; Bishop *et al*, 1992; Shinohara *et al*, 1992). RecA is a key factor in bacterial homologous recombination mechanism and can promote invasion of a duplex DNA by a homologous single-stranded molecule. Consistent with the fact that they are part of the RecA-like family, *in vitro* experiments have shown that such strand exchange reactions are promoted by Rad51 and Dmc1 (Sung, 1994; Hong *et al*, 2001). In addition to Dmc1, two Rad51 paralogs, Rad55 (Lovett, 1994) and Rad57 (Kans and Mortimer, 1991), have been identified in yeast. Rad55 and Rad57 form a heterodimer (Hays *et al*, 1995; Johnson and Symington, 1995; Sung, 1997), which is involved in Rad51-dependent recombination events (Sung, 1997). Yeast mutants impaired in any of the Rad51-like proteins are defective in meiotic recombination and exhibit reduced spore viability (reviewed by Paques and Haber, 1999; Symington, 2002).

Homologues of Dmc1 have been identified and shown to be involved in the meiotic process of several organisms (Diener and Fink, 1996; Pittman *et al*, 1998; Couteau *et al*, 1999; Mikosch *et al*, 2001), but unequivocal homologues of Rad55 and Rad57 have not been characterized in higher eukaryotes. Five Rad51 paralogs (Xrcc2, Xrcc3, Rad51B/Rad51L1, Rad51C/Rad51L2 and Rad51D/Rad51L3) have been identified in mammals, with 20–30% protein sequence identity to Rad51 and to each other. Physical interactions between the five human Rad51 paralogs have been demonstrated using the yeast two-hybrid assay (Schild *et al*, 2000), and coexpression of Xrcc3–Rad51C and Xrcc2–Rad51D in *E. coli*, and Rad51B–Rad51C in *S. cerevisiae* has shown that they can be purified as stable complexes (Kurumizaka *et al*, 2001, 2002; Sigurdsson *et al*, 2001). *In vivo* the five paralogs are associated in two complexes, one containing Rad51B, Rad51C, Rad51D and Xrcc2, and the other containing Rad51C and Xrcc3 (Masson *et al*, 2001; Liu *et al*, 2002; Wiese *et al*, 2002). Interestingly, Rad51 was never found associated with its paralogs, although it can interact with Xrcc3 in a yeast two-hybrid assay (Schild *et al*, 2000). The absence of these two complexes in cells lacking Rad51C suggests that it is a key factor in mammalian homologous recombination processes (French *et al*, 2002; Miller *et al*,

*Corresponding author. CNRS UMR6547, BIOMOVE, Université Blaise Pascal, 24 Avenue des Landais, F-63177 Aubière, France.
Tel.: +33 4 73407978; Fax: +33 4 73407777;
E-mail: chwwhite@univ-bpclermont.fr

Received: 28 August 2003; accepted: 3 December 2003; Published online: 15 January 2004

2002). As is the case for the *RAD51* knockout (Tsuzuki *et al.*, 1996), targeted disruption of mouse *RAD51B*, *RAD51D* and *XRCC2* leads to embryonic lethality (Shu *et al.*, 1999; Deans *et al.*, 2000; Pittman and Schimenti, 2000). Recently, the *Drosophila* genes *Spindle-B* (*Spn-B*) and *Spindle-D* (*Spn-D*) have been related to *XRCC3* and *RAD51C*, respectively (Abdu *et al.*, 2003). Mutants impaired for *Spn-B* and *Spn-D* are partially sterile, defective for meiotic recombination, but not hypersensitive to DSB-inducing agents (Ghabrial *et al.*, 1998; Abdu *et al.*, 2003). Individual knockouts of each of the five paralogs in chicken B-lymphocyte DT40 cell lines are viable, but show spontaneous chromosomal aberrations, hypersensitivity to DNA-damaging treatments and reduced levels of homologous recombination (Takata *et al.*, 2000, 2001). *In vitro* studies have shown that Xrcc3–Rad51C and Xrcc2–Rad51D complexes possess homologous pairing activities similar to those of RecA or Rad51 (Kurumizaka *et al.*, 2001, 2002). Furthermore, the Rad51B–Rad51C complex possesses a mediator role in promoting the assembly of the presynaptic Rad51 nucleofilament, in *in vitro* Rad51-dependent strand exchange reactions, that is similar to that of the Rad55–Rad57 heterodimer in yeast (Sung, 1997; Sigurdsson *et al.*, 2001). These data suggest that Rad51 paralogs are important factors for homologous recombination processes and the maintenance of the integrity of the genetic material.

In the plant *Arabidopsis thaliana*, homologues of *RAD51* (*AtRAD51*) and *DMC1* (*AtDMC1*) have been identified (Klimyuk and Jones, 1997; Doutriaux *et al.*, 1998). Unlike yeast *DMC1*, *AtDMC1* is expressed not only in reproductive tissues but also in leaves and cultured cell suspensions. Cytological observation of a T-DNA insertion mutant has shown that the *AtDmcl* protein is required for bivalent formation and proper chromosome segregation during meiosis (Couteau *et al.*, 1999). The completion of the *Arabidopsis* genome sequence has revealed genes related to the five Rad51 paralogs: AT2G28560 (*RAD51B*), AT2G45280 (*RAD51C*), AT1G07745 (*RAD51D*), AT5G64520 (*XRCC2*) and AT5G57450 (*XRCC3*). Predicted proteins have 14.2–26.7% sequence identity to *AtRad51* and to each other, and 20.1–36.4% sequence identity to their human counterpart (EMBOSS Stretcher, default parameters). *AtXRCC3* and *AtRAD51C* cDNAs have been recently cloned and sequenced, and two-hybrid analyses have confirmed that *AtXrcc3* interacts with *AtRad51* and *AtRad51C* (Osakabe *et al.*, 2002). Furthermore, γ -irradiation has shown that transcription of *AtXRCC3* and *AtRAD51C* is induced in response to DNA damage (Osakabe *et al.*, 2002). These data strongly suggest that *AtXRCC3* and *AtRAD51C* are the functional homologues of *XRCC3* and *RAD51C*, but no further analyses have thus far investigated the roles of these Rad51 paralogs in plants.

To understand the role of *AtXRCC3*, most probably a key factor in homologous recombination and recombinational repair, we have characterized a T-DNA insertion line disrupting the *AtXRCC3* coding sequence. Here we demonstrate that *atxrc3* mutant cells and plants are hypersensitive to DNA crosslinking agents and less so to DSB-inducing agents. This hypersensitivity is consistent with the defects previously reported for vertebrate cells mutant for Xrcc3. Furthermore, we show that *atxrc3* mutant plants undergo aberrant meiosis with extensive chromosome fragmentation seen in postpachytene stages, leading to gametophytic lethality. This finding gives a new insight

concerning the meiotic role of Xrcc3 and probably the other Rad51 paralogs.

Results

Identification and molecular characterization of an *AtXRCC3* T-DNA insertion mutant

To investigate the *AtXRCC3* gene function in plants, we searched for mutants in public T-DNA insertion line collections. A single line, Salk_045564, carrying an insertion at the 3' end of the *AtXRCC3* coding sequence was found in the SIGnAL T-DNA express database and the corresponding allele was named *atxrc3*. The *AtXRCC3*/T-DNA junctions were amplified and PCR products were sequenced to determine the exact position and some structural features of the insertion (Figure 1A and B). The T-DNA insertion disrupts the *AtXRCC3* coding sequence at position +704. The *AtXRCC3* T-DNA insertion is surrounded by two incomplete left borders designated as LB1 and LB2. These two left borders have opposite orientations, which indicate the presence of at least two T-DNAs inserted at the *AtXRCC3* locus. LB1 has a 7 bp deletion and an insertion of an adenine just upstream the T-DNA, generating a TAA STOP codon in-frame with the *AtXRCC3* ORF. LB2 has a 22 bp deletion and a deleted guanidine two bases after LB2. Therefore, a truncated *AtXrcc3* protein, missing the last 70 amino acids, might possibly be produced from the *atxrc3* allele. Primers upstream to the T-DNA insertion were used in semiquantitative RT-PCR amplification to check whether a transcript coding for a truncated *AtXrcc3* protein could be detected in *atxrc3* mutant cells (Figure 1C). We were not able to detect any *AtXRCC3* transcript in *atxrc3* samples. Thus, *AtXRCC3* T-DNA insertion might destabilize or produce an aberrant *atxrc3* transcript, suggesting that *atxrc3* is a null allele.

atxrc3 plants are sterile

A sterility phenotype was observed in the progeny of self-fertilized heterozygous *atxrc3*^{+/-} plants. Over 36 plants screened, eight (22.3%) were sterile and 28 (87.7%) were fertile, which correspond to the 3:1 segregation expected for a single Mendelian locus (X^2 , 1 d.f. = 0.148). Using a PCR genotyping assay, we were able to follow the transmission of the *atxrc3* allele in these plants. Eight of the 36 plants (22.3%) were homozygous for the *atxrc3* T-DNA insertion, 19 of 36 (52.7%) were heterozygous and nine of 36 (25%) were wild type. This analysis of individual plants confirmed that sterile plants were exclusively those homozygous for the *atxrc3* allele. Compared with wild type, *atxrc3* plants produce atrophied siliques (Figure 2B and C) and almost all these siliques are devoid of any seed. *atxrc3* mutants produce an average of 2.25 seeds per plant ($n = 8$), compared to approximately 4500 seeds per fertile plant ($n = 4$), and this represents a residual fertility of 0.05%. Heterozygous plant fertility was similar to wild-type plants, and all plants, regardless of their *AtXRCC3* genotype, had normal vegetative development (Figure 2A and B). Thus, *AtXrcc3* is absolutely required for fertility, but not for vegetative development.

atxrc3 plants are defective in both male and female gametophytic development

To determine the origin of the sterility phenotype observed in *atxrc3* plants, we first examined male and female

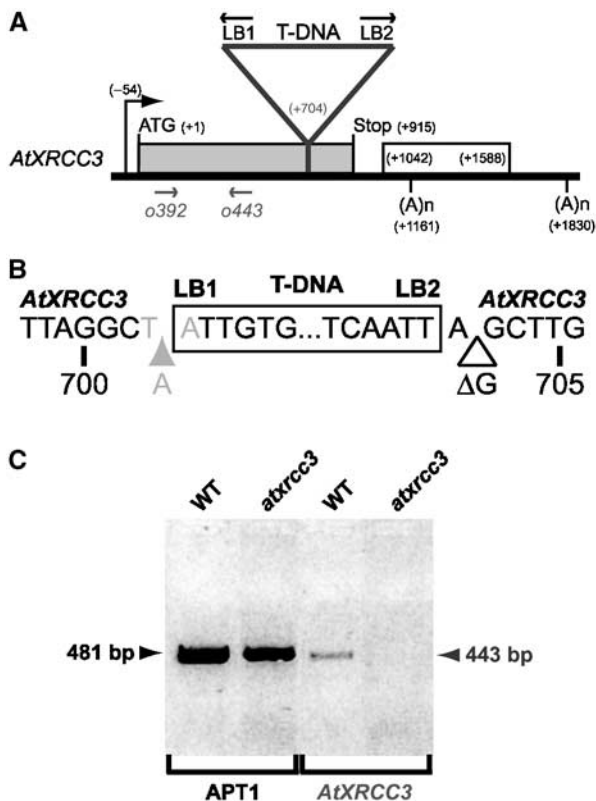


Figure 1 Molecular characterization of *atxrcc3* T-DNA insertion. (A) Diagram showing the position of the T-DNA insertion at the *AtXRCC3* locus. The black arrow indicates the *AtXRCC3* transcriptional start site, the grey box represents the *AtXRCC3* coding sequence and the white box the intron spliced in *AtXRCC3 alpha* mRNA. The triangle represents the T-DNA inserted in position +704 of the *AtXRCC3* coding sequence. (B) Sequence of the *atxrcc3* insertion site. LB1 and LB2 indicate the two left borders surrounding the T-DNA insertion, and their orientations are indicated in (A). The grey and white triangles represent base insertion and deletion, respectively. The in-frame TAA STOP codon resulting from LB1 integration is indicated in grey. The numbers represent positions relative to the start codon. (C) RT-PCR detection of *AtXRCC3* transcript. WT and *atxrcc3* indicate RT-PCR amplifications performed on wild-type and *atxrcc3* total mRNAs extracted from cell suspensions. *AtXRCC3* is the product amplified using primers o392 and o443 represented in (A). Amplification of the APT1 mRNA has been used as a control for reverse transcription.

gametophytic development. To assess pollen grain viability, anthers were dissected from wild-type and mutant flower buds (Figure 3A and B) and stained as described by Alexander (1969). None of the 60 observed *atxrcc3* anthers contained any mature pollen grains, suggesting that male gametophytic development is arrested after meiosis. Heterozygous plants' anthers could not be differentiated from those of wild-type plants in terms of viability and number of pollen grains produced in a single anther. Male gametogenesis is thus strongly affected in the *atxrcc3* mutant.

To check whether female gametogenesis is also affected, we monitored embryo sac development in wild-type and *atxrcc3* ovules ($n = 200$). In wild-type ovules, the megaspore mother cell (Figure 3C) undergoes meiosis and three of the four products degenerate to preserve a single functional megaspore (Figure 3D). This megaspore then undergoes

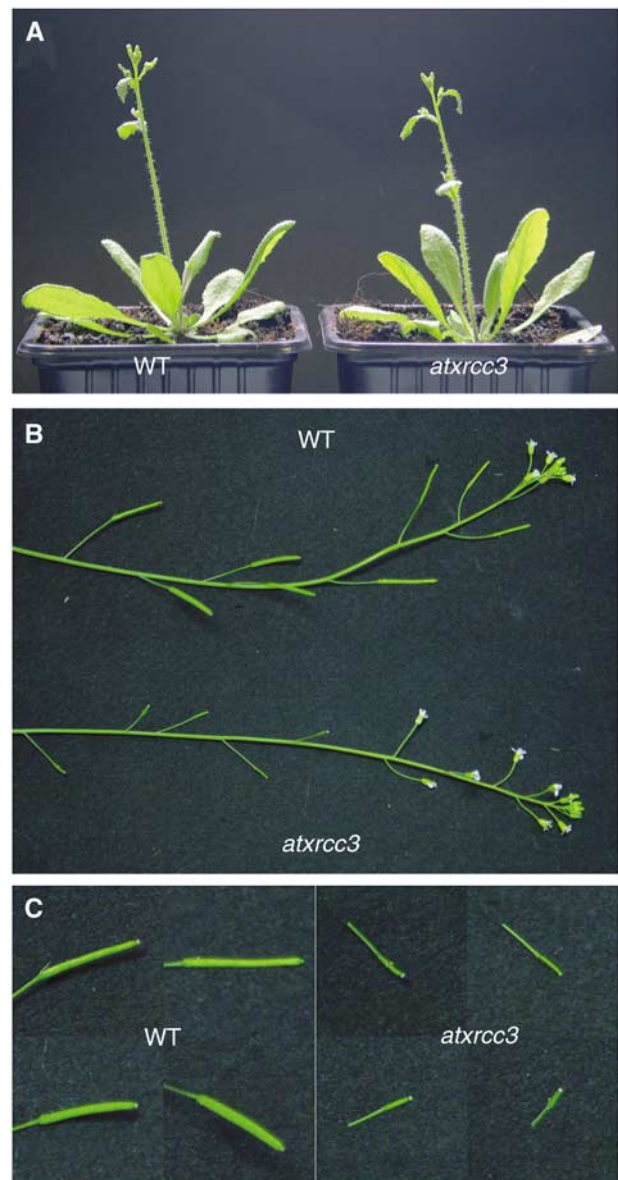


Figure 2 *atxrcc3* mutant plants are sterile. (A) Wild-type (left) and *atxrcc3* (right) 3-week-old plants. Flowering stems (B) and siliques (C) of the wild-type and *atxrcc3* plants.

three rounds of nuclear division to produce the eight nuclei embryo sac, which is the mature female gametophyte. Intermediate stages from the functional megaspore to the eight nuclei embryo sac can be observed in wild-type plants, while the simultaneous organization of integuments and growth around the megaspore can be used to determine the stage of megagametogenesis (Figure 3C-F). Observation of *atxrcc3* mutants revealed that embryo sac development is disrupted after meiosis. The megaspore mother cell could not be differentiated from the wild type (Figure 3G), but the majority of *atxrcc3* ovules do not preserve a functional megaspore, with only a degenerative cell being visible and persisting during embryo sac development (Figure 3H and I). In some cases one of the meiotic products survived, but precise staging of the ovules with growth of integuments indicated that these products did not undergo the nuclear divisions (Figure 3J). Thus, the *atxrcc3* mutation disrupts

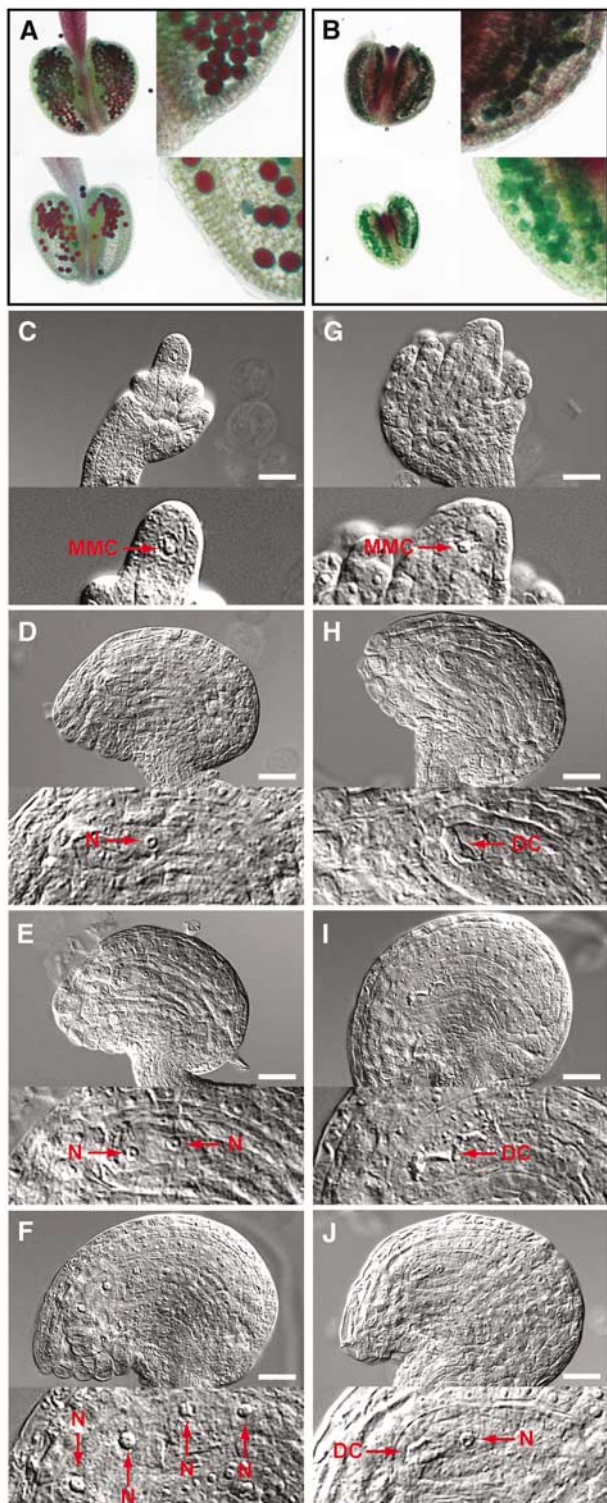


Figure 3 Gametophytic lethality in *atxrcc3* mutant plants. Anthers of wild-type (A) and *atxrcc3* (B) plants have been stained according to Alexander (1969). Anthers from 2 mm buds (up) and mature flower (down) have been compared. The right of each panel presents enlargement of some pollen grains. The red-purple-stained cytoplasm indicates viability, while the pollen cell wall is counterstained in green. (C–J) Differential interference contrast (DIC) microscopy observations of embryo sac development in wild-type (C–F) and *atxrcc3* (G–J) ovules, after clearing. The bottom of each panel presents an enlargement of the nuclei. MMC = megaspore mother cell, N = nucleus, DC = degenerative cell, bars = 20 μ m.

both male and female gametophytic development, leading to a severe sterility phenotype.

Meiosis is severely disrupted in *atxrcc3* plants

To further characterize the *atxrcc3* sterility phenotype, we followed meiosis in pollen mother cells (PMCs). Meiotic progression in wild-type (Figure 4) and *atxrcc3* (Figure 5) PMCs was examined by fluorescence microscopy after 4',6-diamidino-2-phenylindole (DAPI) staining of chromosomes. The Arabidopsis genome consists of five pairs of chromosomes ($2n = 10$) that can be observed as bivalents by the end of prophase I to metaphase I (Figure 4C–E). During meiotic prophase I, individual chromosomes become visible at leptotene, while the beginning of pairing is observable at zygotene (Figure 4A). The pachytene stage is characterized by fully synapsed homologous chromosomes (Figure 4B) and indicates the end of meiotic recombination events initiated in leptotene. Then, chromosomes gradually condense and separate, remaining linked by chiasmata at diplotene and diakinesis (Figure 4C and D).

In *atxrcc3* PMCs, prophase I seems to occur normally up to pachytene, as fully synapsed chromosomes can be observed (Figure 5A and B). To eliminate the possibility that the apparent synapsis results from nonhomologous interactions, fluorescent *in situ* hybridization (FISH) was performed on *atxrcc3* pachytene chromosomes (Figure 6). Hybridization with a 5S rDNA probe yielded the expected three signals for normally paired homologous chromosomes, corresponding to the three major 5S rDNA loci in the Columbia ecotype (Murata *et al*, 1997; Fransz *et al*, 1998). The absence of univalents at metaphase I finally confirmed that homologous chromosome pairing and synapsis take place normally in *atxrcc3* mutants (Figure 5E). At diplotene, the presence of chromosome fragments (red arrows) in addition to bivalents (yellow arrows) (Figure 5C) underlines the severe meiotic deficiency. At diakinesis, bivalents are difficult to differentiate from chromosome fragments (Figure 5D).

At metaphase I in the wild type, chromosomes reach their maximum condensation state, and can be seen as five bivalents orientated on the spindle (Figure 4E). Homologous chromosomes separate from each other and migrate to the opposite poles of the cell in anaphase I (Figure 4F). The first meiotic division ends with partial decondensation of chromosomes at telophase I (Figure 4G). In *atxrcc3* PMCs, in addition to the five bivalents, a variable number of chromosome fragments are seen (Figure 5E). Chromosome fragmentation continues during anaphase I, and bridges between separating chromosomes are frequently observed (Figure 5F). First division finally produces two pools of 'chromosomes' containing more than the five chromosomes expected for Arabidopsis (Figure 5G), presumably due to random assortment of acentric chromosome fragments at anaphase I.

The second meiotic division starts with rapid condensation and alignment of chromosomes (metaphase II, Figure 4H). In anaphase II, sister chromatids separate and migrate to the opposite poles of the cell (Figure 4I), resulting in four groups of five chromosomes. Telophase II ensues, chromosomes decondense (Figure 4J) and cytoplasm is partitioned to produce a tetrad containing four haploid microspores (Figure 4K and L). In *atxrcc3* metaphase II, most visible fragments are aligned on the spindle, with some fragments

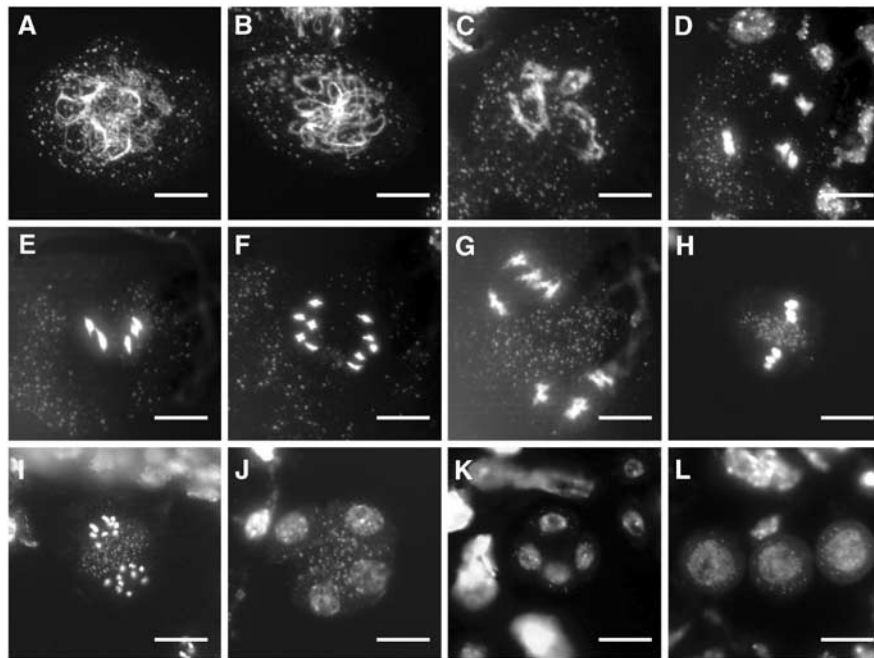


Figure 4 Meiosis in wild-type *Arabidopsis*: (A) zygotene, (B) pachytene, (C) diplotene, (D) diakinesis, (E) metaphase I, (F) anaphase I, (G) telophase I, (H) metaphase II, (I) anaphase II, (J) telophase II, (K) tetrad and (L) microspores. Bars = 10 μ m.

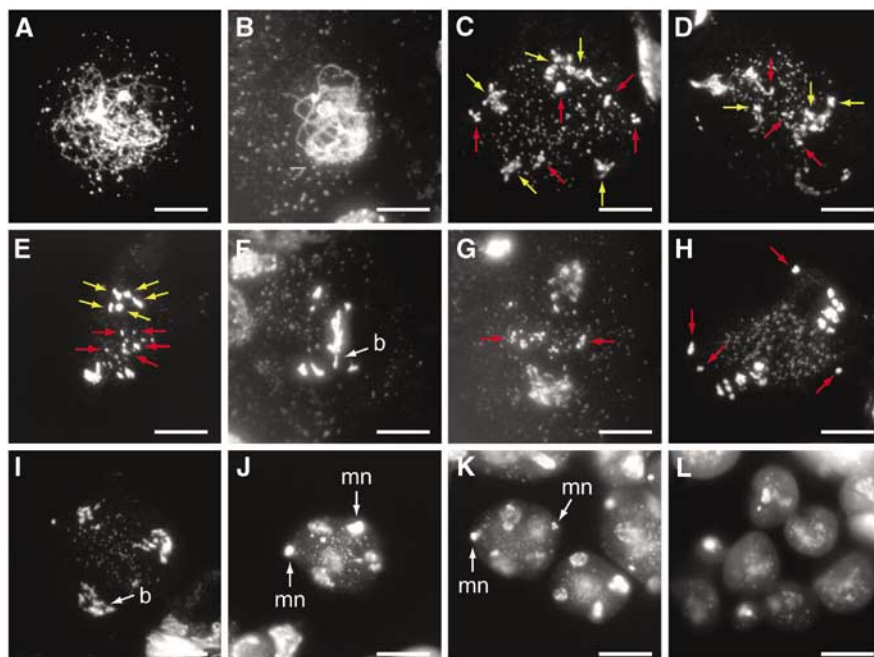


Figure 5 Meiosis is severely disrupted in *atxrcc3* mutant plants: (A) zygotene, (B) pachytene, (C) diplotene, (D) diakinesis, (E) metaphase I, (F) anaphase I, (G) telophase I, (H) metaphase II, (I) anaphase II, (J) telophase II, (K) polyads and (L) microspores. Yellow arrows = bivalents, red arrows = chromosome fragments, b = bridge, mn = micronucleus, bars = 10 μ m.

being isolated (Figure 5H). During anaphase II, four groups of 'chromosomes' are separated, with fragments being scattered throughout the cytoplasm (Figure 5I). At telophase II, more than the four normal nuclei are observed (Figure 5J) and polyads containing three or four major products, associated with several micronuclei, are generated (Figure 5K). These meiotic products finally give rise to microspores with varying size and DNA content (Figure 5L). This cytological analysis of

meiosis in *atxrcc3* PMCs demonstrates that AtXrcc3 is required to ensure chromosome integrity during meiosis.

***atxrcc3* cells and plants are hypersensitive to mitomycin C and less so to bleomycin**

Previous studies with CHO (Chinese hamster ovary) and DT40 (chicken B-lymphocyte) mutant cells have shown that Xrcc3 is required for efficient repair of DNA damage and

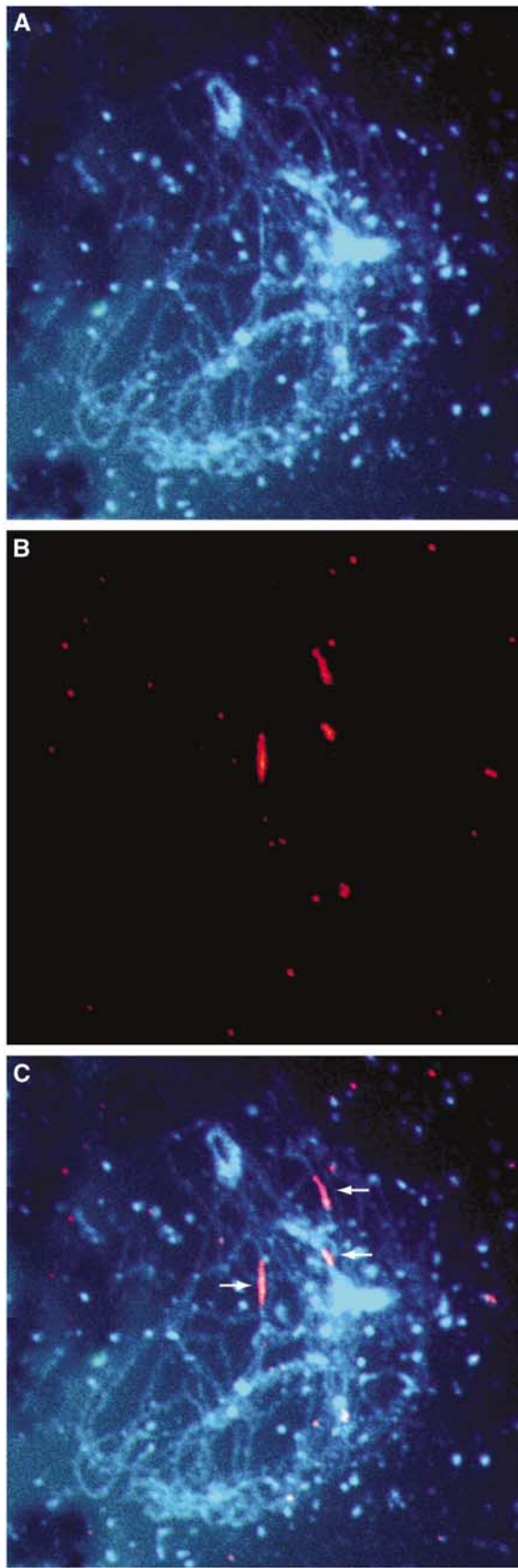


Figure 6 Pachytene chromosomes of *atxrcc3* hybridized with 5S rDNA (red) and DAPI counterstained: (A) DAPI, (B) 5S rDNA signals and (C) merged images. Arrows = 5S rDNA signals.

especially DNA crosslinks (Tebbs *et al.*, 1995; Liu *et al.*, 1998; Cui *et al.*, 1999; Pierce *et al.*, 1999; Brenneman *et al.*, 2000; Takata *et al.*, 2000, 2001). To investigate the role of AtXrcc3 in DNA damage repair, we first determined whether the *atxrcc3* mutation confers hypersensitivity to mitomycin C (MMC), a DNA crosslinking agent (Warren *et al.*, 1998). Cultured cells derived from wild-type and *atxrcc3* plants were transferred to plates containing from 0 to 40 μM of MMC and growth was scored visually after 3 weeks. As shown in Figure 7A, *atxrcc3* cells are highly sensitive to MMC, as a dose of 5 μM is sufficient to reduce callus growth. In all, 10 μM MMC severely affected the growth of mutant cells, while wild-type cells grow normally at this MMC concentration. Doses of 20 μM MMC and above strongly reduce the growth of both mutant and wild-type cells. Thus, AtXrcc3 is involved in the repair of DNA crosslinks.

To confirm the MMC hypersensitivity phenotype observed with *atxrcc3* cells, seeds from wild-type and self-fertilized heterozygous *atxrcc3*^{+/-} plants were sown on plates containing germination medium and increasing doses of MMC. After 14 days the plants were scored for MMC hypersensitivity. Without treatment, most of the plants developed at least four true leaves (excluding the cotyledons); thus, plants with three true leaves or less, in the presence of MMC, were considered as hypersensitive. A dose-response curve was produced for the percentage of plants with three leaves or less at 14 days (Figure 7B), showing a clear MMC hypersensitivity of the *atxrcc3* plants. In the wild type, the percentage of hypersensitive plants rises from 5.8% at 10 μM MMC to 11.9% at 40 μM MMC, with most of them having two or three leaves. The MMC hypersensitivity of *atxrcc3* plants was assayed on the progeny of an *atxrcc3*^{+/-} plant (*atxrcc3* mutants are sterile), and the *atxrcc3* mutant plants thus represent only one quarter of the plants sown. In the presence of MMC, the percentage of hypersensitive plants in the progeny of an *atxrcc3*^{+/-} plant rises from 10.4% at 10 μM MMC to 29% at 40 μM MMC, with half of them having 0 or 1 leaves. That these hypersensitives correspond to the *atxrcc3* mutants was verified by PCR genotyping in one experiment: of 103 plants scored, 28 (27.2%) were hypersensitive to MMC and 26 of these were *atxrcc3* mutant plants. These results confirm that AtXrcc3 is involved in the repair of DNA crosslinks and show that AtXrcc3 is required for plant development in the presence of such lesions.

To further investigate the role of AtXrcc3 in DNA damage repair and especially in DSB repair, hypersensitivity to bleomycin was scored on cell cultures and plants. Bleomycin is a γ -ray mimetic agent known to induce DNA DSBs (Favaudon, 1982). Cells and plants were grown on plates containing from 0 to 5 $\mu\text{g}/\text{ml}$ of bleomycin. As shown in Figure 7C, a dose of 0.2 $\mu\text{g}/\text{ml}$ bleomycin does not affect callus growth in either wild-type or mutant cell cultures. For doses of 0.5 $\mu\text{g}/\text{ml}$ bleomycin and above, the growth of *atxrcc3* cells was significantly more affected than wild type. The highest dose of 5.0 $\mu\text{g}/\text{ml}$ bleomycin was fully lethal for *atxrcc3* cells and considerably affects wild-type callus growth. However, in contrast to the MMC data, no significant difference was found between wild-type and *atxrcc3* plants germinated on medium containing the same range of doses of bleomycin (data not shown). We have also observed the sensitivity of *atxrcc3* cells for doses of 100 ppm methylmethane sulphonate (MMS) and above, while the sensitivity of the wild type is

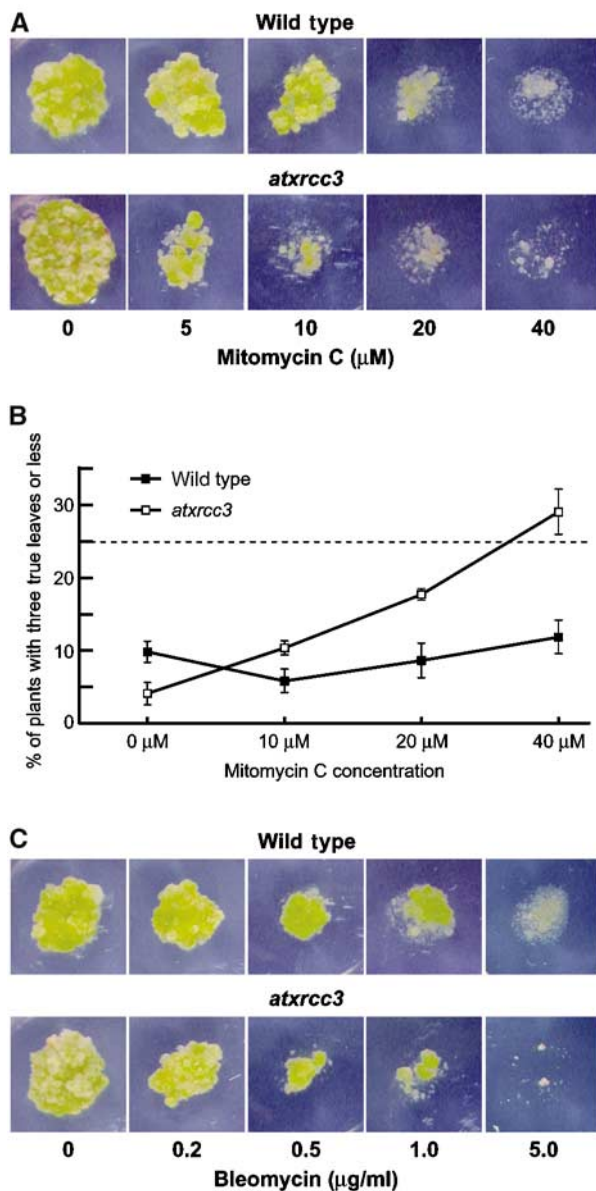


Figure 7 *atxrcc3* cells and plants are hypersensitive to DNA cross-linking agents, but not to DSB-inducing agents. (A, C) Five-day-old wild-type and *atxrcc3* cell cultures were transferred and grown on plates containing increasing doses of mitomycin C (A) or bleomycin (C). After 3 weeks, callus growth was used to score sensitivity to DNA-damaging agent. (B) Seeds of wild-type and *atxrcc3*^{+/-} heterozygote plants were sown on plates containing increasing doses of mitomycin C. After 14 days, the percentage of hypersensitive plants (plants with three leaves or less) was used to produce a mitomycin C dose-response curve. Values represent three replicates, each replicate containing an average of 100 plants per dose. Error bars = standard deviation.

only seen at 166 ppm MMS and above (data not shown). These findings suggest that, like its vertebrate counterpart, AtXrcc3 is involved in repair of DNA DSBs, but is not required for plant survival in the presence of DSB-inducing agent.

Discussion

The sterility of *atxrcc3* mutant is due to a meiotic defect

The most visible defect of the *atxrcc3* mutant is the almost complete absence of seed following flowering (Figure 2).

Cytological observations of male and female gametogenesis show a very high lethality of gametophytes (Figure 3), which is consistent with the strong sterility phenotype. Although we did not observe any mature gametophytes, the presence of arrested female gametophytes (Figure 3J) suggests that a few viable gametophytes can be produced, thus explaining the very low residual fertility of mutant plants.

Arabidopsis mutants defective for genes coding proteins involved in recombination (*AtDMC1* and *AtSPO11-1*) and DNA damage repair (*AtATM* and *MEI1*) during meiosis have reduced gametophytic viability and fertility (Couteau *et al.*, 1999; Grelon *et al.*, 2001, 2003; Garcia *et al.*, 2003). In these mutants, the gametophytic lethality has been correlated with a strong meiotic defect, without meiotic arrest. We show here that the *atxrcc3* mutation leads to aberrant meiosis, with extensive chromosome fragmentation (Figure 5). The production of such (acentric) chromosome fragments would lead to aneuploid gametes and so explain the high gametophytic lethality.

AtXrcc3 is not required for synapsis

Rad51 paralogs play significant roles in homologous recombination and recombinational repair (reviewed by Thompson and Schild, 2001), but the embryonic lethality of mice defective in the Rad51 paralogs has precluded the investigation of their role in meiotic recombination and meiosis in a more general way. Our results demonstrate the requirement for AtXrcc3 to ensure the integrity of the genetic material during meiosis in Arabidopsis, suggesting a role for the AtXrcc3 protein in meiotic recombination. Bishop *et al.* (1998) have shown that Xrcc3 is required for assembly of damage-induced Rad51 foci in CHO cells. Rad51 foci are also formed along meiotic chromosomes, presumably to promote recombinational repair of Spo11-induced DSBs (Barlow *et al.*, 1997); however, the inviability of mouse *xrcc3* mutants has precluded determining whether Xrcc3 plays a role in this. Thus, AtXrcc3 could be required for early steps of meiotic recombination. Previous studies have reported the meiotic phenotypes of Arabidopsis mutants defective for *DMC1* and *SPO11* homologues, two proteins involved in early steps of meiotic recombination (Couteau *et al.*, 1999; Grelon *et al.*, 2001). AtSpo11-1 is presumably required to introduce DSBs along meiotic chromosomes, while AtDmc1 might promote strand exchange between homologous chromosomes (Couteau *et al.*, 1999; Grelon *et al.*, 2001). These two mutants are both deficient for meiotic homologous chromosome synapsis, attested to by the 10 univalents, rather than five bivalents, observed at metaphase I (Couteau *et al.*, 1999; Grelon *et al.*, 2001). These results are consistent with the phenotypes reported for yeast and mice defective for either *SPO11* or *DMC1* (Giroux *et al.*, 1989; Bishop *et al.*, 1992; Rockmill *et al.*, 1995; Bergerat *et al.*, 1997; Keeney *et al.*, 1997; Schwacha and Kleckner, 1997; Pittman *et al.*, 1998; Yoshida *et al.*, 1998; Romanienko and Camerini-Otero, 2000). The *atxrcc3* mutant phenotype differs markedly from those observed for *atdmc1* and *atspo11-1*. The presence of normal pachytene figures (Figures 5B and 6A) and the observation of bivalents at metaphase I (Figure 5E) show that synapsis takes place normally in *atxrcc3* mutants and was confirmed by FISH analysis of the 5S rDNA loci (Figure 6). AtXrcc3 is thus not required for the establishment of inter-homologue synapsis and presumably not for early steps of meiotic recombination.

AtXrcc3 is essential for postsynaptic events in meiosis

Diplotene and later stages of *atxrc3* meiosis show the presence of chromosome fragments (Figure 5), while *atdmc1* and *atspo11-1* do not accumulate chromosomal breaks (Couteau *et al*, 1999; Grelon *et al*, 2001). The chromosome fragmentation observed in the *atxrc3* mutant is more similar to the meiotic phenotypes reported for Arabidopsis *atam* and *mei1* mutants (Garcia *et al*, 2003; Grelon *et al*, 2003). These two proteins are involved in DNA damage repair, AtAtm being required for the repair of DSBs (Garcia *et al*, 2003) and Meil presumably for premeiotic repair of damage associated with replication (Grelon *et al*, 2003). As we show here for *atxrc3*, *atam* and *mei1* mutations lead to extensive chromosome fragmentation associated with bridges in both anaphase I and II (Garcia *et al*, 2003; Grelon *et al*, 2003). Such phenotypes are also seen in the Arabidopsis *dif1/syn1* cohesin mutant (Bai *et al*, 1999; Bhatt *et al*, 1999; Peirson *et al*, 1997) and an *atrad50* mutant (J-Y Bleuyard and C White unpublished). As reported for *mei1* (Ross *et al*, 1997; Grelon *et al*, 2003) and in contrast to *atam* (Garcia *et al*, 2003), *atxrc3* chromosome fragmentation is observed prior to anaphase I, as early as the diplotene stage (Figure 5C). The chromosome fragmentation seen in *atxrc3* thus cannot be solely explained by the breakage of anaphase bridges (as for *atam*), but must derive from the nonrepair of DNA breaks. Liu *et al* (2003) have recently shown that *in vitro*, Rad51C and Xrcc3 are involved in the resolution of Holliday junctions, supporting a late role in recombination for Rad51 paralogs. A lack of resolvase activity in *atxrc3* mutants would explain the presence of bridges at anaphase I and II, as well as the early fragmentation of chromosomes, when synapsed homologues start to separate at diplotene. In the case of *mei1*, Grelon *et al* (2003) showed that the chromosome fragmentation phenotype is not *Atspo11* dependent and thus the DNA breaks presumably are of premeiotic origin. We are performing the cross between *atxrc3*^{+/-} and *atspo11-1*^{+/-} heterozygous plants to produce and characterize an *atxrc3/atspo11-1* double mutant.

AtXrcc3 is also important for somatic DNA repair

The fragmentation of meiotic chromosome in *atxrc3* mutant thus derives from the breakage of anaphase bridges and the nonrepair of (previously existing?) DNA breaks. Vertebrate Rad51 paralogs are required for efficient DNA damage repair in somatic cells (reviewed by Thompson and Schild, 2001). The hypersensitivity of *atxrc3* cells and/or plants to MMC and bleomycin clearly demonstrates that AtXrcc3 is involved in the repair of DNA crosslinks and DSBs in Arabidopsis (Figure 7), supporting the hypothesis that AtXrcc3 is the Arabidopsis functional homologue of vertebrate Xrcc3.

In conclusion, Arabidopsis AtXrcc3 plays essential roles in DNA damage repair in both somatic and meiotic cells. As is the case with *Drosophila* and in contrast to the situation in mice, AtXrcc3 is not required for normal development in Arabidopsis. Further studies will be necessary to understand fully at a mechanistic level the roles of AtXrcc3 in Arabidopsis, in particular in meiotic and somatic recombination and recombinational repair.

Materials and methods

Plant material, growth conditions and mutant screening

A. thaliana seeds (Columbia ecotype) were sown directly into damp compost, and plants were grown in a greenhouse under standard conditions.

The *atxrc3* T-DNA insertion line (Salk_045564) was found in the public searchable database established by the Salk Institute Genomic Analysis Laboratory (Alonso *et al*, 2003). The T-DNA Express database is accessible from the SIGnAL website at <http://signal.salk.edu>.

As the kanamycin resistance gene present in *atxrc3* T-DNA insertion is silenced, plants heterozygous and homozygous for the *atxrc3* mutation were identified by a PCR genotyping assay. For each individual plant, the following primer combinations were used to amplify the wild-type *ATXRCC3* locus, o438 (5'-ATGCCAAAATGGGAAAATTAAGCCG-3') and o439 (5'-CTACGCTTGAACCGCACAAA TC-3'), and the mutant locus, o447 (5'-GGATTTGGTTGAACTTCTGATGG-3') and o405 (5'-TGTTTCACGTAGTGGGCCATCG-3'). Mutant identification was confirmed based on their sterility phenotype.

Sequencing of T-DNA insertion sites

The following primer combinations were used to amplify DNA flanking the T-DNA: at the LB1 left border, o392 (5'-CGAATCGTAAACTAACACAGGC-3') and o406 (5'-GCGTGGACCGCTTGCTGCAACT-3'), and at the LB2 left border, o439 (5'-CTACGCTTGAACCGCACAAAATCG-3') and o405 (5'-TGTTTCACGTAGTGGGCCATCG-3').

The PCR products were then purified on a QIAquick column (Qiagen) and directly sequenced. Sequence reactions were performed using one of the primers used for amplification and the CEQ DTCS Quick Start Kit (Beckman Coulter), and analysed on a CEQ 2000 DNA Analysis System (Beckman Coulter).

Semiquantitative RT-PCR

For semiquantitative RT-PCR, total RNAs extracted from 7-day-old wild-type and *atxrc3* cell suspensions were treated with RNase-free DNase I (Roche). In all, 1 µg of DNA-free total RNA was reverse transcribed in 20 µl of reaction mixture containing 50 U of Expand Reverse Transcriptase (Roche), 1 × random hexanucleotide mix (Roche), 1 mM of each deoxyribonucleotide triphosphate and 20 U of RNasin ribonuclease inhibitor (Promega). PCR was carried out in 25 µl of reaction mixture containing 2 µl of RT reaction mixture, 1 U of HotStarTaq DNA polymerase (Qiagen), 2.5 mM MgCl₂, 100 µM of each deoxyribonucleotide triphosphate and 0.4 µM of gene-specific primers. The gene-specific primers were o392 (5'-CGAATCGTAAACTAACACAGGC-3') and o443 (5'-CAAATCCGATCTAAACAATGC-3') for *ATXRCC3*, and apt1 (5'-TCCCAGAATCGCTAAGATTGC-3') and apt2 (5'-CCTTCCCTTAAGCTCTG-3') for *APT1* (adenine phosphoribosyl transferase) (Moffatt *et al*, 1994). The initial denaturation was carried out at 94°C for 15 min, and then amplification was performed for 35 cycles with a denaturation time of 30 s at 94°C, followed by annealing for 30 s at 52°C and extension for 1 min at 72°C.

Light and fluorescence microscopy

Mature pollen grain viability was assayed according to Alexander (1969). Anthers from fixed flowers were isolated, stained and observed using a Leica MZFLIII stereomicroscope, and photographs were taken using a JVC digital camera.

Embryo sac development was monitored according to Motamayor *et al* (2000). Full inflorescences were collected, fixed and cleared in lactic acid/phenol. Ovules from different sized pistils were then dissected on a slide in a drop of the lactic acid/phenol solution and mounted in a drop of the same mix. Slides were observed with differential interference contrast (DIC) using a Zeiss Axioplan 2 Imaging microscope.

DAPI staining of meiotic chromosomes was performed as originally described by Maluszynska and Heslop-Harrison (1991), and modified as follows. Whole inflorescences were collected and fixed in fresh ethanol:acetic acid (3:1) for 1 h on ice. Fixative solution was renewed several times, until it remained clear. Fixed inflorescences were washed for 5 min at room temperature in 10 mM citrate buffer (pH 4.8) and digested in citrate buffer with 0.2% (w/v) cellulase (Sigma #C-1794) and 2% (v/v) pectinase

(Sigma 1#P-4716) for 30 min at 37°C, in a moist chamber. Enzymes solution was carefully removed and inflorescences were washed for 5 min and then kept in citrate buffer. Buds of 0.1–0.5 mm were selected and anthers were dissected on a slide, in 10 µl of 45% acetic acid. Anthers were slightly squashed under a coverslip by gently tapping them with the base of a needle (release of the meiocytes was monitored under a stereomicroscope). Slides were frozen for 30 s in liquid nitrogen, and the coverslip was quickly removed with a razor blade. The slides were air dried and mounted in VECTASHIELD mounting medium with 1.5 µg/ml DAPI. UV fluorescence microscopy observations were performed using a Zeiss Axioplan 2 Imaging microscope.

Fluorescent in situ hybridization

FISH experiments were performed according to Schubert *et al* (2001). 5S rDNA was amplified with primers 5Suniv1 (5'-CTTTTCGGGCNTTTTNGTG-3') and 5Suniv2 (5'-CGAAAAGG TATCAGATGCC-3'), and labelled using the DIG-Nick Translation Mix (Roche). Mouse antidigoxigenin (1:250, Roche) followed by rabbit anti-mouse conjugated with fluorescein isothiocyanate (FITC) (1:1000, Sigma) and goat anti-rabbit conjugated with Alexa 488 (1:200, Molecular Probes) antibodies were used for the detection of the digoxigenin-labelled probe.

MMC, bleomycin and MMS sensitivity tests

Callus cultures were derived from leaves and maintained in SIM medium as described previously by Gallego and White (2001). A

droplet of a 5-day-old liquid culture was transferred onto the surface of agar plates containing fresh solid SIM medium and different concentrations of bleomycin (Sigma #B-5507), MMC (Sigma #M-0503) or MMS (Sigma #M-4016). The plates were then incubated (23°C, 16 h light), and resistance or sensitivity was scored visually 3 weeks later.

Seeds were surface-sterilized with a 7% calcium hypochlorite solution (w/v). After sterilization, seeds were sown on plates containing fresh solid germination medium with different concentrations of bleomycin (Sigma #B-5507) or MMC (Sigma #M-0503). The plates were then incubated for 14 days (23°C, 16 h light), and resistance or sensitivity was scored by the number of true leaves (excluding the cotyledons) per plant.

Images processing

All images were further processed with Adobe Photoshop 6.0 to enhance their quality.

Acknowledgements

We thank members of BIOMOVE for their help and discussions. Olivier Mathieu is thanked for his help with Arabidopsis cytology and FISH. We thank the Salk Institute Genomic Analysis Laboratory for providing the sequence-indexed Arabidopsis TDNA insertion mutants. This work was partly financed by a European Union research grant (QLG2-CT-2001-01397).

References

- Abdu U, Gonzalez-Reyes A, Ghabrial A, Schupbach T (2003) The *Drosophila* spn-D gene encodes a RAD51C-like protein that is required exclusively during meiosis. *Genetics* **165**: 197–204
- Aboussekhra A, Chanet R, Adjiri A, Fabre F (1992) Semidominant suppressors of Srs2 helicase mutations of *Saccharomyces cerevisiae* map in the RAD51 gene, whose sequence predicts a protein with similarities to prokaryotic RecA proteins. *Mol Cell Biol* **12**: 3224–3234
- Alexander MP (1969) Differential staining of aborted and non-aborted pollen. *Stain Technol* **44**: 117–122
- Alonso JM, Stepanova AN, Leisse TJ, Kim CJ, Chen H, Shinn P, Stevenson DK, Zimmerman J, Barajas P, Cheuk R, Gadrinab C, Heller C, Jeske A, Koesema E, Meyers CC, Parker H, Prednis L, Ansari Y, Choy N, Deen H, Geralt N, Hazari N, Hom E, Karnes M, Mulholland C, Ndubaku R, Schmidt I, Guzman P, Aguilar-Henonin L, Schmid M, Weigel D, Carter DE, Marchand T, Risseuw E, Brogden D, Zeko A, Crosby WL, Berry CC, Ecker JR (2003) Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science* **301**: 653–657
- Bai X, Peirson BN, Dong F, Xue C, Makaroff CA (1999) Isolation and characterization of SYN1, a RAD21-like gene essential for meiosis in *Arabidopsis*. *Plant Cell* **11**: 417–430
- Barlow AL, Benson FE, West SC, Hulten MA (1997) Distribution of the Rad51 recombinase in human and mouse spermatocytes. *EMBO J* **16**: 5207–5215
- Bergerat A, de Massy B, Gabelle D, Varoutas PC, Nicolas A, Forterre P (1997) An atypical topoisomerase II from Archaea with implications for meiotic recombination. *Nature* **386**: 414–417
- Bhatt AM, Lister C, Page T, Fransz P, Findlay K, Jones GH, Dickinson HG, Dean C (1999) The DIF1 gene of *Arabidopsis* is required for meiotic chromosome segregation and belongs to the REC8/RAD21 cohesin gene family. *Plant J* **19**: 463–472
- Bishop DK, Ear U, Bhattacharyya A, Calderone C, Beckett M, Weichselbaum RR, Shinohara A (1998) Xrcc3 is required for assembly of Rad51 complexes *in vivo*. *J Biol Chem* **273**: 21482–21488
- Bishop DK, Park D, Xu L, Kleckner N (1992) DMC1: a meiosis-specific yeast homolog of *E. coli* recA required for recombination, synaptonemal complex formation, and cell cycle progression. *Cell* **69**: 439–456
- Brenneman MA, Weiss AE, Nickoloff JA, Chen DJ (2000) XRCC3 is required for efficient repair of chromosome breaks by homologous recombination. *Mutat Res* **459**: 89–97
- Connelly JC, Leach DR (2002) Tethering on the brink: the evolutionarily conserved Mre11–Rad50 complex. *Trends Biochem Sci* **27**: 410–418
- Couteau F, Belzile F, Horlow C, Grandjean O, Vezon D, Doutriaux MP (1999) Random chromosome segregation without meiotic arrest in both male and female meiocytes of a *dmc1* mutant of *Arabidopsis*. *Plant Cell* **11**: 1623–1634
- Cui X, Brenneman M, Meyne J, Oshimura M, Goodwin EH, Chen DJ (1999) The XRCC2 and XRCC3 repair genes are required for chromosome stability in mammalian cells. *Mutat Res* **434**: 75–88
- Deans B, Griffin CS, Maconochie M, Thacker J (2000) Xrcc2 is required for genetic stability, embryonic neurogenesis and viability in mice. *EMBO J* **19**: 6675–6685
- Diener AC, Fink GR (1996) DLH1 is a functional *Candida albicans* homologue of the meiosis-specific gene DMC1. *Genetics* **143**: 769–776
- Doutriaux MP, Couteau F, Bergounioux C, White C (1998) Isolation and characterisation of the RAD51 and DMC1 homologs from *Arabidopsis thaliana*. *Mol Gen Genet* **257**: 283–291
- Favaudon V (1982) On the mechanism of reductive activation in the mode of action of some anticancer drugs. *Biochimie* **64**: 457–475
- Fransz P, Armstrong S, Alonso-Blanco C, Fischer TC, Torres-Ruiz RA, Jones G (1998) Cytogenetics for the model system *Arabidopsis thaliana*. *Plant J* **13**: 867–876
- French CA, Masson JY, Griffin CS, O'Regan P, West SC, Thacker J (2002) Role of mammalian RAD51L2 (RAD51C) in recombination and genetic stability. *J Biol Chem* **277**: 19322–19330
- Gallego ME, White CI (2001) RAD50 function is essential for telomere maintenance in *Arabidopsis*. *Proc Natl Acad Sci USA* **98**: 1711–1716
- Garcia V, Bruchet H, Camescasse D, Granier F, Bouchez D, Tissier A (2003) AtATM is essential for meiosis and the somatic response to DNA damage in plants. *Plant Cell* **15**: 119–132
- Ghabrial A, Ray RP, Schupbach T (1998) *okra* and *spindle-B* encode components of the RAD52 DNA repair pathway and affect meiosis and patterning in *Drosophila* oogenesis. *Genes Dev* **12**: 2711–2723
- Giroux CN, Dresser ME, Tiano HF (1989) Genetic control of chromosome synapsis in yeast meiosis. *Genome* **31**: 88–94
- Grelon M, Gendrot G, Vezon D, Pelletier G (2003) The Arabidopsis MEI1 gene encodes a protein with five BRCT domains that is involved in meiosis-specific DNA repair events independent of SPO11-induced DSBs. *Plant J* **35**: 465–475

- Grelon M, Vezon D, Gendrot G, Pelletier G (2001) AtSPO11-1 is necessary for efficient meiotic recombination in plants. *EMBO J* **20**: 589–600
- Hays SL, Firmenich AA, Berg P (1995) Complex formation in yeast double-strand break repair: participation of Rad51, Rad52, Rad55, and Rad57 proteins. *Proc Natl Acad Sci USA* **92**: 6925–6929
- Hong EL, Shinohara A, Bishop DK (2001) *Saccharomyces cerevisiae* Dmc1 protein promotes renaturation of single-strand DNA (ssDNA) and assimilation of ssDNA into homologous supercoiled duplex DNA. *J Biol Chem* **276**: 41906–41912
- Johnson RD, Symington LS (1995) Functional differences and interactions among the putative RecA homologs Rad51, Rad55, and Rad57. *Mol Cell Biol* **15**: 4843–4850
- Kans JA, Mortimer RK (1991) Nucleotide sequence of the RAD57 gene of *Saccharomyces cerevisiae*. *Gene* **105**: 139–140
- Keeney S (2001) Mechanism and control of meiotic recombination initiation. *Curr Top Dev Biol* **52**: 1–53
- Keeney S, Giroux CN, Kleckner N (1997) Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. *Cell* **88**: 375–384
- Klimyuk VI, Jones JD (1997) AtDMC1, the Arabidopsis homologue of the yeast DMC1 gene: characterization, transposon-induced allelic variation and meiosis-associated expression. *Plant J* **11**: 1–14
- Kurumizaka H, Ikawa S, Nakada M, Eda K, Kagawa W, Takata M, Takeda S, Yokoyama S, Shibata T (2001) Homologous-pairing activity of the human DNA-repair proteins Xrcc3.Rad51C. *Proc Natl Acad Sci USA* **98**: 5538–5543
- Kurumizaka H, Ikawa S, Nakada M, Enomoto R, Kagawa W, Kinebuchi T, Yamazoe M, Yokoyama S, Shibata T (2002) Homologous pairing and ring and filament structure formation activities of the human Xrcc2*Rad51D complex. *J Biol Chem* **277**: 14315–14320
- Liu N, Lamerdin JE, Tebbs RS, Schild D, Tucker JD, Shen MR, Brookman KW, Siciliano MJ, Walter CA, Fan W, Narayana LS, Zhou ZQ, Adamson AW, Sorensen KJ, Chen DJ, Jones NJ, Thompson LH (1998) XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. *Mol Cell* **1**: 783–793
- Liu N, Schild D, Thelen MP, Thompson LH (2002) Involvement of Rad51C in two distinct protein complexes of Rad51 paralogs in human cells. *Nucleic Acids Res* **30**: 1009–1015
- Liu Y, Masson JY, Shah R, O'Regan P, West SC (2003) Rad51C is required for Holliday junction processing in mammalian cells. *Science*, In Press
- Lovett ST (1994) Sequence of the RAD55 gene of *Saccharomyces cerevisiae*: similarity of RAD55 to prokaryotic RecA and other RecA-like proteins. *Gene* **142**: 103–106
- Maluszynska J, Heslop-Harrison JS (1991) Localization of tandemly repeated DNA sequences in *Arabidopsis thaliana*. *Plant J* **1**: 159–166
- Masson JY, Tarsounas MC, Stasiak AZ, Stasiak A, Shah R, McIlwraith MJ, Benson FE, West SC (2001) Identification and purification of two distinct complexes containing the five RAD51 paralogs. *Genes Dev* **15**: 3296–3307
- Masson JY, West SC (2001) The Rad51 and Dmc1 recombinases: a non-identical twin relationship. *Trends Biochem Sci* **26**: 131–136
- Mikosch TS, Sonnenberg AS, Van Griensven LJ (2001) Isolation, characterization, and expression patterns of a DMC1 homolog from the basidiomycete *Pleurotus ostreatus*. *Fungal Genet Biol* **33**: 59–66
- Miller KA, Yoshikawa DM, McConnell IR, Clark R, Schild D, Albala JS (2002) RAD51C interacts with RAD51B and is central to a larger protein complex *in vivo* exclusive of RAD51. *J Biol Chem* **277**: 8406–8411
- Moffatt BA, McWhinnie EA, Agarwal SK, Schaff DA (1994) The adenine phosphoribosyltransferase-encoding gene of *Arabidopsis thaliana*. *Gene* **143**: 211–216
- Motamayor JC, Vezon D, Bajon C, Sauvanet A, Grandjean O, Marchand M, Bechtold N, Pelletier G, Horlow C (2000) Switch (swi1) an *Arabidopsis thaliana* mutant affected in the female meiotic switch. *Sex Plant Reprod* **12**: 209–218
- Murata M, Heslop-Harrison JS, Motoyoshi F (1997) Physical mapping of the 5S ribosomal RNA genes in *Arabidopsis thaliana* by multi-color fluorescence *in situ* hybridization with cosmid clones. *Plant J* **12**: 31–37
- Osakabe K, Yoshioka T, Ichikawa H, Toki S (2002) Molecular cloning and characterization of RAD51-like genes from *Arabidopsis thaliana*. *Plant Mol Biol* **50**: 71–81
- Paques F, Haber JE (1999) Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev* **63**: 349–404
- Peirson BN, Bowling SE, Makaroff CA (1997) A defect in synapsis causes male sterility in a T-DNA-tagged *Arabidopsis thaliana* mutant. *Plant J* **11**: 659–669
- Petronczki M, Siomos MF, Nasmyth K (2003) Un ménage à quatre: the molecular biology of chromosome segregation in meiosis. *Cell* **112**: 423–440
- Pierce AJ, Johnson RD, Thompson LH, Jasin M (1999) XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. *Genes Dev* **13**: 2633–2638
- Pittman DL, Cobb J, Schimenti KJ, Wilson LA, Cooper DM, Brignull E, Handel MA, Schimenti JC (1998) Meiotic prophase arrest with failure of chromosome synapsis in mice deficient for Dmc1, a germline-specific RecA homolog. *Mol Cell* **1**: 697–705
- Pittman DL, Schimenti JC (2000) Midgestation lethality in mice deficient for the RecA-related gene, Rad51d/Rad51l3. *Genesis* **26**: 167–173
- Rockmill B, Sym M, Scherthan H, Roeder GS (1995) Roles for two RecA homologs in promoting meiotic chromosome synapsis. *Genes Dev* **9**: 2684–2695
- Romanienko PJ, Camerini-Otero RD (2000) The mouse Spo11 gene is required for meiotic chromosome synapsis. *Mol Cell* **6**: 975–987
- Ross KJ, Fransz P, Armstrong SJ, Vizir I, Mulligan B, Franklin FC, Jones GH (1997) Cytological characterization of four meiotic mutants of *Arabidopsis* isolated from T-DNA-transformed lines. *Chromosome Res* **5**: 551–559
- Schild D, Lio YC, Collins DW, Tsomondo T, Chen DJ (2000) Evidence for simultaneous protein interactions between human Rad51 paralogs. *J Biol Chem* **275**: 16443–16449
- Schubert I, Fransz PF, Fuchs J, de Jong JH (2001) Chromosome painting in plants. *Methods Cell Sci* **23**: 57–69
- Schwacha A, Kleckner N (1997) Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway. *Cell* **90**: 1123–1135
- Shinohara A, Gasior S, Ogawa T, Kleckner N, Bishop DK (1997) *Saccharomyces cerevisiae* recA homologues RAD51 and DMC1 have both distinct and overlapping roles in meiotic recombination. *Genes Cells* **2**: 615–629
- Shinohara A, Ogawa H, Ogawa T (1992) Rad51 protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein. *Cell* **69**: 457–470
- Shu Z, Smith S, Wang L, Rice MC, Kmiec EB (1999) Disruption of muREC2/RAD51L1 in mice results in early embryonic lethality which can be partially rescued in a p53(–/–) background. *Mol Cell Biol* **19**: 8686–8693
- Sigurdsson S, Van Komen S, Bussen W, Schild D, Albala JS, Sung P (2001) Mediator function of the human Rad51B–Rad51C complex in Rad51/RPA-catalyzed DNA strand exchange. *Genes Dev* **15**: 3308–3318
- Sun H, Treco D, Schultes NP, Szostak JW (1989) Double-strand breaks at an initiation site for meiotic gene conversion. *Nature* **338**: 87–90
- Sun H, Treco D, Szostak JW (1991) Extensive 3'-overhanging, single-stranded DNA associated with the meiosis-specific double-strand breaks at the ARG4 recombination initiation site. *Cell* **64**: 1155–1161
- Sung P (1994) Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. *Science* **265**: 1241–1243
- Sung P (1997) Yeast Rad55 and Rad57 proteins form a heterodimer that functions with replication protein A to promote DNA strand exchange by Rad51 recombinase. *Genes Dev* **11**: 1111–1121
- Symington LS (2002) Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. *Microbiol Mol Biol Rev* **66**: 630–670
- Takata M, Sasaki MS, Sonoda E, Fukushima T, Morrison C, Albala JS, Swagemakers SM, Kanaar R, Thompson LH, Takeda S (2000) The Rad51 paralog Rad51B promotes homologous recombinational repair. *Mol Cell Biol* **20**: 6476–6482
- Takata M, Sasaki MS, Tachiiri S, Fukushima T, Sonoda E, Schild D, Thompson LH, Takeda S (2001) Chromosome instability and

- defective recombinational repair in knockout mutants of the five Rad51 paralogs. *Mol Cell Biol* **21**: 2858–2866
- Tebbs RS, Zhao Y, Tucker JD, Scheerer JB, Siciliano MJ, Hwang M, Liu N, Legerski RJ, Thompson LH (1995) Correction of chromosomal instability and sensitivity to diverse mutagens by a cloned cDNA of the XRCC3 DNA repair gene. *Proc Natl Acad Sci USA* **92**: 6354–6358
- Thompson LH, Schild D (2001) Homologous recombinational repair of DNA ensures mammalian chromosome stability. *Mutat Res* **477**: 131–153
- Tsuzuki T, Fujii Y, Sakumi K, Tominaga Y, Nakao K, Sekiguchi M, Matsushiro A, Yoshimura Y, Morita T (1996) Targeted disruption of the Rad51 gene leads to lethality in embryonic mice. *Proc Natl Acad Sci USA* **93**: 6236–6240
- Warren AJ, Maccubbin AE, Hamilton JW (1998) Detection of mitomycin C-DNA adducts *in vivo* by 32P-postlabeling: time course for formation and removal of adducts and biochemical modulation. *Cancer Res* **58**: 453–461
- Wiese C, Collins DW, Albala JS, Thompson LH, Kronenberg A, Schild D (2002) Interactions involving the Rad51 paralogs Rad51C and XRCC3 in human cells. *Nucleic Acids Res* **30**: 1001–1008
- Yamamoto A, Hiraoka Y (2001) How do meiotic chromosomes meet their homologous partners? Lessons from fission yeast. *Bioessays* **23**: 526–533
- Yoshida K, Kondoh G, Matsuda Y, Habu T, Nishimune Y, Morita T (1998) The mouse RecA-like gene Dmc1 is required for homologous chromosome synapsis during meiosis. *Mol Cell* **1**: 707–718