Journal of Experimental Botany, Vol. 62, No. 5, pp. 1565–1579, 2011
doi:10.1093/jxb/erq357 Advance Access publication 16 November, 2010

Journal of Experimental Botany
www.jxb.oxfordjournals.org

REVIEW PAPER

The role of DNA helicases and their interaction partners in genome stability and meiotic recombination in plants

Alexander Knoll and Holger Puchta*

Botanical Institute II, Karlsruhe Institute of Technology, D-76131 Karlsruhe, Germany

* To whom correspondence should be addressed. E-mail: Holger.Puchta@kit.edu

Received 28 August 2010; Revised 15 October 2010; Accepted 20 October 2010

Abstract

DNA helicases are enzymes that are able to unwind DNA by the use of the energy-equivalent ATP. They play essential roles in DNA replication, DNA repair, and DNA recombination in all organisms. As homologous recombination occurs in somatic and meiotic cells, the same proteins may participate in both processes, albeit not necessarily with identical functions. DNA helicases involved in genome stability and meiotic recombination are the focus of this review. The role of these enzymes and their characterized interaction partners in plants will be summarized. Although most factors are conserved in eukaryotes, plant-specific features are becoming apparent. In the RecQ helicase family, *Arabidopsis thaliana* RECQ4A has been shown before to be the functional homologue of the well-researched baker's yeast Sgs1 and human BLM proteins. It was surprising to find that its interaction partners *At*RMI1 and *At*TOP3α are absolutely essential for meiotic recombination in plants, where they are central factors of a formerly underappreciated dissolution step of recombination intermediates. In the expanding group of anti-recombinases, future analysis of plant helicases is especially promising. While no *FBH1* homologue is present, the *Arabidopsis* genome contains homologues of both *SRS2* and *RTEL1*. Yeast and mammals, on the other hand. only possess homologues of either one or the other of these helicases. Plants also contain several other classes of helicases that are known from other organisms to be involved in the preservation of genome stability: FANCM is conserved with parts of the human Fanconi anaemia proteins, as are homologues of the Swi2/Snf2 family and of PIF1.

Key words: Chromatin remodelling, DNA repair, double strand break, genome stability, helicase, homologous recombination, meiosis, translocase.

Introduction

The genome of all organisms is in necessary balance between variation for natural selection and the suppression of change harmful to cells or even the whole organism. Genome stability, the interplay between accidental and deliberate modifications of DNA on the one hand and the mechanisms that act to preserve DNA sequence on the other hand, was the focus of genetics even before the discovery of the structure of DNA. Several repair mechanisms exist for basically all types of chemical modifications that can occur on DNA. Interestingly, the most severe damage—the DNA double strand break (DSB)—has to be repaired in order for the cell to survive, but is also induced by sexually propagating eukaryotes to initiate the mixing of their parental genetic material in meiosis (for recent reviews on meiotic recombination in plants see Mercier and Grelon, 2008; Sanchez-Moran *et al.*, 2008; Ronceret and Pawlowski, 2010). Thus, DSB repair is essential for somatic and meiotic cells, and the process itself is characterized by common steps but also by interesting differences in both cell types.

When looking at the protein composition of sequenced genomes, proteins annotated as helicases comprise ~1%. For example, in *Arabidopsis thaliana* (L.) Heynh., 163 gene products have been annotated with the Gene Ontology (GO) term 'helicase activity' (GO:0004386). This may seem a surprisingly large number, since the textbook understanding of a helicase is an enzyme that separates a DNA double

strand into two single strands utilizing the energy generated by hydrolysing nucleoside triphosphates (NTPs). There are several reasons why the genome databases contain >100 protein entries for this task per organism. First, there are genes that were bioinformatically identified as helicases as they show sequence similarity to domains of known helicase families, but they do not have any intrinsic ATPase or DNA-unwinding activity. Secondly, not every protein with a predicted helicase domain and ATPase activity will actually unwind a DNA double strand. The so-called translocases use the energy from hydrolysing NTPs to move along single- or double-stranded nucleic acid strands. Thirdly, because the backbone of DNA–DNA, RNA–RNA, and DNA–RNA duplexes differs sterically, different proteins are needed to bind to and to unwind such duplexes. Finally, some different 'true' helicases are present in the nucleus to process various non-standard DNA structures, for example Holliday junctions (HJs) or G-quadruplexes. All of those helicases can be grouped into superfamilies and families based on the sequence and structure of their helicase domains, but broad discussion of this aspect goes beyond the scope of this review.

DSB repair and homologous recombination (HR)

The most simple and, in multicellular eukaryotes such as plants the most prevalent, way to repair a DSB is via non-homologous end-joining (NHEJ) (for a review, see Puchta, 2005). Here, the ends of the break are joined, and eventual gaps or overhangs are processed until finally the backbones of the two strands are ligated to seal the break. In a surprisingly large number of cases, repair of a DSB by NHEJ is associated with deletions or insertions of foreign sequences at the break site, making NHEJ an effective but mutagenic pathway in plants (Salomon and Puchta, 1998; Kirik *et al.*, 2000).

When direct repeat sequences are present on both sides of a DSB, repair can occur via the mechanism of single strand annealing (SSA). Here, single-strand resection on both sides of the break exposes the repeated region, which makes annealing possible. The remaining gaps need to be filled up and overhangs cut off for ligation to finish repair. Since it is inherent to the SSA mechanism that all DNA between the two repeats is lost, it is always associated with deletions (Siebert and Puchta, 2002).

While most of our understanding of how HR proceeds comes from the study of meiosis in yeast, the resulting picture can still be transferred to somatic cells and to the HR of other organisms. Today's view of the repair of DNA DSBs by HR is best described as an amalgam of originally competing pathways. The first steps of HR (Fig. 1, steps I–V) are commonly shared between all the following models (Szostak *et al.*, 1983; Nassif *et al.*, 1994). Following a DSB, induced either by a protein like Spo11 in meiosis or by accident through irradiation or a chemical agent in somatic cells, the ends are resected to produce single-stranded 3' overhangs. The free single-stranded ends will then be bound by multiple units of the Rad51 recombinase to form a Rad51–ssDNA filament. This filament is able to invade a homologous donor dsDNA molecule, and base pairing with the complementary strand will form a heteroduplex molecule, thereby displacing the second strand of the donor (Fig. 1, step IV). The resulting structure is called a displacement loop (D-loop). The free 3' end of the invading single strand can then be elongated by a DNA polymerase, using the donor strand as template. The D-loop can either be enlarged by this polymerase action (Fig. 1, step V), or move along with the elongating end (not shown). In the double strand break repair (DSBR) model of HR (Szostak *et al.*, 1983), the D-loop will reach a point where the sequence in the displaced strand of the donor molecule is complementary to the second single-stranded end of the DSB, which will bind to it (Fig. 1, step VIII). After closing the gaps through the action of DNA polymerases and ligases, a so-called double Holliday junction (dHJ) structure is formed (Fig. 1, step IX). There are no free ends left at this step; however, it is still essential to separate the two dsDNA molecules. According to the DSBR model, this step (Fig. 1, step X) is carried out by special endonucleases, so-called resolvases, that can bind to a HJ and make symmetrically opposing cuts in the DNA strands. After a final ligation step, the two dsDNA molecules are separated. Depending on the orientation of the resolvase cuts on the two HJs, the HR reaction will result either in a crossover (CO) or in a gene conversion (non-crossover, NCO).

Alternatively, at the D-loop step (Fig. 1, step V) repair can proceed according to the synthesis-dependent strand annealing (SDSA) model (Nassif *et al.*, 1994). Here, instead of capturing the second end of the break and forming a dHJ structure, the invaded strand is released from the D-loop after elongation. This enables it to anneal to the second end (Fig. 1, step VI). After the closing of gaps by DNA polymerases and ligases, the DSB has been repaired and the damaged double strand is intact again (Fig. 1, step VII). Following this route, COs are not possible. About 10 years ago, with meiotic recombination data from *Saccharomyces cerevisiae*, these two conflicting models were combined into one consolidated scheme, called the revised model (Allers and Lichten, 2001; Hunter and Kleckner, 2001).

Research in the past 20 years has shown that a number of different helicase families are involved in several steps of the HR reaction, and their loss can lead to critical problems in the cell. In humans, mutations in those helicases have been implicated in development of cancer and debilitating hereditary diseases. Meiotic recombination is affected as well, when some of these helicases are missing. From a basic research point of view, it is important to decipher the similarities and differences in the functions of the proteins involved in genome stability and HR. For the most part, research in those fields has been restricted to the animal/fungi clade of eukaryotes. Since DNA repair and recombination is thought to have evolved very early, the research on those mechanisms on the plant side of eukaryotes can enable the comparison of these pathways in different kingdoms. Moreover, some DNA repair and recombination mutants that are

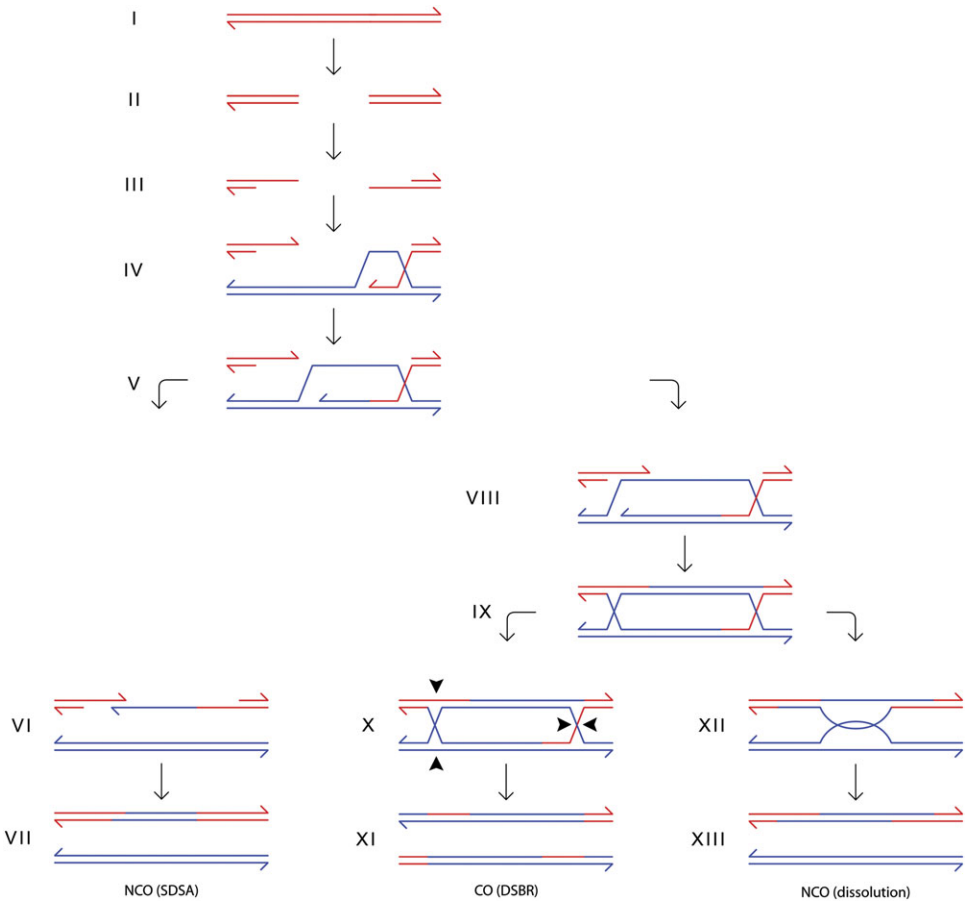

Fig. 1. Homologous recombination (HR) repair of a DNA double strand break (DSB). Following a DSB (step II), the free ends of the break are resected to produce 3' single-stranded overhangs (step III). Such a single-stranded region can invade a homologous duplex region, thereby displacing one of the strands and producing a so-called D-loop (step IV). With the intact donor strand as template, the invading strand can be elongated (step V). The revised model of HR proposes a bifurcation in the pathway here: one route follows steps similar to the synthesis-dependent strand annealing (SDSA) model, where the now elongated single strand is removed from the D-loop so that it can anneal with the second end of the break (step VI), which is repaired after the remaining gaps have been closed (step VII). In this manner, only non-crossover (NCO) products can occur. In the other route, second end capture (step VIII) and closing of gaps produces a double Holliday junction (dHJ; step IX). Specialized endonucleases (resolvases) make symmetrical cuts at the junction points (step X), thereby leading either to CO or NCO products depending on the orientation of the cut (step XI). This branch of the revised model is reminiscent of the DSBR model of HR. In recent years, the dHJ dissolution model has been added in yeast and human somatic HR as an alternative to resolution. There, a hemicatenane structure is formed through branch migration of the two HJs (step XII). This can be repaired by a type I topoisomerase to form only NCO products (step XIII). The same mechanism seems to be an important pathway in *A. thaliana* meiosis.

embryo lethal in mammals are viable in plants, making meiotic studies possible (W. Li *et al.*, 2004; Reidt *et al.*, 2006). There is also an applied aspect to this research in plants: taking control of the recombination reactions could enable researchers to improve plant biotechnology by using directed methods of genome modification.

In the following sections, different classes of helicases will be discussed that are present in plants and play a role in somatic and meiotic recombination (see Table 1 for homologues in humans and yeast, and Table 2 for published *in vivo* and *in vitro* data of the plant helicases discussed below). The review will mainly focus on recent data found in plants. However, not all the enzymes discussed in this review have been characterized in detail in plants yet. As their presence in various plant species indicates their general importance, data obtained in other eukaryotes will also be referred to in order to describe their function.

RecQ helicases

First described in a screen for thymineless death-resistant mutants in *Escherichia coli* (Nakayama *et al.*, 1984), RecQ was only the first of many members of a subfamily of 3'–5' DNA helicases conserved across all domains of life with important roles in the maintenance of genome stability. Virtually all organisms tested so far possess at least one RecQ helicase gene, with increasing numbers of copies found in multicellular eukaryotes (see Fig. 2 for a schematic overview of the protein structure of model RecQ helicases;

Table 1. Fungal and human homologues of plant helicases

Where sequence comparisons, structural or functional data allow identification, homologues or analogues of the plant DNA helicases discussed in this review are given.

Plant helicase	Homologous gene
AtFANCM	ScMPH1
	HsFANCM
AtINO80	ScINO80
	HsINO80
AtMER3/AtRCK	ScMER3
	HsHFM1
AtPIF1	ScPIF1
	ScRRM3
	HsPIF1
AtRAD54	ScRAD54
AtRAD5A	ScRAD5
	HsHLTF
	HsSHPRH
AtRECQ2	HsWRN
AtRECQ4A	ScSGS1
	HsBLM
AtSRS2	ScSRS2
AtSWR1	ScSWR1
	HsSRCAP

for recent reviews see Ouyang *et al.*, 2008; Ashton and Hickson, 2010; Rossi *et al.*, 2010). The expression of *E. coli* RecQ was shown to increase the somatic HR rate in rice (H. Q. Li *et al.*, 2004), which was surprising since RecQ helicases generally are involved in the suppression of HR.

In the yeast *S. cerevisiae*, knocking out its unique RecQ helicase *SGS1* leads to a hyper-recombination phenotype, sensitivity against several genotoxic agents, and also meiotic defects (Watt *et al.*, 1995, 1996; Sinclair and Guarente, 1997).

In humans, three of the five RecQ helicases are associated with severe hereditary diseases. Mutations in the *BLM* gene result in Bloom syndrome (BS), which is characterized by an elevated susceptibility to all types of cancers and several developmental defects (Ellis *et al.*, 1995). On the cellular level, BS cells display genome instability due to an increased rate of sister chromatid exchanges (SCEs) and DNA repair defects (Chaganti *et al.*, 1974). Werner syndrome (WS) is found in persons with mutations in the *WRN* gene (Yu *et al.*, 1996). Here, the occurrence of some types of cancer is more prominent than in the normal population. More interestingly, WS patients show a type of segmental progeria. From the middle of the second decade on, their ageing is accelerated, with typical age-related malignancies such as osteoporosis, diabetes mellitus, and cataracts occurring earlier in life (Epstein *et al.*, 1966; Goto *et al.*, 1996). Finally, mutations in the *RECQL4* gene have been shown to be the basis of at least a subset of cases of the hereditary diseases Rothmund–Thomson syndrome (RTS), Baller–Gerold syndrome (BGS), and RAPADILINO syndrome (Kitao *et al.*, 1999; Siitonen *et al.*, 2003; Van Maldergem *et al.*, 2006). There are intriguing results that open up the possibility that RECQL4 is important for the initiation of replication and could be a second replicative helicase beside the MCM2-7 complex in vertebrates (Matsuno *et al.*, 2006; Xu and Liu, 2009; X. Xu *et al.*, 2009; Capp *et al.*, 2010). The two remaining human RecQ helicases, RECQL and RECQL5, have not been associated with hereditary diseases so far. This does not exclude a role for these proteins in DNA repair or recombination, though. It was recently shown that sequence variants of *RECQL* can influence the outcome of pancreatic cancer (Li *et al.*, 2006), and knockdown of *RECQL5* when BLM is missing will increase the already elevated level of SCEs even more (Hu *et al.*, 2005).

In plants, at least seven different RecQ helicases can be found (Hartung *et al.*, 2000; Hartung and Puchta, 2006). *Arabidopsis thaliana* possesses six of them, one duplicated in the Brassicaceae for a total of seven genes: RECQ1, RECQ2, RECQ3, and RECQ5 are small proteins of 600–700 amino acids, while RECQ4A and RECQ4B are larger at >1100 amino acids. RECQsim is of intermediate length, but is unique compared with RecQ helicases in other kingdoms in that it has an insertion of mainly acidic amino acids in its helicase domain. In rice and many other plant species, there is a RecQ helicase not found in *Arabidopsis*, RECQ886 (Hartung and Puchta, 2006). Additionally, in plants there is a small protein, WRNexo, with only an exonuclease domain that is most similar to the exonuclease domain found in the human WRN RecQ protein.

Of these plant RecQ helicases and related proteins, still very little is known. Os*RECQ1* has been shown to be required for transcription from loci that are able to form cruciform structures, possibly pointing to a role in RNA silencing (H. Chen *et al.*, 2008). Rice *RECQ1*, Os*RECQ886*, and Os*RECQsim* are expressed in meristematic rice tissues. The expression of these three genes, and of rice *RECQ2*, can be induced by genotoxic agents (Saotome *et al.*, 2006). The study also showed the localization of green fluorescent protein (GFP) fusion proteins of rice RECQ2 and RECQ886 in the nucleus, while rice RECQ1 and RECQsim were detected in plastids. Whether the latter two proteins indeed have a function in genome stability of chloroplast DNA awaits future mutant analysis. *Arabidopsis* RECQ2 and RECQ3 have been studied *in vitro* (Kobbe *et al.*, 2008, 2009, 2010). Both are DNA helicases that unwind partial duplex DNA in a 3' to 5' fashion. Testing more complex DNA substrates that resemble intermediates of DNA repair and recombination, the authors found differences in the activities of the two enzymes: RECQ2 resolves a partially mobile HJ structure in a way reminiscent of branch migration. Additionally, both enzymes act on nicked HJs, but interestingly with different preferences concerning the outcome of the reaction. RECQ2 and RECQ3 also act on substrates that mimic replication forks. Here, RECQ2 can regress the fork into a so-called 'chicken foot', which is a cruciform DNA intermediate similar to a HJ proposed for repair and recombination reactions at the replication fork (see Fig. 3 for a model of replication fork regression). RECQ3, however, will unwind the lagging strand of a replication fork structure.

Table 2. Phenotypes of plant DNA helicases

Given are all plant DNA helicases involved in somatic or meiotic homologous recombination that are mentioned in this review. Where applicable, the published phenotypes of the helicases are given, together with the relevant references. See text for details.

Helicase	*In vivo* phenotype	*In vitro* activity	References
AtRECQ1	NA	NA	Hartung *et al.* (2000)
AtRECQ2	NA	Unwinds partial duplex structures *in vitro*; branch migrates a partially mobile HJ structure *in vitro*; promotes fork regression of a model replication fork *in vitro*	Hartung *et al.* (2000); Kobbe *et al.* (2008)
AtRECQ3	NA	Unwinds partial duplex and replication fork structures *in vitro*	Hartung *et al.* (2000); Kobbe *et al.* (2009)
AtRECQ4A	Involved in DNA repair of several types of damage; elevated HR rate in mutant; part of the RTR complex with TOP3α and RMI1; dissolves telomere interactions between non-homologous chromosomes in meiotic prophase I	NA	Bagherieh-Najjar *et al.* (2005); Hartung *et al.* (2000, 2006, 2007, 2008); Higgins and Franklin (personal communication)
AtRECQ4B	Reduced HR rate in mutant	NA	Hartung *et al.* (2000, 2007)
AtRECQ5	NA	NA	Hartung and Puchta (2006)
AtRECQsim	Suppresses yeast *sgs1* mutant MMS sensitivity	NA	Bagherieh-Najjar *et al.* (2003); Hartung *et al.* (2000)
OsRECQ1	Expressed in meristematic tissues; induction of expression by genotoxic agents; GFP fusion protein detected in plastids; involved in gene silencing	NA	H. Chen *et al.*, (2008); Saotome *et al.* (2006)
OsRECQ2	Induction of expression by genotoxic agents; GFP fusion protein detected in nucleus	NA	Saotome *et al.* (2006)
OsRECQsim	Expressed in meristematic tissues; induction of expression by genotoxic agents; GFP fusion protein detected in plastids	NA	Saotome *et al.* (2006)
OsRECQ886	Expressed in meristematic tissues; induction of expression by genotoxic agents; GFP fusion protein detected in nucleus	NA	Saotome *et al.* (2006)
AtSRS2	NA	Unwinds nicked and partial HJs *in vitro*; anneals two single strands into a dsDNA molecule *in vitro*	Blanck *et al.* (2009)
AtFANCM	NA	NA	Our unpublished data
AtINO80	Strong reduction of HR rate in mutant	NA	Fritsch *et al.* (2004)
AtSWR1	Involved in plant development, flowering time regulation, and immunity	NA	March-Diaz and Reyes (2009)
AtRAD54	Partially complements DNA repair defects of yeast *rad54* mutant; sensitivity against γ-irradiation; strong reduction of HR rate in mutant	Interacts with AtRAD51 *in vitro*	Klutstein *et al.* (2008); Osakabe *et al.* (2006)
AtRAD5A	Mutant is sensitive against cross-linking and methylating genotoxins; reduced HR rate in mutant after induction of DSBs; most probably the SDSA branch of HR is affected	NA	I.P. Chen *et al.*, (2008); Mannuss *et al.* (2010)
AtRAD5B	Mutant not different from the wild type in the assays also used with AtRAD5A	NA	I.P. Chen *et al.*, (2008)
AtMER3/AtRCK	Mutant has reduced fertility, reduced number of COs; only class I COs are affected	NA	Chen *et al.* (2005); Mercier *et al.* (2005)
AtPIF1	NA	NA	Bochman *et al.* (2010)

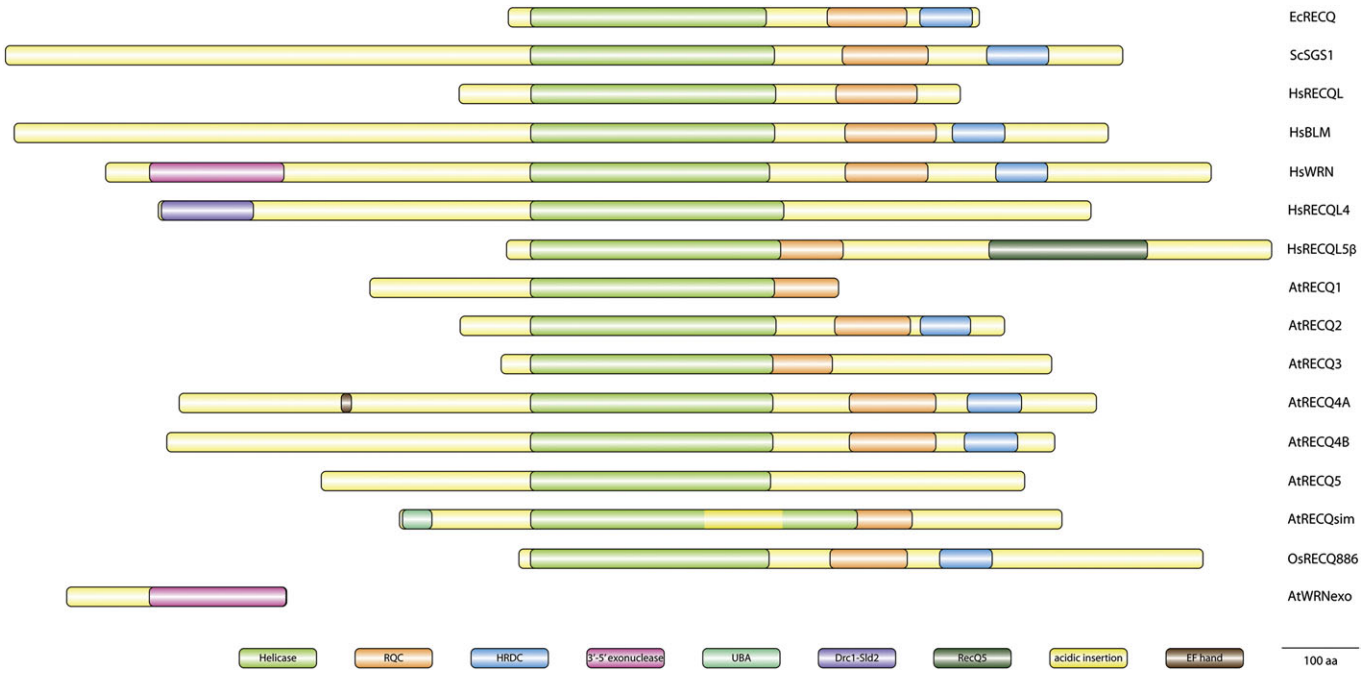

Fig. 2. Domain composition of selected RecQ family helicases. The proteins are aligned at their helicase domains. Two further domains can be found in the RecQ helicase family, the RecQ C-terminal domain (RQC) and the helicase and RNase D C-terminal domain (HRDC). Both are most probably needed for the recognition of and binding to unconventional DNA structures, e.g. Holliday junctions. The RQC domain is composed of a zinc finger motif followed by a winged helix, and while most RecQ helicases possess a RQC domain, in *Hs*RECQL5β, *At*RECQ1, *At*RECQ3, and *At*RECQsim only the zinc finger is conserved. The HRDC domain is only found in about half of the RecQ helicases. For individual RecQ helicases, further domains have been described. In the human WRN protein, a 3' to 5' exonuclease domain can be found that is also present in a small *Arabidopsis* protein WRNexo. The HsRECQL4 helicase has recently been shown to contain a region similar to the *S. cerevisiae* Sld2 protein at the N-terminus which is thought to connect the RecQ helicase with replication initiation. In animal homologues of RECQ5, a large C-terminal region seems to be conserved, although no function could yet be assigned. Finally, in plant RecQ helicases two domains can be identified bioinformatically. In *Arabidopsis* RECQ4A an EF-hand motif might be present at the N-terminus, while *At*RECQsim possesses a UBA domain which has been shown to interact with ubiquitin. RECQsim also contains an insertion of mainly acidic residues in its helicase domain. Ec, *Escherichia coli*; Sc, *Saccharomyces cerevisiae*; Hs, *Homo sapiens*; At, *Arabidopsis thaliana*; Os, *Oryza sativa*.

Arabidopsis RECQ4A and *RECQ4B* arose from a recent duplication of a chromosome segment in the family Brassicaceae which resulted in two genes very similar in their protein sequence (Hartung *et al.*, 2007). However, despite these similarities, the two gene products seem to perform very different functions in the cell. RECQ4A is comparable in many phenotypes with the yeast Sgs1 and the human BLM RecQ helicases; a knockout leads to hyper-recombination and increased sensitivity against a number of genotoxins. Expression of *RECQ4A* in a yeast *sgs1* mutant suppresses its methylmethane sulphonate (MMS) sensitivity and brings its increased recombination rate down to wild-type levels (Bagherieh-Najjar *et al.*, 2005). A *recq4B* mutant, on the other hand, has so far not shown any defect in DNA repair, but has a reduced HR rate, a phenotype not known for any other RecQ helicase described so far (Hartung *et al.*, 2007). Despite the insertion of ~100 amino acids in its helicase domain, *RECQsim* is able to suppress the MMS sensitivity of a yeast *sgs1* mutant (Bagherieh-Najjar *et al.*, 2003), indicating that it is functional despite the insertion.

Looking at the various RecQ homologues in plants and animals, the question arises of whether one could define functional 'one to one' homologues for both clades. Unfortunately, the sequence homologies are not strong enough for an unequivocal classification of all homologues. However, due to the fact that interactions are genetically conserved, at least for WRN and BLM the respective homologues in plants can be identified.

The small protein *At*WRNexo was shown to possess *in vitro* exonuclease activities very similar to the exonuclease domain of *Hs*WRN (Plchova *et al.*, 2003), and it interacted with *Arabidopsis RECQ2*, but not *RECQ1* or *RECQ3*, in a yeast-two-hybrid assay (Hartung *et al.*, 2000). Taken together, it could be interpreted as two separate proteins in *Arabidopsis*, WRNexo and RECQ2, performing the functions of one human protein, WRN. This interpretation is underpinned by similar biochemical properties of *At*RECQ2 and the helicase domain of WRN (Kobbe *et al.*, 2008) as well as by a recent description of a coiled-coil region in *Hs*WRN in front of its helicase domain. Of all seven RecQ helicase proteins in *A. thaliana*, only in RECQ2 can a similar coiled-coil region be found in the same place *in silico* (F. Hartung, personal communication). Similar to the known functions of the human WRN protein in recombination at

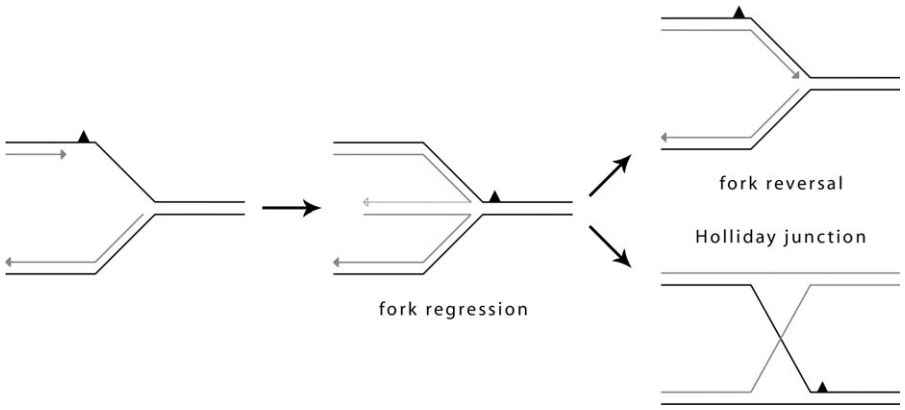

Fig. 3. Bypass of leading strand lesions by replication fork regression. When the replication fork encounters a lesion in the leading strand that cannot be overcome by the replicative DNA polymerase, one pathway to bypass the lesion and proceed with replication is post-replicative repair (PRR; see text for details). In the undamaged lagging strand, overshoot synthesis will elongate the nascent daughter strand past the position of the lesion in the leading strand. Yeast Rad5 and its human homologue HLTF have been shown to use their DNA translocase activity to then regress the fork. In the resulting structure, a so-called 'chicken foot' which resembles the Holliday junction, the two daughter strands can anneal to each other. Because of the overshoot synthesis, the shorter of the strands can now be elongated. After a reversal of the fork, the lesion on the leading strand has been bypassed without introducing a point mutation. Alternatively, the chicken foot can also initiate a homologous recombination reaction in S-phase.

the telomeres, *Arabidopsis* WRNexo and the NHEJ factor KU70 interact with each other *in vitro* (Li *et al.*, 2005), as do human WRN and KU70 (Cooper *et al.*, 2000).

A yeast double mutant of *SGS1* and the endonuclease *MUS81*, which has been shown to act in DNA repair and recombination, is lethal (Mullen *et al.*, 2001). In yeast meiosis, both proteins are needed for the proper progression through meiotic recombination, and their loss leads to the accumulation of meiotic intermediates (Jessop and Lichten, 2008; Oh *et al.*, 2008). This synthetic lethality is also conserved in *Arabidopsis*, where a *recq4A mus81* double mutant will die shortly after germination (Hartung *et al.*, 2006), and recently the lethality was shown to be dependent on HR, as further mutation of the *RAD51C* gene can rescue this phenotype (Mannuss *et al.*, 2010). Thus, the helicase RECQ4A as well as the nuclease MUS81 (Geuting *et al.*, 2009) are involved in the processing of replicative recombination intermediates in *Arabidopsis*.

Furthermore, it was demonstrated in recent years that a protein complex consisting of a RecQ helicase (Sgs1 in yeast, BLM in human), a topoisomerase 3 homologue (Top3 and TOPO3α in yeast and human, respectively), and the structural protein RMI1 can perform the dissolution reaction of dHJs *in vitro* and *in vivo* (Gangloff *et al.*, 1994; Wu *et al.*, 2000, 2005; Wu and Hickson, 2002). Dissolution was theoretically proposed as an alternative model for DSB repair by Thaler and Stahl (1988), and it has received a form of renaissance in recent years. The dissolution model is very similar to the DSBR model in its first steps, and it also leads to a dHJ structure. However, instead of a dHJ resolution by an endonuclease, the dissolution reaction will produce a hemicatenane structure by using a DNA helicase to branch migrate the two HJs together (Fig. 1, step XII). A specialized type I topoisomerase can then open the hemicatenane to release the two dsDNA molecules (Fig. 1, step XIII).

The RecQ helicase is needed to transform two HJs into a hemicatenane that can then be resolved by the type I topoisomerase. Through protein interactions, RMI1 will stabilize the complex and indirectly enhance the dHJ dissolution reaction (Chang *et al.*, 2005; Mullen *et al.*, 2005; Yin *et al.*, 2005; Raynard *et al.*, 2006; Wu *et al.*, 2006). This so-called RTR complex has been shown to be conserved in plants, too. There are homologues of TOP3α and RMI1 in *Arabidopsis*, and the functional homologue of Sgs1 and BLM, RECQ4A, is the most probable partner in the *Arabidopsis* RTR complex. All three genes share common DNA repair and recombination phenotypes consistent with the action of the RTR complexes in other organisms (Hartung *et al.*, 2008). For the first time in any eukaryote, an important role for members of the RTR complex in meiotic recombination could be shown *in planta*. Both *top3α* and *rmi1* mutants are homozygous sterile, and display very similar cytological phenotypes. After apparent chromosome fragmentation in prophase I, fragmented DNA stays at the metaphase plate and is not moved to the poles in anaphase I, with DNA bridges connecting the chromosome fragments. Both mutants never enter meiosis II and arrest at the end of meiosis I as dyads (Chelysheva *et al.*, 2008; Hartung *et al.*, 2008). While *recq4A* mutants do not display similar defects in meiotic recombination, a meiotic role for the helicase has been elucidated recently: RECQ4A localizes to telomeric foci during prophase I and is thought to dissolve telomere interactions between non-homologous chromosomes (J. D. Higgins and F.C. Franklin, personal communication).

Thus, dHJ dissolution by TOP3α plays a very important role in meiotic recombination, although dissolution products can only lead to NCOs. In contrast to previous thoughts, the decision as to which of the DSBs will become COs and NCOs might therefore not be made in all cases

early in meiotic HR at the stage of the D-loop. Later, at the stage of the dHJ, for a second time during the recombination reaction a choice in direction to a NCO outcome becomes possible. To describe meiotic HR fully, the revised model therefore has to be amended by the dissolution pathway, at least for *A. thaliana* (see Fig. 1 for a HR scheme uniting the revised model with the dissolution reaction of the RTR complex).

Anti-recombinases

DNA helicases that suppress HR pathways leading to COs are collectively called anti-recombinases. The best understood eukaryotic anti-recombinase is the *S. cerevisiae* Srs2 protein, which promotes alternative pathways to repair lesions at the replication fork by inhibiting COs (Macris and Sung, 2005). Srs2 is recruited to a stalled replication fork by SUMOylated proliferating cell nuclear antigen (PCNA). There it has been shown to bind to the displaced strand of a D-loop and to translocate along it with a 3′–5′ polarity. When it reaches the end of the D-loop, Srs2 starts to unwind the double strand. This generates a single-stranded region on the template strand, where Srs2 can switch to. Stimulated by Rad51 bound to the invading strand, Srs2 will disrupt the heteroduplex strand by its helicase activity, thus enabling the now free extended end to anneal to the second end of the DSB, resulting in an NCO outcome via the SDSA pathway (Dupaigne *et al.*, 2008).

While most of the work on Srs2 has been done in yeast, it seems to be conserved across most eukaryotes, with potential homologues also found in animals (though missing in teleostei and mammals) and in all plants. An *in vitro* study recently showed that *A. thaliana* SRS2 is a 3′–5′ DNA helicase that acts on recombinogenic DNA intermediates (Blanck *et al.*, 2009). The preferentially unwound substrates, nicked and especially partial HJs, resemble DNA structures early in HR and their unwinding would lead to an NCO outcome via SDSA *in vivo*. Therefore, *At*SRS2 resembles in its basic function the yeast model Srs2 protein. The same study showed, however, a new activity for *At*SRS2, which is capable of annealing of two single strands of DNA to a double strand. This can also be integrated into a recombination model: after the release of the single strand from the D-loop by SRS2, it can be annealed by the same protein to the second free end, which would be the following step in the SDSA pathway. However, no reports on the role of AtSRS2 *in vivo* have been published yet.

Apart from *SRS2* homologues, other proteins have been described as anti-recombinases in eukaryotes. FBH1, which is found in many fungi (but not *S. cerevisiae*) as well as animals, is a unique protein in that it contains a helicase domain as well as an F-box domain. Via this F-box domain, the human FBH1 has been shown to form an SCF complex with SKP1 and Cullin and to display ubiquitin ligase activity (Kim *et al.*, 2002, 2004). Human FBH1 is able to rescue the DNA repair and recombination defects of yeast *srs2* mutants. Interestingly, both the helicase and the F-box domain are needed for this function (Chiolo *et al.*, 2007). Not only are single mutant phenotypes similar between *SRS2* and *FBH1*, but also genetic and physical interactions with other genes or their products, respectively. Knocking out the single RecQ helicase of *S. cerevisiae* in an *srs2* mutant background is lethal in a recombination-dependent fashion, as is an *rqh1* mutation (the *Schizosaccharomyces pombe SGS1* functional homologue) when Sp*FBH1* is missing (Morishita *et al.*, 2005), or a *BLM*/*FBH1* double mutant in vertebrates (Kohzaki *et al.*, 2007). Since there is no *SRS2* homologue in mammals, it has been proposed on the basis of so much similarity between SRS2 and FBH1 that FBH1 could be an evolutionarily unrelated but functional homologue performing those essential SRS2 functions. In *Arabidopsis*, a *FBH1* homologue seems to be missing. There is only one protein with both an F-box and a helicase domain in the most recent release of the *Arabidopsis* genome, At3g54460 (G. Xu *et al.*, 2009). The helicase domain, however, is more similar to the RAD5/RAD16 family of helicases, and not FBH1 (I. P. Chen *et al.*, 2008).

Recently, a further functional homologue of yeast Srs2 has been described in animals. RTEL1 is a DNA helicase related to the Fanconi anaemia (FA) protein FANCJ/BRIP1. Both share functions in unwinding G-quadruplex DNA at telomeres, hence the telomere defects in *RTEL1* mutants. Single nucleotide polymorphisms (SNPs) in *RTEL1* have been associated with increased susceptibility to glioma and survival span in glioblastoma (Shete *et al.*, 2009; Wrensch *et al.*, 2009; Liu *et al.*, 2010). More basic studies on the role of *RTEL1* in DNA repair and recombination have been done in *Caenorhabditis elegans* and in human cell culture, where it has been shown to share many phenotypes with *SRS2*: a knockout or knockdown leads to defective DNA repair and hyper-recombination, and as is the case with *FBH1*, lethal double mutants are also conserved (Barber *et al.*, 2008). The same group showed that human RTEL1 *in vitro* disrupts pre-formed D-loops, but cannot remove RAD51 from ssDNA filaments. Recently, a role in meiotic recombination was identified for *C. elegans* RTEL-1. Without it, the overall number of COs is increased, and CO interference is compromised (Youds *et al.*, 2010). Taken together with the somatic functions, one could speculate whether RTEL-1 is the helicase to lead those meiotic DSBs not destined to become COs into NCO products via the SDSA pathway. Like many other DNA repair and recombination proteins, RTEL1 is conserved in most eukaryotes, but missing in baker's yeast. In *Arabidopsis*, it is most similar to a putative homologue of the yeast CHL1 protein and FANCJ/BRIP1, which was duplicated in the evolution of the Brassicaceae (our own unpublished data). The study of the anti-recombinases SRS2 and RTEL1 and their interplay in plants promises interesting results, because yeast and mammals only possess homologues of either one or the other of these helicases.

FANCM/Mph1

The human hereditary disease Fanconi anemia (FA) correlates with a broad spectrum of clinical phenotypes,

including reduced numbers of all types of blood cells, leukaemia, and other forms of cancer and developmental defects. FA is caused by mutations in one of 13 different genes called *FANCA*, *FANCB*, *FANCC*, *FANCD1*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCI*, *FANCJ*, *FANCL*, *FANCM*, and *FANCN*. Common cellular phenotypes are disturbances in the regulation of the cell cycle and apoptosis, spontaneous chromosome breaks and radial chromosomes, as well as sensitivity against interstrand cross-link-inducing genotoxins such as mitomycin C (Nevelling *et al.*, 2009). Most of the FA proteins are grouped into the so-called core complex, which binds to stalled replication forks at sites of DNA interstrand cross-links. There, it is thought to ubiquitinate a heterodimer of FANCD2 and FANCI, which are in turn recruited to the damaged DNA. This enables the pair to activate downstream effectors, including the FA genes FANCD1 (BRCA2), FANCJ, and FANCN, but also other known proteins of the DNA repair and recombination pathways, for example the kinase ATR or the RecQ helicase BLM (Wang, 2007; Deans and West, 2009; Moldovan and D'Andrea, 2009). Of the 13 human genes, five are conserved in plants—*FANCM* and *FANCL* of the core complex, *FANCD2* and *FANCD1*, and *FANCJ* of the downstream effectors (our own unpublished data). Interestingly, these are the FA genes for whose products a biochemical function could be shown. *FANCM* stands out among the FA genes as it is the only one of the 13 that is also conserved in baker's yeast, there named *MPH1*, and archaea, where the homologue is called Hef.

Human FANCM is the only protein in the FA core complex with DNA-interacting domains, and it has been shown to bind to DNA. Located at the N-terminus is a helicase domain for which ATPase activity, but no true unwinding activity, could be demonstrated. Therefore, FANCM is thought to act as a dsDNA translocase that binds to DNA and transports the FA core complex along it. Interestingly, this translocase activity is able to branch migrate replication forks and HJs and to dissociate D-loops *in vitro* (Meetei *et al.*, 2005; Gari *et al.*, 2008*a*, *b*). Similar to the archaeal Hef protein, mammalian FANCM homologues possess an additional endonuclease domain at the C-terminus; mutations in key residues in this domain indicate that it is most probably inactive. A comparable protein in the human genome with both helicase and endonuclease domains is XPF/ERCC4, which is associated with the nucleotide excision repair pathway. The XPF endonuclease family also contains MUS81, and both interact with a shorter protein—ERCC1 and EME1, respectively—via their C-terminal endonuclease domains. This interaction is also conserved in FANCM, with the recently identified small interaction partner FAAP24, which is needed to suppress FA phenotypes in cells (Ciccia *et al.*, 2007).

The baker's yeast FANCM homologue Mph1 is shorter than its mammalian counterpart, lacking the C-terminal region including the endonuclease domain. A FAAP24 homologue could not be identified so far. Mph1 also seems to be involved in the repair of DNA damage at stalled replication forks. In contrast to FANCM, a DNA-unwinding activity has been shown for Mph1, making it a true DNA helicase (Prakash *et al.*, 2005). There are several differences between the *in vitro* substrates that FANCM and Mph1 bind to and act on (for an overview, see Fig. 2 in Whitby, 2010). Knockouts of *MPH1* are sensitive against a series of different genotoxins and produce a spontaneous mutator phenotype (hence the name Mutator phenotype 1) which is dependent on the error-prone translesion synthesis DNA polymerases REV1 and REV3 (see below; Scheller *et al.*, 2000; Schurer *et al.*, 2004). Mutations in several HR genes have been shown to be epistatic to *mph1*, and the mitotic HR rate is slightly increased when Mph1 is missing. Knocking out the yeast RecQ helicase *SGS1*, which by itself produces a strong hyper-recombination phenotype, leads to an even stronger increase in the HR rate in the *mph1* background (Schurer *et al.*, 2004). Since homozygous *mph1* diploids show normal spore survival, Mph1 is not thought to play an important role in meiotic recombination (Scheller *et al.*, 2000). The combination of HR and translesion synthesis pathways points to a regulatory role for Mph1 for the repair of damage at the replication fork, to focus repair into either one of the two pathways. Such a function is very reminiscent of the role its human homologue FANCM plays in the FA core complex at stalled replication forks, as is the genetic and physical interaction with the RecQ helicases Sgs1 and BLM, respectively. Recently, it has been shown that Mph1 can bind to and disrupt D-loops formed *in vitro* by a dsDNA molecule and a Rad51-coated single-stranded oligonucleotide (Prakash *et al.*, 2009). Taken together with results on *mph1 srs2* double mutants (Panico *et al.*, 2010), both helicases seem to play separate non-overlapping roles in the first steps of HR, especially at the replication fork.

As indicated above, homologues of HsFANCM/ScMph1 are also conserved in plants. They are generally of intermediate length, but also lack the C-terminal endonuclease domain found in mammalian homologues. Pilot experiments with T-DNA insertion lines of the presumed *A. thaliana FANCM* homologue showed elevated recombination rates in spontaneous somatic HR assays compared with the wild type (our own unpublished results).

Swi2/Snf2 family helicases

The members of the Swi2/Snf2 family of helicases are commonly known as chromatin remodellers; several Snf2-related proteins have been shown to use the energy generated from their ATPase activity to move along DNA as a translocase, rather than unwinding it (reviewed in Hopfner and Michaelis, 2007). By interacting with proteins bound to DNA, they can exercise their remodelling activity.

For a number of Swi2/Snf2-family proteins, a role in HR has been demonstrated in plants (Shaked *et al.*, 2006), which shows the importance of chromatin remodelling for making DNA accessible for HR factors in the context of protein-bound chromatin. The yeast INO80 and SWR1 chromatin-remodelling complexes, for example, are recruited to the vicinity of a DSB, where they affect the first

steps of HR and/or NHEJ. Conversely, loss of function of these complexes leads to increased sensitivity to DNA damage (van Attikum *et al.*, 2007). Both complexes are probably conserved in plants, but until now a role in HR has only been demonstrated for INO80. A mutant of the *A. thaliana INO80* gene showed a strong reduction of somatic HR, although no increased sensitivities to DNA damage were found (Fritsch *et al.*, 2004). The *Arabidopsis* SWR1 complex, on the other hand, seems to be involved in plant development, flowering time regulation, and immunity (reviewed in March-Diaz and Reyes, 2009).

The recombinase Rad51 is supported by a number of proteins in the early steps of HR. Another member of the Swi2/Snf2 family is Rad54. As part of the Rad52 epistasis group (Symington, 2002), it performs multiple functions in chromatin remodelling, homology search, stabilizing Rad51–ssDNA filaments in D-loop formation, and finally in the removal of Rad51 from DNA after heteroduplex formation (Heyer *et al.*, 2006). Like other members of the Swi2/Snf2 family, Rad54 also functions as a translocase. The yeast Rad54 and Rad51 proteins have been shown to interact with each other, and they stimulate each other's activity (Clever *et al.*, 1997).

Plants also possess a homologue of Rad54, and studies with yeast Rad54 and *A. thaliana* RAD54 showed the strong conservation of this protein's function. As is the case in yeast, *Arabidopsis* RAD51 and RAD54 proteins interact with each other *in vitro*. Additionally, heterologous interactions (*Sc*Rad51 with *At*RAD54 and *At*RAD51 with *Sc*Rad54) were also possible in yeast two-hybrid assays (Osakabe *et al.*, 2006; Klutstein *et al.*, 2008). Therefore, it is not surprising that *At*RAD54 could complement some DNA repair deficiencies of yeast *rad54* mutant cells, and ScRad54 enhanced resistance to γ-radiation when transformed into *Arabidopsis* plants (Klutstein *et al.*, 2008). A T-DNA insertion mutant of At*RAD54* showed increased sensitivities to γ-irradiation, and the somatic HR rate was strongly reduced compared with wild-type plants (Osakabe *et al.*, 2006). Although At*RAD54* is highly expressed in young flower buds that contain microspore mother cells undergoing meiosis, the At*RAD54* T-DNA line was fertile so, even if the *Arabidopsis* Rad54 homologue functions in meiotic recombination, it is not essential. Gene targeting, which is too rare an event to be useful in plant biotechnology at the moment, was reported to be enhanced by up to two orders of magnitude by expression of yeast Rad54 in *Arabidopsis* plants (Shaked *et al.*, 2005).

Although Rad5 also belongs to the Swi2/Snf2 helicase family, it is generally not considered to be a chromatin remodeller. In yeast, DNA lesions that cannot be overcome by the replisome in S-phase are handled by two different processes—either they are repaired by HR and other pathways, or they enter a damage tolerance pathway where the lesion will not be repaired, but replication can proceed. This damage tolerance is further split into two branches (Unk *et al.*, 2010). In translesion synthesis, specialized DNA polymerases ζ and η are able to incorporate bases opposite to the lesion, leading to error-prone or error-free bypass depending on the respective polymerase and type of lesion. The second branch, called post-replication repair (PRR), takes advantage of DNA synthesis on the complementary strand. Here, due to the absence of the lesion, synthesis can proceed further before the replication fork stalls. By regression of the fork, both newly synthesized daughter strands can anneal, and template switching then allows for the error-free elongation across the lesion. Rad5 is part of the PRR branch of damage tolerance, through signalling and its translocase function. The regulation of which branch to take—translesion synthesis or PRR—occurs via modification of the replicative polymerase sliding clamp PCNA. After DNA damage, the Rad6/Rad18 heterodimer monoubiquitinates PCNA, which promotes a polymerase switch and therefore enables the potentially error-prone translesion synthesis. Rad5, together with the ubiquitin-conjugating heterodimer Mms2/Ubc13, adds Lys63-linked ubiquitins to the one already attached to PCNA. This modification suppresses translesion synthesis and promotes PRR. During PRR, Rad5 has been shown to use its helicase domain to regress the replication fork to allow template switching (Fig. 3) (Blastyak *et al.*, 2007). Model vertebrates, including humans, possess two Rad5 homologues, SHPRH and HLTF. In both proteins, the ubiquitin ligase activity in cooperation with Mms2 and Ubc13 homologues could be reproduced. Loss of either HLTF or SHPRH leads to an increased mutagenesis, probably via translesion synthesis, elevated sensitivity against genotoxins, and an increase in the formation rate of gross chromosomal rearrangements. At least for HLTF, a fork regression activity similar to that of yeast Rad5 could be shown (Blastyak *et al.*, 2010).

In *Arabidopsis*, two homologues, *RAD5A* and *RAD5B*, were described recently (I. P. Chen *et al.*, 2008). T-DNA insertion mutants of the two genes displayed differing phenotypes: while *rad5a* mutants are hypersensitive against cross-linking and alkylating genotoxins, *rad5b* mutants did not show an increased sensitivity against any agent tested. Moreover, in a DSB-induced somatic HR assay, *rad5a* mutants displayed a reduced HR rate, while *rad5b* mutants were not different from the wild type. Epistasis analysis of double mutant combinations of *RAD5A*, *RECQ4A*, and *MUS81* demonstrated that the three proteins are part of three parallel pathways to repair cross-linked and methylated DNA (Mannuss *et al.*, 2010). The same study also defined the role of *RAD5A* in the SDSA and SSA pathways using specially constructed assay lines (Orel *et al.*, 2003). While a strong reduction in the SDSA-like HR rate was found, the *rad5a* mutant line was not different from the wild type in SSA. Thus, RAD5A seems to be involved in either D-loop formation or resolution in somatic cells.

Mer3

As the responses to DNA DSBs are similar in somatic and meiotic cells, it is not surprising that many of the proteins presented above play roles in both contexts. Nonetheless, meiosis-specific proteins have been described as well, including the DNA helicase Mer3. First described in yeast,

this helicase is part of the ZMM epistasis group (Zip1, Zip2, Zip3, Mer3, Msh4, and Msh5) of proteins (Borner *et al.*, 2004) involved in CO formation in meiotic recombination. As shown in Fig. 1, HR reactions leading to COs pass through a dHJ intermediate. In meiotic recombination, there are at least two parallel pathways to reach a CO outcome from a dHJ, based on the observed distribution of COs along a chromosome. While the less common class II COs follow a random distribution and are called interference insensitive, class I COs reduce the probability of another CO occurring nearby, showing so-called CO interference. The ZMM epistasis group proteins, including Mer3, promote these class I COs (Borner *et al.*, 2004). Mutating Mer3 led to a reduction of the CO frequency in *S. cerevisiae*, with the remaining COs showing no interference. An *in vitro* study with Mer3 protein demonstrated that the helicase stabilizes the invaded Rad51–ssDNA filament in the D-loop by extending the length of the heteroduplex region through branch migration (Mazina *et al.*, 2004). This reduces the chance for disruption of the invading ssDNA from the D-loop and a following SDSA-like NCO outcome, and conversely makes second-end capture for dHJ formation more probable.

Homologues of Mer3 seem to be conserved across all eukaryotes. Sequence comparisons led to the identification of HFM1 in humans, which is expressed in meiotic tissues (Tanaka *et al.*, 2006), and further homologues in *Trichomonas vaginalis* and many more protists (Malik *et al.*, 2007). Apart from yeasts, the only functional studies on Mer3 homologues were done in *Arabidopsis*, where the alternative name ROCK-N-ROLLERS (*RCK*) is also in use (Chen *et al.*, 2005; Mercier *et al.*, 2005). Mutants of *Arabidopsis MER3* display reduced fertility, defects in the progression of meiotic recombination, and a strong reduction of CO number, while the number of DSBs is unchanged. As in yeast, the remaining COs are interference insensitive, pointing to a role for MER3 in class I CO formation. Partners of Mer3 in the ZMM epistasis group have been described in *Arabidopsis* as well. Both MSH4 and MSH5 belong to the class I CO pathway of meiotic HR (Higgins *et al.*, 2004, 2008; Lu *et al.*, 2008).

Pif1

The yeast 5'–3' DNA helicases Pif1 and Rrm3 belong to a eukaryote-wide family of helicases that function in the promotion of both nuclear and mitochondrial genome stability. Pif1 was first described to be required for the recombination of mtDNA of different strains (Foury and Kolodynski, 1983). A few years later, it was shown that Pif1 regulates the action of telomerase in the nucleus (Schulz and Zakian, 1994), mainly as a function to suppress *de novo* telomere addition at DSBs (Mangahas *et al.*, 2001; Myung *et al.*, 2001). G-rich stretches of DNA are prone to form G-quadruplexes via Hoogsteen base pairing. They are found at telomeres, but also at other regions throughout the genomes of prokaryotes and eukaryotes. Ribeyre and colleagues analysed the fate of the G-quadruplex-forming human minisatellite CEB1 when inserted into the yeast genome (Ribeyre *et al.*, 2009). Inactivation of *PIF1* led to an increased rate of rearrangements in the element, while a mutation of the RecQ helicase *SGS1* had no effect. Pif1 also was able to unwind CEB1 G-quadruplex structures *in vitro*. Pif1 has been shown to be important in the replication context, as well: it is needed for Okazaki fragment maturation and for arresting replication forks at rDNA replication fork blocks (reviewed in Bochman *et al.*, 2010). Yeast Rrm3 helicase, on the other hand, promotes fork progression at the replication fork barrier. This is best explained as a function of Rrm3 as a component of the replisome, strengthening the force of the replicative helicase MCM2-7 for fork movement through pausing sites (Torres *et al.*, 2004).

Animals, which generally possess only one *PIF1* homologue, use the helicase for functions similar to those described for yeast. Human PIF1 is also localized in nuclei and mitochondria, and there is first evidence pointing to roles in telomere biology, for example binding to telomeric DNA and telomere shortening when *PIF1* is overexpressed (Zhang *et al.*, 2006).

To our knowledge, no studies have been performed on Pif1 homologues in plants. A recent review of the Pif1 family of helicases (Bochman *et al.*, 2010) proposed one putative homologue in rice and three in *A. thaliana*. Our own *in silico* studies demonstrated that there could be up to 11 *PIF1* helicase-related genes in the *Arabidopsis* genome, because a *PIF1* gene was captured by and multiplied with a helitron family transposable element (our own unpublished results).

Conclusions

DNA helicases play diverse and important roles in HR in somatic genome stability as well as in the meiotic mixing of the parental genomes. In almost every step of DSB repair, helicases are involved, and their loss results in very adverse effects for the cell. Nevertheless, compared with other enzyme classes, still very little detailed knowledge has been gained about their functions, especially in plants. With plant biotechnology becoming increasingly important for agriculture, hopefully the study of helicases will be intensified, helping us to set up new molecular tools for plant breeding.

Acknowledgements

The authors would like to thank Manfred Focke, Daniela Kobbe, and Michael Pacher for critical reading of the manuscript and the members of the Puchta lab for many insightful discussions. Due to size limitations, we regretfully were not able to cite all the relevant literature.

References

Allers T, Lichten M. 2001. Differential timing and control of noncrossover and crossover recombination during meiosis. *Cell* **106,** 47–57.

Ashton TM, Hickson ID. 2010. Yeast as a model system to study RecQ helicase function. *DNA Repair* **9,** 303–314.

Bagherieh-Najjar MB, de Vries OM, Hille J, Dijkwel PP. 2005. Arabidopsis RecQl4A suppresses homologous recombination and modulates DNA damage responses. *The Plant Journal* **43,** 789–798.

Bagherieh-Najjar MB, de Vries OM, Kroon JT, Wright EL, Elborough KM, Hille J, Dijkwel PP. 2003. Arabidopsis RecQsim, a plant-specific member of the RecQ helicase family, can suppress the MMS hypersensitivity of the yeast sgs1 mutant. *Plant Molecular Biology* **52,** 273–284.

Barber LJ, Youds JL, Ward JD, *et al*. 2008. RTEL1 maintains genomic stability by suppressing homologous recombination. *Cell* **135,** 261–271.

Blanck S, Kobbe D, Hartung F, Fengler K, Focke M, Puchta H. 2009. A SRS2 homolog from *Arabidopsis thaliana* disrupts recombinogenic DNA intermediates and facilitates single strand annealing. *Nucleic Acids Research* **37,** 7163–7176.

Blastyak A, Hajdu I, Unk I, Haracska L. 2010. Role of double-stranded DNA translocase activity of human HLTF in replication of damaged DNA. *Molecular and Cellular Biology* **30,** 684–693.

Blastyak A, Pinter L, Unk I, Prakash L, Prakash S, Haracska L. 2007. Yeast Rad5 protein required for postreplication repair has a DNA helicase activity specific for replication fork regression. *Molecular Cell* **28,** 167–175.

Bochman ML, Sabouri N, Zakian VA. 2010. Unwinding the functions of the Pif1 family helicases. *DNA Repair* **9,** 237–249.

Borner GV, Kleckner N, Hunter N. 2004. Crossover/noncrossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. *Cell* **117,** 29–45.

Capp C, Wu J, Hsieh TS. 2010. RecQ4 the second replicative helicase? *Critical Reviews in Biochemistry and Molecular Biology* **45,** 233–242.

Chaganti RS, Schonberg S, German J. 1974. A manyfold increase in sister chromatid exchanges in Bloom's syndrome lymphocytes. *Proceedings of the National Academy of Sciences, USA* **71,** 4508–4512.

Chang M, Bellaoui M, Zhang C, Desai R, Morozov P, Delgado-Cruzata L, Rothstein R, Freyer GA, Boone C, Brown GW. 2005. RMI1/NCE4, a suppressor of genome instability, encodes a member of the RecQ helicase/Topo III complex. *EMBO Journal* **24,** 2024–2033.

Chelysheva L, Vezon D, Belcram K, Gendrot G, Grelon M. 2008. The Arabidopsis BLAP75/Rmi1 homologue plays crucial roles in meiotic double-strand break repair. *PLoS Genetics* **4,** e1000309.

Chen C, Zhang W, Timofejeva L, Gerardin Y, Ma H. 2005. The Arabidopsis ROCK-N-ROLLERS gene encodes a homolog of the yeast ATP-dependent DNA helicase MER3 and is required for normal meiotic crossover formation. *The Plant Journal* **43,** 321–334.

Chen H, Samadder PP, Tanaka Y, *et al*. 2008. OsRecQ1, a QDE-3 homologue in rice, is required for RNA silencing induced by particle bombardment for inverted repeat DNA, but not for double-stranded RNA. *The Plant Journal* **56,** 274–286.

Chen IP, Mannuss A, Orel N, Heitzeberg F, Puchta H. 2008. A homolog of ScRAD5 is involved in DNA repair and homologous recombination in Arabidopsis. *Plant Physiology* **146,** 1786–1796.

Chiolo I, Saponaro M, Baryshnikova A, Kim JH, Seo YS, Liberi G. 2007. The human F-box DNA helicase FBH1 faces *Saccharomyces cerevisiae* Srs2 and postreplication repair pathway roles. *Molecular and Cellular Biology* **27,** 7439–7450.

Ciccia A, Ling C, Coulthard R, *et al*. 2007. Identification of FAAP24, a Fanconi anemia core complex protein that interacts with FANCM. *Molecular Cell* **25,** 331–343.

Clever B, Interthal H, Schmuckli-Maurer J, King J, Sigrist M, Heyer WD. 1997. Recombinational repair in yeast: functional interactions between Rad51 and Rad54 proteins. *EMBO Journal* **16,** 2535–2544.

Cooper MP, Machwe A, Orren DK, Brosh RM, Ramsden D, Bohr VA. 2000. Ku complex interacts with and stimulates the Werner protein. *Genes and Development* **14,** 907–912.

Deans AJ, West SC. 2009. FANCM connects the genome instability disorders Bloom's syndrome and Fanconi anemia. *Molecular Cell* **36,** 943–953.

Dupaigne P, Le Breton C, Fabre F, Gangloff S, Le Cam E, Veaute X. 2008. The Srs2 helicase activity is stimulated by Rad51 filaments on dsDNA: implications for crossover incidence during mitotic recombination. *Molecular Cell* **29,** 243–254.

Ellis NA, Groden J, Ye TZ, Straughen J, Lennon DJ, Ciocci S, Proytcheva M, German J. 1995. The Bloom's syndrome gene product is homologous to RecQ helicases. *Cell* **83,** 655–666.

Epstein CJ, Martin GM, Schultz AL, Motulsky AG. 1966. Werner's syndrome a review of its symptomatology, natural history, pathologic features, genetics and relationship to the natural aging process. *Medicine (Baltimore)* **45,** 177–221.

Foury F, Kolodynski J. 1983. pif mutation blocks recombination between mitochondrial rho+ and rho–genomes having tandemly arrayed repeat units in *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences, USA* **80,** 5345–5349.

Fritsch O, Benvenuto G, Bowler C, Molinier J, Hohn B. 2004. The INO80 protein controls homologous recombination in Arabidopsis thaliana. *Molecular Cell* **16,** 479–485.

Gangloff S, McDonald JP, Bendixen C, Arthur L, Rothstein R. 1994. The yeast type I topoisomerase Top3 interacts with Sgs1, a DNA helicase homolog: a potential eukaryotic reverse gyrase. *Molecular and Cellular Biology* **14,** 8391–8398.

Gari K, Decaillet C, Delannoy M, Wu L, Constantinou A. 2008*a*. Remodeling of DNA replication structures by the branch point translocase FANCM. *Proceedings of the National Academy of Sciences, USA* **105,** 16107–16112.

Gari K, Decaillet C, Stasiak AZ, Stasiak A, Constantinou A. 2008*b*. The Fanconi anemia protein FANCM can promote branch migration of Holliday junctions and replication forks. *Molecular Cell* **29,** 141–148.

Geuting V, Kobbe D, Hartung F, Durr J, Focke M, Puchta H. 2009. Two distinct MUS81–EME1 complexes from Arabidopsis process Holliday junctions. *Plant Physiology* **150,** 1062–1071.

Goto M, Miller RW, Ishikawa Y, Sugano H. 1996. Excess of rare cancers in Werner syndrome (adult progeria). *Cancer Epidemiology Biomarkers and Prevention* **5,** 239–246.

Hartung F, Plchova H, Puchta H. 2000. Molecular characterisation of RecQ homologues in *Arabidopsis thaliana*. *Nucleic Acids Research* **28,** 4275–4282.

Hartung F, Puchta H. 2006. The RecQ gene family in plants. *Journal of Plant Physiology* **163,** 287–296.

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

Hartung F, Suer S, Knoll A, Wurz-Wildersinn R, Puchta H. 2008. Topoisomerase 3alpha and RMI1 suppress somatic crossovers and are essential for resolution of meiotic recombination intermediates in Arabidopsis thaliana. *PLoS Genetics* **4,** e1000285.

Hartung F, Suer S, Puchta H. 2007. Two closely related RecQ helicases have antagonistic roles in homologous recombination and DNA repair in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **104,** 18836–18841.

Heyer WD, Li X, Rolfsmeier M, Zhang XP. 2006. Rad54: the Swiss Army knife of homologous recombination? *Nucleic Acids Research* **34,** 4115–4125.

Higgins JD, Armstrong SJ, Franklin FC, Jones GH. 2004. The Arabidopsis MutS homolog AtMSH4 functions at an early step in recombination: evidence for two classes of recombination in Arabidopsis. *Genes and Development* **18,** 2557–2570.

Higgins JD, Vignard J, Mercier R, Pugh AG, Franklin FC, Jones GH. 2008. AtMSH5 partners AtMSH4 in the class I meiotic crossover pathway in Arabidopsis thaliana, but is not required for synapsis. *The Plant Journal* **55,** 28–39.

Hopfner KP, Michaelis J. 2007. Mechanisms of nucleic acid translocases: lessons from structural biology and single-molecule biophysics. *Current Opinion in Structural Biology* **17,** 87–95.

Hu Y, Lu X, Barnes E, Yan M, Lou H, Luo G. 2005. Recql5 and Blm RecQ DNA helicases have nonredundant roles in suppressing crossovers. *Molecular and Cellular Biology* **25,** 3431–3442.

Hunter N, Kleckner N. 2001. The single-end invasion: an asymmetric intermediate at the double-strand break to double-Holliday junction transition of meiotic recombination. *Cell* **106,** 59–70.

Jessop L, Lichten M. 2008. Mus81/Mms4 endonuclease and Sgs1 helicase collaborate to ensure proper recombination intermediate metabolism during meiosis. *Molecular Cell* **31,** 313–323.

Kim J, Kim JH, Lee SH, Kim DH, Kang HY, Bae SH, Pan ZQ, Seo YS. 2002. The novel human DNA helicase hFBH1 is an F-box protein. *Journal of Biological Chemistry* **277,** 24530–24537.

Kim JH, Kim J, Kim DH, Ryu GH, Bae SH, Seo YS. 2004. SCFhFBH1 can act as helicase and E3 ubiquitin ligase. *Nucleic Acids Research* **32,** 2287–2297.

Kirik A, Salomon S, Puchta H. 2000. Species-specific double-strand break repair and genome evolution in plants. *EMBO Journal* **19,** 5562–5566.

Kitao S, Shimamoto A, Goto M, Miller RW, Smithson WA, Lindor NM, Furuichi Y. 1999. Mutations in RECQL4 cause a subset of cases of Rothmund–Thomson syndrome. *Nature Genetics* **22,** 82–84.

Klutstein M, Shaked H, Sherman A, Avivi-Ragolsky N, Shema E, Zenvirth D, Levy AA, Simchen G. 2008. Functional conservation of the yeast and Arabidopsis RAD54-like genes. *Genetics* **178,** 2389–2397.

Kobbe D, Blanck S, Demand K, Focke M, Puchta H. 2008. AtRECQ2, a RecQ helicase homologue from Arabidopsis thaliana, is able to disrupt various recombinogenic DNA structures *in vitro*. *The Plant Journal* **55,** 397–405.

Kobbe D, Blanck S, Focke M, Puchta H. 2009. Biochemical characterization of AtRECQ3 reveals significant differences relative to other RecQ helicases. *Plant Physiology* **151,** 1658–1666.

Kobbe D, Focke M, Puchta H. 2010. Purification and characterization of RecQ helicases of plants. *Methods in Molecular Biology* **587,** 195–209.

Kohzaki M, Hatanaka A, Sonoda E, *et al*. 2007. Cooperative roles of vertebrate Fbh1 and Blm DNA helicases in avoidance of crossovers during recombination initiated by replication fork collapse. *Molecular and Cellular Biology* **27,** 2812–2820.

Li B, Conway N, Navarro S, Comai L. 2005. A conserved and species-specific functional interaction between the Werner syndrome-like exonuclease atWEX and the Ku heterodimer in Arabidopsis. *Nucleic Acids Research* **33,** 6861–6867.

Li D, Frazier M, Evans DB, Hess KR, Crane CH, Jiao L, Abbruzzese JL. 2006. Single nucleotide polymorphisms of RecQ1, RAD54L, and ATM genes are associated with reduced survival of pancreatic cancer. *Journal of Clinical Oncology* **24,** 1720–1728.

Li HQ, Terada R, Li MR, Iida S. 2004. RecQ helicase enhances homologous recombination in plants. *FEBS Letters* **574,** 151–155.

Li W, Chen C, Markmann-Mulisch U, Timofejeva L, Schmelzer E, Ma H, Reiss B. 2004. The Arabidopsis AtRAD51 gene is dispensable for vegetative development but required for meiosis. *Proceedings of the National Academy of Sciences, USA* **101,** 10596–10601.

Liu Y, Shete S, Etzel CJ, *et al*. 2010. Polymorphisms of LIG4, BTBD2, HMGA2, and RTEL1 genes involved in the double-strand break repair pathway predict glioblastoma survival. *Journal of Clinical Oncology* **28,** 2467–2474.

Lu X, Liu X, An L, Zhang W, Sun J, Pei H, Meng H, Fan Y, Zhang C. 2008. The Arabidopsis MutS homolog AtMSH5 is required for normal meiosis. *Cell Research* **18,** 589–599.

Macris MA, Sung P. 2005. Multifaceted role of the *Saccharomyces cerevisiae* Srs2 helicase in homologous recombination regulation. *Biochemical Society Transactions* **33,** 1447–1450.

Malik SB, Pightling AW, Stefaniak LM, Schurko AM, Logsdon JM Jr. 2007. An expanded inventory of conserved meiotic genes provides evidence for sex in Trichomonas vaginalis. *PLoS ONE* **3,** e2879.

Mangahas JL, Alexander MK, Sandell LL, Zakian VA. 2001. Repair of chromosome ends after telomere loss in Saccharomyces. *Molecular Biology of the Cell* **12,** 4078–4089.

Mannuss A, Dukowic-Schulze S, Suer S, Hartung F, Pacher M, Puchta H. 2010. RAD5A, RECQ4A and MUS81 have different functions in homologous recombination and define different pathways of DNA repair in *Arabidopsis thaliana*. *The Plant Cell* doi: 10.1105/tpc.110.078568.

March-Diaz R, Reyes JC. 2009. The beauty of being a variant: H2A.Z and the SWR1 complex in plants. *Molecular Plant* **2,** 565–577.

Matsuno K, Kumano M, Kubota Y, Hashimoto Y, Takisawa H. 2006. The N-terminal noncatalytic region of Xenopus RecQ4 is required for chromatin binding of DNA polymerase alpha in the

initiation of DNA replication. *Molecular and Cellular Biology* **26,** 4843–4852.

Mazina OM, Mazin AV, Nakagawa T, Kolodner RD, Kowalczykowski SC. 2004. *Saccharomyces cerevisiae* Mer3 helicase stimulates 3′–5′ heteroduplex extension by Rad51; implications for crossover control in meiotic recombination. *Cell* **117,** 47–56.

Meetei AR, Medhurst AL, Ling C, *et al*. 2005. A human ortholog of archaeal DNA repair protein Hef is defective in Fanconi anemia complementation group M. *Nature Genetics* **37,** 958–963.

Mercier R, Grelon M. 2008. Meiosis in plants: ten years of gene discovery. *Cytogenetic and Genome Research* **120,** 281–290.

Mercier R, Jolivet S, Vezon D, *et al*. 2005. Two meiotic crossover classes cohabit in Arabidopsis: one is dependent on MER3, whereas the other one is not. *Current Biology* **15,** 692–701.

Moldovan GL, D'Andrea AD. 2009. How the Fanconi anemia pathway guards the genome. *Annual Review of Genetics* **43,** 223–249.

Morishita T, Furukawa F, Sakaguchi C, Toda T, Carr AM, Iwasaki H, Shinagawa H. 2005. Role of the *Schizosaccharomyces pombe* F-Box DNA helicase in processing recombination intermediates. *Molecular and Cellular Biology* **25,** 8074–8083.

Mullen JR, Kaliraman V, Ibrahim SS, Brill SJ. 2001. Requirement for three novel protein complexes in the absence of the Sgs1 DNA helicase in *Saccharomyces cerevisiae*. *Genetics* **157,** 103–118.

Mullen JR, Nallaseth FS, Lan YQ, Slagle CE, Brill SJ. 2005. Yeast Rmi1/Nce4 controls genome stability as a subunit of the Sgs1–Top3 complex. *Molecular and Cellular Biology* **25,** 4476–4487.

Myung K, Chen C, Kolodner RD. 2001. Multiple pathways cooperate in the suppression of genome instability in *Saccharomyces cerevisiae*. *Nature* **411,** 1073–1076.

Nakayama H, Nakayama K, Nakayama R, Irino N, Nakayama Y, Hanawalt PC. 1984. Isolation and genetic characterization of a thymineless death-resistant mutant of *Escherichia coli* K12: identification of a new mutation (recQ1) that blocks the RecF recombination pathway. *Molecular and General Genetics* **195,** 474–480.

Nassif N, Penney J, Pal S, Engels WR, Gloor GB. 1994. Efficient copying of nonhomologous sequences from ectopic sites via P-element-induced gap repair. *Molecular and Cellular Biology* **14,** 1613–1625.

Neveling K, Endt D, Hoehn H, Schindler D. 2009. Genotype–phenotype correlations in Fanconi anemia. *Mutation Research* **668,** 73–91.

Oh SD, Lao JP, Taylor AF, Smith GR, Hunter N. 2008. RecQ helicase, Sgs1, and XPF family endonuclease, Mus81–Mms4, resolve aberrant joint molecules during meiotic recombination. *Molecular Cell* **31,** 324–336.

Orel N, Kyryk A, Puchta H. 2003. Different pathways of homologous recombination are used for the repair of double-strand breaks within tandemly arranged sequences in the plant genome. *The Plant Journal* **35,** 604–612.

Osakabe K, Abe K, Yoshioka T, Osakabe Y, Todoriki S, Ichikawa H, Hohn B, Toki S. 2006. Isolation and characterization of the RAD54 gene from Arabidopsis thaliana. *The Plant Journal* **48,** 827–842.

Ouyang KJ, Woo LL, Ellis NA. 2008. Homologous recombination and maintenance of genome integrity: cancer and aging through the prism of human RecQ helicases. *Mechanisms of Ageing and Development* **129,** 425–440.

Panico ER, Ede C, Schildmann M, Schurer KA, Kramer W. 2010. Genetic evidence for a role of *Saccharomyces cerevisiae* Mph1 in recombinational DNA repair under replicative stress. *Yeast* **27,** 11–27.

Plchova H, Hartung F, Puchta H. 2003. Biochemical characterization of an exonuclease from Arabidopsis thaliana reveals similarities to the DNA exonuclease of the human Werner syndrome protein. *Journal of Biological Chemistry* **278,** 44128–44138.

Prakash R, Krejci L, Van Komen S, Anke Schurer K, Kramer W, Sung P. 2005. *Saccharomyces cerevisiae* MPH1 gene, required for homologous recombination-mediated mutation avoidance, encodes a 3' to 5' DNA helicase. *Journal of Biological Chemistry* **280,** 7854–8760.

Prakash R, Satory D, Dray E, Papusha A, Scheller J, Kramer W, Krejci L, Klein H, Haber JE, Sung P, Ira G. 2009. Yeast Mph1 helicase dissociates Rad51-made D-loops: implications for crossover control in mitotic recombination. *Genes and Development* **23,** 67–79.

Puchta H. 2005. The repair of double-strand breaks in plants: mechanisms and consequences for genome evolution. *Journal of Experimental Botany* **56,** 1–14.

Raynard S, Bussen W, Sung P. 2006. A double Holliday junction dissolvasome comprising BLM, topoisomerase IIIalpha, and BLAP75. *Journal of Biological Chemistry* **281,** 13861–13864.

Reidt W, Wurz R, Wanieck K, Chu HH, Puchta H. 2006. A homologue of the breast cancer-associated gene BARD1 is involved in DNA repair in plants. *EMBO Journal* **25,** 4326–4337.

Ribeyre C, Lopes J, Boule JB, Piazza A, Guedin A, Zakian VA, Mergny JL, Nicolas A. 2009. The yeast Pif1 helicase prevents genomic instability caused by G-quadruplex-forming CEB1 sequences *in vivo*. *PLoS Genetics* **5,** e1000475.

Ronceret A, Pawlowski WP. 2010. Chromosome dynamics in meiotic prophase I in plants. *Cytogenetic and Genome Research* **129,** 173–183.

Rossi ML, Ghosh AK, Bohr VA. 2010. Roles of Werner syndrome protein in protection of genome integrity. *DNA Repair* **9,** 331–344.

Salomon S, Puchta H. 1998. Capture of genomic and T-DNA sequences during double-strand break repair in somatic plant cells. *EMBO Journal* **17,** 6086–6095.

Sanchez-Moran E, Osman K, Higgins JD, Pradillo M, Cunado N, Jones GH, Franklin FC. 2008. ASY1 coordinates early events in the plant meiotic recombination pathway. *Cytogenetic and Genome Research* **120,** 302–312.

Saotome A, Kimura S, Mori Y, Uchiyama Y, Morohashi K, Sakaguchi K. 2006. Characterization of four RecQ homologues from rice (Oryza sativa L. cv. Nipponbare). *Biochemical and Biophysical Research Communications* **345,** 1283–1291.

Scheller J, Schurer A, Rudolph C, Hettwer S, 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.

Schulz VP, Zakian VA. 1994. The Saccharomyces PIF1 DNA helicase inhibits telomere elongation and de novo telomere formation. *Cell* **76,** 145–155.

Schurer KA, Rudolph C, Ulrich HD, Kramer W. 2004. Yeast MPH1 gene functions in an error-free DNA damage bypass pathway that requires genes from homologous recombination, but not from postreplicative repair. *Genetics* **166,** 1673–1686.

Shaked H, Avivi-Ragolsky N, Levy AA. 2006. Involvement of the Arabidopsis SWI2/SNF2 chromatin remodeling gene family in DNA damage response and recombination. *Genetics* **173,** 985–994.

Shaked H, Melamed-Bessudo C, Levy AA. 2005. High-frequency gene targeting in Arabidopsis plants expressing the yeast RAD54 gene. *Proceedings of the National Academy of Sciences, USA* **102,** 12265–12269.

Shete S, Hosking FJ, Robertson LB, *et al*. 2009. Genome-wide association study identifies five susceptibility loci for glioma. *Nature Genetics* **41,** 899–904.

Siebert R, Puchta H. 2002. Efficient repair of genomic double-strand breaks by homologous recombination between directly repeated sequences in the plant genome. *The Plant Cell* **14,** 1121–1131.

Siitonen HA, Kopra O, Kaariainen H, Haravuori H, Winter RM, Saamanen AM, Peltonen L, Kestila M. 2003. Molecular defect of RAPADILINO syndrome expands the phenotype spectrum of RECQL diseases. *Human Molecular Genetics* **12,** 2837–2844.

Sinclair DA, Guarente L. 1997. Extrachromosomal rDNA circles—a cause of aging in yeast. *Cell* **91,** 1033–1042.

Symington LS. 2002. Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. *Microbiology and Molecular Biology Reviews* **66,** 630–670.

Szostak JW, Orr-Weaver TL, Rothstein RJ, Stahl FW. 1983. The double-strand-break repair model for recombination. *Cell* **33,** 25–35.

Tanaka K, Miyamoto N, Shouguchi-Miyata J, Ikeda JE. 2006. HFM1, the human homologue of yeast Mer3, encodes a putative DNA helicase expressed specifically in germ-line cells. *DNA Sequence* **17,** 242–246.

Thaler DS, Stahl FW. 1988. DNA double-chain breaks in recombination of phage lambda and of yeast. *Annual Review of Genetics* **22,** 169–197.

Torres JZ, Bessler JB, Zakian VA. 2004. Local chromatin structure at the ribosomal DNA causes replication fork pausing and genome instability in the absence of the *S. cerevisiae* DNA helicase Rrm3p. *Genes and Development* **18,** 498–503.

Unk I, Hajdu I, Blastyak A, Haracska L. 2010. Role of yeast Rad5 and its human orthologs, HLTF and SHPRH in DNA damage tolerance. *DNA Repair* **9,** 257–267.

van Attikum H, Fritsch O, Gasser SM. 2007. Distinct roles for SWR1 and INO80 chromatin remodeling complexes at chromosomal double-strand breaks. *EMBO Journal* **26,** 4113–4125.

Van Maldergem L, Siitonen HA, Jalkh N, *et al*. 2006. Revisiting the craniosynostosis–radial ray hypoplasia association: Baller–Gerold syndrome caused by mutations in the RECQL4 gene. *Journal of Medical Genetics* **43,** 148–152.

Wang W. 2007. Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins. *Nature Reviews Genetics* **8,** 735–748.

Watt PM, Hickson ID, Borts RH, Louis EJ. 1996. SGS1, a homologue of the Bloom's and Werner's syndrome genes, is required for maintenance of genome stability in *Saccharomyces cerevisiae*. *Genetics* **144,** 935–945.

Watt PM, Louis EJ, Borts RH, Hickson ID. 1995. Sgs1: a eukaryotic homolog of *E.coli* RecQ that interacts with topoisomerase II *in vivo* and is required for faithful chromosome segregation. *Cell* **81,** 253–260.

Whitby MC. 2010. The FANCM family of DNA helicases/translocases. *DNA Repair* **9,** 224–236.

Wrensch M, Jenkins RB, Chang JS, *et al*. 2009. Variants in the CDKN2B and RTEL1 regions are associated with high-grade glioma susceptibility. *Nature Genetics* **41,** 905–908.

Wu L, Bachrati CZ, Ou J, Xu C, Yin J, Chang M, Wang W, Li L, Brown GW, Hickson ID. 2006. BLAP75/RMI1 promotes the BLM-dependent dissolution of homologous recombination intermediates. *Proceedings of the National Academy of Sciences, USA* **103,** 4068–4073.

Wu L, Chan KL, Ralf C, Bernstein DA, Garcia PL, Bohr VA, Vindigni A, Janscak P, Keck JL, Hickson ID. 2005. The HRDC domain of BLM is required for the dissolution of double Holliday junctions. *EMBO Journal* **24,** 2679–2687.

Wu L, Davies SL, North PS, Goulaouic H, Riou JF, Turley H, Gatter KC, Hickson ID. 2000. The Bloom's syndrome gene product interacts with topoisomerase III. *Journal of Biological Chemistry* **275,** 9636–9644.

Wu L, Hickson ID. 2002. The Bloom's syndrome helicase stimulates the activity of human topoisomerase IIIalpha. *Nucleic Acids Research* **30,** 4823–4829.

Xu G, Ma H, Nei M, Kong H. 2009. Evolution of F-box genes in plants: different modes of sequence divergence and their relationships with functional diversification. *Proceedings of the National Academy of Sciences, USA* **106,** 835–840.

Xu X, Liu Y. 2009. Dual DNA unwinding activities of the Rothmund–Thomson syndrome protein, RECQ4. *EMBO Journal* **28,** 568–577.

Xu X, Rochette PJ, Feyissa EA, Su TV, Liu Y. 2009. MCM10 mediates RECQ4 association with MCM2-7 helicase complex during DNA replication. *EMBO Journal* **28,** 3005–3014.

Yin J, Sobeck A, Xu C, Meetei AR, Hoatlin M, Li L, Wang W. 2005. BLAP75, an essential component of Bloom's syndrome protein complexes that maintain genome integrity. *EMBO Journal* **24,** 1465–1476.

Youds JL, Mets DG, McIlwraith MJ, Martin JS, Ward JD, O'Neill NJ, Rose AM, West SC, Meyer BJ, Boulton SJ. 2010. RTEL-1 enforces meiotic crossover interference and homeostasis. *Science* **327,** 1254–1258.

Yu CE, Oshima J, Fu YH, *et al*. 1996. Positional cloning of the Werner's syndrome gene. *Science* **272,** 258–262.

Zhang DH, Zhou B, Huang Y, Xu LX, Zhou JQ. 2006. The human Pif1 helicase, a potential *Escherichia coli* RecD homologue, inhibits telomerase activity. *Nucleic Acids Research* **34,** 1393–1404.