the plant journal

SEB Society for Experimental Biology

The Plant Journal (2014) **78,** 822–833 doi: 10.1111/tpj.12507

MHF1 plays Fanconi anaemia complementation group M protein (FANCM)-dependent and FANCM-independent roles in DNA repair and homologous recombination in plants

Natalie J. Dangel, Alexander Knoll and Holger Puchta*

Botanical Institute II, Karlsruhe Institute of Technology, Hertzstr. 16, Karlsruhe 76187, Germany

Received 29 January 2014; revised 28 February 2014; accepted 6 March 2014; published online 17 March 2014.
*For correspondence (e-mail holger.puchta@kit.edu).

SUMMARY

Fanconi anaemia complementation group M protein (FANCM), a component of the human Fanconi anemia pathway, acts as DNA translocase that is essential during the repair of DNA interstrand cross-links. The DNA-damage-binding function of FANCM is strongly enhanced by the histone fold-containing FANCM-associated protein MHF1. We identified a single homologue of *MHF1* in the genome of *Arabidopsis thaliana*. Similar to the loss of At*FANCM*, the loss of At*MHF1* leads to several meiotic defects, such as chromosome bridges between bivalents and an unequal distribution of chromosomes. Moreover, MHF1, together with FANCM, is involved in interstrand cross-link repair in plants. This phenotype is detectable only in double mutants of the RecQ helicase and BLM homologue RECQ4A, which appears to function in a parallel pathway to the FANCM/MHF1 complex. However, in somatic cells, FANCM has an MHF1-independent function in replicative repair in a parallel pathway to the endonuclease MUS81. Furthermore, MHF1 is required for efficient somatic homologous recombination (HR) – a role antagonistic to FANCM. FANCM and RECQ4A define two parallel pathways of HR suppression in Arabidopsis. Hyperrecombination in the *fancm* but not the *recq4A* mutant can be abolished by *MHF1* mutations. This finding indicates that MHF1 and FANCM act at different steps of a single, common, HR pathway.

Keywords: MHF1, Fanconi anaemia complementation group M protein, DNA repair, homologous recombination, *Arabidopsis thaliana*.

INTRODUCTION

Fanconi anaemia (FA) was first described in 1927 by the paediatrician Dr Guido Fanconi (Fanconi, 1927) and is characterised by chromosomal abnormalities, haematopoietic dysfunction and a predisposition to cancer. Although there is a wide range of symptoms, all FA cells possess the same phenotype, which is also used for diagnosis of the disease: They exhibit a characteristic sensitivity to interstrand cross-link (CL)-inducing agents, such as mitomycin C (MMC; Moldovan and D'Andrea, 2009). FA is caused by the mutation of any one of 16 known *FANC* genes, all of which participate in a common pathway, called the Fanconi anaemia pathway, to repair interstrand CLs. Following a lesion search step by the Fanconi anaemia complementation group M protein (FANCM), the orthologue of yeast Mph1 (Scheller *et al.*, 2000), FANCM is able to recognise and bind to the damaged site, leading to the recruitment of the FA core complex formed by seven of the FANC proteins (FANCA, -B, -C, -E, -F, -G, and -L). The FA core complex then activates further FA proteins and other repair factors to eliminate the lesion. In addition to the *FANC* genes, several FA-associated factors, essential for the correct functioning of the pathway, have been described (Kim and D'Andrea, 2012; Kottemann and Smogorzewska, 2013).

The Mph1-associated histone fold-containing proteins MHF1 and MHF2 form a DNA remodelling complex that has been reported to interact with FANCM. Both proteins are involved in the damage-dependent DNA binding of FANCM and in the subsequent recruitment of the FA core complex. *In vitro*, the MHF heterodimer enhances the DNA translocase (Singh *et al.*, 2010) and replication fork remodelling activities of FANCM (Yan *et al.*, 2010). Both MHF proteins exhibit a typical histone fold domain, a feature of proteins that mediate both protein–protein and protein–DNA interactions (Arents and Moudrianakis, 1993). The crystal structure of the MHF complex shows that MHF1 and MHF2 form a heterotetramer in a manner that resembles the histone H3–H4 heterotetramer, although MHF1 and MHF2 belong to the H2A–H2B family (Tao *et al.*, 2012). Interestingly, MHF1 and

MHF2 also have FANCM-independent functions: they are components of the constitutive centromere-associated network (CCAN) and are also known as CENP-S and CENP-X, respectively (Amano *et al.*, 2009; Nishino *et al.*, 2012). In this respect, MHF1 and MHF2, together with other CENP-proteins, assume important roles in the establishment of kinetochore function at the centromeres to ensure proper chromosome segregation. Mutations in *MHF1* and *MHF2* in chicken DT40 cells and the deletion of *MHF2* in HeLa cells result in several defects during mitosis, such as a longer progression time from prophase to anaphase compared with wild-type (WT) cells and anaphase bridging of chromosomes (Amano *et al.*, 2009). In fission yeast, Mhf1 and Mhf2 support the orthologue of FANCM, Fm11, by transforming crossover (COs) into non-crossover (NCO) recombination events (Lorenz *et al.*, 2012), and indicate a role of MHF during meiosis as well. The deletion of the MHF complex in humans leads to reduced FA pathway activation, an increase in chromosomal aberrations and the affected cells exhibit a hypersensitivity to CL-inducing agents and to camptothecin (CPT; Singh *et al.*, 2010). In yeast, both MHF proteins seem to be important in the repair of alkylated bases caused by methyl methanesulfonate (MMS; Yan *et al.*, 2010). Unlike other *FANC* genes and FA-associated factors, which are only conserved in vertebrates, MHF1, MHF2 and FANCM are widely conserved, with orthologues in yeast and in plants as well (Scheller *et al.*, 2000; Singh *et al.*, 2010; Yan *et al.*, 2010; Knoll *et al.*, 2012), suggesting a functional importance of FANCM-MHF1/2 for DNA repair in all eukaryotes.

Recently, the biological role of a FANCM homologue in plants was characterised. It was shown that AtFANCM is involved in the suppression of spontaneous HR events in somatic cells (Knoll *et al.*, 2012). Moreover, AtFANCM is important for HR during meiosis to ensure proper synapsis and MUS81-dependent CO formation (Crismani *et al.*, 2012; Knoll *et al.*, 2012). Surprisingly, there is no indication of AtFANCM involvement in CL repair as its single mutants are not hypersensitive to CL-inducing agents (Knoll *et al.*, 2012). In fact, CL repair differs considerably between plants and animals. Homologues of only a small number of the multiple human *FANC* genes can be found in plant genomes. Whereas the FA pathway is the major CL repair pathway in mammals, it was shown that the RECQ4A and MUS81 proteins are involved in two independent branches of CL repair in *Arabidopsis thaliana* (Mannuss *et al.*, 2010).

The RecQ family of helicases is highly conserved throughout evolution and is important for ensuring genome stability. Three of the human RecQ helicases are associated with severe genetic diseases due to the fact that the mutations in BLM, WRN or RECQ4 lead to Bloom (German *et al.*, 1965; Ellis *et al.*, 1995), Werner (Epstein *et al.*, 1966) or Rothmund–Thomson (Kitao *et al.*, 1999) syndromes, respectively. It was demonstrated previously that the *A. thaliana* RECQ4A is the functional homologue of the human BLM and yeast Sgs1 helicases (Hartung *et al.*, 2007). Furthermore, AtRECQ4A is involved in the repair of methylated bases and intrastrand CLs induced by the genotoxins MMS and cisplatin, respectively (Mannuss *et al.*, 2010). As a part of the RTR complex (RecQ helicase, a type IA topoisomerase and the structural protein RMI1), AtRECQ4A is involved in the suppression of spontaneous HR in somatic cells (Hartung *et al.*, 2007; Bonnet *et al.*, 2013; Schröpfer *et al.*, 2013).

Together with its interacting partner, EME1, the endonuclease MUS81 forms a nuclease complex, that resolves DNA intermediates such as stalled replication fork structures, displacement loops and Holliday junctions (Boddy *et al.*, 2001; Chen *et al.*, 2001; Kaliraman *et al.*, 2001). Previously, a homologue of *MUS81* in *A. thaliana* has been identified and it was reported that *mus81* mutant lines exhibited a strong sensitivity to cisplatin, MMC and MMS, respectively (Hartung *et al.*, 2006). Additionally, the mutation of the RecQ helicase *RECQ4A* in At*mus81* mutant lines leads to several developmental defects and synthetic lethality, and indicates that MUS81 and RECQ4A act in two parallel pathways to resolve stalled replication forks (Mannuss *et al.*, 2010).

Here, we report the identification of a *MHF1* homologue in the *A. thaliana* genome and its relation to FANCM, RECQ4A and MUS81 in DNA repair and recombination. Similar to FANCM, MHF1 is important for the regulation of HR during meiosis. Moreover, we discovered a hidden role of both MHF1 and FANCM in the repair of interstrand CLs in plants. There, both proteins act in a common pathway in parallel to the pathway that involves the RECQ4A helicase. MHF1 is also required for the promotion of somatic HR in a step that does not involve FANCM. Both proteins seem to act in the same pathway, whereas RECQ4A and MHF1 most likely act in different HR pathways.

RESULTS

Identification of a single *MHF1* gene in *Arabidopsis thaliana*

Through BLAST analyses, we identified a single homologue of human *MHF1* on chromosome 5 at locus AT5G50930 in *A. thaliana.* We were surprised to find that alignments of the predicted amino acid (aa) sequence of AtMHF1 with animal MHF1 homologues revealed that the putative plant open reading frame (ORF) had a long N–terminus that was not conserved in animals. The genomic sequence of the At*MHF1* locus and a predicted transcript sequence found in online databases (e.g., the Arabidopsis Information Resource, AT5G50930, accession number 4515117436) indicated that the N-terminus contained four putative in-frame start codons, with the most 5′ codon predicted to be the translation start site.

We analysed the 5′ end of the AtMHF1 cDNA using 5′ rapid amplification of cDNA ends (5′ RACE) polymerase chain reaction (PCR) followed by sequencing (Method S1). The result revealed that, in contrast with the predicted mRNA sequence, the 5′ end of the cDNA was much shorter and lacked three of the four potential start codons (Figure 1a). Only the proximal ATG was present in the AtMHF1 cDNA and, therefore, the genomic sequence of At*MHF1* consists of four exons and three introns, with a total length of 1600 bp from start to stop codon (Figure 1b) and encodes a protein of 144 aa without a longer N-terminus. In addition, by searching for publicly available expressed sequence tag (EST) sequences available in the Arabidopsis Information Resource TAIR, we could not find any ESTs located upstream of the transcription start found in our RACE experiment. AtMHF1 exhibits an overall protein similarity to HsMHF1 of 25.8%. Similar to HsMHF1 (138 aa) and ScMhf1 (90 aa), the Arabidopsis homologue is predicted to contain a histone fold-like domain named CENP-S [PFAM15630, (Punta *et al.*, 2012)], which shares several conserved amino acids with the CENP-S domain of the human and yeast MHF1 (Figure 1c), respectively.

In humans, MHF1 forms a heterodimer with MHF2, of which we also found a single homologue in Arabidopsis located on chromosome 1 at locus AT1G78790 with an overall protein similarity to HsMHF2 of 44.8%.

To analyse the biological function of *MHF1* in *A. thaliana*, we used two different T-DNA insertion lines named *mhf1-1* (SALK_119435) from the SALK collection (Alonso *et al.*, 2003) and *mhf1-2* (WiscDsLox507A09) from the WiscDsLox collection (Woody *et al.*, 2007) and characterised their insertion sites and the expression levels of different parts of the ORF [for details see Figure S1(a,b) and Method S2].

According to AtGenExpress microarray experiments, the transcript level of At*MHF1* is only elevated in stamens of flowers stages 12 and 15 and in mature pollen compared with control values (Schmid *et al.*, 2005). In a number of abiotic stress treatments, including genotoxic stress, *MHF1* expression was not changed more than two-fold compared with control values.

At*MHF1* single mutants have no detectable DNA repair defects, but they exhibit a defect in somatic homologous recombination

In humans it has been shown that the deletion of *MHF1*, similar to knockouts of other members of the FA pathway, results in an increased sensitivity to CLs, leading to the suggestion that MHF1 is involved in the repair of this type of DNA lesion (Singh *et al.*, 2010; Yan *et al.*, 2010). To analyse whether MHF1 plays a comparable role in *A. thaliana*, we tested both *mhf1* mutant lines for hypersensitivity to the interstrand CL-inducing agent MMC and to cisplatin,

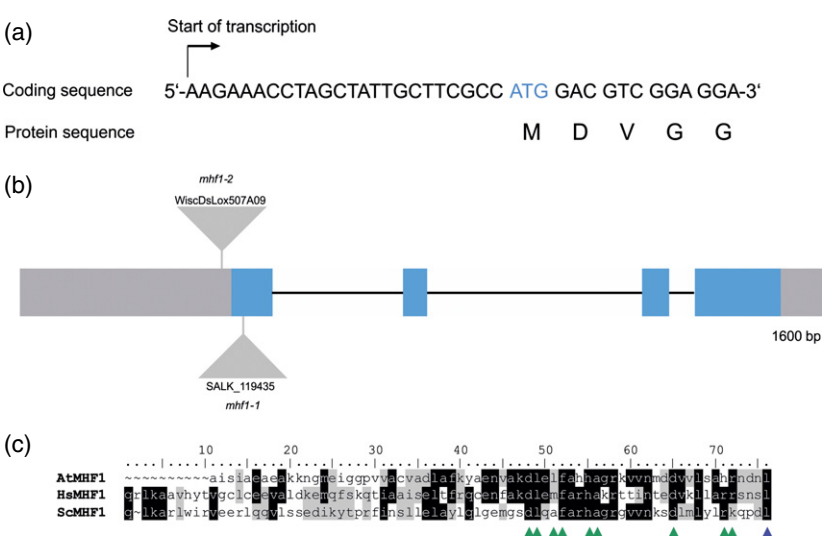

Figure 1. The transcription start site and gene structure of At*MHF1* as well as the alignment of the CENP-S domain of MHF1 homologues in *Arabidopsis thaliana*, *Homo sapiens* and *Saccharomyces cerevisiae*.
(a) The sequence of the 5′ end of the transcript, containing the start codon ATG (highlighted in blue) was determined by 5′ RACE PCR (rapid amplification of cDNA ends, polymerase chain reaction) analysis. The N-terminal sequence of the protein is displayed under the coding sequence.
(b) At*MHF1* is located at locus AT5G50930 and consists of four exons (blue) and three introns (black lines). The 5′ and 3′ untranslated regions (UTRs) are highlighted in grey. The total length of the gene from start to stop codon is 1600 bp. The insertion site of the T-DNA in the *mhf1-1* mutant (SALK_119435) is located in exon 1, whereas the T-DNA insertion in the *mhf1-2* mutant line (WiscDsLox507A09) is located shortly upstream of the start codon in the 5′ UTR.
(c) Alignment of the CENP-S domains. Identical amino acids are highlighted in black and similar amino acids are highlighted in grey. The residues marked with a green triangle have been shown to be involved in the formation of the human $(MHF1–MHF2)_2$ tetramer (Tao *et al.*, 2012). The blue triangle marks the HsMHF1 residue that resides in the hydrophobic core of HsMHF2 (Tao *et al.*, 2012).

which mostly induces intrastrand CLs (Rink *et al.*, 1996; Boulikas and Vougiouka, 2003). After 1 week of growth under standard conditions, the plantlets were incubated for 2 weeks in different concentrations of the genotoxin, followed by the determination of the fresh weight. To evaluate the results, we compared the fresh weights of the mutant lines with the fresh weights of WT plants that had been treated similarly. Compared with the WT plants, the *mhf1-1* and *mhf1-2* single mutants did not exhibit an elevated sensitivity to the cross-linking agents cisplatin and MMC (Figure 2a,b). Furthermore, neither mutant was hypersensitive to bleomycin, hydroxyurea or MMS, respectively (Figure S2). This result, although different from the phenotype of the animal MHF1 homologue, was not particularly surprising because the mutant of the putative complex partner FANCM in Arabidopsis also did not exhibit increased sensitivity to these agents (Knoll *et al.*, 2012).

It has been shown that the orthologue of MHF1 in *Schizosaccharomyces pombe* is involved in the suppression of CO formation and that it plays an essential role during the promotion of NCO recombination events in somatic cells, in a pathway together with the *S. pombe* orthologue of FANCM, Fm11 (Bhattacharjee *et al.*, 2013). To test the influence of AtMHF1 on HR in somatic cells, we crossed both *mhf1* mutant lines with the reporter line IC9 (Molinier *et al.*, 2004). This reporter line possesses a β-glucuronidase (GUS) reporter gene, which is separated into two non-functional parts that share a homologous segment of the gene. Only after interchromosomal homologous recombination (HR), the GUS gene *uidA* can be restored fully and converts the substrate 5-bromo-4-chloro-3-indolyl glucuronide (X-GlucA) into a blue stain. Thus, every recombination event results in a quantifiable blue sector on the plant (Puchta and Hohn, 2012). To exclude transcriptional or post-transcriptional repression of the GUS gene, the expression of the gene was tested in both *mhf1* IC9 mutants (Method S2) and was compared with its expression in WT IC9, which revealed no silencing.

AtMHF1 appears to promote somatic HR because both *mhf1* mutant lines showed a decrease in the frequency of spontaneous recombination compared with WT. The relative HR frequency was reduced to less than half in *mhf1-1* IC9 and *mhf1-2* IC9 compared with WT IC9 plants (Figure 2c). This result was surprising because in *A. thaliana*, *FANCM* mutants exhibited a hyper-recombination phenotype (Knoll *et al.*, 2012). Thus, AtMHF1 is involved in the promotion of spontaneous HR events, which is antagonistic to the function of AtFANCM.

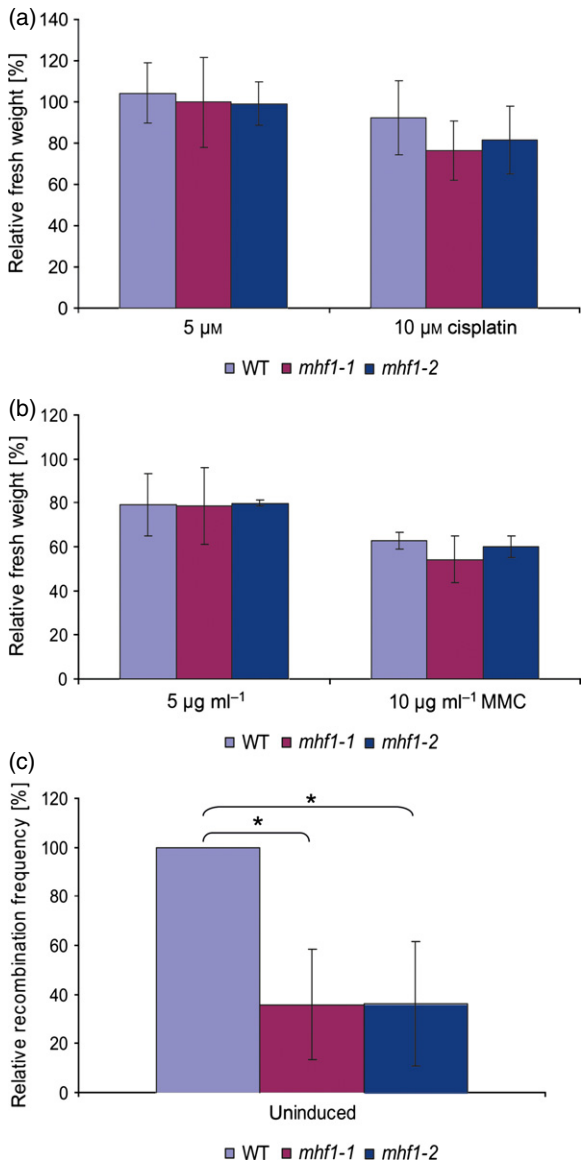

Figure 2. The relative fresh weights after cisplatin and mitomycin C (MMC) treatment and spontaneous homologous recombination (HR) frequencies of the At*mhf1-1* and At*mhf1-2* single mutants.
The relative fresh weights of the tested lines were calculated as the fresh weight of the line after genotoxin treatment in relation to the fresh weight of untreated control plants of the same line. Each assay was performed at least three times to calculate the standard deviations (SD; error bars).
(a) The relative fresh weights of *mhf1-1* and *mhf1-2* after 5 or 10 μM cisplatin treatment. Compared with the wild-type (WT) plants, neither *mhf1* mutant line exhibited a reduction in fresh weight after cisplatin incubation.
(b) The relative fresh weights of *mhf1-1* and *mhf1-2* after 5 and 10 μg ml^{-1} MMC treatment. Neither *mhf1-1* nor *mhf1-2* showed a difference in fresh weight compared with the WT plants.
(c) The relative recombination frequencies of both *mhf1* mutants. *mhf1-1* and *mhf1-2* displayed similar significant reductions in the recombination frequency compared with WT plants. *$P < 0.05$.

Role of AtMHF1 during meiosis

In fission yeast, MHF, together with the FANCM orthologue Fm11, is not only involved in somatic HR but also plays a role in meiotic recombination by processing joint DNA molecules and directing NCO recombination events (Lorenz *et al.*, 2012; Bhattacharjee *et al.*, 2013). The interaction between MHF1 and FANCM in animals and in fission yeast prompted us to test whether the *mhf1* mutants would

exhibit meiotic defects similar to those of *fancm* mutants in *A. thaliana.* Therefore, we analysed the meiotic stages of DAPI-stained chromosome spreads from *mhf1-1* and *mhf1-2* pollen mother cells and WT cells that were undergoing meiosis using fluorescence microscopy. In the pachytene stage, the DAPI-positive chromosomes seem to be fully synapsed as in the WT cells. In later stages of meiosis I, such as diplotene, the chromosomes of both *mhf1* mutants were arranged as five bivalents linked by chiasmata, as in the WT meiotic cells (Figure 3d–f). Additionally, *mhf1-1* and *mhf1-2* exhibited connections between bivalents that had been shown before in *fancm* mutants (Knoll *et al.*, 2012). These connections were also apparent in early anaphase I (Figure 3h) and resulted in defects in chromosome distribution at the end of meiosis II (Figure 3n,o). As our results are reminiscent of the phenotype of the *fancm* mutant (Knoll *et al.*, 2012), it seems likely that MHF1 indeed controls proper meiotic recombination in a complex with FANCM.

AtMFH1 has an AtRECQ4A-independent role in interstrand cross-link repair

In *A. thaliana* it has been shown previously that CLs are repaired differently than in mammals and that the RecQ family helicase RECQ4A, a homologue of the human BLM and yeast Sgs1 proteins, and the endonuclease MUS81 are important during this repair and act in parallel pathways (Mannuss *et al.*, 2010). Although neither *mhf1* single mutant was sensitive to MMC or cisplatin, we created and tested different double mutants by crossing *mhf1-1* mutants with the mutants of the CL repair genes *recq4A-4* and *mus81-1*, respectively. If MHF1 plays only a minor role, its function might be discernable only in the absence of another important factor in CL repair. As MHF1 interacts with FANCM in humans, we also created a double mutant of At*fancm-1* with At*mhf1-1.*

We created the double mutant of *mhf1-1* and *recq4A-4* by crossing the respective homozygous single mutant lines. The corresponding double mutant, which was homozygous for *mhf1-1* and *recq4A-4*, was identified in the F2 generation through PCR-based genotyping (Method S3). The plants were viable and showed no obvious differences in growth compared with WT plants. To characterise the sensitivity of *mhf1-1 recq4A-4*, we determined the fresh weights of the double mutant, both single mutants and the WT plants after the induction of interstrand crosslinks by MMC and intrastrand crosslinks by cisplatin. Two different concentrations of each genotoxin were tested.

Both single mutants *recq4A-4* and *mhf1-1* showed no greater sensitivity to MMC treatment than the WT plants, as the fresh weights of both lines were similar to those of the WT plantlets. Surprisingly, the corresponding double mutant exhibited a strong hypersensitivity to MMC. After 10 μg ml^{-1} MMC treatment, the fresh weight of the double mutant was reduced to a third of the untreated control, whereas both single mutants still had a significantly higher fresh weight compared with the double mutant (Figure 4a). A similar effect was observed after 15 μg ml^{-1} MMC treatment. Thus, although it was not measurable in the single mutants, both AtMHF1 and AtRECQ4A play roles in interstrand CL repair and they act in different pathways.

In contrast with the results of MMC treatment, we did not find any evidence of an involvement of AtMHF1 in the repair of intrastrand cross-links induced by cisplatin. The double mutant showed the same level of hypersensitivity as the *recq4A-4* single mutant (for details see Figure S3a). Taken together, our results indicated that, in addition to the involvement of RECQ4A in intrastrand CLs, both MHF1 and RECQ4A are also involved in independent pathways of interstrand CL repair in *A. thaliana.*

In *A. thaliana*, MUS81 acts as an endonuclease and is involved in the resolution of both intra- and interstrand CLs (Hartung *et al.*, 2006). Our discovery of a function of MHF1 in interstrand CL repair due to the analysis of the *mhf1-1 recq4A-4* double mutant prompted us to test the sensitivity of the double mutant of *mhf1-1* with *mus81-1.* The plants, which were homozygous for both *mhf1-1* and *mus81-1*, were viable and exhibited no obvious growth defects compared with WT plants. This result was surprising because it has been demonstrated previously that a double mutant of *fancm* and *mus81* showed severe growth defects [see below and Crismani *et al.* (2012)]. Similar to the analysis of *mhf1-1 recq4A-4*, we also tested the sensitivities of the *mhf1-1 mus81-1* double mutant, both single mutants and WT plants to two different concentrations of the cross-linking agents MMC and cisplatin by measuring the fresh weights of the plantlets. However, although *mus81-1* single mutants showed a notable sensitivity against both agents, neither treatment revealed a statistically significant enhancement of sensitivity in the double mutant at the various concentrations tested (Figures 4b and S3b,c).

Because the animal MHF1 and FANCM homologues interact, it was interesting to test the double mutant line in Arabidopsis for CL sensitivity. Therefore, we crossed both single mutants and identified the corresponding double mutant line in the F2 generation through PCR-based genotyping (Method S3). The deletion of both genes in *A. thaliana* did not lead to any differences in growth or viability compared with the WT plants. Similar to the analysis of the other double mutants, we determined the fresh weight of the plantlets after 2 weeks of incubation with MMC or cisplatin, respectively. We did not detect any sensitivity compared with the WT plants (Figures 4c and S3d). These results indicated that if FANCM has a role in interstrand CL repair, it should act in the same pathway as MHF1.

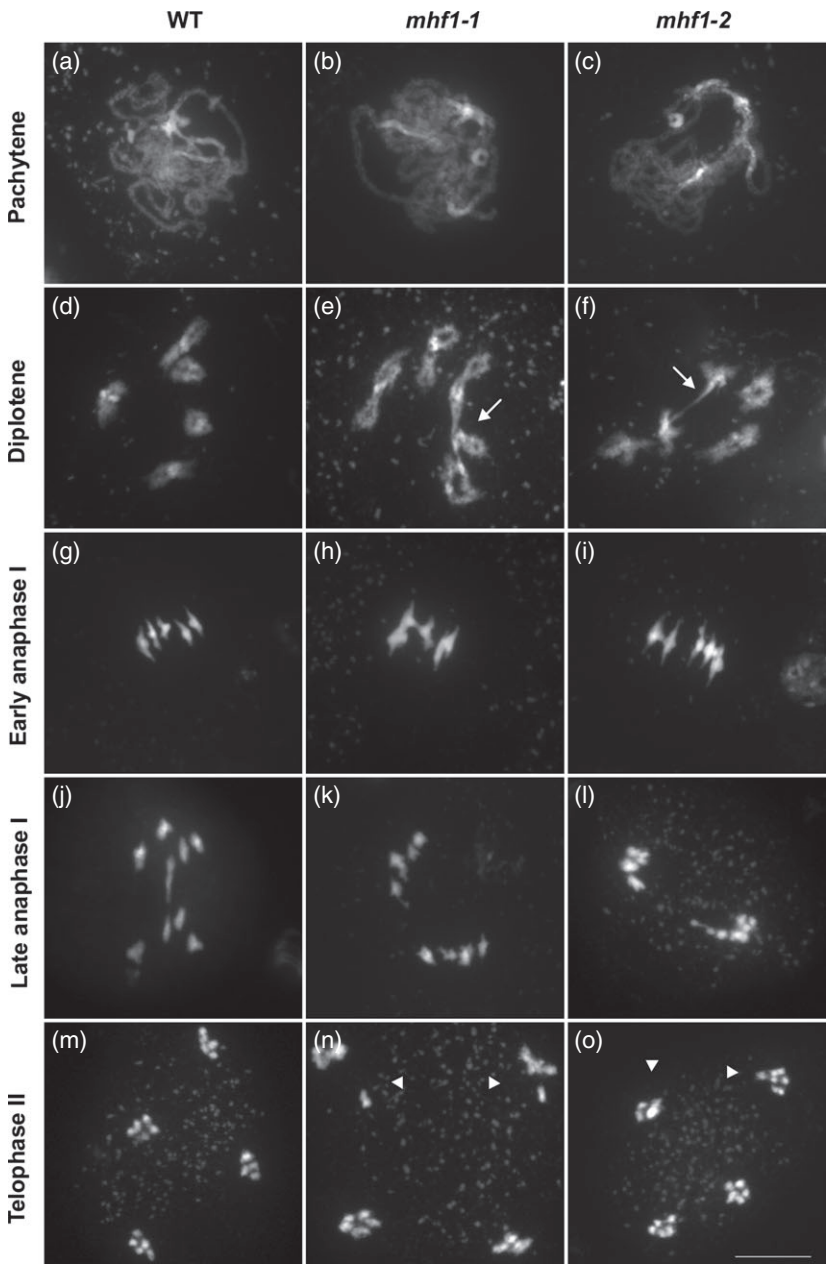

Figure 3. 4′,6-Diamidino-2-phenylindole (DAPI)-stained chromatin spreads of *Arabidopsis thaliana* cells undergoing meiosis of wild-type (WT) (a, d, g, j, m), *mhf1-1* (b, e, h, k, n) and *mhf1-2* (c, f, i, l, o) meiocytes.
In the pachytene stage (a–c), neither *mhf1* mutant exhibited defects compared with WT meiocytes. In the diplotene stage (d–f), connections between the five bivalents in *mhf1-1* and *mhf1-2* were observed (arrows). In early anaphase I (g–i), those bridges were also apparent, resulting in unequal chromosome distributions in both *mhf1* mutants at the end of the second meiotic division in telophase II (m–o, arrowheads). Scale bar = 10 μm.

AtFANCM has an AtRECQ4A-independent role in interstrand cross-link repair

Our analysis of double mutants revealed a hidden role for MHF1 in interstrand CL repair. To test whether FANCM is indeed involved in the same pathway, we generated and evaluated the sensitivity to MMC of the corresponding double mutants of *fancm-1* with *recq4A-4* and *mus81-1.*

The *fancm-1 recq4A-4* double mutant was created by crossing both single mutant lines. In the F2 generation, the double mutant was identified through PCR-based genotyping (Method S3) and its seeds were used for the sensitivity analysis. The *fancm-1 recq4A-4* double mutant was viable and had no obvious differences in growth or viability compared with the WT plants. Similar to the analysis of *mhf1-1 recq4A-4*, we tested two concentrations of the interstrand cross-linking agent MMC.

After 10 μg ml^{-1} MMC induction, the double mutant had a fresh weight of only a fifth of the untreated control and this result was also observed after 15 μg ml^{-1} MMC treatment, although neither single mutant exhibited this strong hypersensitivity (Figure 4d). Thus, FANCM, similar to MHF1, seems to be involved in interstrand CL repair in a pathway parallel to that of RECQ4A.

Contrary to the results of the *mhf1-1 mus81-1* double mutant, we were unable to produce any plants that were

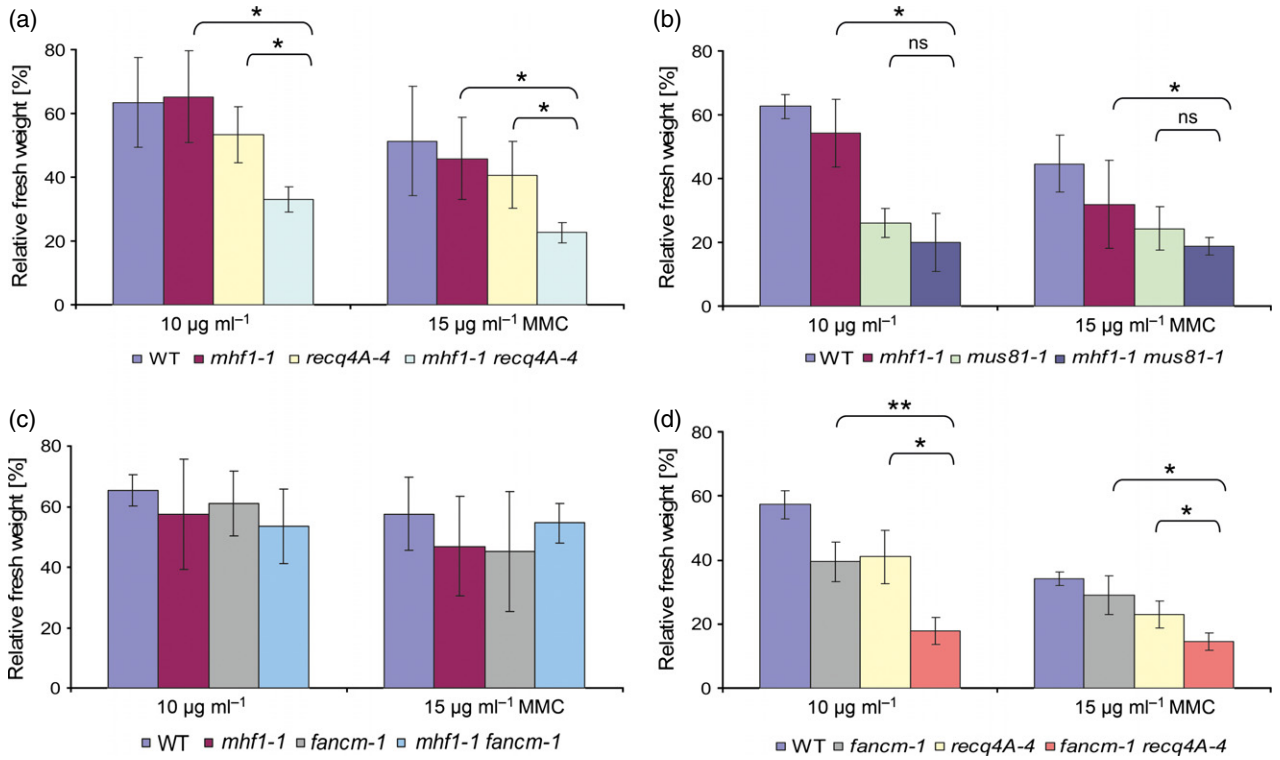

Figure 4. Relative fresh weights of different double mutants after mitomycin C (MMC) incubation.
The relative fresh weights of the tested lines were calculated as the fresh weight of the line after genotoxin treatment in relation to the fresh weight of untreated control plants of the same line. Each assay was performed at least three times to calculate the standard deviations (SD, error bars).
(a) The *mhf1-1 recq4A-4* double mutant displayed a significantly lower fresh weight compared with the fresh weights of both single mutants and wild-type (WT) plantlets.
(b) The fresh weight of *mhf1-1 mus81-1* showed a reduced relative fresh weight similar to that of the *mus81-1* single mutant.
(c) Neither the *mhf1-1* or *fancm-1* single mutant, nor the *mhf1-1 fancm-1* double mutant exhibited a sensitivity that differed from that of the WT plants.
(d) The *fancm-1 recq4A-4* double mutant exhibited a relative fresh weight that was significantly lower than those of both single mutants and the WT plants.
*$P < 0.05$; **$P < 0.01$; ns = not significant.

homozygous for both the *fancm-1* and *mus81-1* mutations, as the plantlets died shortly after germination. To confirm this observation, we quantified seedling viability of progeny from a line homozygous for *mus81-1* and segregating for *fancm-1* and revealed that, based on normal Mendelian segregation, the one-quarter of the progeny that were homozygous for both *fancm-1* and *mus81-1* dies very early during vegetative growth (Table S1). This result indicated that *FANCM* and *MUS81* are indispensable in *A. thaliana* because the plants are unable to survive if both genes are missing. A similar observation has been reported previously, made based on other alleles of *fancm-9* and *mus81-2.* In this case the double mutant showed severe growth defects but was viable (Crismani *et al.*, 2012). As the double mutant phenotype is much more severe than either single mutant phenotype, one must assume that these genes act in separate pathways. Because the *mhf1-1 mus81-1* double mutant was viable (see above), FANCM seems to have an MHF1-independent function in the repair of replicative damage that arises in the absence of MUS81.

The AtMHF1-dependent defect in somatic HR can be suppressed by mutations in At*RECQ4A*, but not in At*FANCM*

Our analysis indicated that MHF1 and FANCM differ in their somatic recombination behaviour. MHF1, similar to MUS81 (Hartung *et al.*, 2006), promotes somatic HR, however, FANCM, similar to RECQ4A, suppresses it (Knoll *et al.*, 2012) and both helicases are involved in independent pathways. It was therefore of interest to define the function of MFH1 in relation to the other factors. For epistasis analysis we tested the recombination frequency of the generated double mutants of *mhf1-1* with *recq4A-4*, *mus81-1* and *fancm-1*, respectively, in the IC9 recombination reporter background.

The recombination frequency of the *mhf1-1 recq4A-4* double mutant was strongly elevated compared with the recombination frequency of WT IC9 and it was similar to the recombination frequency of *recq4A-4* IC9 mutant plants (Figure 5a). Thus, the recombination defect of *mhf1* mutants can be suppressed by the elimination of the RECQ4A helicase.

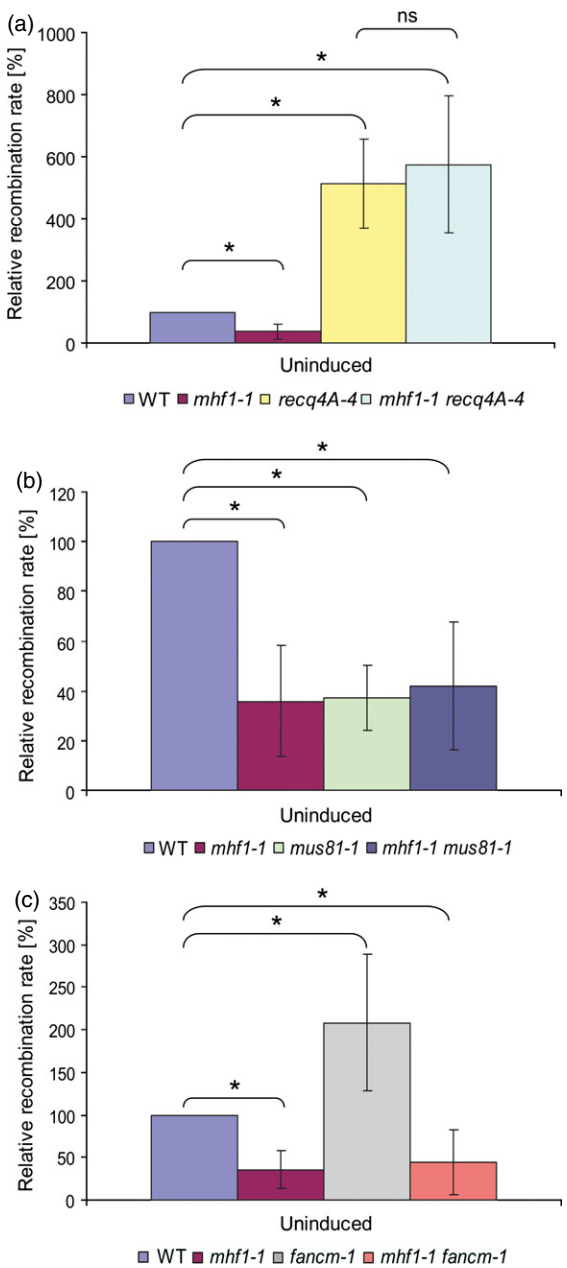

Figure 5. Spontaneous somatic homologous recombination (HR) frequencies of the different double mutants.

The spontaneous HR frequencies of the tested lines were calculated as the HR frequency normalised to the HR frequency of the wild-type (WT) plants. Each assay was performed at least three times to calculate the standard deviations (SD, error bars).

(a) The HR rate of the *mhf1-1* mutants was significantly lower than that of the WT plants, whereas the HR frequency of *recq4A-4* was elevated compared with that of the WT plants. The generated double mutant displayed an HR frequency that did not differ significantly from the *recq4A-4* single mutant.

(b) The HR frequencies of both the *mhf1-1* and *mus81-1* single mutants and the corresponding double mutant were reduced to a similar extent compared with WT plants.

(c) Whereas the HR frequency was decreased in *mhf1-1* mutants and increased slightly in *fancm-1* mutants, the *mhf1-1 fancm-1* double mutant exhibited an HR frequency similar to that of the *mhf1-1* single mutant. $*P < 0.05$.

In the case of MUS81, the spontaneous recombination frequency of both single mutants in the IC9 background was reduced to less than half in comparison with WT (Figure 5b). Because the double mutant showed a decrease in HR frequency that was almost identical to that of both single mutants, the simplest explanation is that AtMUS81 and AtMHF1 act in a common epistatic pathway to repair spontaneous double-stranded breaks via HR in somatic cells.

As FANCM and MHF1 appear to have opposing functions in HR in *A. thaliana*, it was of special interest to test the double mutant of both genes. Surprisingly, and in contrast with the data obtained with the *RECQ4A* mutant, the loss of *FANCM* did not suppress the recombination defect of the *mhf1-1* IC9 mutant (Figure 5c).

DISCUSSION

Our analyses revealed a quite complex interaction between FANCM and its putative complex partner MHF1. On the one hand, it is obvious that both proteins act together in Arabidopsis in certain functions. We supplied strong evidence that this hypothesis is the case for CL repair and, additionally, the meiotic phenotype of the *mhf1* mutants argues for a common complex with FANCM during meiosis. Conversely, we clearly demonstrated that, in somatic cells, these proteins have functions that are independent from each other: MHF1 and FANCM assume opposing roles in HR and only FANCM is essential for replicative repair in the absence of the endonuclease MUS81. A detailed discussion of these findings is presented below.

AtMHF1 is involved in meiotic HR

The deletion of AtFANCM is accompanied by a reduction in fertility and several defects during meiotic recombination repair. Similar to FANCM, MHF1 seems to play a role in the regulation of meiotic HR as we observed several defects during meiosis, as well, such as connections between bivalents and an unequal chromosome distribution at the end of meiosis II. The defects observed during meiosis resembled the defects associated with *FANCM* mutations in *A. thaliana* and indicated the possibility that MHF1 and FANCM act together during meiotic recombination. This hypothesis is supported by observations of the fission yeast orthologues of MHF1 and FANCM, which also function together in meiosis by promoting NCO recombination events (Lorenz *et al.*, 2012; Bhattacharjee *et al.*, 2013).

AtMHF1 and AtFANCM work in interstrand cross-link repair in a parallel pathway to AtRECQ4A

The mammalian histone fold protein MHF1, together with its partner MHF2, ensures the correct binding of the DNA translocase FANCM to DNA lesions during the repair of interstrand CLs by the Fanconi anaemia pathway. Mutations in *MHF1*, similar to mutations in other FA genes, lead to a

strong hypersensitivity of the affected cells to interstrand CL-inducing agents (Singh *et al.*, 2010). Although in *A. thaliana*, single mutants of *MHF1* and *FANCM* do not exhibit a detectable sensitivity phenotype, we revealed here that both proteins do play a role in interstrand CL repair as was demonstrated for their orthologues in humans. The RecQ helicase RECQ4A was described previously as being involved only in the repair of intrastrand CLs induced by cisplatin in *A. thaliana* (Mannuss *et al.*, 2010). Our analysis of the double mutants of *RECQ4A* with *mhf1-1* and *fancm-1*, respectively, revealed that AtRECQ4A also plays a role in interstrand CL repair and that both MHF1 and FANCM act in a pathway parallel to that of RECQ4A. Interestingly, these mutants did not exhibit a comparable phenotype for intrastrand CLs induced by the genotoxin cisplatin as the *mhf1-1 recq4A-4* double mutant did not show a further reduction in fresh weight compared with *recq4A-4*. In humans, it has been shown that the RecQ helicase BLM, the functional orthologue of AtRECQ4A, interacts with ubiquitinated FANCD2 and that these proteins colocalise at interstrand CL damage sites to facilitate repair (Pichierri *et al.*, 2004; Hirano *et al.*, 2005). Furthermore, HsFANCM has been shown to interact with the RTR complex (Deans and West, 2009; Hoadley *et al.*, 2012), which consists of the BLM helicase, a type IA topoisomerase and two structural proteins, RMI1 and RMI2. Recently, a similar interaction was shown for FancM in the silkworm *Bombyx mori*, where FancM interacts with Mhf1 and Rmi1 of the RTR complex, as well (Sugahara *et al.*, 2013). Such an interaction has not been shown for *A. thaliana* FANCM. As our results indicated, FANCM, MHF1 and RECQ4A are involved in the repair of interstrand CLs but the FA proteins act in a different pathway than does RECQ4A. Thus, the situation in plants seems to differ from that in animals.

Similar to the situation in mammals, MHF1 and FANCM seem to act in the same pathway to repair interstrand CLs in *A. thaliana*, as the sensitivity of the corresponding double mutant did not differ from that of either single mutant or the WT plants. Because AtMHF1, similar to its animal and fungal homologues, contains a histone fold-like domain, one could speculate that Arabidopsis MHF1 is also involved in supporting the binding of FANCM to damaged DNA.

Our analysis of the double mutant of *mhf1-1* and *mus81-1* did not reveal any growth defects compared with WT plants. This result was surprising because it had been shown previously that the deletion of *FANCM* and *MUS81* in *A. thaliana* was lethal (Crismani *et al.*, 2012), an observation we could confirm by creating the double mutant of *fancm-1* and *mus81-1.* The double mutant of At*mhf1-1* and At*mus81-1* had not been characterised previously but if AtFANCM and AtMHF1, similar to the situation in mammals, always work together, we would have expected that *mhf1-1 mus81-1* would also exhibit a lethal phenotype. Because the double mutant *mhf1-1 mus81-1* was viable and exhibited a hypersensitivity to interstrand CLs similar to that of the *mus81-1* single mutant, it is tempting to speculate that MHF1 and MUS81 function in the same repair pathway. Due to its lethal phenotype, we could not analyse the behaviour of the *fancm mus81* double mutant in interstrand CL repair. Therefore, we cannot draw any final conclusions about the role of both factors in interstand CL repair. Nevertheless, taking into account the fact that MHF1 and FANCM, as well as MHF1 and MUS81, seem to act in the same pathway, it is tempting to speculate that all three proteins are members of a single pathway of interstrand CL repair. A respective model is depicted in Figure 6(a).

The deletion of At*MHF1* can suppress the hyper-recombination caused by At*FANCM* but not by At*RECQ4A* mutation

In addition to its function in interstrand CL repair, AtMHF1 seems to play an important role during somatic HR, as *mhf1* mutants exhibited a reduced recombination frequency compared with WT plants. Therefore, AtMHF1 is required to promote a step of HR in somatic plant cells. Interestingly, this phenotype is antagonistic to that of FANCM in *A. thaliana* (Knoll *et al.*, 2012). Thus, MHF1 and FANCM do have independent functions during somatic HR in Arabidopsis. This result is in contrast with what is known for MHF1 and FANCM in the fission yeast, where Mhf1 and Fm11 act together in the same pathway to promote NCO recombination events (Bhattacharjee *et al.*, 2013). In the At*mhf1-1 fancm-1* double mutant, the HR phenotype of the *mhf1-1* single mutant is dominant. Thus, MHF1 is able to suppress the hyper-recombination phenotype caused by a mutation in *FANCM.* This finding indicates strongly that MHF1 may be involved in the same somatic HR pathway as FANCM, but in a step before FANCM becomes active. If FANCM and MHF1 would act in two different pathways, the HR frequency of the

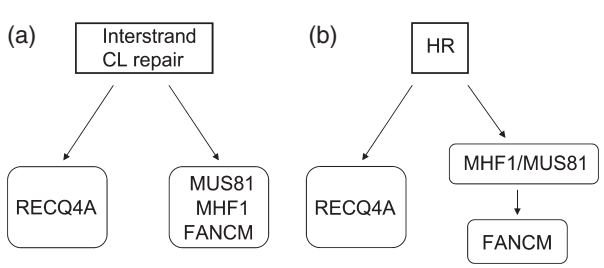

Figure 6. Models of the involvement of MHF1, FANCM, MUS81 and RECQ4A in interstrand CL repair (a), and somatic homologous recombination (HR) (b) in *Arabidopsis thaliana.*
Our analyses indicated that, in both cases, MHF1 acts in the same pathway as MUS81 and in a parallel pathway to RECQ4A. As MHF1 is able to suppress the hyper-recombination phenotype of the *fancm* mutant, MHF1 seems to act before the helicase in HR.

corresponding double mutant should be intermediate to the HR frequencies of both single mutants.

The analysis of the *mhf1-1 mus81-1* double mutant revealed a reduced spontaneous recombination frequency at the same level of both single mutants. As we could not detect a further reduction in the HR frequency of the double mutant compared with both single mutants, the fact that MHF1 and MUS81 act in two different pathways of spontaneous HR in somatic cells is unlikely. Thus, the most obvious hypothesis is that MHF1 and MUS81 act in the same pathway of somatic HR.

Similar to FANCM, the RecQ helicase RECQ4A is involved in the suppression of spontaneous recombination events in *A. thaliana*, however it was demonstrated that RECQ4A functions in a parallel pathway to FANCM (Knoll *et al*., 2012). An investigation of the relationship between AtMHF1 and AtRECQ4A in somatic HR could lead to three different outcomes when comparing the HR frequency of the double mutant with that of each single mutants: the double mutant could show an HR frequency at the level of either the *mhf1* or the *recq4A* single mutant, respectively, if both genes act in a common pathway with one mutation suppressing the second. Alternatively, if the genes act in two parallel pathways one would expect the double mutant to exhibit an intermediate phenotype compared with the single mutant. However, the decrease in HR frequency due to the *MHF1* mutation in relation to the increase observed in *recq4A* mutants is rather small. Therefore, in the case of two parallel pathways, a small reduction in the high HR frequency of the *mhf1 recq4A* double mutant might not be apparent as significantly different from the HR frequency of the *recq4A* single mutant. Because the HR frequency of the *mhf1 recq4A* double mutant is higher than the WT and similar to the *recq4A* mutant, we can exclude the possibility that MHF1 acts before RECQ4A in a single pathway. It is not possible, however, to distinguish between the other two options. AtMHF1 might therefore function in a RECQ4A-dependent pathway at a later step or it might act in a parallel pathway to RECQ4A. Because our data show that FANCM and MHF1 function in a common pathway and that FANCM and RECQ4A are also involved in different pathways of HR suppression (Knoll *et al*., 2012), we favour the hypothesis that MHF1 and RECQ4A act in two parallel pathways. Figure 6(b) depicts our hypothesis on the role of MHF1 in somatic HR in relation to RECQ4A, FANCM and MUS81.

AtMHF1 and its relation to AtMUS81 and AtRECQ4A

The MUS81 endonuclease and the RECQ4A helicase are key actors in different DNA repair and recombination pathways in Arabidopsis. In the absence of both proteins, Arabidopsis is not viable (Mannuss *et al*., 2010), this result indicated that they act in different pathways that are essential for DNA replication. It is, therefore, especially interesting to evaluate the outcome of our analysis of MHF1 functions in relation to these two genes. All of our results indicated that MUS81 and MHF1 act in the same pathway in plants. There is no evidence that MHF1 acts independently of MUS81 in interstrand CL repair. In addition, the double mutant exhibited an HR defect similar to that of both single mutants, this result indicated an epistatic relationship between the two genes. The fact that the double mutants are viable, in contrast with the combinations of *mus81* with either *fancm* or *recq4A* mutants, indicates that MHF1 is not involved in an essential MUS81-independent pathway of replicative DNA repair. In contrast, we found little evidence that RECQ4A shares pathways with MHF1. On the one hand we demonstrated that MHF1 functions in the repair of interstrand CLs in a different pathway than RECQ4A. Conversely, the suppression of the MHF1 HR defect by the additional mutation of *RECQ4A* could be explained by the hypothesis that these proteins act in two parallel pathways, as discussed above. Therefore, all the data obtained in this study indicated that AtMHF1 functions in pathways together with MUS81, but parallel to RECQ4A (Figure 6a,b).

Both MHF1 and FANCM are conserved in all eukaryotes and are involved in different important cellular processes. In *S. pombe*, Mhf1 and Fm11 are involved in the processing of joint DNA molecules and directing NCO recombination events during meiosis (Bhattacharjee *et al*., 2013). Similar to that finding, our analyses revealed the possibility that, in *A. thaliana*, MHF1 and FANCM could also work together in terms of meiotic recombination, as both single mutants showed comparable defects.

During HR in somatic cells, the situation seemed to differ between the orthologues of MHF1 and FANCM in yeast and in plants. Contrary to fission yeast, in which Mhf1 and Fm11 act together to promote NCO recombination events (Bhattacharjee *et al*., 2013), we showed that MHF1 and FANCM have opposing functions during somatic HR in *A. thaliana.*

Interstrand CLs in humans are processed by the Fanconi anaemia pathway, including FANCM and MHF1 which work together to recognise and bind the damage site. The deletion of *FANCM* or *MHF1* in humans leads to a hypersensitivity to interstrand CL-inducing agents (Singh *et al*., 2010). Contrary, in yeast, the mutation of Mhf1 or the FANCM orthologue Mph1 does not lead to a hypersensitivity to MMC but the mutants are sensitive to the genotoxin MMS that leads to replication stress (Scheller *et al*., 2000; Yan *et al*., 2010). The situation in plants seems to be similar to that in humans, as we could show that *mhf1* and *fancm*, at least in the double mutants with *recq4A*, exhibited an increased sensitivity to the interstrand CL-inducing agent MMC, as well.

As homologues of some other *FANC* genes can be found in the Arabidopsis genome (Patel and Joenje, 2007; Knoll

and Puchta, 2011), it will be interesting to define the biological functions of these proteins in DNA repair and recombination in relation to FANCM and MHF1.

EXPERIMENTAL PROCEDURES

Plant materials and growth conditions

For the characterisation of *MHF1* in *A. thaliana* two mutant lines *mhf1-1* (SALK_119435) and *mhf1-2* (WiscDsLox507A09) were used (Alonso *et al.*, 2003; Woody *et al.*, 2007). For the generation of double mutants, the mutant lines *fancm-1* (SALK_069784), *mus81-1* (GABI_113F11) and *recq4A-4* (GABI_203C07) were used and which have been described previously (Hartung *et al.*, 2006, 2007; Knoll *et al.*, 2012). For this purpose and for the cytological methods, the plants were grown as described previously (Schröpfer *et al.*, 2013). For sensitivity and HR assays, an axenic plant culture was used as described recently (Schröpfer *et al.*, 2013).

Sensitivity assays

The sensitivity assays were performed as described previously (Hartung *et al.*, 2007). The genotoxins tested in this assay were bleomycin (Selleckchem, http://www.selleckchem.com), cisplatin (Sigma-Aldrich Chemie, http://www.sigmaaldrich.com), hydroxyurea (Sigma-Aldrich Chemie), MMC (Duchefa Biochemie, http://www.duchefa-biochemie.com) and MMS (Sigma-Aldrich Chemie).

HR assays

The HR assays were performed as described recently (Hartung *et al.*, 2007).

Preparation of DAPI-stained pollen mother cells

The preparation of DAPI-stained pollen mother cells was performed as described previously (Armstrong *et al.*, 2009; Bonnet *et al.*, 2013).

ACKNOWLEDGEMENTS

The authors wish to thank Manfred Focke for a thorough reading of the manuscript and Jana Bender and Sabrina Wagner for technical assistance. This work was funded by the European Research Council (ERC) Advanced Grant 'COMREC' and the Deutsche Forschungsgemeinschaft (DFG) grant Pu 137-11.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1. Characterisation of At*mhf1-1* and At*mhf1-2* mutant lines.

Figure S2. Relative fresh weight of At*mhf1-1* and At*mhf1-2* after the treatment with bleomycin (bleo) (a), hydroxyurea (HU) (b) or methyl methanesulfonate (MMS) (c), respectively.

Figure S3. Relative fresh weights of the generated double mutants after *cis*-Platin treatment.

Table S1. Segregation analysis of the *fancm-1 mus81-1* double mutant.

Methods S1. Rapid amplification of 5′ cDNA ends (RACE).

Methods S2. Quantitative Real-Time Expression Analysis.

Methods S3. Primers used for PCR-based genotyping of T-DNA insertion lines.

REFERENCES

Alonso, J.M., Stepanova, A.N., Leisse, T.J. *et al.* (2003) Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science*, **301**, 653–657.

Amano, M., Suzuki, A., Hori, T., Backer, C., Okawa, K., Cheeseman, I.M. and Fukagawa, T. (2009) The CENP-S complex is essential for the stable assembly of outer kinetochore structure. *J. Cell Biol.* **186**, 173–182.

Arents, G. and Moudrianakis, E.N. (1993) Topography of the histone octamer surface: repeating structural motifs utilized in the docking of nucleosomal DNA. *Proc. Natl Acad. Sci. USA*, **90**, 10489–10493.

Armstrong, S.J., Sanchez-Moran, E. and Franklin, F.C. (2009) Cytological analysis of *Arabidopsis thaliana* meiotic chromosomes. *Methods Mol. Biol.* **558**, 131–145.

Bhattacharjee, S., Osman, F., Feeney, L., Lorenz, A., Bryer, C. and Whitby, M.C. (2013) MHF1-2/CENP-S-X performs distinct roles in centromere metabolism and genetic recombination. *Open Biology*, **3**, 130102.

Boddy, M.N., Gaillard, P.H., McDonald, W.H., Shanahan, P., Yates, J.R. and Russell, P. (2001) Mus81-Eme1 are essential components of a Holliday junction resolvase. *Cell*, **107**, 537–548.

Bonnet, S., Knoll, A., Hartung, F. and Puchta, H. (2013) Different functions for the domains of the *Arabidopsis thaliana* RMI1 protein in DNA cross--link repair, somatic and meiotic recombination. *Nucleic Acids Res.* **41**, 9349–9360.

Boulikas, T. and Vougiouka, M. (2003) Cisplatin and platinum drugs at the molecular level. *Oncol. Rep.* **10**, 1663–1682.

Chen, X.B., Melchionna, R., Denis, C.M., Gaillard, P.H., Blasina, A., van de Weyer, I., Boddy, M.N., Russell, P., Vialard, J. and McGowan, C.H. (2001) Human Mus81-associated endonuclease cleaves Holliday junctions in vitro. *Mol. Cell*, **8**, 1117–1127.

Crismani, W., Girard, C., Froger, N., Pradillo, M., Santos, J.L., Chelysheva, L., Copenhaver, G.P., Horlow, C. and Mercier, R. (2012) FANCM limits meiotic crossovers. *Science*, **336**, 1588–1590.

Deans, A.J. and West, S.C. (2009) FANCM connects the genome instability disorders Bloom's syndrome and Fanconi anemia. *Mol. Cell*, **36**, 943–953.

Ellis, N.A., Groden, J., Ye, T.Z., Straughen, J., Lennon, D.J., Ciocci, S., Proytcheva, M. and German, J. (1995) The Bloom's syndrome gene product is homologous to RecQ helicases. *Cell*, **83**, 655–666.

Epstein, C.J., Martin, G.M., Schultz, A.L. and Motulsky, A.G. (1966) Werner's syndrome a review of its symptomatology, natural history, pathologic features, genetics and relationship to the natural aging process. *Medicine*, **45**, 177–221.

Fanconi, G. (1927) Familiäre infantile perniziosaartige Anämie (perniziöses Blutbild und Konstitution). *Jahrbuch für Kinderheilkunde (Wien)*; **117**, 257–280.

German, J., Archibald, R. and Bloom, D. (1965) Chromosomal breakage in a rare and probably genetically determined syndrome of man. *Science*, **148**, 506–507.

Hartung, F., Suer, S., Bergmann, T. and Puchta, H. (2006) The role of AtMUS81 in DNA repair and its genetic interaction with the helicase AtRecQ4A. *Nucleic Acids Res.* **34**, 4438–4448.

Hartung, F., Suer, S. and Puchta, H. (2007) Two closely related RecQ helicases have antagonistic roles in homologous recombination and DNA repair in *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA*, **104**, 18836–18841.

Hirano, S., Yamamoto, K., Ishiai, M. *et al.* (2005) Functional relationships of FANCC to homologous recombination, translesion synthesis, and BLM. *EMBO J.* **24**, 418–427.

Hoadley, K.A., Xue, Y., Ling, C., Takata, M., Wang, W. and Keck, J.L. (2012) Defining the molecular interface that connects the Fanconi anemia protein FANCM to the Bloom syndrome dissolvasome. *Proc. Natl Acad. Sci. USA*, **109**, 4437–4442.

Kaliraman, V., Mullen, J.R., Fricke, W.M., Bastin-Shanower, S.A. and Brill, S.J. (2001) Functional overlap between Sgs1-Top3 and the Mms4-Mus81 endonuclease. *Genes Dev.* **15**, 2730–2740.

Kim, H. and D'Andrea, A.D. (2012) Regulation of DNA cross-link repair by the Fanconi anemia/BRCA pathway. *Genes Dev.* **26**, 1393–1408.

Kitao, S., Shimamoto, A., Goto, M., Miller, R.W., Smithson, W.A., Lindor, N.M. and Furuichi, Y. (1999) Mutations in RECQL4 cause a subset of cases of Rothmund-Thomson syndrome. *Nat. Genet.* **22**, 82–84.

Knoll, A. and Puchta, H. (2011) The role of DNA helicases and their interaction partners in genome stability and meiotic recombination in plants. *J. Exp. Bot.* **62**, 1565–1579.

Knoll, A., Higgins, J.D., Seeliger, K., Reha, S.J., Dangel, N.J., Bauknecht, M., Schropfer, S., Franklin, F.C. and Puchta, H. (2012) The Fanconi anemia ortholog FANCM ensures ordered homologous recombination in both somatic and meiotic cells in *Arabidopsis*. *Plant Cell*, **24**, 1448–1464.

Kottemann, M.C. and Smogorzewska, A. (2013) Fanconi anaemia and the repair of Watson and Crick DNA crosslinks. *Nature*, **493**, 356–363.

Lorenz, A., Osman, F., Sun, W., Nandi, S., Steinacher, R. and Whitby, M.C. (2012) The fission yeast FANCM ortholog directs non-crossover recombination during meiosis. *Science*, **336**, 1585–1588.

Mannuss, A., Dukowic-Schulze, S., Suer, S., Hartung, F., Pacher, M. and Puchta, H. (2010) RAD5A, RECQ4A, and MUS81 have specific functions in homologous recombination and define different pathways of DNA repair in *Arabidopsis thaliana*. *Plant Cell*, **22**, 3318–3330.

Moldovan, G.-L. and D'Andrea, A.D. (2009) How the Fanconi anemia pathway guards the genome. *Annu. Rev. Genet.* **43**, 223–249.

Molinier, J., Ries, G., Bonhoeffer, S. and Hohn, B. (2004) Interchromatid and interhomolog recombination in *Arabidopsis thaliana*. *Plant Cell*, **16**, 342–352.

Nishino, T., Takeuchi, K., Gascoigne, K.E., Suzuki, A., Hori, T., Oyama, T., Morikawa, K., Cheeseman, I.M. and Fukagawa, T. (2012) CENP-T-W-S-X forms a unique centromeric chromatin structure with a histone-like fold. *Cell*, **148**, 487–501.

Patel, K.J. and Joenje, H. (2007) Fanconi anemia and DNA replication repair. *DNA Repair*, **6**, 885–890.

Pichierri, P., Franchitto, A. and Rosselli, F. (2004) BLM and the FANC proteins collaborate in a common pathway in response to stalled replication forks. *EMBO J.* **23**, 3154–3163.

Puchta, H. and Hohn, B. (2012) In planta somatic homologous recombination assay revisited: a successful and versatile, but delicate tool. *Plant Cell*, **24**, 4324–4331.

Punta, M., Coggill, P.C., Eberhardt, R.Y. *et al.* (2012) The Pfam protein families database. *Nucleic Acids Res.* **40**, D290–D301.

Rink, S.M., Lipman, R., Alley, S.C., Hopkins, P.B. and Tomasz, M. (1996) Bending of DNA by the mitomycin C-induced, GpG intrastrand cross-link. *Chem. Res. Toxicol.* **9**, 382–389.

Scheller, J., Schürer, A., Rudolph, C., Hettwer, S. and Kramer, W. (2000) MPH1, a yeast gene encoding a DEAH protein, plays a role in protection of the genome from spontaneous and chemically induced damage. *Genetics*, **155**, 1069–1081.

Schmid, M., Davison, T.S., Henz, S.R., Pape, U.J., Demar, M., Vingron, M., Scholkopf, B., Weigel, D. and Lohmann, J.U. (2005) A gene expression map of *Arabidopsis thaliana* development. *Nat. Genet.* **37**, 501–506.

Schröpfer, S., Kobbe, D., Hartung, F., Knoll, A. and Puchta, H. (2013) Defining the roles of the N-terminal region and the helicase activity of RECQ4A in DNA repair and homologous recombination in *Arabidopsis*. *Nucleic Acids Res.* **42**(3), 1684–1697.

Singh, T.R., Saro, D., Ali, A.M. *et al.* (2010) MHF1-MHF2, a histone-fold-containing protein complex, participates in the Fanconi anemia pathway via FANCM. *Mol. Cell*, **37**, 879–886.

Sugahara, R., Mon, H., Lee, J.M. and Kusakabe, T. (2013) Middle region of FancM interacts with Mhf and Rmi1 in silkworms, a species lacking the Fanconi anaemia (FA) core complex. *Insect Mol. Biol.* **23**(2), 185–198.

Tao, Y., Jin, C., Li, X., Qi, S., Chu, L., Niu, L., Yao, X. and Teng, M. (2012) The structure of the FANCM–MHF complex reveals physical features for functional assembly. *Nat. Commun.* **3**, 782.

Woody, S.T., Austin-Phillips, S., Amasino, R.M. and Krysan, P.J. (2007) The WiscDsLox T-DNA collection: an Arabidopsis community resource generated by using an improved high-throughput T-DNA sequencing pipeline. *J. Plant. Res.* **120**, 157–165.

Yan, Z., Delannoy, M., Ling, C. *et al.* (2010) A histone-fold complex and FANCM form a conserved DNA-remodeling complex to maintain genome stability. *Mol. Cell*, **37**, 865–878.

