Published online 30 March 2012 *Nucleic Acids Research, 2012, Vol. 40, No. 13* ***5795–5818***
doi:10.1093/nar/gks270

SURVEY AND SUMMARY

Homologous recombination and its regulation

Lumir Krejci[1,2,3,*], Veronika Altmannova[1,2,3], Mario Spirek[1] and Xiaolan Zhao[4]

[1]Department of Biology, Masaryk University, Brno, Czech Republic, [2]National Centre for Biomolecular Research, Masaryk University, Brno, Czech Republic, [3]International Clinical Research Center, Center for Biomolecular and Cellular Engineering, St. Anne's University Hospital in Brno, Brno, Czech Republic and [4]Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, USA

Received January 12, 2012; Revised March 7, 2012; Accepted March 8, 2012

ABSTRACT

Homologous recombination (HR) is critical both for repairing DNA lesions in mitosis and for chromosomal pairing and exchange during meiosis. However, some forms of HR can also lead to undesirable DNA rearrangements. Multiple regulatory mechanisms have evolved to ensure that HR takes place at the right time, place and manner. Several of these impinge on the control of Rad51 nucleofilaments that play a central role in HR. Some factors promote the formation of these structures while others lead to their disassembly or the use of alternative repair pathways. In this article, we review these mechanisms in both mitotic and meiotic environments and in different eukaryotic taxa, with an emphasis on yeast and mammal systems. Since mutations in several proteins that regulate Rad51 nucleofilaments are associated with cancer and cancer-prone syndromes, we discuss how understanding their functions can lead to the development of better tools for cancer diagnosis and therapy.

INTRODUCTION—RAD51 NUCLEOFILAMENTS AND HOMOLOGOUS RECOMBINATION PATHWAYS

Cells are under constant genotoxic pressure from both endogenous and exogenous sources. It has been estimated that more than tens of thousands of DNA lesions occur in a single human cell every day (1). These lesions need to be repaired to avoid deleterious mutations, blockage of replication and transcription, and chromosomal breakage. The importance of DNA repair to human health is highlighted by the fact that failure to repair damaged DNA increases the likelihood of developing tumours and other diseases. In this review, we focus on homologous recombination (HR), a mechanism that repairs a variety of DNA lesions, including double-strand DNA breaks (DSBs), single-strand DNA gaps and interstrand crosslinks. Among these lesions, DSBs are highly toxic as a single unrepaired DSB can lead to aneuploidy, genetic aberrations or cell death. DSBs can be generated by a number of sources, including treatment with genotoxic chemicals and ionizing radiation, collapsed replication forks, and other endogenous DNA breaks. On the other hand, repair of DSBs is essential for the first meiotic division where it contributes to the formation of chiasmata, required for proper pairing and segregation of homologous chromosomes, and the generation of genetic diversity in most organisms (2).

A central player in HR is the strand-exchange protein, called Rad51 in eukaryotic cells (RecA in *Escherichia coli*). Rad51 functions in all three phases of HR: presynapsis, synapsis and post-synapsis [Figure 1A, (3)]. In the presynaptic phase, Rad51 is loaded onto single-strand DNA (ssDNA) that either is generated by degrading 5′-strands at DSBs or arises from replication perturbation. The resulting Rad51–ssDNA filament (presynaptic filament) is right-handed and comprises six Rad51 molecules and 18 nucleotides per helical turn. The ssDNA within the filament is stretched as much as half the length of B-form dsDNA (4). The stretching of the filament is essential for fast and efficient homology search (5,6). During synapsis, Rad51 facilitates the formation of a physical connection between the invading DNA substrate and homologous duplex DNA template, leading to the generation of heteroduplex DNA (D-loop). Here, Rad51–dsDNA filaments are formed by accommodating both the invading and donor ssDNA strands within the filament. Finally, during post-synapsis when DNA is synthesized using the invading 3′-end as a primer, Rad51 dissociates from dsDNA to expose the 3′-OH required for DNA synthesis.

At least three different routes can be used once DNA synthesis is initiated (Figure 1B–D). First, as envisioned in

*To whom correspondence should be addressed. Tel: +420 549493767; Fax: +420 549491327; Email: lkrejci@chemi.muni.cz

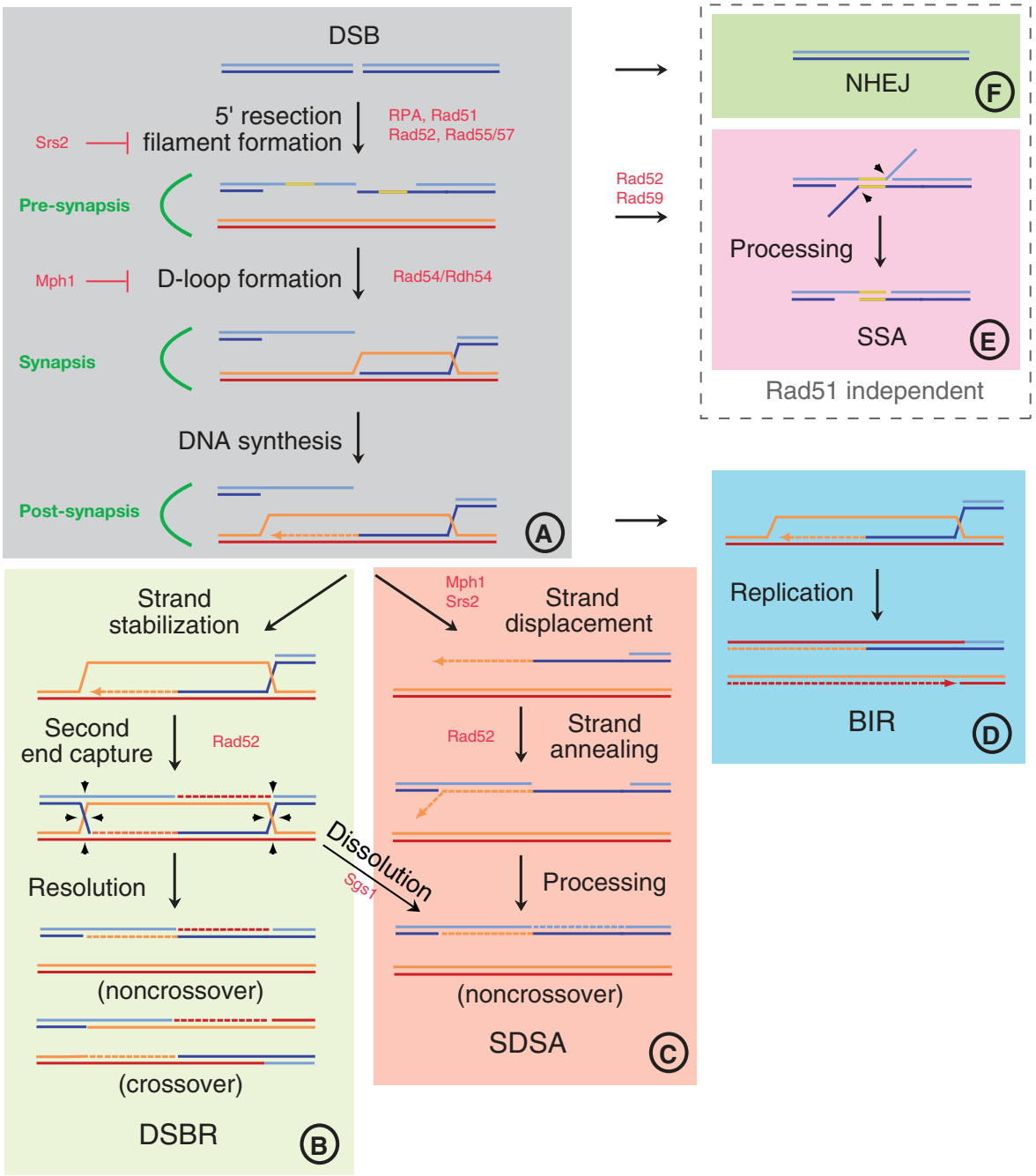

Figure 1. Models for the repair of DNA double-strand breaks. DNA DSBs are resected to generate 3′-protruding ends followed by formation of Rad51 filaments that invade into homologous template to form D-loop structures. (**A**) After priming DNA synthesis, three pathways can be invoked. In the DSBR pathway, the second end is captured and a dHJ intermediate is formed. (**B**) Resolution of dHJs can occur in either plane to generate crossover or non-crossover products. Alternatively, dHJs can be dissolved by the action of Sgs1–Top1–Rmi1 complex to generate only non-crossovers. In the SDSA pathway (**C**), the extended nascent strand is displaced, followed by pairing with the other 3′-single-stranded tail, and DNA synthesis completes repair. Nucleolytic trimming might be also required. In the third pathway of BIR (**D**), which can act when the second end is absent, the D-loop intermediate turns into a replication fork capable of both lagging and leading strand synthesis. Two other Rad51-independent recombinational repair pathways are also depicted. In SSA (**E**), extensive resection can reveal complementary sequences at two repeats, allowing annealing. The 3′-tails are removed nucleolytically and the nicks are ligated. SSA leads to the deletion of one of the repeats and the intervening DNA. Finally, the ends of DSB can be directly ligated resulting in NHEJ. (**F**) Newly synthesized DNA is represented by dashed lines.

the double-strand break repair model (DSBR), the second end of DSB can be engaged to stabilize the D-loop structure (second-end capture), leading to the generation of a double-Holliday Junction (dHJ) [(7), reviewed in Ref. (8); Figure 1B]. A dHJ is then resolved to produce crossover or non-crossover products (Figure 1B) or dissolved to exclusively generate non-crossover products. Second, the invading strand can be displaced from the D-loop and anneals either with its complementary strand as in gap repair or with the complementary strand associating

with the other end of the DSB. This represents the synthesis-dependent strand-annealing mode of HR (SDSA) [(9), Figure 1C]. SDSA mechanism is preferred over DSBR during mitosis. During meiosis, crossovers are formed by resolution of dHJs via the DSBR mechanism, while non-crossovers are primarily produced via SDSA mechanism (10,11). In the third mode, the D-loop structure can assemble into a replication fork and copy the entire chromosome arm in a process called break-induced replication (BIR) [(12), Figure 1D]. This mechanism is evoked more often when there is only one DNA end, either due to the loss of the other end or in the process of lengthening telomeres in telomerase-deficient cells.

All the above pathways require Rad51, with the exception of some forms of BIR. However, DSBs can also be sealed by pathways independent of Rad51 (Figure 1E and F). One of these pathways is the single-strand annealing pathway (SSA). In SSA, ssDNA sequences generated during DSB processing contain regions of homology at both sides of DSB and can be annealed and ligated [(13), Figure 1E]. SSA does not require Rad51 but requires other HR proteins that mediate annealing. Another Rad51-independent pathway that operates at DSBs is non-homologous end joining (NHEJ), which ligates ends of DSBs with little or no requirement for homology [reviewed in Ref. (8), Figure 1F].

THE MANY FACETS OF HR REGULATION

The presence of multiple Rad51-dependent pathways and other alternative pathways suggests the existence of regulatory mechanisms that determine the choices of pathways and manner of execution. Many important decisions need to be made to control the outcome of repair of different types of lesions. For example, whether both ends of DSBs are used for repair, how DNA synthesis is initiated and terminated, whether SSA and BIR pathways are used only when other repair attempts fail. Considering the central role of Rad51 in HR, it is only logical that much of the regulation impinges on this protein and its regulators. Here, we provide a comprehensive and up-to-date overview of how this multi-layered regulation affects the formation, maintenance and disassembly of Rad51 nucleofilaments. There are both positive and negative regulators of Rad51 function, some of which play a general role in both mitotic and meiotic cells, whereas others are specific to only one (Table 1). In addition, HR regulation employs protein modifications, such as phosphorylation and SUMOylation, to provide the required flexibility and dynamics.

We also envision that coordination and hierarchies exist among the large number of Rad51 regulation modules. In a sense, the system of HR regulation may be considered as a 'quality control' scheme in which the optimal output requires specific interplay between all regulatory modules (Figure 2). An understanding of such an 'HR quality control' system requires better characterization of each regulation module, their relationships, and dynamics. As many facets of this regulation are best studied in the model organism budding yeast, examples in this system are often used to illustrate the principles of HR regulation. Additional regulation in mammalian cells and occasionally in other organisms is described in the later part of the text, though some are mentioned early on where they can be helpful to illustrate the point. While this review presents HR regulation from a Rad51-centric view, additional information on HR mechanisms can be found in several reviews (3,8,14–16).

COMPETITION AND COLLABORATION BETWEEEN RAD51 AND RPA

One level of HR regulation occurs at the interplay between Rad51 and the ssDNA-binding factor, replication protein A (RPA) complex. RPA has higher affinity for ssDNA than Rad51, and the presence of RPA on ssDNA prevents Rad51 from binding *in vitro*, suggesting that RPA–ssDNA formation precedes Rad51 presynaptic filament formation (17,18). In line with this, RPA was found to arrive at DSB sites prior to Rad51 based on both cytology data and Chromatin immunoprecipitation analysis (19–22). For recombination to proceed, however, it is critical that RPA is subsequently replaced by Rad51 with the help of other proteins known as mediators (Figure 2). Mutations in RPA can impair HR by slowing this replacement step. For example, the recombination-deficient RPA mutant *rfa1-t11* is displaced more slowly from ssDNA by Rad51 than wild-type RPA and consequently inhibits Rad51 protein-mediated DNA strand exchange (23).

On the other hand, RPA also promotes recombination by removing secondary structures formed on ssDNA that could impede Rad51 filament formation (3). In addition, RPA can aid Rad51 by preventing the reversal reaction of Rad51-mediated D-loop formation. This is mediated by the sequestration and scavenging of free ssDNA, thereby preventing DNA from entering the second DNA-binding site of Rad51 (24,25). RPA's contributions to HR extend beyond its interplay with Rad51. For example, it promotes DSB resection by stimulating the Sgs1 helicase, directing Dna2 nucleolytic activity towards the 5′-terminus and protecting the 3′-end from degradation (26,27). In addition, the amount of RPA–ssDNA is sensed by checkpoint kinases to elicit cell-cycle arrest allowing sufficient time for repair (28–30).

RECOMBINATION MEDIATORS: POSITIVE REGULATORS OF RAD51

The proteins that can overcome the inhibitory effect of RPA on Rad51 nucleofilament formation are referred to as recombination mediators. In yeast, these include at least two types of proteins: Rad52 and the Rad51 paralogues, Rad55 and Rad57 that share the RecA core sequences with Rad51 (Figure 2). Mediators can facilitate Rad51 loading on ssDNA, increase intrinsic stability of Rad51 presynaptic filament and protect Rad51 from removal by factors such as helicases. Their roles in mammals and other eukaryotes will be described later in the text.

Table 1. The effect of post-translational modifications (PTMs) on Rad51 and its regulators

	Human	*S. cerevisiae*	Function	PTM	Effect of the PTM modification
Recombinase	RAD51	Rad51	Homology search and DNA strand exchange	P	Mec1-mediated phosphorylation of Rad51 at S192 is required for ATPase and DNA binding activities (133) Phosphorylation of RAD51 T309 by Chk1 is essential for Rad51 foci formation (319) RAD51 is phosphorylated on Y54 and Y315 in c-Abl dependent manner (320) BCR-ABL1-mediated phosphorylation of RAD51 at Y315 promotes error-prone HR in leukemia cells (131,132) Plk1- and CK2-dependent phosphorylation facilitates RAD51 recruitment to damage sites and its binding to NBS1 (134)
Positive regulators	RAD52	Rad52	Recombination mediator, SSA	SUMO	SUMOylation of Rad52 at K43,44,253 promotes protein stability, disfavors nucleolar localization, and inhibits DNA binding and annealing activity (138,141,142) SUMOylation of RAD52 alters its subcellular localization (143)
				P	Phosphorylation of RAD52 mediated by c-ABL enhances RAD52 annealing activity (146)
	BRCA2		Recombination mediator	P	CDK-dependent phosphorylation at S3291 of BRCA2 regulates its interaction with RAD51 (321)
				Ub	Ubiquitylation of BRCA2 after MMC treatment leads to its proteasomal degradation (322)
	RAD51B-RAD51C RAD51D-XRCC2 RAD51C-XRCC3	Rad55-Rad57	Recombination mediator	P	Phosphorylation of Rad55 at S2,8,14 is required for efficient recombination after replication fork stalling (136)
	Psy3 = RAD51D, Shu2 = SWS1 Shu1 = XRCC2 Csm2 = not identified	Shu1-Psy3-Shu2-Csm2	Regulation of Srs2 activity, stabilization Rad51 filament	P	XRCC2 is phosphorylated upon DNA damage, probably by ATM or ATR (323)
		Rad59	ssDNA annealing, Rad51 filament stability	SUMO	Deletion of *SLX5/8* decreases Rad59 sumoylation and increases SSA and BIR (119)
	RAD54	Rad54	ATP-dependent dsDNA translocase, stabilization of Rad51 filament	P	Mek1-mediated phosphorylation of Rad54 down-regulates Rad51 recombinase activity (157)
				Ub	APC/C-dependent ubiquitylation of *S.pombe* Rdh54 in G1 phase is required for efficient HR (156)
	RAD54B	Rdh54/Tid1	ATP-dependent dsDNA translocase, stabilization of Dmc1 filament		
		Tid4/Uls1	SUMO-dependent ubiquitin ligase, stabilization of Rad51 filament		
	RAD51AP1		Stabilization of D-loop formation		
	SWI5-MEI5	Mei5-Sae3*	Mediator activity		
	MND1-HOP2*	Mnd1-Hop2*	Stabilization of Rad51- and Dmc1-presynaptic filaments		
Negative regulators		Srs2	Helicase activity, disruption of Rad51 presynaptic filament, promotes SDSA	SUMO	Srs2 SUMOylation at K1081,1089,1142 is responsible for toxicity seen in cells lacking Cdk1-mediated phosphorylation of Srs2 (147)
				P	Cdk1-dependent phosphorylation of Srs2 promotes accurate DSB repair (147)
		Hed1*	Inhibition of Rad54 recruitment to Rad51 presynaptic filament		
	FANCM	Mph1	Helicase and branch migration activity, dissociation of D-loops formed by Rad51, promotes SDSA	P	FANCM is phosphorylated after DNA damage (324)
	BLM	Sgs1	RecQ-like DNA helicase, multiple roles in HR and DNA replication (resolution dHJ)	SUMO	BLM SUMOylation promotes RAD51 function (153) Sgs1 sumoylation promotes telomere recombination (152)
				P	Sgs1 is phosphorylated *in vivo* (325) BLM phosphorylation at T99 causes its dissociation from Top3a (326) MPS1-dependent BLM phosphorylation is essential for accurate chromosome segregation (327)
	RTEL1		ATP-dependent DNA helicase, inhibition of D-loop formation, promoting SDSA		
	PARI		Inhibition of HR, binds PCNA and Rad51		
Other regulators	RPA	RPA	Binding to resected ssDNA ends (competition with Rad51)	SUMO	SUMOylation of RPA70 facilitates RAD51 foci formation and promotes HR (120) Deletion of *SLX5/8* decreases sumoylation of Rfa1 and Rfa2 and affects SSA and BIR (119)
				P	Hyperphosphorylated RPA2 is required for RAD51 recruitment to DSB sites (123)

* meiosis specific proteins, PTM—post-translational modifications: P—phosphorylation, Ub—ubiquitylation, SUMO—sumoylation

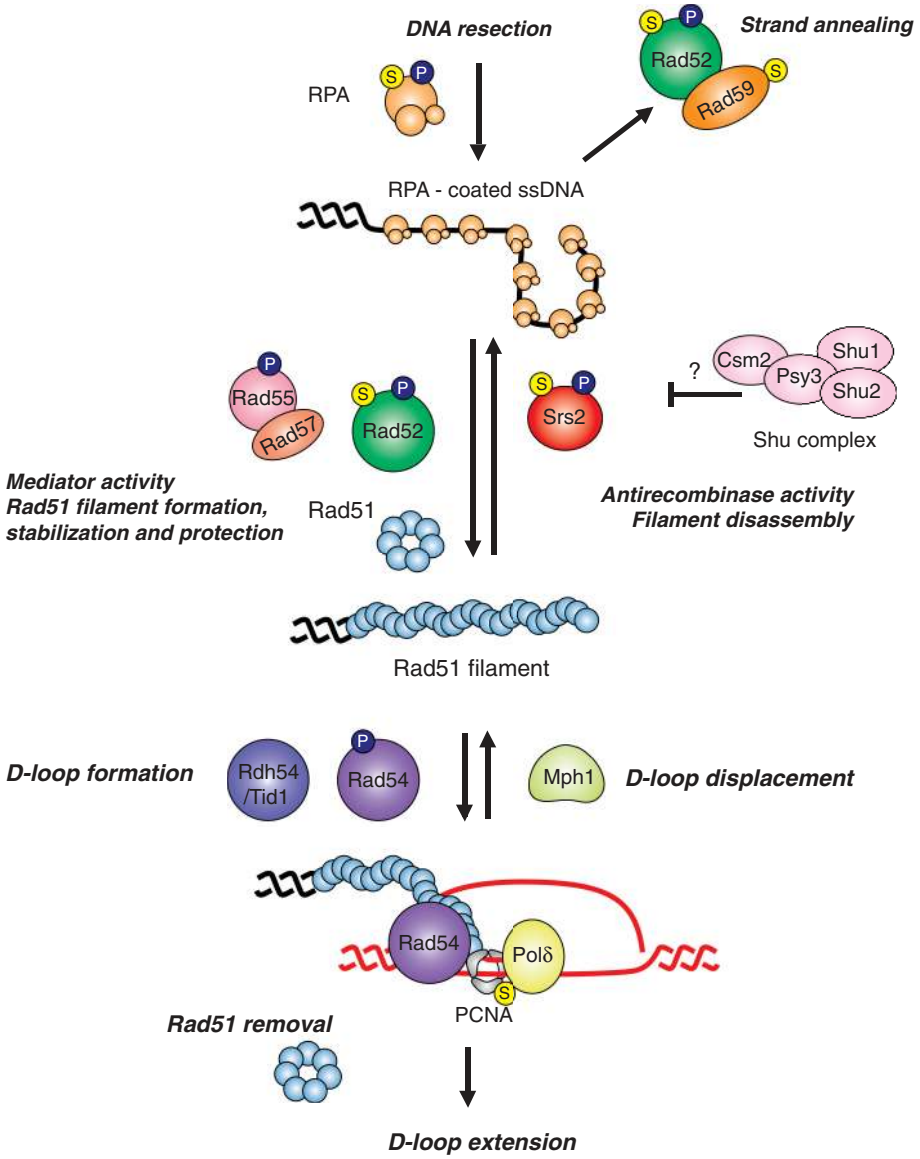

Figure 2. Rad51 filament formation and regulation. RPA can be replaced by Rad51 from ssDNA with the help of recombination mediators, including Rad52 and Rad55/57. These proteins can promote both the formation and stability of Rad51 presynaptic filaments and they themselves also bind DNA during this process (not drawn here for simplicity). Rad51 presynaptic filaments perform homology search with help from Rad54 and Rhd54. Mph1 can promote the SDSA pathway by unwinding D-loop intermediates. Srs2 is capable of dismantling Rad51 filaments in an ATP-dependent manner, leading to the displacement of Rad51 by RPA. This prevents untimely or unwanted recombination. However, Rad52 and Rad55/57 can antagonize Srs2 activity. The Shu complex promotes Rad51 function during replication-associated repair but may also function by antagonizing Srs2. RPA–ssDNA complex can also lead to Rad51-independent repair wherein Rad52 and Rad59 replace RPA from DNA and anneal complementary strands. The balancing act of proteins with antagonistic roles as depicted here determines the fate of Rad51 nucleofilaments and the recombination outcome. Most proteins regulating Rad51 are modified by phosphorylation (P) and/or SUMOylation (S). The modifications are depicted on one form of the protein for simplicity; future work is needed to determine when the modification takes place. These modifications can dynamically change in response to DNA damaging agents and regulate the functions of the target proteins.

Rad52

Rad52 interacts with Rad51 and can also bind RPA once the latter coats ssDNA (31,32). The Rad51–Rad52 interaction is required to recruit and nucleate Rad51 onto RPA-coated DNA (33,34). Only catalytic amounts of Rad52 are needed for presynaptic filament formation (3), suggesting that RPA is not displaced from DNA directly by Rad52, but rather as a consequence of filament extension by the polymerization of nucleated Rad51 molecules (35,36). The mediator function of Rad52 is largely attributable to its C-terminus where the Rad51 and DNA interacting domains are located. However, other Rad52 domains also contribute to recombination (31). The middle part of Rad52 interacts with RPA and is essential for the localization of Rad52 to repair centres (31,37). The N-terminal part of the protein possesses several activities, including oligomerization, DNA binding and annealing, and binding to a

homologous protein Rad59 (34,38,39). The DNA annealing function of yeast Rad52 protein can promote second-end DNA capture in the DSBR pathway, as well as in SSA and possibly other forms of HR (40,41). Similar functions were found for mammalian Rad52 (more in a later section) (40,41). The importance of this function is supported by the observation that most defective *rad52* mutations are found in the N-terminal part of the protein. For example, *rad52-R70A* is defective in DNA binding and annealing, but is proficient for mediator functions and does not affect the recruitment of Rad51 and itself to DSBs. Since *rad52-R70A* cells are γ-radiation sensitive, Rad52's roles in the steps of HR that do not entail Rad51-loading play important roles in DSB repair (42,43).

The Rad55–Rad57 heterodimer

Like Rad51, the Rad55 and Rad57 heterodimer exhibits ATPase activity and binds ssDNA; but unlike Rad51, it cannot catalyse the strand-exchange reaction (44–46). It is noteworthy that while a Rad57 ATPase-deficient mutant confers little sensitivity to irradiation, the corresponding mutation in Rad55 has a much stronger effect (45), indicating that the two proteins are not equivalent. The Rad55–Rad57 heterodimer directly interacts with Rad51 and can load Rad51 onto RPA-coated ssDNA (Figure 2). It can also form co-filaments with Rad51 and the resulting nucleofilament is more resistant to Srs2 anti-recombinase activity (47). These functions are in line with previous data suggesting a role for this complex in the stabilization or protection of Rad51 nucleofilament that is required for downstream HR steps. For example, Rad51 overexpression, gain-of-function Rad51 mutations, or Srs2 removal can rescue the DSB repair defects and DNA damage sensitivity of *rad57*Δ or *rad55*Δ (48–50). However, Rad55 and Rad57 may have other roles besides being Rad51 mediators. For example, spontaneous sister chromatid recombination (SCR) is more defective in *rad51Δ rad57Δ* double mutant than *rad51Δ* and this phenotype cannot be suppressed by *RAD51* overexpression or deletion of *SRS2* (51). This suggests a specialized role for Rad55/Rad57 in SCR that is distinct from its role in modulating Rad51 function.

A LESS UNDERSTOOD POSITIVE REGULATOR OF RAD51—THE SHU COMPLEX

The Shu complex is composed of Shu1, Psy3, Shu2 and Csm2 proteins with Shu1 and Psy3 being Rad51 paralogues. This complex is conserved in *Schizosaccharomyces pombe* and likely also in humans (52,53). The precise role of the Shu complex is not well understood, but available data indicate that it functions as a positive regulator of Rad51 (Figure 2). Like *rad51Δ*, mutants of Shu subunits suppress the DNA damage sensitivity and defects in dissolution of recombination structures associated with mutants such as *sgs1Δ* and *top3Δ*, suggesting that the complex can facilitate Rad51 function (54,55). However, lack of this complex leads to sensitivity only to replication blocking agents and not DSB-inducing agents, indicating a specialized role in dealing with HR during replication stress, such as the facilitation of Rad51 loading onto DNA containing lesions or a function in ssDNA gap repair (54–58). Another suggestion is that the Shu complex, like Rad55 and Rad57, may promote recombination by inhibiting Srs2 since *shu1Δ* results in the accumulation of Srs2 foci (59).

MULTIFACETED REGULATORS OF RAD51—THE SNF2/SWI2 FAMILY MEMBERS

Two members of the Snf2/Swi2 family of DNA-dependent ATPases, Rad54 and Rdh54/Tid1, play multiple roles in regulating Rad51. They serve as positive regulators of Rad51 at early stages of recombination by stabilizing presynaptic filaments, stimulating Rad51-mediated strand invasion, and promoting migration of the branch point of D-loops/HJs, though the latter activity has not yet been demonstrated for Rdh54/Tid1 [Figure 2, reviewed in Refs (3,60)]. Furthermore, these activities promote Rad51-mediated homology search within the chromatin context (61–64). However, they both also work as negative regulators of Rad51 at later stages of recombination by preventing non-specific binding of Rad51 to dsDNA or by removing Rad51 from dsDNA to expose a free 3′-OH primer terminus for DNA synthesis (65–68) (Figure 2). Mutants lacking Rad54 or Rdh54 accumulate Rad51 foci, with *rad54Δ* more defective in the removal of the DNA damage-associated Rad51 foci and *rdh54Δ* in spontaneous ones (69). The sequential execution of positive and negative regulation by Rad54 or Rdh54 is important for efficient recombination.

More recently, another member of this family, Uls1 in budding yeast and Rfp1/2 in fission yeast, was found to genetically interact with recombination factor such as mediator proteins and Sgs1, and to be required for efficient replication of damaged genomes (70). Since mutants lacking Rad54, Rdh54 and Uls1 exhibit more severe defects in Rad51 foci accumulation, slow growth and chromosome loss than any single mutant, these homologues may partially substitute for each other in removing Rad51–DNA complexes (69). Unlike Rad54 and Rdh54, Uls1 was also proposed to be a SUMO-targeted ubiquitin ligase (STUbL) (71). It will be interesting to determine how this and the ATPase functions of Uls1 contribute to HR.

NEGATIVE REGULATORS OF RAD51

There are at least three reasons to remove Rad51 from DNA or block its action. First, recombination can be harmful in certain situations such as stalled replication forks, which may be more safely restored using translesion synthesis. Also, nucleoprotein intermediates generated by the HR machinery can trigger cell-cycle arrest and even cause cell death in certain genetic backgrounds (72–74). This means that recombination events that can interfere with replication progression and DNA repair need to be prevented at an early step, such as presynaptic filament formation. Second, it is important to choose the right

forms of HR in different types of cells and at specific times of the cell cycle. For example, SDSA should be preferentially used during mitosis to avoid potentially harmful events such as loss of heterozygosity. To achieve this, Rad51–ssDNA filaments need to be efficiently displaced from D-loops. Third, Rad51 needs to be removed from post-synaptic filaments to allow subsequent DNA synthesis, resolution and chromatin assembly. These three types of regulation at three different stages of HR require several helicases and translocases, each with properties attuned to a special task.

(i) Srs2, an anti-recombinase that disassembles Rad51 presynaptic filaments

The Srs2 protein belongs to the superfamily I of DNA helicases with the strongest homology to the *E. coli* protein UvrD. Genetic studies suggest that the Srs2 protein can counteract Rad51 function. For example, *srs2Δ* leads to hyper-recombination and sensitizes other helicase mutations, and these defects are suppressed by removal of recombination proteins or mutation of the active site of Rad51 [reviewed in Ref. (75)]. Direct evidence for an anti-Rad51 function came from biochemical studies showing that catalytic amounts of Srs2 efficiently dismantle Rad51 presynaptic filaments (76,77) (Figure 2). The disassembly of Rad51 presynaptic filaments by Srs2 requires both its translocase activity and interaction with Rad51, and is enhanced in the presence of RPA that prevents re-nucleation of Rad51 (76–80). The protein interaction between Srs2 and Rad51 serves two purposes, one to target Srs2 to Rad51 and the other to trigger ATP hydrolysis within the Rad51 filament, causing a weakening of the Rad51–DNA interaction, thus allowing more efficient clearing of the nucleoprotein filament by Srs2 (81). Importantly and as described above, mediator proteins can suppress the action of Srs2 anti-recombinase indicating that the relative strength of the two types of regulation determines the fate of presynaptic filaments (47,76). Although Srs2 is often referred to as an anti-recombinase due to its ability to disassemble presynaptic filaments it also plays a pro-recombination role to promote SDSA. The mechanisms underlying this latter function are not well understood, though three non-mutually exclusive possibilities can be proposed. Srs2 may remove Rad51 filaments from D-loops, prevent second-end capture, or collaborate with nucleases to cleave DNA tails or other intermediates after annealing.

Although no mammalian homologue of Srs2 has been identified, several helicases appear to have acquired a similar function. For example, RecQ5, BLM and FANCJ were reported to disrupt unstable RAD51–ssDNA filaments (82–84). In addition, the human FBH1 protein, which has both helicase and SCF ubiquitin ligase domains, can carry out a subset of the Srs2 functions in yeast, suggesting that it could represent a functional Srs2 homologue in human cells (85). Functional studies in human and *S. pombe* are in agreement with this prediction (86,87). Finally, another human protein, PARI, which lacks ATPase activity, can also suppress inappropriate recombination via its interaction with SUMOylated PCNA and Rad51 (88). Notably, both Srs2 and an FBH1-like protein are present in *Ustilago maydis* and *S. pombe*, and the *fbh1 srs2* double mutant shows more than additive reduction in growth due to unrestrained recombination in *S. pombe* indicating that overlapping systems could exist in some organisms to keep recombination under control (89,90).

(ii) Translocases that unwind D-loop intermediates

Mph1 and its homologues, Fml proteins in fission yeast and FANCM in humans, are translocases. They share several activities, including disrupting Rad51-coated D-loops and catalyzing branch migration (91–94). Mph1 can also displace the extended primer in D-loop-based DNA synthesis (95) (Figure 2). These functions underlie the role of Mph1, and likely its homologues, in favouring SDSA over DSBR, thereby suppressing crossover in mitotic cells (92). The function of these helicases appears to be regulated by accessory proteins. For example, the histone-fold proteins Mhf1 and Mhf2 appear to cooperate with Mph1 in DNA damage and replication fork repair and are suggested to facilitate Mph1 activity (96).

It is likely that similar mechanisms can be used to disrupt telomere specific D-loops (also called T-loops) to facilitate telomere maintenance (97). Indeed, the human RTEL1 protein was shown to efficiently disassemble D-loops (98). Correspondingly, *RTEL1*-deficient cells undergo DSBR at telomeres, resulting in telomere loss, chromosomal rearrangements and formation of telomere circles (99).

(iii) Helicases that dissolve dHJs and channel D-loops into SDSA

Sgs1, a RecQ family helicase, forms a complex with Top3 and Rmi1 proteins in yeast (100,101). Sgs1 has five orthologues in humans, including the cancer syndrome-associated proteins BLM, WRN and RTS, with BLM being the functional Sgs1 homolog. Sgs1 and its homologues have several roles in regulating Rad51 nucleofilaments. Since *sgs1Δ*, like *srs2Δ*, shows hyper-recombination and Sgs1 overexpression can rescue *srs2Δ* recombination defects, Sgs1 may directly dismantle presynaptic filaments, an activity that has been observed for BLM (82,102,103). Additional mechanisms include elimination of aberrant invasion events and resolution of recombination intermediates. This is supported by the ability of Sgs1 to prevent the formation of multi-chromatid joint molecules (104,105). In addition, Sgs1–Top3–Rmi1 can dissolve dHJs in a non-crossover configuration; both Sgs1 and BLM promote the formation of hemicatenane structures by branch migrating two HJs between paired duplexes and this is followed by dissolution using topoisomerase III to produce non-crossover products (106,107). Finally, Sgs1 and similar proteins may also prevent the channelling of D-loop intermediates into the crossover-forming DSBR pathway (Figure 1B). For example, genetic studies in *Drosophila melanogaster* suggest that the BLM orthologue, MUS-309, can free the invading ssDNA tail from D-loops, thereby

channelling it into the strand-annealing step of SDSA (108,109). Combination of these functions likely underlies the increased crossover levels in cells lacking Sgs1 (102,110,111). We note that Sgs1 can also indirectly promote Rad51 filament formation by generation of 3′-overhangs during end processing (102,112). For more details about Sgs1 and its homologues, see review by Ashton and Hickson (113).

(iv) Removal of Rad51 from dsDNA

Several studies suggest that removal of Rad51 from dsDNA is required to promote downstream recombination events. This may occur in multiple steps with the initial ejection of Rad51 from the 3′-end of the invading strand to promote extension of the D-loop by DNA repair synthesis (Figure 2). But complete removal of Rad51 from dsDNA may be required for the resolution of recombination intermediates and chromatin assembly. A function in Rad51 removal from dsDNA was first reported for yeast Rad54 protein as described above. Recently, *Caenorhabditis elegans* proteins ceHELQ-1 and ceRFS-1 were also shown to promote post-synaptic Rad51 filament disassembly from strand invasion intermediates (114). This is in agreement with the persistence of ceRad51 foci at meiotic DSBs in *helq-1* and *rfs-1* mutants, and the biochemical evidence that these proteins can remove ceRad51 from dsDNA but not ssDNA. The disruption activity of the ceRFS-1 peptide requires ceRad51 ATP hydrolysis, as dsDNA–ceRad51 filaments formed in the presence of nonhydrolysable analog of ATP are resistant to disruption. Since ceRFS-1 is a ceRad51 paralogue, it might integrate into and stabilize the ceRad51 filament on ssDNA, but inhibit ceRad51 binding to dsDNA (114).

REGULATING RAD51 AND ITS REGULATORS BY POST-TRANSLATIONAL MODIFICATIONS

The regulatory mechanisms governing HR involve not only the aforementioned positive and negative regulator proteins, but also an intricate network of post-translational modifications (PTMs) (Table 1). Genetic studies provided the first clues for the important roles of PTMs, particularly phosphorylation and SUMOylation, in HR regulation. For example, lack of cyclin-dependent kinase (CDK) and the DNA damage checkpoint severely diminishes HR in yeast and higher eukaryotic cells [reviewed in Ref. (72)]. In addition, mutating the SUMOylation or deSUMOylation enzymes in yeast leads to a range of phenotypes indicative of HR defects, such as hypersensitivity to DNA damaging agents and accumulation of recombination intermediates (115–118). Recent advances in this field provide some degree of detailed understanding of how CDK and checkpoint-mediated phosphorylation and SUMOylation affect the functions of Rad51 and its regulators.

Modifications of RPA, Rad51 and Rad55

The large subunit of RPA in both budding yeast and humans is SUMOylated upon genotoxic treatment (119,120). Genetic data in yeast suggest that RPA SUMOylation may disfavour Rad51-independent pathways, such as SSA and BIR. In human cells, DNA damage triggers the dissociation of the RPA subunit, RPA70, from the deSUMOylating enzyme SENP6, resulting in RPA modification by SUMO-2/3. SUMOylated RPA70 facilitates Rad51 foci formation, and promotes HR and DNA damage resistance (120). Depletion of PIAS1 or PIAS4, the human SUMO E3 ligases, impairs human RPA accumulation at damage sites and causes a decrease in HR levels, indicating that SUMOylation is also important for RPA recruitment to DSB sites (121,122).

RPA is phosphorylated both by checkpoint kinases, ATM/Mec1, and by cell-cycle kinases CDKs. Phosphorylation of RPA by these kinases is critical for Rad51 recruitment to DSB sites or for HR during replication stress (30,123). *In vitro* studies provide some mechanistic understanding of this modification. Phosphorylation of RPA increases the binding affinity of Rad52 for ssDNA, thus promoting the mediator function of Rad52 (124). In line with this idea, Rad52 recruitment is dependent upon RPA during S and G2/M phases, and CDK1 activity (20,125). The role of CDK1 in this case may be both to generate ssDNA by enabling resection and to modify RPA to facilitate Rad52 recruitment (126). Dephosphorylation of RPA is also important, as depletion of PP4C or PP4R2, components of the heterodimeric phosphatase that controls dephosphorylation of RPA, also impairs HR (127).

Rad51 was identified as a SUMO and Ubc9 interactor (128,129). In further support of the connection between Rad51 and SUMOylation is the observation that mislocalization of UBC9 or depletion of SUMO E3 ligase MMS21 disrupts RAD51 trafficking, resulting in marked inhibition of DNA damage-induced RAD51 nuclear foci formation (130). However, it is not clear whether this is mediated by a direct effect on RAD51. Rad51 was shown to be phosphorylated by several kinases (Table 1). Phosphorylation of Tyr-315 by BCR/ABL appears to be essential for enhanced DSB repair and drug resistance, and phosphorylation of Tyr-54 by c-Abl inhibits Rad51 binding to DNA and its ATP-dependent DNA strand-exchange reaction (131,132). Recent work also uncovered the phosphorylation of Ser-192 in a Mec1-dependent manner in response to DNA damage (133). This residue is required for Rad51 ATPase and DNA-binding activity *in vitro*, suggesting that the modification can affect Rad51 activity (133). Moreover, human RAD51 has been shown to be phosphorylated at Ser-14 by Plk1 in a cell-cycle- and DNA-damage-responsive manner. Ser-14 phosphorylation triggers phosphorylation at Tyr-13 by casein kinase (CK2) leading to direct binding to the MRN component, Nbs1. This process helps RAD51 to be recruited to DNA damage sites, thus allowing accurate HR (134). Phosphorylation also affects the formation of Rad51 nucleofilaments by modifying the mediator Rad55 (135). Rad55 is phosphorylated by DNA damage checkpoint kinases at three residues (serine 2, 8 and 14), and the unphosphorylable mutant displays increased sensitivity to genotoxic stress and replication fork stalling, indicating that this modification promotes Rad51 function (136).

Rad52 and its modifications

Rad52 proteins in fission yeast, budding yeast and human cells are all SUMOylated (137,138). In budding yeast, Rad52 SUMOylation is induced after DSB generation in meiotic cells or genotoxic treatment of S-phase cells (139,140). SUMOylation of Rad52 likely precedes Rad51 filament formation based on the observations that RPA-bound ssDNA enhances Rad52 SUMOylation and that SUMOylation inhibits Rad52 DNA binding and strand-annealing activity (141). Since *rad51Δ* leads to the accumulation of Rad52 foci, it is likely that Rad52 SUMOylation can be attenuated upon Rad51 filament formation. Studies using mutants affecting the three SUMOylation sites Rad52 (lysines 10, 11 and 220) suggest that the role of Rad52 SUMOylation can be diverse depending on the state of the DNA substrates. First, this modification can shelter Rad52 from proteasome-mediated degradation when recombination intermediates accumulate in *sgs1Δ srs2Δ* background (138). This was extrapolated to suggest that SUMOylation may serve to protect the active forms of Rad52 from degradation (138). Second, SUMOylation of Rad52 is important for damage-induced interchromosomal recombination and recombination pathway choices, with a bias towards gene conversion and against BIR and SSA (139,141). Furthermore, Rad52 SUMOylation appears to facilitate the exclusion of Rad52 foci from the rDNA locus thereby inhibiting rDNA recombination (142). It will be interesting to determine whether the different effects seen for Rad52 SUMOylation are mediated by the same or different molecular mechanisms.

In contrast to yeast Rad52, SUMOylation does not seem to affect the biochemical activities of human Rad52 nor is it induced by DNA damage. Rather, SUMOylation alters RAD52 subcellular localization (143). In addition to SUMOylation, Rad52 also undergoes phosphorylation. While phosphorylation of yeast Rad52 occurs constitutively, that of RAD52 at tyrosine 104 is mediated by c-ABL and activated upon exposure to various types of DNA damage (144,145). The phosphotyrosine analogue of Y104 of RAD52 enhances ssDNA annealing activity by attenuating dsDNA binding, suggesting that this modification can direct RAD52 to DNA repair intermediates that undergo annealing (146).

Modifications of translocases in HR regulation

The dual functions of Srs2, both as a negative regulator of HR by dismantling presynaptic filaments and as a positive regulator by processing recombination intermediates in favour of the SDSA pathway, suggest that the protein may be regulated by different modifications to serve different functional purposes. Indeed, Srs2 is regulated by both phosphorylation and SUMOylation in response to DNA damage. Cdk1-mediated phosphorylation of Srs2 appears to promote the SDSA pathway (147). A pro-SDSA function may provide an explanation for the requirement of this phosphorylation in HR-dependent recovery after chronic exposure to low doses of UV irradiation (148). Additionally, Srs2 is SUMOylated near the C-terminus of the protein [(147) and our unpublished data], a region that interacts with both SUMO and PCNA and is required to prevent unscheduled recombination events at replication forks (149,150). The function of Srs2 SUMOylation is less clear, but genetic data suggest an important role for this modification. In particular, inhibition of Srs2 phosphorylation results in the accumulation of SUMOylated Srs2. In addition, SUMOylation of Srs2 is responsible for the DSB repair defects associated with non-phosphorylable Srs2, as eliminating its SUMOylation is able to rescue the phenotype of the latter. Understanding how SUMOylation can cause toxicity to cells when Srs2 is not phosphorylated will provide important clues about the function of Srs2 SUMOylation.

Additionally, both Sgs1 and BLM proteins are SUMOylated (115,151). While SUMOylation of yeast Sgs1 appears to specifically promote recombination at telomeres, that of BLM was shown to increase its binding to RAD51 and promote HR at stalled replication forks (152,153). Cells expressing a SUMO-deficient mutant of BLM display defects in RAD51 localization to stalled replication forks and failure to induce sister chromatid exchanges (SCEs), indicating that SUMOylation of BLM controls the recruitment and/or retention of RAD51 at damaged replication forks (153). Additionally, SUMOylation of another RecQ-like helicase, WRN, was suggested to be involved in multiple processes, such as co-localization with RAD51, stabilization of stalled replication forks, and telomere maintenance (154,155). It remains to be determined whether these functions reflect a more fundamental effect of WRN SUMOylation that is manifested in different cell lines or conditions.

The function of Rad54 is also regulated by at least two types of PTMs. Its activity during the G1 phase of the cell cycle in *S. pombe* seems to be regulated by ubiquitin-mediated proteolysis (156). In meiosis, Rad54 undergoes Mek1-dependent phosphorylation that abrogates its interaction with Rad51, thus preventing inter-sister recombination (157). Recently, the Rad53 kinase was also shown to target Rad54 for phosphorylation at the same site, suggesting that Rad54 may also be under checkpoint control in the mitotic DNA damage response (158).

In summary, available data suggest that PTMs regulate HR at several levels. A better understanding of how these modifications affect HR proteins will require the integration of biochemical examination of the modified forms of the protein and *in vivo* genetic studies. In addition, it is important to understand the interplay between the different forms of modifications: when they can work in a concerted manner and when they can be antagonistic to each other. A recent work shows that many of the SUMOylation targets are different from the checkpoint substrates, though a number of proteins are subjected to both modifications, indicating both separateness and potential coordination between SUMOylation and checkpoint-mediated phosphorylation (159). Other interconnections between protein modifications likely exist. For example, given the presence of STUbL proteins

such as Uls1 and the Slx5/8 complex that have been implicated in HR, it will not be surprising if the interplay between SUMOylation and ubiquitylation also contributes to HR regulation.

FUNCTIONS OF RAD51 AND ITS REGULATORS IN MAMMALIAN CELLS

The function of Rad51 appears to be largely conserved in higher eukaryotic cells, however, its regulators and their functions are more complex (Table 1). Multiple homologues of the yeast proteins have evolved to affect different aspects of DSB repair or in different tissues, or to link repair with other cellular processes such as checkpoint control and apoptosis. In addition, new mediators and regulators have also appeared. Here, we focus on the core proteins that directly interact with Rad51, including ssDNA-binding proteins, mediators and their regulators, and the Rad54 proteins. Detail information on other translocases and helicase homologues in higher eukaryotic cells can be found in several recent reviews (160–165).

RAD51 and ssDNA-binding proteins

Although the biochemical activities of RAD51 mimic those of yeast Rad51 and bacterial RecA, RAD51 in higher eukaryotic cells is essential for cell survival as demonstrated in both mouse and chicken DT40 cells (166–169). The essentiality of RAD51 in these organisms is likely due to the increased burden of repair associated with the higher number of lesions in larger genomes (166). Another intriguing aspect of RAD51 and its paralogues is that they are implicated in the oxidative stress response in mitochondria (170). Further developments on this front will help answer the long-standing question of recombination in mitochondrial genomes.

While RPA is highly conserved between yeast and humans, human cells have two other ssDNA-binding proteins, human SSB1 and SSB2 that bear a greater resemblance to bacterial SSB than RPA. SSB1 deficiency does not affect replication and S-phase progression, but results in checkpoint activation defects, increased IR sensitivity and impaired HR, implying a role in the DSB response (171). Indeed, SSB1 and SSB2 are part of the sensor ssDNA complex that binds to DSB ends and is required for ataxia telangiectasia mutated (ATM) checkpoint signalling and efficient HR repair (172,173). An additional function was assigned to SSB1 in DSB processing, during which it can recruit and stimulate the activity of MRN complex via its interaction with the NBS1 subunit (174,175).

BRCA2—the main mediator

While the requirement for mediators is universally conserved, the specific proteins can vary between organisms. Despite the presence of human RAD52 protein, the central RAD51 mediator function in humans is carried out by another protein, BRCA2. Although BRCA2 has no homology with yeast Rad52, BRCA2 is its functional equivalent since it controls the assembly of human RAD51 into nucleoprotein filaments as demonstrated both *in vivo* and *in vitro* (15,176). In particular, the structural characterization of the C-terminal part of BRCA2 and its mediator activity was essential in this regard (177). For example, both BRCA2 and RAD52 specifically interact with the corresponding Rad51 proteins, show preferential binding affinity for ssDNA, have the ability to overcome RPA inhibition, and promote RAD51-mediated strand exchange.

As BRCA2 orthologues in various organisms appear to function as mediators, yeast may be an exception in that it uses Rad52 as the mediator. The BRCA2 orthologues differ greatly in size and domain structures, suggesting evolutionary flexibility and explaining the ability of the ssDNA-binding region from RPA or RAD52 proteins to substitute for the BRCA2 DNA-binding domain (DBD) to efficiently suppress the cellular defects of BRCA2-mutant cells (178). The understanding of the role of BRCA2 in HR benefits greatly from studies of its *U. maydis* homolog Brh2, which is much smaller than BRCA2 (179,180). The recent breakthrough with the purification of full-length BRCA2 confirmed previous results seen by using truncated proteins, as well as provides new insights into the biochemical functions of BRAC2, such as its possible dimerization, its capacity to bind approximately six RAD51 proteins, and its stimulation of RAD51 activities without direct interaction with RPA (181–183).

Recent studies have also provided more insight into how BRCA2 interacts with and affects RAD51 function. BRCA2 can interact with RAD51 through two types of domains. The first type includes various conserved BRC repeats that exhibit different capacities for RAD51 interaction. One category of BRC domains performs the mediator function by targeting RAD51 to ssDNA to form a nucleoprotein filament, and by stabilizing this nucleofilament in active form via down-regulation of RAD51 ATP hydrolysis. The other category of BRC domains can prevent the nucleation of RAD51 on dsDNA (184–187). Besides BRC domains, BRCA2 also interacts with RAD51 through its C-terminal part that is encoded by exon 27 of the human BRCA2 gene. Unlike BRC domains, this region can interact with RAD51 only in the nucleoprotein filament form in a cell-cycle-dependent fashion (188,189). Two recent studies suggest that this domain stabilizes RAD51 filament or replication forks. In the first study, mutations within the BRCA2 C-terminus that block its interaction with RAD51 was shown to not affect Rad51 foci formation or HR repair, but instead result in rapid foci disassembly and mitotic entry (190). In another case, the C-terminal domain of BRCA2 is essential for fork protection by stabilizing RAD51 filaments and preventing MRE11-mediated degradation (191). Altogether, the multiple RAD51 interaction domains meet the different demands for BRCA2 function as both a mediator and a scaffold protein that links HR with replication and mitosis. It is noteworthy that inside cells, the interaction between BRCA2 and RAD51 is also subject to regulation by localization, as DNA damage can induce a redistribution of soluble nucleoplasmic BRCA2 available for RAD51 binding (192). In addition, it will be interesting to

understand whether the BRCA2–RAD51 interaction is critical for the newly described role of BRCA2 in preventing the degradation of newly synthesized DNA when replication is interrupted (191).

DSS1—a binding partner of BRCA2

DSS1 interacts with the C-terminal DBD of BRCA2 (177). In *U. maydis, dss1Δ* mutants are phenotypically similar to *rad51Δ* and *brh2Δ*. DSS1 confers allosteric regulation of the Brh2–DNA interaction and prevents the formation of Brh2 homo-oligomers, thereby maintaining it in an active state (193,194). No such effect was observed for the human protein, but rather the human DSS1 facilitates BRCA2 in RAD51–ssDNA filament formation (168). Strangely, the yeast DSS1 homologue, Sem1, is a subunit of the regulatory component of the proteasome as well as signalosome, which is involved in de-neddylation and activation of some types of ubiquitin E3s. This indicates that the Sem1/DSS1 family proteins are versatile proteins regulating the integrity and function of several protein complexes involved in diverse pathways (195). Depletion of DSS1, like BRCA2 depletion, greatly reduces HR efficiency, and this is not via a ubiquitin–proteasome system, suggesting that DSS1 regulates BRCA2 by means other than regulating protein stability (196).

PALB2 and other BRCA2 regulators

Another important regulator of BRCA2 is PALB2. PALB2 interacts with the N-terminus of BRCA2 and plays several roles in HR by regulating BRCA2 and possibly by directly affecting RAD51 function. Several germline BRCA2 mutations identified in breast cancer patients lead to loss of PALB2 binding and BRCA2 function in HR, suggesting that PALB2 is a key regulator of BRCA2's biochemical and tumour suppression function (197). In addition, a germline mutation of PALB2 itself was also identified in breast cancer patients (198). The structure of the PALB2 C-terminus in complex with BRCA2-peptide identifies molecular determinants for the protein–protein interaction and helps to explain the effects of cancer-associated truncations of both proteins (199).

PALB2 colocalizes with BRCA2 in nuclear foci and stabilizes BRCA2 by promoting its chromatin association. In addition, PALB2 and its oligomerization promote the delivery and stabilization of RAD51 to the site of DNA damage (197,200). While this effect likely involves its regulation of BRCA2, PALB2 may also directly affect RAD51 function. PALB2 was recently shown to bind DNA, directly associate with RAD51, and promote RAD51-mediated D-loop formation. Additionally, it also binds to and cooperates with RAD51AP1 (described below) to enhance RAD51-mediated recombination activities, suggesting a role after the assembly of presynaptic filaments (201,202). Both PALB2 and BRCA2 influence cell-cycle checkpoints, as depletion of either prematurely abrogates checkpoint signalling and activates the checkpoint-recovery pathway (203). In addition, p53 interacts with multiple regions of BRCA2 and suppresses HR in a transactivation-independent fashion, whereas overexpression of BRCA2 attenuates p53-mediated apoptosis, suggesting that BRCA2 also connects HR with apoptosis (204).

MCPH1 (microcephalin) is another BRCA2-interacting partner that can reduce the levels of both BRCA2 and RAD51 at damage sites and interfere with BRCA2-dependent HR (205). Similar results were observed for the mouse homologue of microcephalin, BRIT1 (206), suggesting that these proteins provide a means to attenuate RAD51 function.

RAD52 with non-conserved functions

While human RAD52 shares structural and some biochemical similarity with yeast Rad52, it has not been shown to possess recombination mediator activity. This could explain both the minor role of RAD52 in vertebrate HR and its replacement by BRCA2 for loading RAD51 on ssDNA (207). Despite its high homology with yeast Rad52, human RAD52 is functionally more similar to the yeast Rad59 protein, which acts with Rad52 and has both a minor role in Rad51-dependent recombination and a critical role in SSA between direct repeats. Like Rad59, human RAD52 lacks the C-terminal part of the yeast Rad52 that contains Rad51- and RPA-interaction domains, as well as the region responsible for mediator activity (31). Similarly, RAD52 also possesses strand-annealing activity and acts in parallel to BRCA2, and its inactivation is lethal in BRCA2 deficient cells (208–210). However, RAD52 may be able to compensate for BRCA2 under certain circumstances as observed in *U. maydis* (211). RAD52 also has a function in the late stages of DSB repair at stalled or collapsed replication forks that does not appear to be shared by BRCA2 (212). These observations argue that RAD52 has a unique role in catalyzing ssDNA annealing in homology-directed DNA repair. These activities may be toxic in certain genetic backgrounds since RAD52 deletion can partially rescue T cell development and reduce T-cell lymphomas in ATM-deficient mice (213).

Rad51 paralogues and other Rad51 binding factors

The RAD51 paralogues, including RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3, share 20–30% sequence identity with RAD51. Several lines of evidence suggest that they function as mediators or promote and/or stabilize RAD51 nucleofilaments. For example, depletion of these proteins blocks IR-induced RAD51 foci formation, and the defects in each of these RAD51 paralogues are partially suppressed by overproduction of RAD51 in chicken DT40 cell lines (214–216). Two complexes can be formed by RAD51 paralogues, including the RAD51B–RAD51C–RAD51D–XRCC2 complex and the RAD51C–XRCC3 complex (217). The first complex has the highest affinity for branched DNA substrates, which is consistent with a function in formation or stabilization of RAD51 filaments during repair of damaged replication forks (218–221). In addition, this complex can stimulate homologous DNA pairing, likely due to the ability of RAD51C to promote the melting of dsDNA. The

second complex likely plays a role in the later steps of recombination, as suggested by the association of RAD51C–XRCC3 complex with HJ resolution activity in human cell extracts and that RAD51C-deficient cells show phenotype associated with defects in HJ resolution activity (222,223). The RAD51 paralogues may function in parallel with BRCA2 in RAD51 loading, as RAD51C foci are not affected in BRCA2-deficient cell lines (218). While mutations in any of these paralogues in chicken or hamster cells lead to increased sensitivity to DNA damaging agents (224), disruption of RAD51B, RAD51D and XRCC2 in mice leads to embryonic lethality, indicating an increased dependency of these proteins in larger genomes (225–227). Further elucidation of the molecular mechanisms of how RAD51 paralogues function in different steps of HR will illuminate the complex regulation of RAD51.

RAD51AP1

Additional factors are also involved in regulating RAD51. Most noticeably, RAD51AP1 (Rad51-associated protein 1) represents a vertebrate-specific protein that interacts with human RAD51 (228). It enhances RAD51 recombination activity by stabilizing D-loops formed by RAD51, but plays little or no role in the assembly of DNA damage-induced RAD51 foci (229,230). This suggests that the function of RAD51AP1 is limited to the DNA strand invasion step of HR. Meiosis-specific roles of RAD51AP1 are described below.

Human Snf2/Swi2 members involved in HR

Human cells possess two Rad54 homologues, RAD54 and RAD54B, which share similar biochemical activities (231). However, in contrast to the situation in yeast, knockouts of either RAD54 or RAD54B show modest to no HR defects in vertebrates, though the RAD54 RAD54B double knockout displays stronger defects (232). One possible explanation for the different effects of lack of Rad54 in yeast and vertebrates is that yet other members of the Snf2/Swi2 family in the latter case may be able to carry out similar functions, though these factors are yet to be identified.

MEIOSIS-SPECIFIC REGULATION OF RECOMBINATION NUCLEOFILAMENTS

Recombination in meiosis shares similarities with mitotic recombination, but also exhibits many unique features. Unlike mitotic recombination, meiotic recombination is genetically programmed with DNA breaks being endogenously induced by Spo11 (233). The repair of Spo11-generated breaks is essential for homolog pairing in some organisms and for the generation of genetic diversity. Recombination also mediates crossing-over between homologues leading to the formation of chiasmata, which are required for proper segregation of homologous chromosomes at meiosis I. In addition, the process of HR in meiosis needs to be tightly integrated with other DNA–protein structures uniquely required for meiosis such as the synaptonemal complex. Finally, to allow homologous chromosomal pairing and the generation of genetic diversity, the DSBR mode of recombination is more favoured in meiosis than in mitosis by several mechanisms. These specific requirements during meiosis are fulfilled by both having a specialized strand-exchange protein and several meiosis-specific regulators.

Meiosis-specific strand-exchange proteins

Most eukaryotes contain a meiosis-specific Rad51 paralogue, Dmc1. Unlike *rad51Δ*, which leads to severe defects in both mitotic and meiotic recombination, *dmc1Δ* is deficient only in meiotic recombination (8,233,234). The essential role of Dmc1 in this process is demonstrated by the spore inviability, absence of recombination intermediates and dramatic reduction of crossover products in *dmc1* mutants (235–237). A conserved role of Dmc1 is seen in mouse cells, as lack of mouse Dmc1 results in the same phenotype as that of yeast *dmc1* mutants (238,239). However, several organisms such as *D. melanogaster*, *C. elegans* and *Neurospora crassa* lack the Dmc1 protein suggesting the use of alternative mechanisms (233).

There are several similarities and differences between Rad51 and Dmc1. In the absence of DNA, both exist as rings consisting of several protomers. In the presence of DNA, DMC1 forms a helical filament as well as stacked rings (240–243). However, only the filament similar to that formed by Rad51 shows the ability to catalyse DNA pairing and strand exchange (242,244). Several articles have described the differences in the properties of Dmc1 and Rad51. For example, Rad51 and Dmc1 proteins localize differently on meiotic chromosome (245,246). In addition, D-loops formed by DMC1 are more resistant to dissociation by branch-migration proteins such as RAD54 than the ones formed by RAD51 (247). It needs to be noted that interpretations of these observations should consider the different methodologies as well as conditions employed (243,248).

The interplay between Rad51 and Dmc1 is not yet fully understood, and several non-exclusive models have been put forward. The cooperative model suggests the formation of co-filaments composed of both proteins, whereas other models prefer the formation of asymmetric filaments or the assembly of different types of nucleofilaments leading to different HR subpathways (249). However, it does not seem that Rad51 and Dmc1 can form different filament structures with intrinsically distinct biochemical activities. This means that the different effects of the two proteins have to be also influenced by the distinct sets of specific accessory proteins that can differently interact with these proteins.

The Mei5–Sae3 complex—a meiosis-specific mediator only in budding yeast

Mei5 and Sae3 likely represent a meiosis-specific recombination mediator required for Dmc1 recruitment and loading, with no effect on Rad51 filament formation in budding yeast (250). As a typical recombination mediator, the Mei5–Sae3 heterodimer interacts with Dmc1, RPA, and both ssDNA and dsDNA, and is able

to overcome the inhibitory effect of RPA on the Dmc1-mediated strand-exchange reaction (251,252). In addition, similar to other recombination mediators, such as Rad55/Rad57, mutations in the *SAE3* gene result in hyper-resection of DSBs (253). Sae3–Mei5 localization is dependent on Dmc1, suggesting interdependency. This is reminiscent of the relationship between the Rad55–Rad57 complex and Rad51 (20,49,254). The roles of these proteins appear to be conserved, but may exhibit some variation regarding whether they facilitate Rad51 and/or Dmc1.

The Mei5–Sae3 complex also has roles in mitosis in other organisms. The fission yeast homologues (Swi5–Sfr1) function in both mitotic and meiotic cells, and exhibit mediator activity in both Dmc1- and Rad51-mediated strand-exchange reactions (255–258). Recently, the human and mouse homologues of the Swi5–Mei5 complex have been identified and were shown to interact with Rad51. Accordingly, their depletion leads to defects in Rad51 foci formation and increased sensitivity to DNA damaging agents (259,260). It appears that this complex functions in both mitosis and meiosis, and the budding yeast situation is the exception.

The Hop2–Mnd1 complex and its multiple roles in promoting meiotic recombination

The Hop2–Mnd1 complex is another meiosis-specific factor identified in all organisms expressing Dmc1. The absence of both proteins results in non-homologous synapses and persistence of meiotic DSBs (261). This complex likely performs two functions. First, it can stabilize Rad51- and Dmc1-presynaptic filaments (262,263). However this activity is different from a recombination mediator role, as both Rad51 and Dmc1 foci form normally in *mnd1* and *hop2* mutants, and unlike mediators, Mnd1 is not recruited to DSB sites (261,264,265). Second, the Hop2–Mnd1 complex facilitates strand invasion and stimulates D-loop formation by promoting the capture of dsDNA by Dmc1 or Rad51 nucleoprotein filaments (262,263). This function is suggested by the observation that *mnd1* mutants exhibit normal initiation of recombination but fail to form heteroduplex DNA or dHJs (266). A likely mechanism for this function is the reversible dsDNA condensation that allows efficient capture of homologous dsDNA (265,267). This represents a mechanism distinct from Rad54 stimulated synapsis, where dsDNA capture follows ATP hydrolysis-coupled dsDNA translocation (268). Further work is needed to understand how the various biochemical functions of Hop2–Mnd1 contribute to meiotic recombination.

Rad54 and Rdh54/Tid1 and their different roles in meiosis

Rad54 and Rdh54/Tid1 are also important for recombination during meiosis. Their double mutant almost eliminates meiotic HR, whereas each single mutant results in partial defects in both sporulation and spore viability (269,270). Rdh54 seems to be more critical during meiosis than mitosis, likely due to its role in promoting Dmc1-mediated interhomologue recombination (269–271). Indeed, Dmc1 interacts with Rdh54/Tid1, but not Rad54, although Rad51 interacts with both (271–273). In addition, Rdh54 prevents the accumulation of Dmc1 on chromatin in the absence of DSBs in an ATPase-dependent manner, suggesting that Rdh54 can dissociate dead end Dmc1 complexes (274). These activities have also been demonstrated biochemically as purified SpRdh54 can both stimulate the Dmc1 reaction and remove Dmc1 from dsDNA in an ATP-dependent manner (275).

In contrast to the active role of Rdh54/Tid1 in regulating Dmc1, Rad54 fails to disassociate Dmc1-mediated D-loops (247). This may provide a better opportunity for second-end capture of Dmc1 D-loops and promote DSBR. In addition, Rad54 is regulated by Mek1-mediated phosphorylation that inhibits the Rad51–Rad54 interaction, providing another means to favour Dmc1-mediated recombination (157). However, Rad54 does contribute to meiotic progression, likely by promoting sister chromatid or interhomologue recombination (276).

Hed1—a meiotic Rad51 inhibitor

Hed1 mediates another mechanism in favour of Dmc1-mediated recombination in meiosis in budding yeast. Hed1 interacts with Rad51 in yeast two-hybrid assays and colocalizes with Rad51 at meiotic DSBs in a Rad51-dependent manner (277). Hed1 does not affect Rad51 presynaptic filament formation; rather, it interferes with the Rad51–Rad54 interaction thereby restricting Rad54 recruitment to site-specific DSBs (278). In agreement with this, overexpression of both Rad51 and Rad54 in *dmc1* cells can suppress Hed1-mediated inhibition of Rad51 function (279,280). As there are no apparent Hed1 homologues in other higher eukaryotic cells, how Rad51 is inhibited in these systems remains to be elucidated.

Other meiotic recombination factors

Two mammalian Rad51-interacting proteins, RAD51AP1 and RAD51AP2, also regulate meiotic HR. hDmc1-mediated D-loop formation is enhanced by RAD51AP1 and the functional synergy of the two proteins requires their physical interaction (281). RAD51AP2 is a meiosis-specific Rad51-interacting protein as suggested by yeast two-hybrid results, but the possible regulatory role of this protein remains unclear (282).

Besides a critical role in mitotic recombination, BRCA2 is also implicated in meiosis and binds both Rad51 and Dmc1 in *A. thaliana* and humans (283,284). Distinct binding domains could allow coordinated interactions of the two strand-exchange proteins with BRCA2 during meiosis. Genetic data from several organisms also support a role for BRCA2 in meiosis. First, silencing of plant BRCA2 results in meiotic defects and sterility, which could also be related to its role in oocyte nuclear architecture and gametogenesis (285,286). Second, deletion of *Drosophila* BRCA2 leads to recombination defects and checkpoint activation during meiosis (287). Finally, BRCA2-deficient zebrafish and mice cell lines reveal a role for BRCA2 in ovarian development and in tumourigenesis of reproductive tissues and impairment

Table 2. List of diseases linked to or associated with either recombination mediators or their interacting partners, synthetic lethality interactions are also shown (216,290,291)

Genes	Syndromes/disorders	Cancers	Synthetic lethality
BRCA1	–	Breast, prostate, ovarian cancer	PARP1
BRCA2	Fanconi anaemia (FANCD1)	Breast, prostate, ovarian cancer	PARP1, RAD52
RAD54B	–	Colon cancer, non-Hodgkin lymphoma	PARP1, FEN1
RAD51B	–	Lipoma, uterine leiomyoma	PARP1
RAD51C	Fanconi anaemia (FANCO)	Breast, ovarian cancer	–
BLM	Bloom	–	–
WRN	Werner	–	–
RECQL4	Rothmund–Thomson	–	–
p53	Li–Fraumeni	Breast, pancreatic, lung cancer, etc.	–
FANCM	Fanconi anaemia (FANCM)	–	–
PALB2	Fanconi anaemia (FANCN)	Breast, pancreatic cancer	
Potential associations with diseases			
RAD51	–	Breast cancer in BRCA1 and BRCA2 carriers	–
DMC1	Infertility		
XRCC2	–	Breast cancer	–
XRCC3	–	Basal cell carcinoma, malignant melanoma, bladder cancer, breast cancer	RAD52
RAD51D	–	Breast cancer	–
DSS1	Split hand/split foot malformation	Skin squamous cell carcinoma	–

of mammalian gametogenesis, respectively (288,289). Similarly, Brh2 and Dss1 proteins, together with Rad51, are required during meiotic HR in *U. maydis* (180).

RECOMBINATION DEFECTS IN HUMAN DISEASES

Given the important roles of RAD51 and its regulators in repairing DNA lesions and preventing inappropriate recombination, it is not surprising that mutations of these proteins can lead to predisposition to a variety of cancers (Table 2) (290,291). Among the RAD51 regulators, heterozygous mutations in BRCA2 increase susceptibility to breast and ovarian cancers (292). While heterozygous mutations in several HR genes involved in Rad51 filament assembly, including BRCA2, PALB2 and RAD51C increase the risk of breast, pancreatic and ovarian cancer, homozygous mutations cause Fanconi anaemia (FA), a cancer predisposition syndrome characterized by a defect in the repair of DNA interstrand crosslinks (197,198,293–297). Mutations of other RAD51 regulators were also found in cancer cells. For example, translocation of RAD51B was found in uterine leiomyoma and several mutations of RAD54B that reduce or eliminate its activity *in vitro* have been found in primary colon carcinomas and lymphomas (298–300). Inappropriate HR during meiosis due to mutation of RAD51 regulators, results in abnormal numbers of homologous chromosomes, developmental abnormalities, and/or embryonic death (288,301). In addition, mutations in BLM and WRN helicases are associated with cancer-predispose syndromes, genomic instability and premature aging (160,163).

Although mutations in RAD51 have not been linked to any disease, many cancer cell lines show elevated levels of the protein. It has been proposed that high levels of RAD51 may lead to uncontrolled HR and destabilization of the genome in the early events in carcinogenesis (302). Another view is that higher levels of RAD51 help to maintain the genome during tumourigenesis when it experiences some levels of instability (224). Accordingly, it was shown that p53 plays an important role in suppressing RAD51 expression and activity [for review see Ref. (303)]. In addition, constitutive activation of c-ABL due to the BCR–ABL fusion, a key event in the pathogenesis of chronic myeloid leukaemia and other myelo-proliferative diseases, results in higher expression and phosphorylation of RAD51, promoting unfaithful HR events and contributing to secondary aberrations or drug resistance (131). Another example is c-ABL activation that enhances nuclear localization of RAD52 (304), accompanied by upregulation of SSA (305). This suggests that the BCR/ABL kinase may shift the balance from error-free to mutagenic recombination. Finally, a mutation in the other strand-exchange protein, DMC1, has been associated with infertility (306).

DIAGNOSIS AND THERAPEUTIC STRATEGIES

Due to the important roles of HR proteins in tumour progression and their involvement in the resistance to some therapeutic agents, they represent potential targets for diagnosis and therapy. One main concept in devising these strategies is that HR-deficient tumours are more sensitive to killing by DNA damaging agents or by chemicals that inhibit other repair pathways or checkpoint mechanisms (208,307,308). For example, tumour cells that are mutated for the FA repair pathway show hypersensitivity to inhibitors of the main checkpoint kinase CHK1 (309). Another example is the selective killing of RAD54B-deficient colorectal cancers by down-regulation of FEN1, a nuclease involved in replication and excision repair (310). A third promising strategy uses PARP inhibitors. PARP is an enzyme involved in the repair of SSBs, and its inhibition leads to the persistence of DNA lesions normally repaired by homologous recombination. As a

result, inhibition of PARP in HR-deficient cells confers strong lethality. Since PARP inhibition selectively targets HR-defective cells, they have shown good effects in cancers associated with BRCA1 or BRCA2 mutations (307,311).

Diagnosis tools can also be generated based on the interplay between PARP and HR proteins. Since PARP inhibitors can result in RAD51 foci formation only in HR-proficient cells, a diagnostic tool using these inhibitors has been developed in primary cell cultures to identify HR-deficient tumours (312). Similarly, since PARP is hyperactivated in HR-defective cells including RAD54, RAD52, BLM, WRN and XRCC3 (313), a strategy can be devised which uses this feature as predictive biomarkers for PARP inhibition.

More complex therapy strategies that use multiple agents to impair HR and other repair pathways have shown some promise. For example, preclinical and preliminary clinical evidence suggest a potentially broad scope for PARP inhibitors in combination with DNA-damaging agents [for review see Refs (314,315)]. In addition, *in vitro* studies on BRCA2-deficient cells showed synergistic effects for combinations of olaparib with alkylating agents (316). However, as the DNA-damaging agents used to target rapidly dividing cancer cells also affect other proliferating cells, the therapeutic window of the drug cocktail needs to be regulated to minimize toxicity to healthy cells. In addition, BRCA2-deficient cells were shown to gain resistance to PARP inhibitors due to acquired mutations in BRCA2 that restore its activity (317,318). These observations have implications for understanding drug resistance in BRCA mutation carriers (317). The recently observed synthetic lethality of RAD52 and BRCA2 deficient cells could provide a treatment strategy not only in BRCA2-defective tumours, but also in BRCA2 revertants that become treatment resistant (208,317,318). It is clear that further research in this area will contribute to a better understanding of the processes underlying the maintenance of genomic integrity in eukaryotes, with implications for design of innovative treatment strategies.

ACKNOWLEDGEMENTS

We thank Lorraine Symington, Scott Keeney, Hannah Klein, Michael Lisby, Marek Sebesta, Prabha Sarangi and Katerina Krejci for valuable comments, suggestions and critical reading of the article. We apologize to colleagues whose work was not cited because of space limitations. X.Z. acknowledges grant GM080670 from NIGMS and RSG-12-013-01-CCG American Cancer Society Research Scholar Grant.

FUNDING

Funding for open access charge: The Wellcome International Senior Research Fellowship [WT076476]; the Ministry of Education Youth and Sport of the Czech Republic [ME 10048, Mendel Centre for Education in Biology, Biomedicine and Bioinformatics – CZ.1.07/2.3.00/09.0186, MSMT0021622413, LC06030]; Czech Science Foundation [GACR 301/09/1917, GACR 203/09/H046 and GACR P207/12/2323], European Regional Development Fund – (Project FNUSA-ICRC) [No. CZ.1.05/1.1.00/02.0123]; National Institute of General Medical Sciences (NIGMS) [GM080670]; and RSG-12-013-01-CCG American Cancer Society Research Scholar Grant (to X.Z.).

Conflict of interest statement. None declared.

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