Mechanism of homologous recombination: mediators and helicases take on regulatory functions

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Abstract | Homologous recombination (HR) is an important mechanism for the repair of damaged chromosomes, for preventing the demise of damaged replication forks, and for several other aspects of chromosome maintenance. As such, HR is indispensable for genome integrity, but it must be regulated to avoid deleterious events. Mutations in the tumour-suppressor protein BRCA2, which has a mediator function in HR, lead to cancer formation. DNA helicases, such as Bloom's syndrome protein (BLM), regulate HR at several levels, in attenuating unwanted HR events and in determining the outcome of HR. Defects in BLM are also associated with the cancer phenotype. The past several years have witnessed dramatic advances in our understanding of the mechanism and regulation of HR.

Meiosis I
The successful completion of meiosis requires two cell divisions. Meiosis I refers to the first division in which the pairs of homologous chromosomes are segregated into the two daughter cells.

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Homologous recombination (HR) occurs in all life forms. HR studies were initially the domain of a few aficionados who had a desire to understand how genetic information is transferred and exchanged between chromosomes. The isolation and characterization of relevant mutants in *Escherichia coli*[1], and later in the budding yeast *Saccharomyces cerevisiae* (see below), uncovered the role of HR in DNA repair and led to the recognition that HR prevents the demise of damaged DNA replication forks, orchestrates the segregation of homologous chromosomes in meiosis I and functions in telomere maintenance[2–5] (FIG. 1). Several cancer-prone genetic diseases, including Bloom's syndrome and Fanconi anaemia, are associated with HR dysfunction or deficiency[6–8]. Furthermore, HR impairment is probably the underlying cause of breast, ovarian and other cancers in individuals who harbour mutations in the *BRCA1* and *BRCA2* genes[9,10]. Given the link to cancer, research on the mechanism and regulation of HR has received increasing attention.

Programmed DNA double-strand breaks (DSBs) that occur in meiosis and in processes such as mating-type switching in *S. cerevisiae* are strong inducers of HR. Recombination also occurs in response to unscheduled DSBs and other DNA lesions. Certain types of DNA damage pose a strong impediment to the DNA-replication machinery, and recombination of a damaged DNA with its sister chromatid re-establishes the DNA replication fork[3]. Meiotic recombination is 100–1,000-fold more frequent than mitotic recombination, and it usually involves homologous chromosomes and generates chromosome-arm crossovers. These crossovers are essential for proper chromosome segregation at the first meiotic division[2,4]. Mitotic HR differs from meiotic HR in that few events are associated with crossover and indeed, crossover formation is actively suppressed by specialized DNA helicases (see below). Some of these DNA helicases also prevent inappropriate HR events that can cause cell-cycle arrest or interfere with post-replication DNA repair (see below).

Much of our knowledge on HR pathways has come from studies on meiotic recombination that is initiated in specific recombination hotspots, which are targeted by the Spo11 complex to form DSBs[11], and also from HR-reporter systems that involve a site-specific DSB that has been introduced by endonucleases[2,4,12]. The past decade or so has witnessed tremendous progress in deciphering the intricacies of HR. Specifically, the mechanistic understanding of the HR machinery is being advanced by the burgeoning biochemical and structural characterization of recombinases and of the accessory factors and regulators that help tune the efficiency of this machinery. Cell-biological and chromatin immunoprecipitation experiments have helped to elucidate the temporal order of recruitment of HR factors to DSBs in the cell and have validated the biological relevance of results from biochemical experiments. The link between chromatin and HR is also beginning to be appreciated.

In this review, we first provide a primer on the most important HR pathways that are connected with DSB repair (DSBR) and that are relevant for understanding

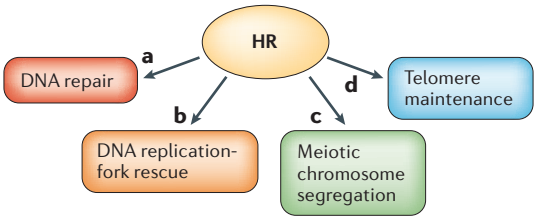

Figure 1 | **Biological roles of HR. a** | The mechanism of homologous recombination (HR) repairs chromosomes that harbour DNA double-strand breaks (DSBs) and other types of damage. In mitotic cells, the repair of DSBs by HR most often involves the use of the intact sister chromatid as an information donor, and therefore occurs primarily in the late S and G2 phases of the cell cycle, when the sister chromatid becomes available. Because the sister chromatid is identical in sequence to the damaged DNA molecule, the repair reaction faithfully restores the genetic configuration of the injured chromosome and is viewed as being error free. DSB repair can also occur by a different mechanism known as non-homologous DNA end-joining, which is much more error prone than HR[2,154]. **b** | The replication of a damaged DNA template, such as one that harbours a DNA nick, can lead to a broken DNA replication fork. The newly formed sister chromatid serves to direct the repair of the damaged DNA so as to prevent fork demise. **c** | Early on in meiosis, programmed DSBs are made in all the chromosomes, and these DSBs trigger HR between chromosome homologues rather than sister chromatids. These meiotic HR events help ensure the proper segregation of the chromosome homologues at the first meiotic division. **d** | Genetic studies, first in *Saccharomyces cerevisiae* and later in other organisms, have unveiled a role for HR proteins in several aspects of telomere maintenance, including the elongation of shortened telomeres without the need for telomerase, which is the enzyme that is responsible for adding the telomeric sequence at the end of chromosomes.

Fanconi anaemia
(FA). A genetically inherited anaemia that leads to bone marrow failure. Patients with FA are also susceptible to acute myelogenous leukaemia and squamous cell carcinomas in multiple organs. The disease is genetically complex.

Mating-type switching
Haploid *S. cerevisiae* cells can be of one of two mating types. Only cells of opposite mating types can mate to form a diploid. Cells can switch their mating type through an HR-dependent process.

Crossover
One of the possible outcomes of a physical exchange between duplex DNA molecules. Crossover can occur between sister chromatids or between the non-sister chromatids of a homologous pair of chromosomes. Crossover between non-sister chromatids results in new combinations of parental alleles on the crossover chromosomes.

DNA helicase
An enzyme that uses the energy from ATP hydrolysis to separate the two DNA strands in a double helix.

Post-replication DNA repair
A process that repairs gaps on newly replicated DNA using a DNA polymerase. The gaps usually occur opposite adducts or other lesions on the template strand. Post-replication repair fills in the gaps but does not remove the lesions from the template strand DNA.

Endonuclease
An enzyme that catalyses hydrolytic cleavage of DNA in the middle of a DNA strand or a double helix.

the mechanism and regulation of recombination in general. Second, we discuss the mechanistic principles that underlie the action of the recombinases, which constitute the catalytic core of the HR machinery. And third, we underline the essence of recently published studies on the functions of various regulatory factors that enhance or attenuate the efficiency of HR or direct the choice of a particular recombination pathway. We highlight some of the most pertinent questions concerning the mechanism and regulation of HR. The role of chromatin in HR-mediated DSBR has been the subject of several comprehensive reviews, and we invite readers to refer to some of these sources[13,14] for information.

HR-mediated DSBR

There are several distinct HR pathways for DSBR. The DSBR model was developed from transformation studies in yeast, which involved linear plasmids that carried yeast chromosomal DNA sequences and the monitoring of their integration into homologous chromosomal sequences[15–17]. The model explains most of the meiotic genetic recombination segregation data of marker alleles from fungi, including the association of gene conversion with crossovers. The details of the DSBR model have been worked out in studies on meiotic recombination that is initiated by programmed DSBs. The broken chromosome ends are first processed to give single-stranded DNA (ssDNA) tails, which invade a homologous chromosome to copy genetic information into the donor chromosome (FIG. 2a). Resolution of the exchanged DNA strands can result in crossover, whereby segments of the interacting chromosomes are exchanged (FIG. 2b). Central to the DSBR model is the formation of a DNA joint molecule that harbours two Holliday junctions (HJs; FIG. 2b). The existence of such a DNA intermediate has been verified in two-dimensional gel analysis of meiotic HR[18–20].

Mitotic DSBR is frequently unassociated with crossover, and to account for this, the synthesis-dependent strand-annealing (SDSA) model has been proposed[21–24]. The SDSA model is similar to the DSBR model in the initial steps of DSB-end processing and invasion into a homologous chromosome (FIG. 2a), but instead of capturing the second end of the DSB into the recombination intermediate, the invading strand is displaced after repair synthesis and reanneals with the single-stranded tail on the other DSB end (FIG. 2c). SDSA probably also accounts for those meiotic DSBR events that do not give rise to crossovers[25].

If a DSB occurs between closely repeated sequences, then it can also be repaired by the HR process of single-strand annealing (SSA). In the SSA pathway, the DSB ends are processed to form ssDNA tails that can anneal with each other. SSA does not require the full repertoire of HR genes, as the repair process does not require strand invasion[2,4].

Last, when a DSB has only one end, as might occur at a collapsed replication fork in which one branch of the fork has been lost, the single end can participate in an HR reaction that is known as break-induced replication (BIR)[2,4,5]. In this reaction, the DNA end is processed to give a single-stranded tail that invades a homologous sequence, followed by DNA synthesis to copy information from the donor chromosome. If the sequence that is used to initiate the BIR mechanism is part of a repeated-sequence family and is located on a non-homologous chromosome, BIR can result in a non-reciprocal translocation, which joins part of one chromosome to a different chromosome. BIR is mostly dependent on the Rad51 recombinase, but can also occur in the absence of Rad51 (REFS 26,27). BIR provides a means for the elongation of shortened telomeres[5].

The basal HR machinery

The RAD52 epistasis group. HR is catalysed by a class of enzyme known as recombinases, the activity of which is tightly regulated by other factors (TABLE 1 and see below). Much of the genetic framework concerning the basal HR machinery and its regulation in eukaryotic cells was initially defined in the budding yeast *S. cerevisiae*. Due to the role of HR proteins in DSBR and other DNA-repair reactions, mutants of the corresponding genes are hypersensitive to DNA-damaging agents, especially those, such as ionizing radiation and

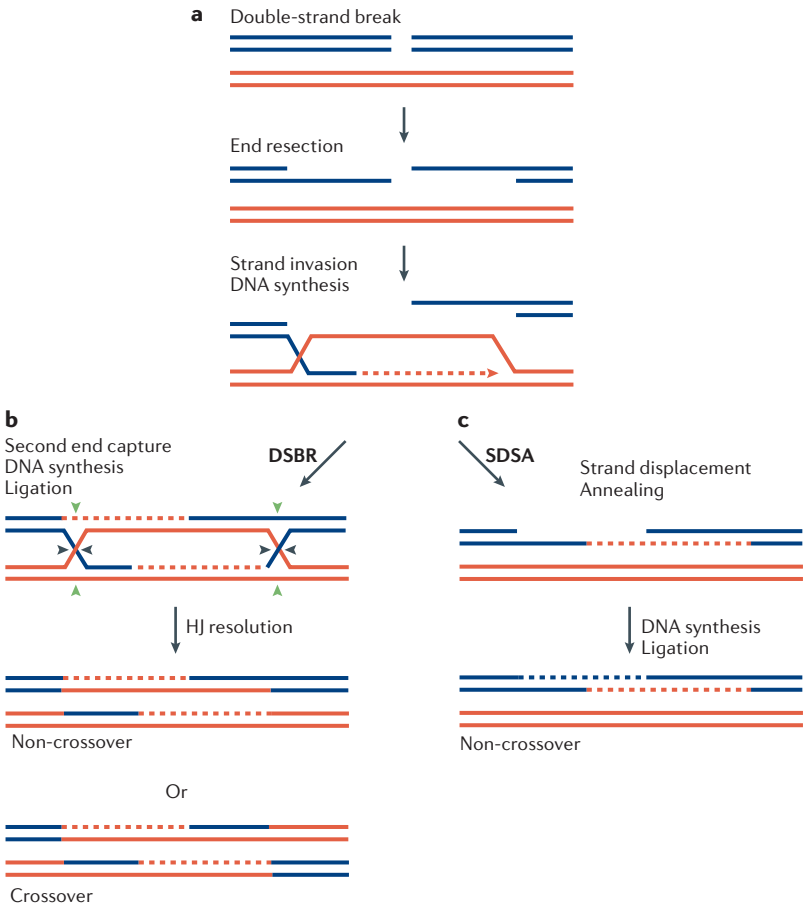

Figure 2 | **Repair of DNA double-strand breaks by DSBR and SDSA.** Double-strand breaks (DSBs) can be repaired by several homologous recombination (HR)-mediated pathways, including double-strand break repair (DSBR) and synthesis-dependent strand annealing (SDSA). **a** | In both pathways, repair is initiated by resection of a DSB to provide 3′ single-stranded DNA (ssDNA) overhangs. Strand invasion by these 3′ ssDNA overhangs into a homologous sequence is followed by DNA synthesis at the invading end. **b** |After strand invasion and synthesis, the second DSB end can be captured to form an intermediate with two Holliday junctions (HJs). After gap-repair DNA synthesis and ligation, the structure is resolved at the HJs in a non-crossover (black arrow heads at both HJs) or crossover mode (green arrow heads at one HJ and black arrow heads at the other HJ). **c** | Alternatively, the reaction can proceed to SDSA by strand displacement, annealing of the extended single-strand end to the ssDNA on the other break end, followed by gap-filling DNA synthesis and ligation. The repair product from SDSA is always non-crossover.

Chromatin immunoprecipitation
A technique for determining whether a protein binds to a particular region of the genome *in vivo*. It involves treating live cells with formaldehyde to form nonspecific crosslinks between the DNA and any associated proteins. The cells are then lysed, the genomic DNA is sheared into small fragments and the protein of interest is immunoprecipitated. Any protein-associated DNA is then removed and analysed by PCR.

DNA-crosslinking chemicals, that directly or indirectly lead to the formation of DSBs. Early work in the 1960s from several laboratories[28–33] led to the isolation of X-ray-sensitive mutants of *S. cerevisiae*. It was decided at the IVth International Yeast Genetics Conference in 1970 to refer to these mutants as *rad* mutants and to reserve the numbers 50 (*rad*50) and onwards for these mutants. A detailed examination of the initial collection of 64 mutants from laboratories around the world resulted in the classification of the various *RAD* genes as the *RAD52* epistasis group[30]. Since then, four additional genes have been added to the *RAD52* epistasis group: *RAD59*, *MRE11*, *XRS2* and *RDH54*/*TID1* (REFS 33–38). Mutation in any of the *RAD52* group of genes results in DNA-damage sensitivity and defective HR.

The known biochemical attributes and functions of the proteins that are encoded by the *RAD52* group genes and their human orthologues are summarized in TABLE 1. Of particular interest is the *RAD51* gene, the product of which is structurally related to the *E. coli* RecA protein and, similarly to RecA, possesses a recombinase activity that mediates the linkage of recombining chromosomes via DNA joint formation[39]. *RAD51* and other members of the *RAD52* epistasis group are needed for meiotic HR and chromosome segregation in meiosis I. The efficiency of HR is enhanced by mediator proteins that promote the loading of Rad51 onto ssDNA and is attenuated by the Srs2 helicase that dismantles the Rad51–ssDNA complex (see below). Other helicases control HR at the level of crossover formation. The Mer3 helicase ensures that enough crossovers are made during meiosis, whereas the Sgs1 and BLM helicases suppress spurious crossovers (see below).

The meiotic recombinase Dmc1 and its associated factors. A study that was aimed at uncovering genes the expressions of which are enhanced or restricted to meiosis led to the isolation of a gene, *DMC1*, that encodes a RecA/Rad51-like recombinase enzyme[40]. Further genetic, cell-biological and biochemical analyses have unveiled protein factors that physically and functionally interact with Dmc1 (TABLE 1). Overall, the mechanistic attributes of the Dmc1-associated HR machinery are not as well defined as the Rad51-associated machinery.

Formation of recombinase filaments on ssDNA

As discussed above (and depicted in FIG. 2a), the substrate of the HR machinery is ssDNA that stems from the processing of a DSB (or another lesion). The ssDNA serves to attract the recombinase, either Rad51 or Dmc1, and its associated ancillary factors. Studies that are directed at deciphering the recombinase mechanism have been guided by knowledge of the prokaryotic prototype *E. coli* RecA protein (BOX 1). RecA and its orthologues, including Rad51 and Dmc1, possess protein motifs that enable them to bind and hydrolyse ATP. Both Rad51 and RecA exist as a protein ring that consists of six and seven monomers, respectively. RecA and Rad51 polymerize on ssDNA to form a highly ordered, right-handed helical protein filament[41–43], which is commonly known as the presynaptic filament. The presynaptic filament has a pitch of 95–100 Å, comprising 6 recombinase molecules and 18 nucleotides of the DNA ligand per helical repeat (FIG. 3). Notably, the ssDNA is held in an extended formation in the presynaptic filament, being stretched by as much as 50% of the length of a B-form duplex DNA molecule[42–45]. Assembly of the presynaptic filament requires ATP binding, but not ATP hydrolysis[46,47].

The catalytic core of the HR machinery. The assembly of the presynaptic filament is the most important step in HR reactions as it provides the catalytic centre for the formation of DNA joints between the two recombining DNA molecules. Extensive biochemical studies with the RecA presynaptic filament have uncovered two DNA-binding sites: the 'primary' site accommodates the

Holliday junction
A cruciform DNA structure that is generated during the synaptic phase of homologous recombination. It is named after Robin Holliday, who proposed its existence in 1964.

Epistasis group
A group of genes that are most frequently defined by double-mutant analyses and function in the same biological pathway.

Orthologue
The structural and functional equivalent of a gene or protein in a different species.

Table 1 | **Mitotic and meiotic homologous-recombination factors**

Saccharomyces cerevisiae	Human	Biochemical function(s)	Notable features
Factors that function with RAD51			
Rad50	RAD50	DNA binding; DNA-dependent ATPase	Member of the SMC protein family; forms a complex with MRE11 and NBS1; involved in DSB end resection; involved in DNA-damage checkpoints
Mre11	MRE11	DNA-structure-specific endonuclease; 3′→5′ exonuclease	Forms a complex with RAD50 and NBS1; involved in DSB-end resection; involved in DNA-damage checkpoints
Xrs2	NBS1	DNA binding	Forms a complex with RAD50 and MRE11; involved in DSB-end resection; involved in DNA-damage checkpoints
Rad52	RAD52	ssDNA binding and annealing; recombination mediator	Interacts with RAD51 and RPA
Rad54	RAD54, RAD54B	dsDNA-dependent ATPase; dsDNA translocase; induces superhelical stress in dsDNA; stimulates the RAD51-mediated D-loop reaction; chromatin remodeller	Member of SWI2/SNF2 protein family; interacts with RAD51; Yeast Rad54 strips Rad51 from dsDNA
Rdh54/Tid1	RAD54, RAD54B	dsDNA-dependent ATPase; dsDNA translocase; induces superhelical stress in dsDNA; stimulates the RAD51-mediated D-loop reaction	Member of SWI2/SNF2 protein family; interacts with RAD51
Rad55–Rad57	XRCC2, XRCC3, RAD51B, RAD51C, RAD51D	Binds ssDNA; recombination mediator (shown for Rad55–Rad57 and RAD51B–RAD51C complexes only)	The human proteins form complexes (see main text for details); might stabilize the presynaptic filament; interacts with RAD51; Human RAD51C associates with Holliday-junction-resolvase activity
Rad59	Unknown	ssDNA binding and annealing	Homology to Rad52; interacts with Rad52
Mer3	Unknown	ssDNA-dependent ATPase; 3′→5′ DNA helicase activity; unwinds the Holliday junction	Extends the DNA joint made by Rad51
Factors that function with DMC1			
Mei5–Sae3	Unknown	Predicted recombination-mediator activity	Interacts with Dmc1
Hop2–Mnd1	HOP2–MND1	Binds ssDNA and dsDNA; stimulates the DMC1-mediated D-loop reaction	Interacts with DMC1
Rdh54/Tid1	RAD54 RAD54B	DNA-dependent ATPase; DNA translocase; induces superhelical stress in dsDNA; predicted to stimulate the DMC1-mediated D-loop reaction; Human RAD54B stimulates the DMC1-mediated D-loop reaction	Yeast Rdh54 was identified by yeast two-hybrid interaction with Dmc1; Human RAD54B interacts with DMC1

For more information on specific homologous-recombination activities, see Refs 2, 54, 61, 97, 98, 155 and 157–159. D-loop, displacement-loop; DSB, double-strand break; dsDNA, double-stranded DNA; Mnd1/MND1, meiotic nuclear division protein-1; RPA, replication protein A; SMC, structural maintenance of chromosomes; ssDNA, single-stranded DNA.

initiating ssDNA substrate, and the 'secondary' site helps capture the donor duplex DNA molecule[44,45]. The synaptic complex refers to the ensemble in which the three DNA strands from the participant DNA molecules are bound within the recombinase protein filament (FIG. 3). The 'search' for homology in the recombining DNA molecules and the DNA joint formation that occurs between them happens within the confines of the synaptic complex (for a detailed discussion of the mechanism of DNA homology search and DNA joint formation, see REFS 44,45). No molecular details concerning the transition of the recombinase protein ring to the presynaptic filament are available to date. Although filamentous forms of RecA and Rad51 have been characterized by X-ray crystallography[48–50], a high-resolution structure of the presynaptic filament or the synaptic complex has not yet been reported.

Presynaptic filaments of DMC1. Human DMC1 exists as an octameric protein ring[51]. The initial characterization of human DMC1 showed only a modest capability for this protein to catalyse DNA joint formation[52,53], and electron microscopy studies did not detect protein-filament formation on DNA. Instead, pairs of stacked protein rings with DNA that passed through the channel of the toroidal structure were observed[51,53]. A more recent study showed a robust recombinase activity in human DMC1 (REF. 54). Importantly, electron microscopy was

Box 1 | **RecA**

Clark and Margulies[1] first isolated mutants of the *Escherichia coli recA* gene on the basis of the inability of these mutants to conduct recombination and on the basis of their sensitivity to DNA-damaging agents. We now know that RecA is central to several DNA-repair and replication-fork-restart pathways. The biochemical functions of RecA have been studied intensely in many laboratories over a period of two decades. The large body of knowledge that has accrued from these endeavours makes RecA the paradigm for understanding the general mechanism of recombinases, including Rad51 and Dmc1. Biochemically, RecA seems to be a more versatile recombinase than its eukaryotic counterparts. For example, the RecA presynaptic filament can bypass regions of DNA heterology in the recombining DNA molecules and also promotes a specialized recombination reaction that involves two duplex DNA molecules (which is known as four-stranded exchange), whereas there is no evidence that either Rad51 or Dmc1 is capable of accomplishing these feats[44].

In addition to its involvement in recombination reactions, RecA also fulfils a pivotal role in other aspects of DNA metabolism. Upon DNA damage, RecA serves as a cofactor in the autocatalytic cleavage of the transcription repressor LexA, which then leads to the coordinated upregulation of more than 40 genes that are required for the tolerance and removal of DNA damage in a process known as the SOS response. In a similar fashion, RecA helps mediate the autocatalytic cleavage of the UmuD protein to promote the assembly of a DNA polymerase, Pol V, that comprises the cleaved UmuD polypeptide (known as UmuD′) and the UmuC protein. Remarkably, RecA also cooperates with Pol V in the replicative bypass of DNA lesions. The RecA–Pol V complex has been dubbed the 'mutasome', and the specialized DNA-damage-tolerance mechanism that is mediated by the mutasome[153] is referred to as translesion DNA synthesis. It is not thought that Rad51 or Dmc1 are involved in any transcriptional regulation event or translesion DNA synthesis.

used to show the formation of helical DMC1–ssDNA nucleoprotein filaments under reaction conditions in which DMC1 is capable of performing the recombination reaction[54] (FIG. 4). Since then, several studies have independently shown that the catalytic form of DMC1 comprises a helical filament of this recombinase on ssDNA[55–57]. That DMC1 also mediates recombination in the context of a nucleoprotein filament helps to establish the presynaptic filament as the universal catalytic core intermediate in recombinase function.

Presynaptic filament assembly and maintenance

The sections above emphasized the importance of the presynaptic filament in the recombination reaction. Paradoxically, nucleation of the recombinase onto ssDNA is a slow process. As a consequence, presynaptic filament assembly is particularly prone to interference by other proteins that bind ssDNA. In the physiological setting, replication protein A (RPA), which is an abundant ssDNA-binding protein, poses a challenge to successful presynaptic filament assembly by its competition for sites on the initiating ssDNA substrate in HR reactions[58–60]. A combination of biochemistry and chromatin immunoprecipitation has been used to identify HR factors within the *S. cerevisiae RAD52* gene group that are capable of overcoming the inhibitory effect of RPA on Rad51 presynaptic filament assembly. Such 'recombination mediators' include the Rad52 protein and the heterodimeric Rad55–Rad57 complex[2,61–65]. More recently, biochemical evidence was presented to support the idea that the tumour suppressor BRCA2 also functions as a recombination mediator in RAD51-mediated reactions[66,67].

Action of recombination mediators. Rad52 physically interacts with Rad51 and possesses a ssDNA-binding activity[2,61,68–70]. Rad52 nucleates Rad51 onto an RPA-coated ssDNA template to facilitate presynaptic filament assembly. A substoichiometric amount of Rad52, relative to Rad51, is sufficient for presynaptic filament assembly, which is indicative of a catalytic mode of action for Rad52 (REFS 62–64). This finding indicates that Rad52 helps gather a limited number of either monomeric Rad51 molecules or Rad51 protein rings to RPA-covered ssDNA in order to seed the assembly of a nascent recombinase filament. But, the subsequent displacement of RPA is linked to the extension of the nascent filament by the polymerization of additional Rad51 molecules that are free from Rad52 (REFS 71,72). The Rad52-mediated delivery of Rad51 to RPA-coated DNA is dependent on complex formation between Rad51 and Rad52 (REFS 63,73). It is expected, although not yet proven, that the DNA-binding activity of Rad52 is critical for its recombination-mediator function. As well as binding Rad51 and ssDNA, Rad52 also seems to interact with RPA[74,75], but the functional relevance of this RPA-binding activity remains to be elucidated. Interestingly, although a Rad52 orthologue exists in vertebrates, its deletion from the genome causes only a mildly affected DNA recombination and repair phenotype in mouse and chicken DT40 cells[2,76,77]. However, combining the RAD52 mutation with a mutation in XRCC3, which is one of the five RAD51 paralogues that are needed for RAD51 presynaptic assembly or maintenance (see below), results in lethality in DT40 cells[78]. These observations indicate that in higher organisms, the promotion of RAD51 presynaptic filament assembly is primarily mediated by factors other than RAD52, with RAD52 having a subsidiary role.

The Rad55–Rad57 complex helps alleviate the inhibition of presynaptic filament assembly that is imposed by RPA[65]. Like Rad52, the Rad55–Rad57 heterodimer physically interacts with Rad51 and has a ssDNA-binding activity[65,79,80], but whether this protein complex also interacts with RPA has not been determined. Results from genetic studies have hinted that Rad55–Rad57 might stabilize the already assembled Rad51 presynaptic filament[81]. If so, this would mark an important functional difference between the mediator activity of Rad55–Rad57 and that provided by Rad52. Five proteins — XRCC2, XRCC3, RAD51B, RAD51C and RAD51D — that are related to Rad55 and Rad57 in structure and probably in function, have been described in higher organisms, including humans[2,4]. These five Rad55- and Rad57-like

Paralogue
A protein that shares some relatedness in sequence with another protein but not necessarily in function. Paralogues arise through gene duplication.

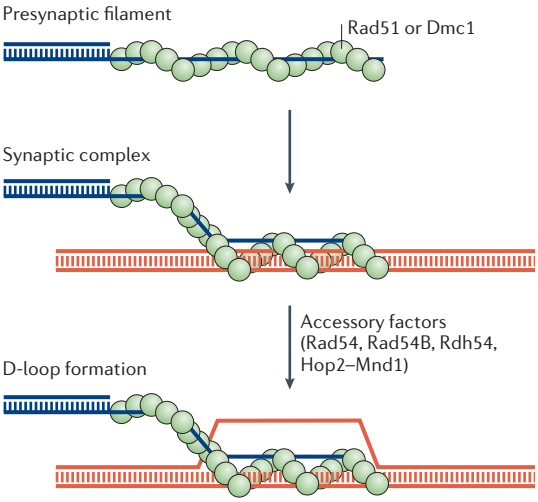

Figure 3 | **Recombinase filament and displacement-loop formation.** The recombinases Rad51 or Dmc1 (green circles) assemble onto the single-stranded DNA (ssDNA) tails that have been derived from the nucleolytic processing of a DNA double-strand break (DSB) to form a helical protein filament, which is known as the presynaptic filament. The presynaptic filament binds duplex DNA to form the synaptic complex and 'searches' for DNA homology in the duplex DNA molecule. With the aid of accessory factors including Rad54, Rad54B, Rdh54 and Hop2–Mnd1 (meiotic nuclear division protein-1), the ssDNA invades the homologous region in the duplex to form a DNA joint, known as the displacement (D)-loop. Rad54, Rad54B and Rdh54 are related proteins that promote the DNA-strand-invasion step by changing the topology of the DNA[2,61,155]. The manner in which the Hop2–Mnd1 complex promotes the D-loop reaction has not yet been delineated.

factors are also referred to as RAD51 paralogues because of their limited sequence resemblance to RAD51. Several complexes of the RAD51 paralogues have been noted: namely, RAD51B–RAD51C, RAD51D–XRCC2 and RAD51C–XRCC3, as well as a tetrameric complex of RAD51B–RAD51C–RAD51D–XRCC2 (REFS 82,83). These protein complexes probably function to assemble and/or preserve the RAD51 presynaptic filament, as the deletion of any one of the RAD51 paralogues results in an inability to deliver RAD51 to recombination substrates in the cell[84,85] and a recombination-mediator activity has been ascribed to the RAD51B–RAD51C complex[86]. Interestingly, results from a recent study have indicated that the RAD51C–XRCC3 complex, apart from its presynaptic role, is involved in the resolution of late DNA intermediates in the recombination process[87].

BRCA2, a tumour suppressor and recombination mediator. Mutations in the human *BRCA2* gene give rise to familial breast and ovarian cancers[9,10], and can also cause the cancer-susceptibility syndrome Fanconi anaemia[7]. BRCA2-deficient cells show a marked hypersensitivity to DNA-damaging agents, including ionizing radiation, DNA-alkylating chemicals and DNA-crosslinking agents, which is indicative of an impairment of the cellular DNA-repair capacity[10,88,89]. BRCA2 is required for efficient HR[90,91], and several observations have provided clues that BRCA2 regulates the RAD51 recombinase activity by providing a recombination-mediator function. First, BRCA2 physically interacts with RAD51 through several copies of a conserved module known as the BRC repeat[89,92] and also through its C terminus[88]. Second, BRCA2 binds ssDNA[67,93]. And third, *BRCA2*-mutant cells are deficient in assembling DNA-damage-induced nuclear RAD51 foci[91], which are thought to represent RAD51 presynaptic filaments on damaged DNA. Indeed, biochemical studies that were conducted with the Brh2 protein, which is a BRCA2-like molecule that is needed for HR and meiosis in the fungus *Ustilago maydis*[94], and with a human BRCA2-derived polypeptide have provided compelling evidence that BRCA2 serves such a recombination-mediator role[66].

Brh2 that had been purified as a complex with a small acidic polypeptide known as Dss1 was found to bind Rad51 and DNA — in particular, partially duplex DNA that harbours a 3′ single-strand overhang[66]. Importantly, the Brh2–Dss1 complex was shown to recruit Rad51 to RPA-coated 3′ ssDNA and to seed presynaptic filament assembly there[66,95] (FIG. 5). More recently, biochemical and electron microscopy studies demonstrated a recombination-mediator activity in a polypeptide, known as BRC3/4–DBD, that harbours the BRC3 and BRC4 repeats and the entire DNA-binding domain (DBD) from human BRCA2 (REF. 67) (FIG. 5). Furthermore, whereas RAD51 alone cannot discriminate between ssDNA and double-stranded DNA (dsDNA), BRC3/4–DBD specifically targets RAD51 to ssDNA[67]. Evidence was presented in these studies that the BRC repeats and the DBD are both required for functional interactions of BRCA2 or Brh2 with RAD51 (REFS 66,67). Taken together, and in accordance with the available cytological and genetic data[9,10], recent biochemical studies have provided compelling evidence that BRCA2 specifically targets RAD51 to ssDNA and also promotes the use of RPA-coated ssDNA for presynaptic filament assembly[66,67].

The C terminus of BRCA2 harbours a RAD51-binding domain that is needed for the efficiency of HR. This RAD51-binding domain is unrelated to the BRC repeat[10], and its phosphorylation by cyclin-dependent kinases negatively regulates the association with RAD51 (REF. 96). The mechanistic role of this RAD51-binding domain in HR has not yet been defined.

Putative recombination mediators of Dmc1. Very little is currently known about how the assembly and maintenance of the Dmc1 presynaptic filament are regulated. Genetic and cytological results have provided clues that the Mei5–Sae3 complex might fulfil a recombination-mediator role in Dmc1-dependent HR[97,98]. Similar to the *dmc1* mutant, the *mei5* and *sae3* mutants are defective in meiotic HR and exhibit meiotic prophase arrest due to an accumulation of unrepaired DSBs[97,98]. In wild-type meiosis, Dmc1 and Rad51 are targeted to recombination sites and colocalize in nuclear foci[99]. In cells that lack Mei5 or Sae3, Dmc1 targeting is impaired, whereas Rad51 targeting remains normal[97,98]. Mei5 and Sae3

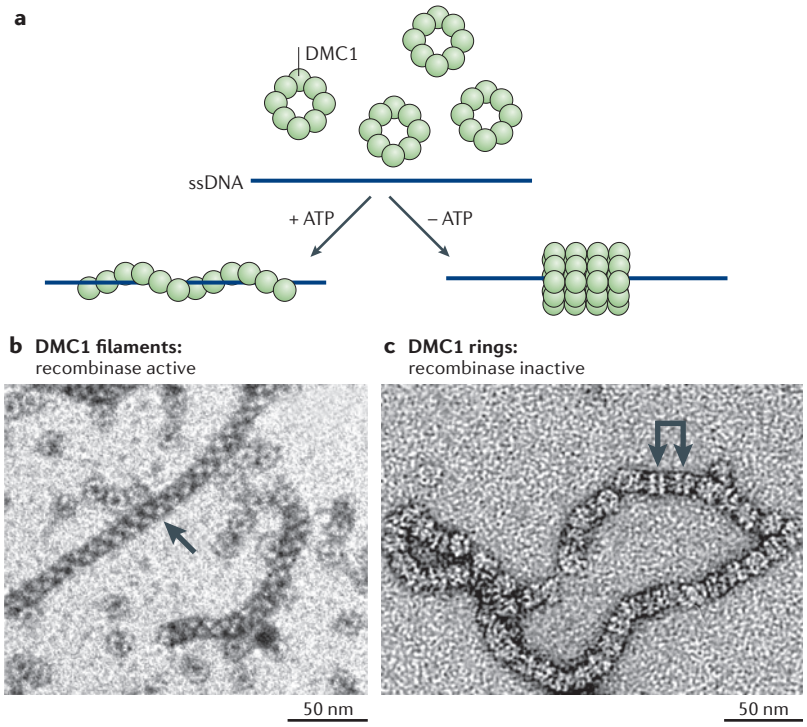

Figure 4 | **DMC1: rings versus filaments. a** | Human DMC1 can form either stacked rings[51,53,54] or helical filaments[54–57] on DNA, depending on whether ATP is present or not[54]. **b** | This electron micrograph shows helical filaments (see arrow) of DMC1 protein on single-stranded DNA (ssDNA). These filaments are the catalytically active form of DMC1 (REF. 54). DNA-free DMC1 protein rings (not marked) were also seen. **c** | This electron micrograph shows stacked DMC1 rings (two such stacked rings are indicated by the double arrow) on ssDNA. These stacked DMC1 rings seem incapable of mediating the HR reaction[54].

form a stable complex[98]. Mei5 interacts with Dmc1 in a yeast two-hybrid assay, and the Mei5–Sae3 complex can be co-immunoprecipitated with Dmc1 from meiotic cell extracts[98]. It is thought that Mei5–Sae3 facilitates the assembly of the Dmc1 presynaptic filament[97,98]. It remains to be determined experimentally whether Mei5–Sae3 has a recombination-mediator activity (that is, whether it mediates Dmc1 presynaptic filament assembly on RPA-coated ssDNA) and whether its interaction with Dmc1 favours the formation of helical filaments over rings[97,98] (FIG. 4). It is interesting to note that the formation of Dmc1 foci depends on Rad51 (REF. 99), which indicates that Rad51 somehow facilitates the delivery of Dmc1 to the HR substrate or enhances the stability of the Dmc1 presynaptic filament.

Recent studies have shown a physical and a functional interaction between the *Arabidopsis thaliana* BRCA2 protein and DMC1. Depletion of BRCA2 by RNA-interference-mediated silencing results in defective meiosis[100], and BRCA2 binds not only RAD51 but also DMC1 through its BRC repeats[101]. Further analyses have provided evidence that the BRCA2–DMC1 axis is indispensable for meiosis[100]. Future studies with purified proteins are needed to validate the hypothesis that BRCA2 works as a recombination mediator in DMC1-mediated HR.

Loss of heterozygosity
(LOH). Loss of biallelic information at a gene or chromosome region through a number of events, including homologous recombination and deletions. LOH can reveal recessive mutations and is often associated with tumours.

DNA-damage checkpoint
A signal-transduction response that is triggered by DNA damage and that results in the arrest (or delay) of cell-cycle progression.

Regulation of HR by DNA helicases

Even though HR is a major DNA-repair apparatus and helps prevent replication fork demise, inappropriate or untimely HR events can have deleterious consequences. For example, the formation of crossovers (via the DSBR pathway) during HR can lead to the loss of heterozygosity (LOH). If the LOH events involve genes that regulate cell growth and differentiation, they could lead to cell transformation and cancer. Furthermore, if crossover recombination is allowed to occur among repetitive DNA sequences dispersed within the genome (this is known as ectopic recombination), chromosome translocations can occur. The significance of such translocations in cancer is well documented[102–106]. Genetic studies in model organisms such as *S. cerevisiae* have provided evidence that HR can interfere with post-replication repair and cause the formation of intermediates that are difficult to resolve and therefore result in the prolonged activation of DNA-damage checkpoint-mediated cell-cycle arrest as well as chromosome rearrangements. For these reasons, HR must be tightly regulated so as to safeguard the cell against the harmful consequences of inappropriate or untimely events[107–111].

Genetic analyses in *S. cerevisiae* have implicated the Srs2 and Sgs1 helicases in the prevention of undesirable HR events[107]. Bloom's syndrome is an autosomal recessive disorder that is marked by growth retardation, sunlight sensitivity and a strong disposition to cancers[112]. The gene that is mutated in Bloom's syndrome encodes BLM helicase[113,114], the orthologue of the *S. cerevisiae* Sgs1 helicase. A hallmark feature of BLM-deficient cells is a genome-wide increase in the frequency of sister-chromatid exchanges and interhomologue recombination[8,115]. Taken together, the existing evidence points to a key role of the BLM helicase in HR modulation, much like what has been described for Sgs1 (REF. 8).

Mechanism of the Srs2 anti-recombinase function. Srs2 protein has a 3′→5′ DNA-helicase activity[116], and the likely functional equivalent in other eukaryotes is FBH1 (REFS 117,118). The *SRS2* gene was initially described after the isolation of mutants that could suppress the severe UV sensitivity of *rad6* and *rad18* mutants[119–121]. It was also described as a hyper-recombination mutant[122,123] and as a suppressor of certain *rad52* mutants[124–126]. The suppression and recombination phenotypes of the *srs2* mutants require HR functions. These results are best explained if Srs2 attenuates recombination and promotes the repair of damaged DNA replication forks. In support of the idea that an important function of Srs2 is to antagonize Rad51, suppressors of *srs2* mutants have proven to be mutations in *RAD51* that reduce Rad51 activity in HR[127,128]. Based on this and other related observations, it was deduced that Srs2 must function to restrict the activity of Rad51. The mechanistic basis of the Srs2 anti-recombinase function was elucidated by biochemical studies that showed that Srs2 can dismantle the Rad51 presynaptic filament in a manner that requires ATP hydrolysis by Srs2 (REFS 129–131) (FIG. 6a).

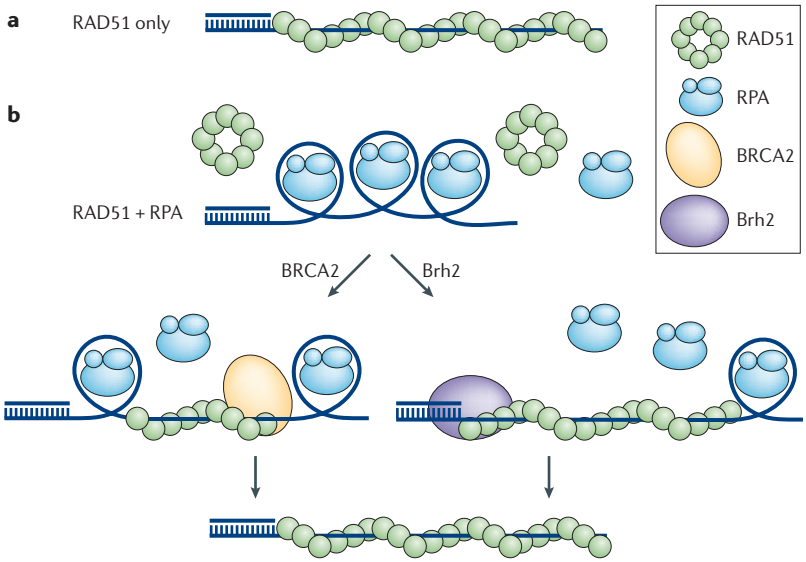

Figure 5 | **Action of recombination mediators. a** | *In vitro*, RAD51 can form the presynaptic filament on single-stranded DNA (ssDNA) without the assistance of another protein. **b** | However, in cells, due to competition for sites on the ssDNA substrate, the formation of the RAD51 presynaptic filament is strongly suppressed by the ssDNA-binding replication protein A (RPA). Several recombination mediators that can help RAD51 to alleviate the inhibitory effect of RPA have been described. In a recent study, a polypeptide that was derived from human BRCA2 protein was shown to nucleate RAD51 onto RPA-coated ssDNA to initiate the assembly of the RAD51 presynaptic filament[67]. Brh2 protein from *Ustilago maydis*, a BRCA2-like protein, binds the duplex–ssDNA junction of the recombination substrates and delivers RAD51 at that locale[66]. Biochemical studies have found a recombination-mediator activity in the *Saccharomyces cerevisiae* Rad52 and Rad55–Rad57 complex as well as in the human RAD51B–RAD51C complex[2,61].

Genetic evidence has implicated Srs2 in preventing the use of ssDNA gaps that arise during stalled replication as HR substrates and in specifically promoting post-replication DNA repair[111,119]. When leading- and lagging-strand synthesis at the replication fork becomes uncoordinated, due to blockage (by a bulky DNA lesion, for example) on one of the template strands, a single-strand gap occurs at the fork. The gap is often filled by a translesion DNA polymerase. To synthesize across the gap, the ssDNA region must be free of Rad51 protein. Srs2 is thought to be brought to stalled DNA-replication complexes to prevent Rad51-filament formation on the ssDNA[132,133]. The recruitment of Srs2 to the DNA replication fork is dependent on PCNA (proliferating cell nuclear antigen), which is an essential component of the DNA-replication apparatus and functions by tethering the DNA polymerase to the DNA template. PCNA is subject to several modifications that affect its interaction with DNA polymerases and other proteins. Srs2 binds PCNA, and the modification of PCNA by SUMO (small ubiquitin-like modifier) further enhances complex formation with Srs2. The targeting of Srs2 to stalled replication forks by SUMO-modified PCNA is thought to promote translesion-synthesis gap repair by removing Rad51 (REFS 132,133). During DSBR by HR, the stages after strand invasion also seem to be subject to regulation by Srs2. Specifically, DSBs in the *srs2Δ* mutant result in a prolonged DNA-damage-checkpoint-mediated cell-cycle arrest[134,135]. This arrest is dependent on Rad51. One explanation for this phenotype is that the capability of Srs2 to disassemble the Rad51 presynaptic filament prevents the accumulation of a Rad51-containing DNA intermediates that can trigger the DNA-damage-checkpoint-mediated arrest response.

Crossover suppression by BLM and Sgs1. In the DSBR pathway of HR, an intermediate with a double HJ is generated (FIG. 2b). The double HJ can be resolved by a specific endonuclease, known as 'resolvase', that can produce crossover recombinants[136] (FIG. 2b). However, some DSBR intermediates that harbour the double HJ never actually give rise to any crossovers[25], which indicates the presence of a mechanism that specifically suppresses crossover formation, especially during mitotic HR. Based on mutant analyses, the two RecQ helicases Sgs1 and BLM have been implicated in this novel mechanism of non-crossover recombination[108,110,137,138]. Acting in partnership with topoisomerase III, Sgs1 or BLM helicase is thought to push the two HJs between the paired duplexes inward in a process called branch migration, to form a hemicatenane structure that is then resolved by topoisomerase III to yield only non-crossover products (FIG. 6b). Direct biochemical evidence that supports this premise has come from recent studies that show an ability of the BLM–topoisomerase-III pair to dissolve a DNA substrate that contains two HJs to generate non-crossover recombinants[108,139,140] (FIG. 6B). Also, genetic studies have shown that the crossover recombinant class is increased in *sgs1Δ* mutants[110].

The suppression of crossovers by Sgs1 is an important regulatory mechanism for ensuring the proper distribution of crossovers for homologue-chromosome segregation at the first meiotic division[141]. There is a rise in crossover but not non-crossover formation in the *sgs1* mutant, which indicates that Sgs1 prevents some HR intermediates from becoming crossovers. The increase in crossovers is accompanied by a meiotic pachytene-checkpoint arrest and poor viability of the meiotic products.

Genetic studies in the fruitfly *Drosophila melanogaster* have shown that the BLM orthologue MUS-309 has an important role in the SDSA pathway of HR. It is thought that MUS-309 promotes the dissociation of the DNA joint that links the recombining DNA molecules, so as to free the invading ssDNA tail for the strand-annealing step of SDSA (FIG. 2c)[142,143]. The result of this putative BLM activity is the prevention of the channelling of the displacement (D)-loop intermediate into the crossover-forming DSBR pathway.

Promotion of meiotic crossover formation by Mer3. Deletion of the *MER3* gene, which encodes a DNA helicase, affects the transition of DSBs to late HR intermediates and diminishes the level of meiotic crossovers several fold without affecting the formation of non-crossovers[144]. The Mer3 protein can unwind different DNA structures, including HJs[145]. Interestingly, the helicase activity of Mer3 extends the DNA joint that is made by Rad51 (REF. 146). This attribute of Mer3 is thought to

Translesion DNA polymerase
A specialized low-fidelity DNA polymerase that is capable of synthesizing past lesions in the DNA template.

Topoisomerase
An enzyme that removes torsional stress from double-stranded DNA by changing the DNA supercoiling. It accomplishes this goal by breaking and rejoining one, or both, of the DNA strands, thereby inserting or removing superhelical twists.

Hemicatenane
A DNA structure that forms when one strand of a DNA duplex is wound around a strand from another duplex DNA molecule. Newly replicated sister chromatids are thought to harbour hemicatenanes.

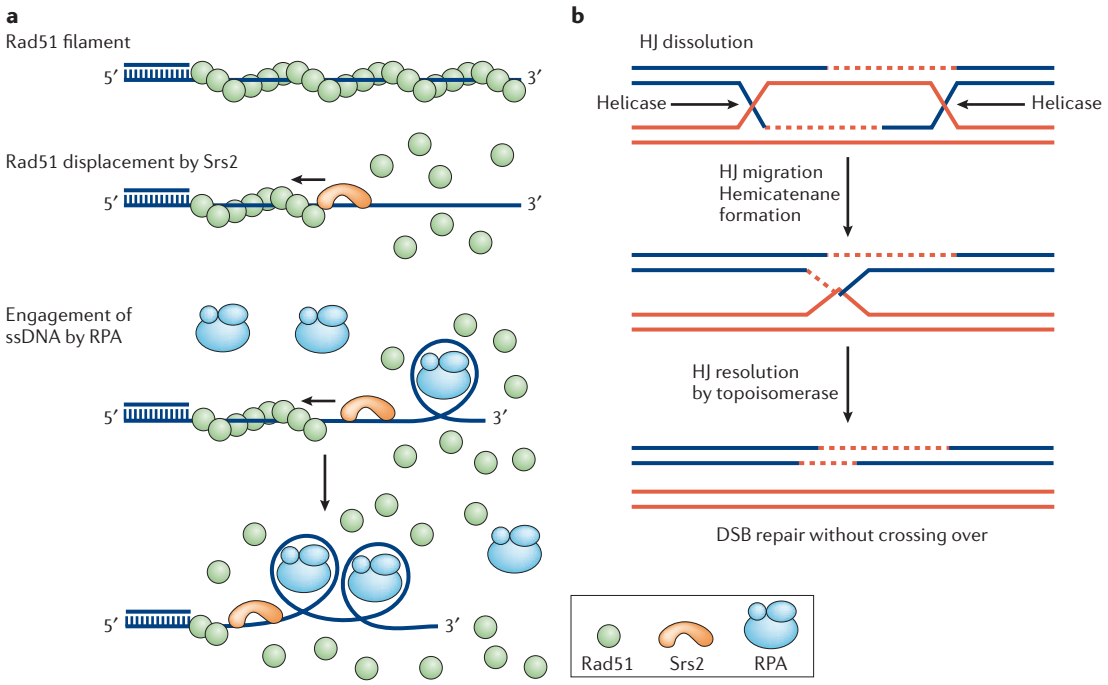

Figure 6 | **Regulation of HR by Srs2 and BLM helicases. a** | Disruption of the Rad51 presynaptic filament by Srs2. Biochemical studies have shown that the Srs2 helicase, at the expense of ATP hydrolysis, dismantles the Rad51 presynaptic filament in the 3′→5′ direction[129–131]. The single-stranded DNA (ssDNA) that becomes available as a result of Rad51 eviction is immediately occupied by the ssDNA-binding replication protein A (RPA) to prevent the reloading of Rad51 (REF. 129). This action of the Srs2 helicase prevents unwanted homologous recombination (HR) events and helps to channel certain DNA lesions into the Rad6-mediated post-replication-repair pathway[107]. **b** | Dissolution of an HR intermediate that harbours two Holliday junctions (HJs). The two HJ structures are pushed inward by a DNA helicase to form a hemicatenane, which is then dissolved by a topoisomerase to give non-crossover recombinants. This regulatory mechanism is thought to prevent undesirable HR outcomes, including the loss of heterozygosity and chromosome rearrangements. Recent studies have found that the BLM helicase, topoisomerase IIIα and the BLAP75 (also known as Rmi) protein, which interacts with both BLM and topoisomerase IIIα, act together to catalyse the HJ dissolution reaction[108,139,140,156]. DSB, double-strand break.

facilitate the formation of the double HJ during meiotic HR. Whether Mer3 functionally synergizes with Dmc1 in a similar fashion has not been determined.

Conclusions and outlook

Drawing on the insights and conceptual frameworks derived from earlier genetic experiments involving model eukaryotes, especially *S. cerevisiae*, studies in vertebrates have revealed a remarkable degree of conservation in the structure and function of the HR machinery. Importantly, a linkage of HR dysfunction to the cancer phenotype is now clear. Being guided by the RecA paradigm, studies that have been conducted in the recent past have made available a considerable body of molecular information on the two recombinases Rad51 and Dmc1, and have helped to establish the universally conserved nature of the presynaptic filament. Through the biochemical and structural characterization of purified proteins and the reconstitution of recombination reactions, the roles that many of the known HR factors fulfil are being clarified. In particular, concerted efforts on the *U. maydis* BRCA2 orthologue Brh2 and polypeptides that harbour functional domains of the human BRCA2 protein have helped to uncover a recombination-mediator function of BRCA2.

We now realize that inopportune HR events can lead to cell-cycle perturbation, genome destabilization and even cancer formation. Genetic evidence has implicated several DNA helicases — Srs2 and Sgs1 in yeast and BLM in vertebrates — in the regulation of HR-pathway choice and the suppression of unwanted HR events. As briefly reviewed in this article, biochemical studies on Srs2 and BLM have uncovered two distinct modes of HR control, with one being exerted at the level of Rad51 filament disassembly (by Srs2) and the other acting on late HR intermediates to limit the formation of DNA crossovers (by BLM and Sgs1).

Many important questions concerning the mechanism and regulation of HR remain. For example, the manner in which the tumour suppressor BRCA1, which binds DNA[147] and possesses a ubiquitin ligase activity[148], enhances HR efficiency has not yet been delineated. As for BRCA2, we still do not know how phosphorylation[96,149,150] or the associated protein factor DSS1 (REFS 93,151) influences the HR function of this tumour suppressor. Does BRCA2 have a recombination-mediator role in DMC1-mediated HR? Aside from its well documented role in the suppression of breast and ovarian cancers, the inactivation of BRCA2 can also cause Fanconi anaemia[6,7,152].

Ubiquitin ligase
An enzyme that facilitates the ligation of ubiquitin to target protein substrates by acting as an intermediary between a ubiquitin-conjugating enzyme and a substrate.

Cells from patients with Fanconi anaemia are extremely sensitive to DNA-crosslinking agents, which is reflective of a failure to remove interstrand DNA crosslinks. It is generally thought that HR contributes toward the elimination of DNA crosslinks and that BRCA2 serves a crucial function in RAD51 recruitment to help effect lesion removal[7]. The molecular details concerning this linkage of HR to DNA-crosslink repair remain to be deciphered. As mentioned earlier, the functional characterization of the meiotic HR machinery that comprises Dmc1 and its associated factors has lagged behind studies on the Rad51-associated HR machinery. This is an area wherein we expect to see rapid progress within the next few years.

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Competing interests statement
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