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Review

DNA double-strand break repair by homologous recombination

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This review is dedicated to Dr. Jela Brozmanová who has been our excellent supervisor over many years

Abstract

DNA double-strand breaks (DSB) are presumed to be the most deleterious DNA lesions as they disrupt both DNA strands. Homologous recombination (HR), single-strand annealing, and non-homologous end-joining are considered to be the pathways for repairing DSB. In this review, we focus on DSB repair by HR. The proteins involved in this process as well as the interactions among them are summarized and characterized. The main emphasis is on eukaryotic cells, particularly the budding yeast *Saccharomyces cerevisiae* and mammals. Only the *RAD52* epistasis group proteins are included.

Keywords: DNA double-strand breaks; DNA double-strand break repair; Homologous recombination; *RAD52* epistasis group; *Saccharomyces cerevisiae*; Vertebrate cells; Mammalian cells; Protein–protein interactions

1. Introduction

DNA damaging agents constantly challenge DNA inside cell. Therefore, between replication rounds DNA tends to accumulate lesions. As they disrupt both DNA strands, DNA double-strand breaks (DSB) are one of the most detrimental DNA lesions. DSB can be induced by exogenous agents, such as ionizing radiation (IR) (for reviews, see [1–3]) and a wide range of chemical compounds, e.g. methyl methanesulfonate (MMS) and bleomycin [4–6], as well as by endogenous agents such as free radicals generated during metabolic processes [2]. Moreover, DSB arise in DNA as intermediates during mitotic and meiotic recombination, DNA replication, transposition of certain mobile elements, transduction, transformation and conjugation in bacteria, mating-type switching in yeast, and V(D)J recombination in the vertebrate immune system. The action of restriction endonucleases and topoisomerases also generates DSB [7–11].

Unrepaired DSB can cause loss of chromosomes and/or cell death. If misrepaired, DSB can give rise to mutations [12,13] and chromosomal rearrangements and hence cancer in multicellular organisms [7,14,15]. To combat these detrimental effects, multiple pathways have evolved for the repair of DSB: homologous recombination (HR), single-strand annealing (SSA) and non-homologous end-joining (NHEJ) (reviewed in [8,10,11,16]). The fundamental difference in these pathways is the requirement for a homologous DNA sequence: as shown in Fig. 1, HR repairs DSB by retrieving genetic information from an undamaged homologue (sister-chromatid or homologous chromosome), and this requires DNA sequence homology. SSA repairs DSB through annealing of complementary sequences on both sites of the break and thus

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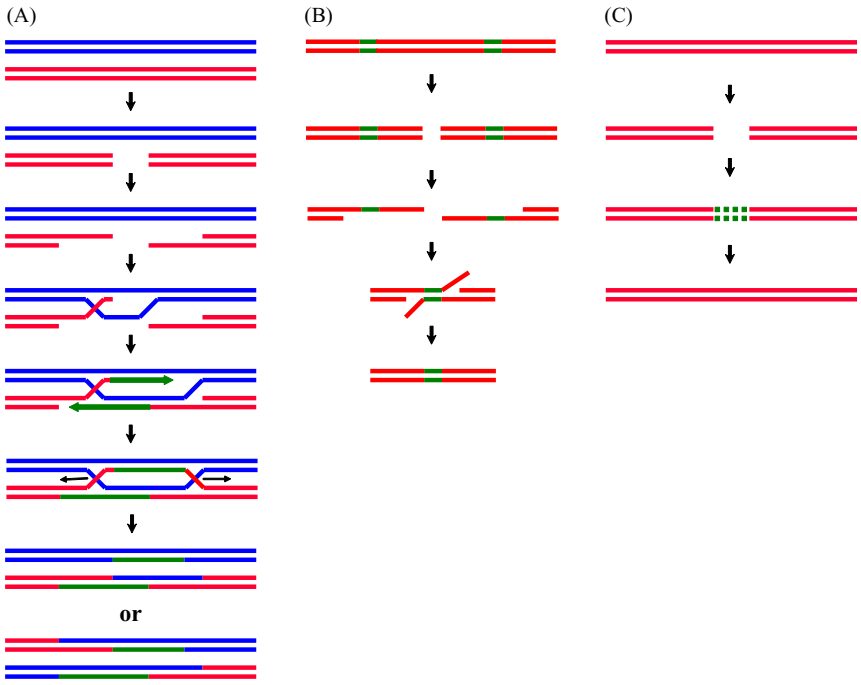

Fig. 1. Models for DNA double-strand break repair. (A) *Homologous recombination*. In this model, red and blue dsDNA represent homologous sequence. At the DSB site, dsDNA (shown in red) is exonucleolytically processed to form 3′ ssDNA tails, which invade homologous intact sequences (shown in blue). DNA strand exchange follows and generates a joint molecule between damaged and undamaged duplex DNAs. Sequence information that is missing at the DSB site is restored by DNA synthesis (resynthesized DNA is shown in green). The interlinked molecules are then processed by branch migration (indicated by right and left arrows), Holliday junction resolution and DNA ligation. (B) *Single-strand annealing*. At the DSB site, 3′ ssDNA tails, consisting of direct repeats (shown in green), are generated. They are aligned and the intervening sequences as well as protruding 3′ ends are removed. (C) *Non-homologous end-joining*. Following DSB formation, broken DNA ends are processed to yield appropriate substrates for direct ligation. No homology is necessary for DSB repair by non-homologous end-joining. Breaks can be joined accurately, but more often, small insertions or deletions are created.

also needs the presence of DNA sequence homology. In contrast to HR and SSA, NHEJ rejoins DSB via direct ligation of the DNA ends without any requirement for sequence homology. As a consequence of the mechanisms, the accuracy of repair constitutes a crucial difference among the three pathways: while HR ensures essentially accurate repair of DSB, SSA and NHEJ rejoin broken DNA in a manner that is almost always mutagenic. In the case of SSA, loss of one of the repeats and the sequence between the repeats is a characteristic of the repair process (reviewed in [11]).

All of these repair mechanisms have been conserved through evolution and operate in a wide range of organisms, including both prokaryotes and eukaryotes. Their relative contribution to DSB repair, however, significantly differs depending on the organism. While HR plays a major role in DSB repair in prokaryotes and lower eukaryotes, it operates rather rarely in somatic cells of higher eukaryotes. Instead, in somatic cells of higher eukaryotes including mammals, DSB are repaired primarily through NHEJ [3,17]. Although the NHEJ machinery has been identified in lower eukaryotes such as the yeast *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* [18–25] and recently in bacteria [26], in these organisms it is presumed to serve only as a backup system for repairing DSB. The importance of each pathway also depends on the phase of the cell cycle and, in multicellular organisms, on the cell type and the stage of development. In addition, the involvement of each pathway may be influenced by the complexity and/or the quality of the break [8,10,11].

In this review, we focus on DSB repair by HR. The proteins involved in this process as well as the

interactions among them are of particular interest. The emphasis is mainly in eukaryotic cells, especially the budding yeast *S. cerevisae* and mammals. The proteins belonging to the *RAD52* epistasis group are solely included in this review.

2. Nomenclature

For consistency, the following nomenclature has been used throughout the text. Using *RAD51*/*rad51*/Rad51 as an example: *RAD51* for the wild type gene or locus, *rad51* for the mutant gene or locus, and Rad51 for the protein. *Homo sapiens*, *Mus musculus*, *S. cerevisiae*, *Kluyveromyces lactis* and *Gallus domesticus* are abbreviated to Hs, Mm, Sc, Kl and Gd, respectively. If none of these is used, then the process is universal: alternatively, it is clear from the context to which animal species a particular protein belongs.

3. Molecular mechanism of homologous recombination

As mentioned above, living organisms have evolved multiple pathways for repairing DSB, namely HR, SSA and NHEJ [8,10,11,16] (Fig. 1). SSA and NHEJ are outside the scope of this review and the reader is referred to a number of excellent reviews on these topics [3,8,10,11,17,27–32].

As shown in Fig. 1, HR can be divided into several distinguishable steps. During initiation, the substrate for HR is generated: both of the 5′ ends of the DSB are resected by the action of a specific nuclease to yield 3′-single-stranded DNA (ssDNA) tails. Subsequently, one of the 3′-ssDNA tails invades an intact homologous duplex and generates a D-loop structure. The second 3′-ssDNA tail could also carry out invasion, or perhaps more likely, it could simply anneal with the displaced strand at the joint. Both 3′ ends then prime new DNA synthesis using the intact duplex as a template. This process, followed by ligation, leads to the formation of two Holliday junctions which are four-stranded branched structures. Holliday junctions can be moved along the DNA by a process of branch migration, thereby extending or shrinking the region of heteroduplexed DNA (hDNA). During the last step of HR, Holliday junctions are cleaved by a resolvase which cuts the crossed or non-crossed strands, resulting in crossover or non-crossover products [33,34].

3.1. Homologous recombination in *Escherichia coli*

The molecular mechanism of HR is best understood in *Escherichia coli* [35,36]. The principal recombination protein in this organism is the RecA protein and almost all DSB repair by HR is through the RecBCD pathway [37]. In the initiating step, DSB are specifically recognized by the RecBCD heterotrimer (exonuclease V), which has both exonuclease and helicase activities. In the presence of Mg^{2+} and ATP, the RecBCD complex binds to the end of a double-stranded DNA (dsDNA) substrate, unwinds processively and degrades preferentially the 3′-ending strand until it encounters a Chi (χ)-site sequences that create recombination hot spots. After interaction with the χ-site, the nuclease activity of the enzyme is altered so that degradation of the 3′-terminal strand is downregulated and the nuclease activity at the 5′-terminated strand is upregulated, whilst helicase activity remains unaltered. Finally, the χ-modified RecBCD enzyme produces resected dsDNA with a 3′-ssDNA tail terminating at the χ-sequence [38,39]. Another helicase, RecQ, has also been shown to be able to initiate recombination by providing the RecA protein with a suitable substrate [40]. In the presence of ATP, RecA coats a 3′-ssDNA tail and polymerizes head-to-tail, forming a right-handed presynaptic helical nucleoprotein filament. Upon modification of nuclease activity, RecBCD enzyme coordinates the preferential loading of RecA onto the resultant 3′-ssDNA tail downstream of χ [41]. In this process, RecBCD promotes displacement of single-strand binding (SSB) protein from ssDNA by RecA. SSB plays a dual role in RecA-mediated strand exchange [42]. Firstly, by binding to ssDNA, it removes any secondary structure and allows RecA to form a continuous filament. Secondly, as strand exchange proceeds, it binds to the released single-stranded DNA, and therefore stabilizes the joint molecule and prevents reinitiation [43]. Additional proteins, RecF, RecO and RecR, help RecA to overcome inhibition of binding by the SSB protein [44,45]. The RecA nucleoprotein filament then recognizes and invades the homologous sequence in duplex DNA, thereby creating hDNA

[46]. Additional factors, including RuvA, RuvB and RecG proteins, bind to the Holliday junctions and promote branch migration [47,48]. The RuvA protein specifically recognizes the Holliday junction, whereas RuvB is necessary for branch migration. RecG protein, like RuvA–RuvB, also promotes branch migration but the reaction is weaker. The Holliday junction can be resolved by the Holliday junction resolvase, RuvC [47]. In the absence of RuvC, another Holliday junction resolvase, RusA, can be activated and act on Holliday junction. Suppression function of RusA, however, critically depends on RecG protein [49]. RuvC endonuclease binds to the RuvA–RuvB complex and then cuts the DNA intermediate at two sites. Subsequent ligation generates recombinant (or non-recombinant) molecules containing a segment of hDNA. Alternatively, the double Holliday junction might be resolved by reverse branch migration [33].

As expected, cells lacking the RecA protein show nearly no recombination events [36,37]. Nevertheless, *recA* mutants have 50% viability when grown in liquid cultures [50]. The *recA* mutants exhibit various defects in recombination, repair and SOS response and are sensitive to a variety of DNA damaging agents (reviewed in [36,37]).

3.2. Homologous recombination in Saccharomyces cerevisiae

As shown by Siede et al. [51], HR is the favored DSB repair pathway in *S. cerevisiae*, whereas NHEJ is only of minor importance. These authors found that in cells competent for HR, deletion of NHEJ component did not result in increased sensitivity to IR or to MMS and DSB repair was not impaired. However, if HR was disabled, inactivation of NHEJ led to an additional sensitization to IR and MMS. Hence, in *S. cerevisiae* NHEJ activity can be demonstrated only in the absence of HR and it may thus serve only as a backup system.

In the *S. cerevisiae*, the genes implicated in HR belong to the *RAD52* epistasis group, which includes *RAD50*, *RAD51*, *RAD52*, *RAD54*, *RAD55*, *RAD57*, *RAD59*, *RDH54*/*TID1*, *MRE11* and *XRS2* genes [8,10,11,16,28,52]. Cells mutated in these genes are in general sensitive to IR, but not to ultraviolet (UV) irradiation, unable to repair DSB, and defective in mitotic and/or meiotic recombination [53]. However, there is a considerable variability among the *RAD52* epistasis group members with respect to the repair defect conferred by the particular mutation. Subdivision of the *RAD52* epistasis group members into several families was therefore suggested [28]. Since the *RAD52* gene is required for virtually all HR events, it stands alone. The uniqueness of the *RAD52* gene product is well established by the IR sensitivity of the *rad52* mutant: the *rad52* single mutant is as IR sensitive as a double mutant of *rad52* combined with a mutation in one of the other *RAD* genes [54]. The *rad51*, *rad54*, *rad55* and *rad57* mutants have common phenotypes, and the corresponding gene products constitute a family that has been designated the *RAD51* family. Contrary to *RAD52*, the *RAD51* family is necessary for some HR events, but dispensable for others. Furthermore, the IR sensitive phenotype of the *RAD51* family mutants is much less severe than that of the *rad52* mutant. The *rad50*, *mre11* and *xrs2* mutants also display identical phenotypes and therefore the respective genes constitute another family. There are homologues of the *RAD52* and *RAD54* genes, designated *RAD59* and *RDH54*/*TID1*, respectively, which may represent the separate subgroups because of their specific functions in HR.

3.3. Homologous recombination in higher eukaryotes

For many years, HR had been considered to be a minor pathway that acts on DSB in higher eukaryotes. This opinion was based on the fact that none of the cell lines displaying an IR sensitive phenotype and/or DSB repair defects were found to be mutated in any of the genes encoding the HR proteins. Moreover, some of the HR mutants are lethal in vertebrate cell lines and therefore they could not be further tested for the particular mutation. It was therefore believed that DSB repair in higher eukaryotes is almost exclusively undertaken by the NHEJ pathway. Recently, however, it has been established that HR also contributes considerably to DSB repair in these cells (recently reviewed in [11,55–57]). This conclusion has come mainly from the identification of relevant mammalian HR genes either through cloning genes that complement cell lines that are sensitive to DNA damaging agents or through homology searches [58–70], or from generating HR gene knockout mutants of the chicken B lymphocyte cell line, DT40. Furthermore,

the generation of homozygous HR gene knockout mice [71–78] and embryonic stem (ES) cells has also highlighted the importance of the HR pathway in DSB repair in higher eukaryotes [71,72,77,79].

In addition to the HR genes described for *S. cerevisiae*, mammalian cells contain some additional HR factors. Thus, there are five *RAD51*-like genes, namely *RAD51B*/*RAD51L1*/*hREC2*/*R51H2*, *RAD51C*/*RAD51L2*, *RAD51D*/*RAD51L3*, *XRCC2* and *XRCC3*. In addition, the breast cancer susceptibility proteins, Brca1 and Brca2, seem to be directly involved in the HR process in mammalian cells (for reviews, see [80,11,55,57]). These are outside the scope of this review and therefore are not discussed further.

4. Mre11, Rad50 and Xrs2

4.1. Mutant phenotypes

The *mre11*, *rad50* and *xrs2* mutants have many phenotypic features in common. They display a mitotic hyperrecombination phenotype, sensitivity to DSB-inducing agents, impairment in meiosis and compromised vegetative growth. The mutants also exhibit telomere shortening, a senescence phenotype, gross chromosomal rearrangements, a reduced frequency of NHEJ and defective checkpoint activation after DNA damage. On the other hand, mating-type switching, gene conversion, SSA and telomere position effect are not affected in any of these mutants (reviewed in [10,11,16]). Based on these common phenotypic features and on the epistasis analysis comparing the phenotypes of the double mutants to those of the respective single mutants, the *MRE11*, *RAD50* and *XRS2* genes were assigned to the same epistasis group [81,82]. Predictably, it was found that in yeast Mre11 interacts with Rad50, Xrs2, and itself, thus forming a stable complex Mre11–Rad50-Xrs2 (MRX), although Rad50 and Xrs2 fail to interact in the absence of Mre11 [81,83–86].

Not surprisingly, an analogous complex, Mre11–Rad50-Nbs1 (MRN), was demonstrated in vertebrates [87,88]. In these organisms, however, the Mre11 and Nbs1 proteins associate directly [89]. In contrast to *S. cerevisiae*, mutations in the *MRE11*, *RAD50* and *NBS1* genes cause more severe defects in vertebrates [75,90–92]. The essential role of the MRN complex was highlighted in mammals by the discovery that mutations in any of these genes can lead to cancer-prone syndromes. In humans, mutations in the *NBS1* and *MRE11* genes are associated with *N*ijmegen *b*reakage *s*yndrome (NBS) and *A*taxia *t*elangiectasia-*l*ike *d*isorder (ATLD), respectively [93–96]. These two syndromes share certain features with *A*taxia *t*elangiectasia (AT) and are associated with an increased risk of cancer. Even though no mutations in the *RAD50* gene have been linked to genetic diseases in humans so far, *RAD50*$^{S/S}$ mice (for details, see text below) have been shown to be predisposed to cancer [97]. For the clinical characteristics of NBS, ATLD, and AT, the reader is referred to several comprehensive reviews [98–102]. Although there are some differences in the clinical features of NBS, ATLD and AT patients, these three diseases are essentially identical at the cellular level. In general, cells of all syndromes exhibit sensitivity to IR, DSB repair defects and impaired checkpoint control.

Mre11-depleted murine and chicken cells are not viable [90,91] and display increased sensitivity to IR, severe HR impairment, an arrest in the G_2 phase of cell cycle, and significantly elevated levels of spontaneous as well as IR-induced chromosomal fragility [91]. Likewise, chicken cells, which are likely to be Nbs1-depleted, exhibit hypersensitivity to IR, abnormal S phase checkpoint control, an increase in spontaneous and IR-induced chromosomal aberrations, and HR impairment [103]. In addition, cells obtained from mice with a deletion of exons 4 and 5 of the *NBS1* gene (a murine model of NBS) are IR and mitomycin C (MMC) sensitive, defective in the intra-S phase checkpoint, and unable to form IR-induced Mre11 and Nbs1 foci [104]. Those derived from mice carrying a deletion of exons 2 and 3 are impaired in proliferation, hypersensitive to IR, aberrant in intra-S and G_2/M checkpoints and prone to premature senescence [105]. On the other hand, a mild phenotype at the cellular level was reported for *RAD50*$^{S/S}$, a hypomorphic *rad50* mutant allele analogous to the *S. cerevisiae rad50S* allele *rad50 R20M* [106]. Mouse embryonic fibroblasts harboring this allele display no growth defects, no defect in IR-induced relocalization of the MRN complex, no sensitivity to spontaneous or DNA damage-induced DSB, and no intra-S phase defect [97,107]. Whole animals are, however, susceptible to partial embryonic lethality and are predisposed

to cancer if long-lived [97]. *RAD50*$^{-/-}$ and *NBS1*$^{-/-}$ knockout mice die during embryonic development [75,92], although mice carrying a deletion of exons 2 and 3 of the *NBS1* gene are viable, but retarded in growth, impaired in lymphocyte development and oogenesis, immunodeficient, hypersensitive to IR and prone to lymphoid tumors [105].

4.2. Genes and proteins

Based on the phenotypes described above, the MRX/MRN complex has been implicated in many important cellular and developmental processes and its function correlates with its structure and biochemical activities. In *S. cerevisiae*, the individual components of the complex consist of 692, 1312 and 854 amino acid residues and have a molecular weight of 77.7, 152.6 and 96.4 kDa, respectively [108–111]. Their human counterparts comprise 708, 1312 and 754 amino acids and have a molecular weight of 81, 153 and 95 kDa, respectively [61,87,112]. Importantly, while HsMre11 and HsRad50 are considered to be structural homologues of their yeast counterparts, Nbs1 represents a functional analogue rather than structural homologue of Xrs2 [93–95]. Consistent with this, the primary amino acid sequences of HsMre11 and HsRad50 display high levels of similarity (61 and 52%, respectively) and identity (39 and 29%, respectively) with the yeast proteins [113]. In contrast, the overall homology between Nbs1 and Xrs2 is significantly lower, being restricted exclusively to the N-terminal region of the proteins.

The Mre11 protein represents the catalytic subunit of the MRX/MRN complex. As demonstrated by the two-hybrid system and gel-filtration column chromatography, it is able to form dimers and multimers that display several biochemical activities [81,85,114,115]. The protein has 3′ to 5′ dsDNA exonuclease and ssDNA endonuclease activities, both of which are specified by four phosphoesterase motifs residing in the N-terminal half of the protein and are dependent on Mn^{2+} as a cofactor [84,115–119]. Predictably, Mre11 exhibits a DNA-binding activity [84,114,119–121]. HsMre11 binds both dsDNA and ssDNA, with a preference for ssDNA, and does not require DNA termini for efficient binding [121]. In the presence of HsRad50 and Nbs1, HsMre11 also possesses DNA duplex unwinding and hairpin cleavage activities [120]. Moreover, HsMre11 mediates the annealing of complementary ssDNA molecules but, in contrast to HsRad52, this activity is abrogated by HsRpa (the human replication protein A) [121].

Rad50 belongs to the family of structural maintenance of chromosome (SMC) proteins (reviewed in [122]). It has two motifs, Walker type A and B, which are responsible, respectively, for ATP binding and hydrolysis at its N- and C-terminal ends. Walker type A and B motifs are separated by a long coiled-coil region. Interestingly, no influence of ATP on the biochemical activities of the MRN complex has been as yet detected [117,120], although the presence of ATP does result in the additional activity of MRX to degrade 3′ overhangs in *S. cerevisiae* [119]. Rad50 possesses a conserved hinge motif in the center of the coiled-coil region, that has a sequence Cys-X-X-Cys (CXXC) [123–125].

Recently, the structure of the Mre11-Rad50 complex has been elucidated. It has been shown that the CXXC motif promotes Zn^{2+}-dependent Rad50 dimerization, as the two CXXC motifs (each present on different Rad50 molecules) form interlocking hooks that bind one Zn^{2+} ion. Coiled coils extend from the Zn^{2+}-binding site in opposite directions, which allows them to link two distinct Mre11 dimers. The Mre11 dimer binds to the coiled coils of two Rad50 molecules adjacent to the ATPase domain, forming a globular head. The globular head is responsible for the DNA-binding and/or DNA end processing activities of the complex. Hence, one Mre11-Rad50 heterodimer (M_2R_2) possesses two DNA-binding/DNA end processing active sites. These two active sites would bind two separate broken DNA ends at a time and, subsequently, align and tether them during the process of NHEJ. In HR, the nuclease activity of the M_2R_2 complex may process broken DNA ends and the two DNA binding sites may mediate sister chromatid interactions, as well as initiating and stabilizing displacement D-loop formation [125,126]. It is clear from this that the Mre11 and Rad50 proteins form a tight complex with remarkable protein architecture [127].

In addition to eukaryotes, orthologues of the Mre11 and Rad50 proteins have been found in all kingdoms of life, including bacteriophage, eubacteria and archaebacteria. The Mre11-Rad50 family of proteins involves gp47-gp46 of bacteriophage T4, SbcD-SbcC of *E. coli*, and Mre11-Rad50 of eukaryotes and archaebacteria [116,127].

Analysis of Nbs1 has revealed three functional regions: the N-terminal region (amino acid residues 1–196), the central region (amino acids 278–343) and the C-terminal region (amino acid residues 665–693). In the N-terminal part of the protein, two domains were identified: a forkhead-associated (FHA) domain (amino acids 24–108) and a Brca1 C-terminus (BRCT) domain (amino acids 108–196) ([95], reviewed in [101]). Like Nbs1, Xrs2 has the FHA domain [93], but none of the typical sequence features of the BRCT domain [115]. This is consistent with the observation that the sequence similarity between Xrs2 and Nbs1 is limited to 115 amino acids at the N-terminus [128]. The FHA and BRCT domains of Nbs1 are essential for IR-induced focus formation and ATM-dependent Nbs1 phosphorylation, as well as for S phase checkpoint activation. On the other hand, they are dispensable for interaction of Nbs1 with Mre11 and Rad50 and, surprisingly, for IR resistance [129–131]. The central region of Nbs1 possesses two serine residues, Ser 278 and Ser 343, which are phosphorylated in response to DNA damage in an ATM-dependent manner [132–135]. Therefore, the DNA damage-triggered signal transduction function of Nbs1 was attributed to this part of the protein. The C-terminus of Xrs2/Nbs1 appears to be a critical region for Mre11-binding [89,129], as this region contains a sequence at codons 682–693 that is highly conserved from yeast to human. Importantly, the Mre11-binding domain is essential for IR resistance and the nuclear localization of the complex [129]. To summarize this, Nbs1 is likely to represent a regulatory subunit of the MRN complex, which is involved in its nuclear localization, signal transduction and catalytic activation [89,136,137]. It is also a regulator of the ATP-driven unwinding and nuclease activities of the MRN complex [120], which it recruits to the vicinity of DNA damage sites by direct binding of phophorylated histone H2AX [101,138].

4.3. Interacting partners

There are interactions of the MRX/MRN complex in addition to those amongst the components of the complex themselves (Table 1, Fig. 2). Based on these interactions, further roles of the MRX/MRN complex have been proposed. In mammalian cells, the MRN complex interacts with Ku70, a subunit of the DNA-dependent protein kinase complex, as well as with Brca1 [139–141]. Furthermore, it associates with the mismatch repair (MMR) protein Mlh1 [142] and co-localizes with the Bloom's syndrome protein [141]. Consistent with this, the MRN complex, Mlh1 and Bloom's syndrome protein were found to be the members of a group of proteins that associate with Brca1 to form a large complex named BASC (*B*rca1-*a*ssociated genome *s*urveillance *c*omplex). Since the BASC complex involves many other DNA repair proteins, it was proposed that it has two roles in the cell: it serves as a sensor of abnormal DNA structures and/or as a regulator of post-replication repair [141].

The MRN complex was also found to be associated with the telomere repeat binding factors, Trf1 and Trf2 (Table 1) [143–145]. This suggests a role of the MRN complex in telomere maintenance in mammalian cells, analogous to the role its homologue plays in yeast. In mammalian cells, telomeres terminate in so-called t-loops, the closure of which involves the invasion of the 3′ single-stranded telomeric DNA overhang into the upstream duplex telomeric repeat region, generating a stable hDNA at the base of the loop [146]. Hence, in this process the function of the MRN complex may involve initiating and stabilizing t-loop formation by tethering the invading overhang to the telomeric duplex.

The *S. cerevisiae ZIP1* and *ZIP2* gene products are required during meiosis to mediate the synapsis of homologous chromosomes. Whereas Zip2 appears to be an initiator of this process and recruits the synaptonemal complex (SC) proteins to the chromosomes [147], Zip1 is an integral part of the SC [148]. Since synapsis starts at the sites of meiotic recombination events, which are initiated by DSB formation, mutants having an impairment in DSB formation do not exhibit localization of Zip1 and Zip2 to meiotic chromosomes [147]. Therefore, it was speculated that Zip2 is attracted to the site of DSB, possibly by interacting with recombination protein(s), and promotes the assembly of the Zip1 [149]. Indeed, Zip2 was found to co-localize extensively with Mre11 (Table 1, Fig. 2) and, by inference, with Rad50 and Xrs2 [147]. Consequently, Zip2 appears to represent a bridge between the initiation of meiotic recombination and SC formation. Both Zip1 and Zip2 interact physically

Table 1
Homologous recombination factors in *Saccharomyces cerevisiae* and humans

S. cerevisiae	Humans	Biochemical activity/function	Interacting partners
Rad50	Rad50	ATP-dependent DNA-binding activity; ATPase activity	Yeast: Mre11; human: Mre11, Nbs1, Rint-1, Trf2, Brca1, Bloom's syndrome protein, PCNA
Mre11	Mre11	3′ to 5′ dsDNA exonuclease and ssDNA endonuclease activities; DNA-binding activity; ssDNA annealing activity; DNA duplex unwinding and hairpin cleavage activities	Yeast: itself, Rad50, Xrs2, Zip2, Zip3; human: Mlh1, Trf2, Ku70, Bloom's syndrome protein
Xrs2	Nbs1	Human: regulates DNA duplex unwinding and nuclease activities of the MRN complex; recruits the MRN complex to vicinity of DNA damage	Yeast: Mre11; human: Sp100, Trf1, Trf2, E2f1
Rad51	Rad51	ATP-dependent DNA-binding activity; ATP-dependent homologous pairing and DNA strand exchange activities	Yeast: Rad52, Rad54, Rad55, Rdh54/Tid1, Sgs1, Rsi1/Apc2, Zip3, Dmc1[a], Mlh1, Sap1, Ubc9, YMR233W, YPL238C, YPR011C; human: Rad51C, Rad52, Rad54, Rad54B[a], Werner's syndrome protein, Xrcc3, c-Abl, p53, Brca1[a], Brca2, Ube2I/Ubc9, Ubl1, Pir51, Rpb1
–	Rad51B	DNA-binding activity (enhanced in complex with Rad51C); protein kinase activity; DNA-stimulated ATPase activity	Human: Rad51C
–	Rad51C	DNA-binding (enhanced in complex with Rad51B, also exhibited in complex with Xrcc3); DNA-stimulated ATPase activity; ATP-independent DNA strand exchange activity; ATP-independent and Mg^{2+}-dependent homologous pairing activity on its own or in complex with Xrcc3; forms filamentous structures in complex with Xrcc3	Human: Rad51, Rad51B, Rad51D, Xrcc3
–	Rad51D	DNA-binding activity on its own or in complex with Xrcc2; DNA-stimulated ATPase activity; ATP-independent and Mg^{2+}-dependent homologous pairing activity in complex with Xrcc2; forms filamentous structures in complex with Xrcc2	Human: Rad51C, Xrcc2
Rad52	Rad52	DNA-binding activity; ssDNA annealing activity; weak DNA strand exchange activity; weak homologous pairing activity	Yeast: Rpa, Rad51, Rad52, Rad59; human: Rpa, Rad51, Rad52, Ube2I/Ubc9, Ubl1, Xpb, Xpd, c-Abl
Rad54	Rad54	DNA-binding activity; dsDNA-dependent ATPase activity; dsDNA unwinding activity; dsDNA topology-modifying activity	Yeast: Rad51, Mus81; human: Rad51, Mus81
Rdh54/Tid1	Rad54B	DNA-binding activity; dsDNA-dependent ATPase activity	Yeast: Rad51, Dmc1; human: Rad51[a]
Rad55	Xrcc2	Yeast: ATPase; human: DNA-binding activity on its own or in complex with Rad51D; ATP-independent and Mg^{2+}-dependent homologous pairing activity in complex with Rad51D; forms filamentous structures in complex with Rad51D	Yeast: Rad51, Rad57; human: Rad51D
Rad57	Xrcc3	Yeast: ATPase; human: DNA-binding activity on its own or in complex with Rad51C; ATP-independent and Mg^{2+}-dependent homologous pairing activity on its own or in complex with Rad51C; forms filamentous structures in complex with Rad51C	Yeast: Rad55, Zip3; human: Rad51, Rad51C
Rad59	–	DNA-binding activity; ssDNA annealing activity	Yeast: Rad52, Rad51[a]

For references, see text.

[a] Means indirect interaction or interaction that was detected by one method but the other failed to confirm it.

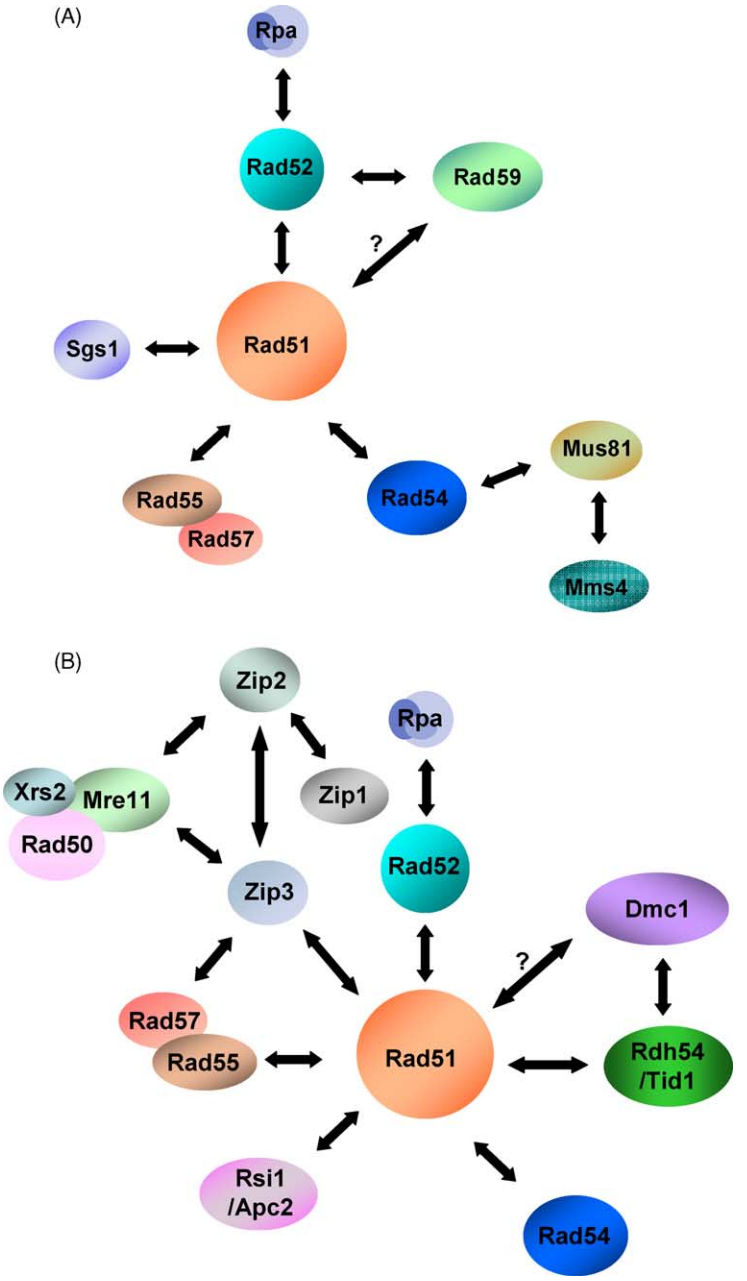

Fig. 2. Protein–protein interaction network among the proteins known to be involved in homologous recombination in *S. cerevisiae*. Interactions that occur or might occur in mitosis (A) and meiosis (B). See text for details. Question mark means that interaction is either indirect or that there is an unsatisfactory evidence for the particular interaction.

with another component of the synapsis initiation complex, Zip3. Zip3 was also shown to associate with a number of other HR factors, including Mre11, Rad51 and Rad57 (Table 1, Fig. 2) [150] and might thus constitute a key component that links HR to SC formation.

The MRN complex, through its Rad50 subunit, also interacts with a novel protein named Rint-1 (Table 1). This interaction seems to be cell cycle regulated and restricted to late S and G_2/M phases, suggesting a role of Rint-1 at these time windows during cell cycle progression. In agreement with this, Rint-1 was shown to affect G_2/M transition upon IR exposure. This indicates an important role of the Rint-1 in DNA damage-induced checkpoint control [151].

Furthermore, the MRN complex associates with the E2f1 transcription factor (Table 1), which can also control the transcription of genes that encode enzymes involved in DNA replication. Both Nbs1 and Mre11 are required for this interaction, as the interaction is abrogated or significantly reduced in NBS and ATLD cells, respectively. There is evidence that the MRN-E2f1 interaction occurs near the origins of DNA replication and is basically restricted to non-stressed S phase cells. This indicates a link between MRN-E2f1 complex formation and the activity of replication origins [152]. Since the MRN complex also co-localizes throughout S phase with the sliding clamp PCNA (*p*roliferating *c*ell *n*uclear *a*ntigen) (Table 1) [141,152], these results collectively suggest that the MRN complex influences both the regulation and progression of DNA replication. This is presumably the mechanism by which it suppresses genomic instability [152].

The *prom*yelocytic *l*eukemia (PML) protein is a nuclear phosphoprotein that localizes to distinct domains in the nucleus, described as PML nuclear bodies (PML-NBs). It has been shown that small amounts of Nbs1 and Mre11 are associated with PML-NBs [143]. Subsequent studies showed that Nbs1 is localized to PML-NBs via interaction with one of its components, Sp100 (Table 1) [153]. Since PML protein is involved in several p53-dependent cellular processes [154,155], it has been speculated that the localization of Nbs1 in PML-NBs via Sp100 might link DSB repair to p53-dependent cellular processes, such as apoptosis and cell cycle arrest [153].

5. Rad51

5.1. Mutant phenotype

The *S. cerevisiae rad51* mutants were isolated by screening for mutants that were sensitive to IR and had reduced mitotic and meiotic recombination [54]. In addition to sensitivity to IR, the *rad51* mutant is sensitive to a number of chemical agents, namely adriamycin [156], bleomycin [156–158], cisplatin [156,158], MMC [156,158], *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) [159], MMS [160,161 our unpublished results], and photoactivated 8-methoxypsoralen (8-MOP) [161,162]. It is also sensitive to many other anticancer drugs representing different alkylating agents, antimetabolites and topoisomerase poisons [158]. Moreover, the *rad51* mutation affects various recombination events [163–166] although the null mutant is viable [167,168]. In meiosis, it accumulates meiosis-specific DSB at recombination hotspots [169], is partially defective in the formation of physical recombinants [167], and has poor sporulation efficiency and very low spore viability [54].

In contrast to yeast, Rad51 is indispensable in higher eukaryotes: *RAD51*$^{-/-}$ knockout mice arrest early during embryonic development [71,72]. *RAD51B*$^{-/-}$ and *RAD51D*$^{-/-}$ (for details on the *RAD51*-like genes, see text below) knockout mice also die during early embryogenesis [76,78], although those with an *XRCC2*$^{-/-}$ mutation survive until later and die only at birth [77]. This suggests that high-fidelity DSB repair by Rad51-dependent HR is required for embryonic development probably because it operates on DSB generated during cell proliferation. Like *RAD51*$^{-/-}$ knockout mice, *RAD51*$^{-/-}$ chicken B lymphocyte DT40 cells are not viable [170]; however, inactivation of other *RAD51*-like genes is tolerated [171–174]. Chicken DT40 cell lines, each deficient in a different *RAD51*-like gene, exhibit very similar phenotypes, as they are moderately and highly sensitive to killing by IR and cross-linking agents (MMC and cisplatin), respectively, and are impaired in HR. Moreover, they display spontaneous chromosomal aberrations and significantly attenuated Rad51 focus formation during HR after IR exposure [174]. The V79 hamster cell lines irs1 and irs1SF, which are mutated in the *XRCC2* and *XRCC3* genes, respectively, show sensitivity to cross-linking agents, ethyl methanesulphonate, UV

light and IR, a defect in rejoining DSB (for a review, see [175]), and a decrease in HR induced by DSB [176–178]. In addition, they exhibit severe chromosomal instability [67,179], which might be a consequence of impaired HR. Derivatives of irs1SF cells having a single, integrated copy of the HR substrate display a radically altered HR product spectra [180]. It has therefore been suggested that genomic instability caused by defects in HR cannot solely be attributed to the failure to initiate HR, but also to the aberrant processing of HR intermediates.

5.2. Gene and protein

The *S. cerevisiae RAD51* gene was cloned and sequenced simultaneously in three laboratories [167,168,181]. The predicted ScRad51 contains 400 amino acid residues and has a molecular weight of 43 kDa [181]. ScRad51 comprises three regions: the N-terminal region (amino acids 1–154), the central core region (amino acids 155–374) and the C-terminal region (amino acids 375–400) [33,182]. Based on the protein sequence, ScRad51 is considered to be a structural and functional homologue of the *E. coli* RecA protein [167,168,181]. The overall amino acid sequence similarity and identity between RecA and ScRad51 is 54 and 29%, respectively [33,167, 168,183]. The most conserved regions are represented by Walker type A and B motifs [33,181] in the central core region [167] where RecA and ScRad51 are 61% similar and 35% identical [33,167]. Compared to RecA, ScRad51 lacks about 112 amino acids at the C-terminus but has an extension of about 120 amino acids at the N-terminus [33,167,168,182,184]. In addition, ScRad51 has a putative leucine zipper motif between amino acid residues 296 and 317 [52].

The human and mouse *RAD51* genes were isolated by Shinohara et al. [59] and Morita et al. [58]. Both genes encode 339-amino acid proteins with a predicted molecular weight of 36.9 kDa. The deduced amino acid sequences of the proteins are 83% similar and 67% identical to that of ScRad51 [58,59], and 55–56% similar and 30% identical to that of RecA [58,185]. Despite the extensive sequence similarity between HsRad51 and ScRad51, the HsRad51 protein cannot complement the defects of yeast *rad51* mutants [59]. Several additional human *RAD51*-like genes, *RAD51B*, *RAD51C*, *RAD51D*, *XRCC2* and *XRCC3*, have been cloned [62,63,66–68,186,187]. The GdRad51 protein was shown to share 68% identical amino acid residues with ScRad51 [188].

As already mentioned, ScRad51 was proposed to be the main RecA-like recombinase in *S. cerevisiae*, playing a crucial role in both mitotic and meiotic recombination as well as in DSB repair. Like RecA, ScRad51 has an ATP-dependent DNA-binding activity and polymerizes on both ssDNA and dsDNA forming helical filaments, only the ScRad51–ssDNA nucleoprotein filament being functionally relevant for DNA strand exchange [189–191]. Nevertheless, there are some important differences between RecA and ScRad51 (for a review, see [182]). The two proteins differ mainly in their requirements for initiating joint molecules and in the polarity of branch migration. Thus, the RecA protein is able to initiate joint molecules from any dsDNA ends and branch migration proceeds only in the 5′ to 3′ direction with respect to the ssDNA substrate. In contrast, ScRad51-catalyzed initiation requires overhanging 5′ or 3′ ends of linear dsDNA and branch migration proceeds in both directions [192,193], although the 5′ end of the ssDNA is more invasive than the 3′ end [194] and branch migration in the 5′ to 3′ direction is favored [192]. Moreover, RecA binds more strongly to ssDNA than to dsDNA, while the preferred DNA substrate for ScRad51 is dsDNA with ssDNA tails [194]. RecA also has higher ATPase activity on ssDNA than ScRad51 [182]. Like RecA and ScRad51, HsRad51 binds both ssDNA and dsDNA and exhibits a DNA-dependent ATPase activity [185,195]. DNA-binding by HsRad51 is mediated by the N-terminal domain (amino acid residues 19–83) of the protein [196,197] and is dependent on ATP hydrolysis [195]. HsRad51 also promotes an ATP-dependent homologous pairing and DNA strand exchange reactions in vitro [195,198–201].

In comparison to RecA, the extent of ScRad51-mediated DNA strand exchange is rather limited [190]. The efficiency of this reaction can be enhanced by the addition of ScRpa (the yeast replication protein A) after ScRad51 has nucleated onto ssDNA [202,203]. When both proteins were added simultaneously, no stimulation was observed [204]. Presumably a stable Rpa-ssDNA complex prevents the formation of Rad51–ssDNA filaments through competition

between Rpa and Rad51 for the same ssDNA-binding site [205]. The rate-limiting step of the displacement reaction is probably the nucleation of the Rad51 protein onto ssDNA. Once nucleation occurs, extensive displacement of Rpa occurs by growth of the Rad51 filament along the ssDNA [205]. The inhibitory effect of Rpa can be overcome by addition of the Rad52 protein or the *S. cerevisiae* Rad55–Rad57 heterodimer (for details on Rad55–Rad57, see text below), suggesting that these proteins aid in the replacement of Rpa by Rad51 [204,206,207]. Like ScRad51, HsRad51 has also limited DNA pairing and strand exchange activity compared to RecA [182,208,209] but this can be augmented by HsRpa [198–200], HsRad52 [210], or the Rad51B–Rad51C heterodimer [211].

Until recently, very little was known about the biochemical properties or precise biological functions of the Rad51-like proteins. Recently, however, significant progress has been made in this field. For example, it has been reported that the Rad51B protein exhibits a protein kinase activity: it phosphorylates and physically interacts with a number of different proteins, including p53, cyclin E, and Cdk2 [212]. Discovering a protein kinase activity for Rad51B represents a first step toward understanding how this protein affects cell cycle progression and causes G_1 delay [213]. Furthermore, some biochemical properties of Rad51B [214,215] and Rad51C [214] as individual proteins, as well as of the Rad51B–Rad51C complex [211,214], have been established. Both Rad51B and Rad51C bind to various DNA substrates [211,214,215], preferentially to dsDNA with 3′-tailed ssDNA [214]. Interestingly, DNA-binding by the Rad51B–Rad51C complex is enhanced over the individual proteins [214], indicating that the physical interaction between Rad51B and Rad51C is required for more efficient DNA-binding. The relative affinities of Rad51B and Rad51C for DNA have been compared to that of Rad51 and found to be Rad51C > Rad51 > Rad51B [214] therefore suggesting that Rad51B and Rad51C bind to the site of the break after 5′ end resection and that Rad51C is very important for this process. The notion that the Rad51-like proteins play a role in the initial steps of HR, mainly in ssDNA-binding, is strengthened by the results of Braybrooke et al. [216], Masson et al. [217] and Kurumizaka et al. [218,219], who together showed that Rad51D alone as well as Xrcc3–Rad51C and Xrcc2–Rad51D complexes also preferentially bind to ssDNA versus dsDNA.

In addition to their DNA-binding activities, Rad51B, Rad51C and Rad51D exhibit DNA-stimulated ATPase activities, which are dependent on the presence of a divalent cation [211,214–216]. Furthermore, Rad51C, but not Rad51B, displays an apparent DNA strand exchange activity, which is comparable to that of Rad51. Contrary to expectations, this activity is not dependent on ATP and results from the ability of Rad51C to melt and separate duplex DNA. Since such activities have not been described for Rad51, DNA melting and strand separation are unique to Rad51C [214]. Unique to Rad51B is that it specifically binds to synthetic Holliday junctions [215]. Such individual biochemical properties of the Rad51-like proteins might explain embryonic lethality in *RAD51*-like gene knockout mice and this suggests that the encoded proteins do not have redundant functions, as originally thought. Therefore, Rad51-like proteins either have highly specific functions in HR or, more likely, each of them functions under certain conditions in certain cell types and/or at certain cell cycle or developmental stages. The latter is supported by the finding that the *XRCC2* and *XRCC3* genes are expressed in the brain [67], where *RAD51* expression is very low [59].

The Xrcc3–Rad51C and Xrcc2–Rad51D complexes were shown to exhibit ATP-independent and Mg^{2+}-dependent homologous pairing activity [218,219]. Homologous pairing was not catalyzed by Rad51B itself, suggesting that this protein may play a role in HR that is different from the other Rad51-like proteins [215]. As Rad51C alone also promoted homologous pairing, although less efficiently than Xrcc3–Rad51C, it may represent a catalytic subunit of Xrcc3–Rad51C in this process [218]. On the other hand, Xrcc3 seems to be a catalytic subunit of the complex that is required for DNA-binding [218]. Unfortunately, experiments by Kurumizaka et al. [218] lacked Xrcc3-only controls in the reactions, and this makes the results difficult to interpret. In the presence of ssDNA, both the Xrcc3–Rad51C and Xrcc2–Rad51D heterodimers form filamentous structures, which markedly differ from that formed by RecA or HsRad51 [218,219].

Despite the universality of the Rad51 proteins, ScRad51 is not indispensable for all forms of

DSB-induced recombination in *S. cerevisiae* [220]. There are at least two pathways for HR that can be distinguished according to their requirement for the Rad51 protein, the Rad51-dependent and the Rad51-independent pathways, both of which, however, share a common requirement for Rad52 [28]. Rad51 is as essential as Rad52 for some processes, such as DSB-induced gene conversion involving two homologous chromosomes. It is, however, less important, or even dispensable, for other types of events such as spontaneous recombination, DSB-induced DNA replication (BIR) and some types of DSB-induced events such as SSA [7].

5.3. Interacting partners

One of the most astonishing features of eukaryotic Rad51 proteins is that they interact with multiple proteins. It has been found that ScRad51 physically associates with itself [160], and with other proteins of the *RAD52* epistasis group including Rad52 [167,221,222], Rad54 [223–225] and Rad55 [226–228]. Furthermore, it weakly interacts with Rdh54/Tid1 [229], a homologue of Rad54 [230,231], and Sgs1 [232], a *S. cerevisiae* homologue of the human Bloom's syndrome and Werner's syndrome proteins [233] (Table 1, Fig. 2).

Like its yeast counterpart, HsRad51 protein interacts physically with itself [234], Rad52 [209,234], Rad54 [235] and the Werner's syndrome protein [236] (Table 1). Although its interaction with Rad54B, a protein that shares homology with ScRad54, was detected by co-immunoprecipitation, the two-hybrid assay failed to confirm this and it was therefore suggested that the Rad51 and Rad54B interaction may be indirect [69]. There had been several indications that the Rad51-like proteins might form complexes with Rad51 and/or with one another. Indeed, it was shown that Rad51 interacts with Xrcc3 [67,237] and that Rad51C interacts with Rad51B, Xrcc3 and weakly with Rad51 [66,214,237–239] (Table 1). Compared to that of Rad51C–HsRad51, the Rad51C–Rad51B and Rad51C–Xrcc3 interactions are highly stable and resemble those of the Rad55–Rad57 heterodimer. By analogy with yeast, where the Rad55–Rad57 heterodimer interacts via Rad55 with Rad51 and enhances Rad51-dependent homologous pairing [204], Rad51C–Rad51B and Rad51C–Xrcc3 heterodimers might have the same role in HR in mammalian cells through a Rad51C-mediated interaction with Rad51 [214,240]. However, this possibility was tested for the Rad51C–Xrcc3 heterodimer and was shown not to be the case [218]. Furthermore, Rad51D associates with Rad51C and Xrcc2 [216,237,239,240] (Table 1). Therefore, there are clearly at least two complexes of the Rad51-like proteins in human cells: Rad51C–Xrcc3 and Rad51B–Rad51C–Rad51D–Xrcc2, both containing Rad51C as a component [237,239,241,242].

Interaction studies clearly demonstrated the formation of a complex involving a large number of proteins during HR in eukaryotes. For the yeast *S. cerevisiae*, this complex was named the *recombinosome* [225,226,243] and it also involves Rpa [244] (Table 1, Fig. 2). The existence of the *recombinosome* in vivo was supported by the observation that Rpa, Rad51, Rad52, Rad55 and Rad57 co-localize to the sites of DSB during meiotic recombination [245]. However, it is not clear whether these interactions occur to form a single complex or rather several smaller complexes each comprising two or more of the interacting proteins. Recent results have indicated that the assembly of the whole complex might be sequential, with competitive binding of substrate, ATP, or the protein components of the recombination–repair complex to Rad51 [246]. Therefore, it is likely that, at any point in time, the composition of the *recombinosome* will depend on the phase of the recombination process.

A meiosis-specific role for the ScRad51 protein might, at least in part, be related to Rsi1/Apc2, the subunit of the anaphase-promoting complex [247], or to Zip3, a protein that might link HR to SC formation (see above) [248], as these proteins were shown to interact physically with ScRad51 [150,229] (Table 1, Fig. 2). ScRad51 is also associated with ScDmc1 (Table 1, Fig. 2), a meiosis-specific RecA homologue involved in reciprocal recombination and formation of the SC [249]. Although two-hybrid analysis suggested that these two proteins do not interact directly in yeast [229], they do co-localize to nuclear foci during meiotic recombination [229,250,251]. This is somewhat in contrast to mammalian cells, where interaction between HsDmc1 and HsRad51 was demonstrated both by the two-hybrid system and by co-immunoprecipitation [252,253].

Systematic identification of protein complexes in *S. cerevisiae* by high-throughput mass spectrometric protein complex identification (HMS–PCI) revealed an interaction between Rad51 and the MMR protein, Mlh1 [86] (Table 1). MMR proteins are known to inhibit recombination between non-identical DNA sequences, and hence prevent genome rearrangements that might result from interactions between repetitive elements (for a review, see [254]). One of the components of MMR, Mlh1, interacts with multiple proteins involved in DNA repair and/or replication [255] and is considered to be a *molecular matchmaker* or coordinator [256]. The Rad51-Mlh1 interaction might therefore be important for coupling HR with MMR and/or for the interconnection of recombination with other cellular processes such as DNA replication. In addition to HMS–PCI, Uetz et al. undertook two genomic-scale two-hybrid screens to identify protein–protein interactions between full-length open reading frames predicted from the *S. cerevisiae* genome sequence [228]. This analysis revealed that the Rad51 protein further interacts with the Sap1 and Ubc9 proteins (see below) (Table 1). Moreover, ScRad51 interacts with the products of three open reading frames, YMR233W, YPL238C and YPR011C [228] (Table 1), whose function is still unknown.

There is compelling evidence in mammalian cells that the recombination machinery is subjected to modulation by gene products that have a tumor suppression function. One of them, c-Abl, a non-receptor tyrosine kinase [257], was shown to associate with Rad51 and Rad52 (Table 1) and phosphorylate both proteins in an ATM-dependent manner after IR exposure. This phosphorylation enhances complex formation between Rad51 and Rad52 [258–260] and is responsible for IR-induced Rad52 nuclear focus formation [261]. Other tumor suppressor proteins, Brca1 and Brca2, co-localize with Rad51 to nuclear foci following DNA damage in mitotic and meiotic cells [262,263]. Moreover, Brca1 and Rad51 were reported to co-immunoprecipitate [262]. Nevertheless, a direct physical interaction between Brca1 and Rad51 was not confirmed in other studies [264] (Table 1). The association of these two proteins is therefore likely to be indirect and perhaps mediated by Brca2 [263]. Brca2 interacts directly with Rad51 (Table 1), and the interaction domain was mapped to the BRCT repeat in Brca2 [263–265]. Like *RAD51*$^{-/-}$ knockout mice, the *BRCA1*$^{-/-}$ and *BRCA2*$^{-/-}$ knockouts show embryonic lethality [72,266,267], suggesting functional similarity and/or cooperation between Rad51 and Brca1 or Brca2 in embryonic development. Interestingly, Brca1 also interacts with c-Abl. This interaction is disrupted following IR in an ATM-dependent process and leads to the increase of c-Abl tyrosine kinase activity [268]. The tumor suppressor protein p53, which is involved in G_1/S and probably G_2/M checkpoints, also associates with Rad51 [269–271] (Table 1) as well as with Brca2 [265] and Brca1 [272]. Taken together, these results indicate that HR may act in concert with tumor suppression and cell cycle checkpoint proteins to maintain genome integrity [273].

Two-hybrid analysis has revealed other proteins that bind to HsRad51. The human *UBE2I*/*UBC9* gene encodes ubiquitin-conjugating enzyme 9 (HsUbc9) [274], an enzyme involved in the conjugation of SUMO-1/Ubl1 to target proteins. In human cells, the SUMO-1 pathway was shown to interact with a number of cellular processes including DNA repair (for a review, see [275]). HsUbc9 and SUMO-1/Ubl1 possess counterparts in *S. cerevisiae* that are highly homologous to the human proteins [274]. This suggests conservation of the SUMO-1 pathway throughout evolution. Both the yeast and the human Ubc9 bind to Rad51 [228,276,277] and HsUbc9 further associates with Rad52 [277] (Table 1). Moreover, non-conjugated SUMO-1/Ubl1 forms a complex with HsRad51 and HsRad52 [278] (Table 1). It is reasonable to conclude that the association of HR with the SUMO-1 pathway is biologically significant. In support of this, it was shown that overexpression of the SUMO-1/Ubl1 protein downregulates DSB-induced HR and reduces cellular resistance to IR in mammalian cells [278]. Hence, Li et al. [278] suggested a regulatory role for SUMO-1/Ubl1 in HR. Pir51 was also isolated as an HsRad51-interacting protein (Table 1) using the two-hybrid system [279]. Consistent with this, a mouse homologue of Pir51, Rab22, was shown to interact with the mouse Rad51 [280]. Purified HsPir51 protein binds ssDNA and dsDNA, as well as RNA, and causes aggregation. For this reason it was proposed that Pir51 may represent a new member of the multiprotein complexes involved in HR in mammalian cells [279]. Finally, the association of HsRad51 with the largest subunit of RNA polymerase II (Rpb1) has been reported [281] (Table 1).

6. Rad52

6.1. Mutant phenotype

The *S. cerevisiae rad52* mutant was originally isolated on the basis of its increased sensitivity to IR and MMS [54]. Subsequent experiments linked IR sensitivity to defective DSB repair [282]. In addition to IR and MMS, the *rad52* mutant is highly sensitive to a wide spectrum of DNA damaging agents, including bleomycin, cisplatin, MNNG, 8-MOP, hexavalent chromium, MMC, actinomycin D and many other alkylating agents, antimetabolites and topoisomerase poisons [156–158,161,283–286]. *rad52* mutant cells exhibit severe defects in various spontaneous and DNA damage-induced mitotic recombination events, such as SSA [287], mating-type switching [288], Rad51-dependent gene conversion [289], BIR [290], BIR coupled to SSA [220] and the formation of Holliday junctions [291]. Moreover, *rad52* mutation affects the process of meiotic recombination: diploid *rad52* mutant cells fail to produce viable spores [54] and accumulate single-strand breaks in parental DNA during meiotic DNA synthesis [292]. Of all the *RAD52* epistasis group mutants, the *rad52* mutants show the most severe recombination defects and sensitivity to DSB-inducing agents. The Rad52 protein therefore has an indispensable role in DSB repair and mitotic and meiotic recombination in *S. cerevisiae* [53,293].

In contrast to *RAD51*$^{-/-}$ knockout mice, *RAD52*$^{-/-}$ mice are viable and fertile and show no gross abnormalities [74]. *RAD52*$^{-/-}$ murine ES cells have a reduced capability of performing HR, indicating that the mammalian Rad52 protein, like its yeast counterpart, plays a role in HR. However, in contrast to *S. cerevisiae*, mammalian Rad52 does not seem to be involved in the repair of lethal DNA damage induced by IR, MMS, cross-linking agents and UV light [74] and similar results were obtained with *RAD52*$^{-/-}$ chicken DT40 cells [294]. These findings were unexpected, considering the severe phenotype of the *S. cerevisiae rad52* mutant cells. Possible explanations for this difference in phenotypes could be that in higher eukaryotes there is an as yet unidentified homologue of the *RAD52* gene [74,294] or the absence of Rad52 can be compensated by an alternative repair pathway. The former is supported by the existence in the fission yeast *S. pombe* of two Rad52 homologues, Rad22A and Rad22B/Rti1 [295,296], the latter resembling the vertebrate Rad52 protein in terms of its ability to confer a mild phenotype when mutated [11].

6.2. Gene and protein

Although the *S. cerevisiae RAD52* gene was cloned and sequenced almost 20 years ago [297], the biochemical function and the structure of the protein have only recently been revealed. Based on the results by Mortensen et al. [298], the size of the ScRad52 protein is likely to be smaller than that proposed originally: the actual transcriptional start site is the third putative initiation codon located within the N-terminus of the original *RAD52* gene [297]. Consequently, ScRad52 consists of 471 amino acid residues and has a molecular weight of 52.4 kDa. Since the 471 amino acid protein did not produce an altered phenotype nor did it change the size and cellular concentration of ScRad52, it indeed represents the actual length of the protein [298]. Moreover, the 471 amino acid protein complements the MMS sensitivity and meiotic recombination defects of the *rad52* mutants [299]. In addition, the Rad52 homologues from *S. pombe* and vertebrates lack the first 33 amino acids of the N-terminus proposed originally to be present in ScRad52 [16].

The human and mouse *RAD52* cDNAs were cloned and shown to encode proteins of 418 and 420 amino acid residues, respectively. The identity between the two proteins is 69% and the overall similarity 80%. Amino acid sequence identity and similarity between ScRad52 and HsRad52 is about 30% and 50%, respectively [113,300–302], being markedly higher within the N-terminus. The chicken *RAD52* counterpart encodes a protein the region between codons 40 and 178 of which is strongly homologous to that of ScRad52 [303].

The Rad52 protein non-cooperatively binds both ssDNA and dsDNA [298,299,304–310], this activity being localized to the conserved N-terminus of the protein [298,304,308–310]. This was supported by mutational analysis of HsRad52 which showed that a positively charged site on the protein (amino acid residues 1–212) is essential for binding [309]. Electron microscopy studies showed that Rad52 bound to ssDNA forms ring-like structures with a diameter of approximately 10 nm each containing a hole at its center [299,305,307–311]. ssDNA–Rad52 complexes,

in which the Rad52 rings are distributed along the length of the DNA substrate, resemble *necklaces* [299] or *beads on a string* [305]. At higher protein concentrations, Rad52 forms 30 nm diameter super rings on ssDNA [305]. Scanning transmission electron microscopy and sedimentation equilibrium studies demonstrated that each ring consists of seven full-length HsRad52 monomers [309,311], whilst others have reported an average of six and ten monomers per ring for the full-size HsRad52 and N-terminal fragment of HsRad52, respectively [308,310]. The crystal structure of the N-terminal fragment of HsRad52 shows a ring of eleven monomers [309,312]. These results together demonstrate the existence of two ring forms of Rad52: a heptamer of the full-length Rad52 and an undecamer of the N-terminal domain of Rad52. It has been suggested that these heptameric and undecameric rings might represent the oligomerization states generally exhibited by the Rad52 homologues [309]. It would thus be interesting to discover what ring structure is adopted by the *S. cerevisiae* Rad59 protein, a homologue of the N-terminal domain of ScRad52 (for details on Rad59, see Section 10.2.) [165,313], or by alternative splice variants of HsRad52 that contain most of the conserved N-terminal region of the protein [314].

The Rad52 protein also promotes annealing of complementary ssDNA [299,304,306,310,312], irrespective of its length [299,304], an activity that in humans is retained by the N-terminal region of the protein [310,312]. ScRad52-promoted strand annealing can be stimulated by the presence of ScRpa [299,306]. However, this depends on both the type and the length of the ssDNA substrate: only with plasmid-sized ssDNA substrates with secondary structure is ScRpa essential for ScRad52-promoted DNA strand annealing. In addition, strand annealing is stimulated only when ScRpa is added to the DNA before ScRad52 [306]. Rad52 also serves as a mediator protein in the Rad51-dependent DNA strand exchange reaction, where Rad52 is proposed to facilitate the loading of Rad51 onto the recombination site [207,210,315,316]. The Rad52 protein plays two separate, successive roles to stimulate both the extent and the rate of DNA strand exchange. Firstly, the presence of ScRad52 is particularly necessary in vitro to overcome the inhibitory effects of ScRpa [207]. This occurs by a mechanism that is dependent on ScRad52-ScRpa and ScRad52-ScRad51 protein–protein interactions in which ScRad52 allows ScRad51 to gain access to the ssDNA by facilitating the displacement of ScRpa. By targeting the ScRad51 protein to ssDNA, ScRad52 also reduces the sequestration of ScRad51 by dsDNA. More recent results indicate that ScRad52 alone cannot displace ScRpa from ssDNA. Instead, ScRad52 forms a co-complex with the ScRpa-ssDNA complex and recruits ScRad51 onto ssDNA: subsequently, ScRad51 displaces ScRpa. Secondly, ScRad52 promotes formation of a single contiguous filament by contributing additional stability through its physical interaction with ScRad51, resulting in a reduced requirement for ScRpa. Since ScRpa is involved in multiple cellular processes, ScRad52 may buffer DNA strand exchange against the competing demands of other ScRpa-requiring processes, especially under conditions of severe genotoxic stress, by substantially expanding the range of ScRpa concentrations over which DSB repair can efficiently occur [317].

The Rad52 protein has also been reported to have a weak homologous pairing activity, although significantly lower than that of RecA [184,209,307,309]. In HsRad52, this activity resides in the conserved N-terminal domain of the protein [209,307,309] and suggests a role for the Rad52 proteins in the homologous pairing step of HR.

In addition to its role in DNA-binding and homologous pairing, the N-terminus is responsible for homodimerization of Rad52 [244,318,319]. Recently, an additional self-association domain (amino acid residues 218–418), distinct from that previously identified [318], has been found in the C-terminal portion of HsRad52 [308], providing evidence for two distinct modes of Rad52 self-association. It seems that the C-terminal self-association domain is responsible for the formation of higher order structures that are larger than the 10 nm-rings [307,308,310]. Interestingly, the N-terminal homodimerization domain of HsRad52 was also assigned to be a Ubc9-interacting domain [277] so that interaction between HsRad52 and HsUbc9 leading either to HsRad52 degradation or to modulation of its function, competes with HsRad52 self-association.

Recently, Asleson and Livingston [320] investigated the stability of ScRad52 and concluded that mutations in the central region of the protein increase its cellular level and half-life. Because such mutations did

not affect *RAD52* mRNA expression or stability it was concluded that amino acid residues in the central region of ScRad52 are important for post-translational modification of the protein. Asleson and Livingston further hypothesized that this region is a domain that is essential for interaction with a protein that affects ScRad52 degradation [320].

The C-terminus of Rad52 is outside the conserved region and is responsible for the physical interaction with Rad51 in both the yeast [221] and human proteins [234,307]. The Rad51-interacting domains of ScRad52 and HsRad52 were mapped to amino acid residues 407–419 [321] and 291–330 [234], respectively. Krejči et al. have produced the *rad52Δ409–412* mutant carrying a four-amino acid deletion within Rad51-interacting region of Rad52. The Rad52Δ409–412 mutant protein is unable to interact with Rad51 and therefore lacks recombination mediator activity, but has a normal ability to bind ssDNA, mediate ssDNA annealing and oligomerize. Although the *rad52Δ409–412* mutant is IR sensitive, its sensitivity can be complemented by overexpression of Rad51 [321]. This suggests that in the Rad51-dependent HR pathway, mediator function is not the main role of Rad52 in the process of DSB repair. The C-terminal domain of HsRad52 (amino acids 302–418) is also involved in interaction with two of the subunits of transcription factor TFIIH, Xpb and Xpd, and possesses transcription activation activity as measured by in vivo transcription assay. Interestingly, the full-length HsRad52 suppresses transcription in this assay [322]. Since the transcription complex also associates with HsRad51 (see Section 5.3) [281], it is reasonable to assume that the HR pathway plays a role in repair DSB encountered by the transcription machinery and that HsRad52 oligomerizes in order to suppress transcription and hence to coordinate transcription with HR [322]. In support of this, an interaction between transcription and recombination, referred to as transcription-associated/stimulated recombination, has been reported in *S. cerevisiae* [323,324].

ScRad52 is induced during meiosis [325], but not in response to DNA damage [326]. In meiosis, the protein forms foci and extensively co-localizes with Rpa and to a lesser extent with Rad51. The assembly of such foci was shown to be closely associated with the formation of meiotic DSB [245]. Studies with ScRad52 fused to green fluorescent protein (ScRad52-GFP) also revealed distinct Rad52 focus formation during meiosis [327]. Furthermore, Lisby et al. showed that Rad52 re-localizes from a diffuse nuclear distribution to distinct foci upon DSB induction by IR and the HO endonuclease, and that this almost exclusively occurs during the S phase of mitotic cells. The fact that spontaneous ScRad52-GFP foci are also most frequently observed in S phase cells and only rarely in G_1 cells implies a coupling between DNA replication and HR [327]. Similarly, HsRad52 and MmRad52 re-localize to form distinctive foci in response to DNA damage [243,328] resulting in co-localization with other HR components [243,328] and providing evidence that these proteins function together in DSB repair in mammalian cells.

6.3. Interacting partners

In both yeast and human cells, the Rad52 protein interacts with Rad51 [160,167,209,221,222,226,234,246,305,321], itself [244,305,308,318], Rpa [206,207,210,222,245,299,308,316,329,330] (Table 1, Fig. 2), and Ubc9 and SUMO-1/Ubl1 [277,331] (Table 1) [277]. HsRad52 also associates with c-Abl tyrosine kinase, as well as with two of the subunits of the transcription factor TFIIH, i.e. Xpb and Xpd [322]. ScRad52 forms a complex with Rad59, a homologue of Rad52 [332] (Table 1, Fig. 2) [86]. Interactions of Rad52 that are common with those of Rad51 are discussed in Section 5.3.

7. Rad54

7.1. Mutant phenotype

Mutations in *RAD54* confer sensitivity to the DSB-inducing agents, IR [333,334], MMS [230,231] and bleomycin [157], and this was ascribed to defective DSB repair [335,336]. *rad54* mutants are not defective in SSA [337], spontaneous intergenic recombination [338], BIR [339] and NHEJ [340]. On the other hand, they are severely impaired in gene conversion [231,338,339] and exhibit significantly increased genome-wide chromosome loss [338]. Meiotic defects of *rad54* mutants are rather mild compared to those of other members of the *RAD52* epistasis

group, resulting in spore viability ranging from 26 to 67% [230,231,341]. Diploid *rad54* mutant strains have significantly reduced levels of spontaneous and induced mitotic recombination [342].

RAD54$^{-/-}$ knockout mice are viable and exhibit normal V(D)J and immunoglobulin class-switch recombination. Mouse *RAD54*$^{-/-}$ ES cells display defects in HR and are sensitive to IR, MMC, and MMS, but not to UV light [73]: direct evidence that these cells are defective in DSB repair was provided later [343]. Similarly, *RAD54*$^{-/-}$ chicken DT40 cells have been reported to be highly sensitive to IR and to have a reduced rate of HR [344,345]. Moreover, they show elevated levels of spontaneous cell death and spontaneous chromosomal aberrations (isochromatid and chromatid breaks and gaps, and exchanges) [346]. However, Sonoda et al. [345] reported significantly reduced levels of spontaneous and MMC-induced sister chromatid exchanges (SCE) in the *RAD54*$^{-/-}$ chicken DT40 cells. In mouse *RAD54*$^{-/-}$ ES cells, a slightly lower level of spontaneous SCE was observed by Dronkert et al. [343].

By generating *RAD54*$^{-/-}$/*KU70*$^{-/-}$ double knockout mice Takata et al. were able to investigate the contribution of NHEJ and HR to DSB repair in vertebrate cells. Based on the sensitivity of the *RAD54*$^{-/-}$, *KU70*$^{-/-}$ and *RAD54*$^{-/-}$/*KU70*$^{-/-}$ cells to IR, the authors showed that the NHEJ and HR pathways are complementary and that NHEJ plays a major role in DSB repair in G_1/early S phase, while HR acts in late S/G_2 phase [346]. Consistent with this are the findings of Fukushima et al. who demonstrated that *KU70*$^{-/-}$ knockouts are markedly sensitive to IR in G_1/early S phase, but are as resistant as wild type cells to IR in late S/G_2 phase [347]. In contrast, a pulsed-field gel electrophoresis study, measuring DSB rejoining up to 8 h following IR exposure in the same knockouts [348], failed to show a contribution of HR. Therefore, it was speculated that the contribution of HR to DSB repair is at a stage following the initial rejoining of the broken strands.

7.2. Gene and protein

The *S. cerevisiae RAD54* gene encodes a protein of 898 amino acid residues with a molecular weight of 101.8 kDa [349]. The human and mouse *RAD54* genes encode 83.4 kDa proteins of 747 amino acids that are 94% identical and the chicken Rad54 protein has 733 amino acid residues [344]. HsRad54 shares with its yeast counterpart 48% identity and 68% similarity [350,351].

The *S. cerevisiae RAD54* gene is inducible in both haploid and diploid cells in response to IR, UV light, MMS, and monofunctional and bifunctional furocoumarins [326,349,352]. This induction was shown to be cell cycle regulated, increasing in the G_1 phase [353]. Consistent with this, expression of the *RAD54* gene in HeLa cells is increased in late G_1 phase [350] and in mouse ES cells, the amount of the Rad54 protein is higher in the S and G_2 phases of the cell cycle than in the G_1 phase [243]. Hence, cell cycle regulation of the *RAD54* expression, matching the requirement for the HR proteins during DNA replication, appears to be conserved from yeast to humans. In contrast to cell cycle regulated *RAD54* induction, DNA damage induced *RAD54* induction is not evolutionary conserved: in HeLa cells there is no increase in *RAD54* expression following IR [350]. Interestingly, deletions that eliminate induction of *RAD54* transcription by DNA damage have no effect on growth or survival of non-inducible cells relative to wild type cells following DNA damage. Furthermore, these deletions do not affect mating-type switching, induction of *RAD54* during meiosis, meiotic recombination, and spontaneous or IR-induced mitotic recombination [334]. Consequently, the biological relevance of *RAD54* induction by DNA damage remains unclear.

The Rad54 protein shares seven conserved sequence motifs with the Swi2/Snf2 superfamily of dsDNA-stimulated ATPases and DNA/RNA helicases [354]. This superfamily includes proteins involved in chromatin remodeling, chromosome segregation, transcriptional regulation, and DNA repair and recombination [355,356]. Biochemical analysis of the Swi2/Snf2 superfamily failed to demonstrate helicase activity, but identified a dsDNA-stimulated ATPase activity (reviewed in [355]). Consistent with this are findings that whilst neither ScRad54 nor HsRad54 have DNA helicase activity they both have DNA-stimulated ATPase activity [225,357,358]. The dsDNA-stimulated ATPase activity is required for DNA repair and recombination in vivo [224,356,359–361] and it is indispensable for heteroduplex joint (D-loop) formation mediated by Rad54 in vitro [225,358]. It has

been suggested that Rad54 uses the free energy from ATP hydrolysis to remodel DNA structure so that both negatively and positively supercoiled domains are generated in the DNA template [358,362]: this may be the result of Rad54 translocation along the DNA [359,362,363]. Negative supercoils probably lead to DNA strand opening. The dsDNA-stimulated ATPase and dsDNA unwinding activities of Rad54 have been shown to be stimulated by Rad51 and by the Rad51-Rpa-ssDNA complex [358,363,364]. The ability of Rad51 to stimulate the Rad54-mediated DNA remodeling activity requires DNA-binding by Rad51 [358,362]. In vitro experiments showed that this effect is reciprocal: Rad54 significantly increases Rad51-mediated homologous pairing as well as heteroduplex extension [225,359,360,362,363,365]. Collectively, these findings indicate that efficient homologous pairing requires cooperation between Rad54 and Rad51.

Recent results have demonstrated additional roles for Rad54 in HR. Thus, the Rad54 protein can move nucleosomes along DNA in either of the two possible directions and remove them from the DNA, processes that are dependent on ATP hydrolysis and enhanced by a pre-assembled Rad51–ssDNA complex. It has therefore been suggested that nucleosome sliding is the mechanism by which HR overcomes the inhibition imposed by nucleosomes, a process that would integrate Rad54 function into the pre-synaptic events of HR [366]. This contribution of Rad54 to presynaptic events has been supported by the discovery that the binding of Rad54 to the Rad51–ssDNA nucleoprotein filament substantially increases its stability [367]. Additional synaptic and postsynaptic functions of Rad54 have recently been suggested by the observation that Rad54 dissociates Rad51 from dsDNA in an ATP-dependent manner. Rad54 would thus represent an excellent candidate for mediating the turnover of Rad51 nucleoprotein filament in the process of HR [368,369].

7.3. Interacting partners

Both ScRad54 and HsRad54 interact with Rad51 [223–225,235,358,370] (Table 1, Fig. 2) and this requires the N-terminal domain of Rad54 [223,235]. Rad54 also interacts with Mus81 [371] (Table 1, Fig. 2), a structure-specific endonuclease that resolves DNA junctions formed either during recombination or at stalled replication forks [372–375]. Mus81 further interacts with Mms4 [374,376] (Fig. 2), and this interaction is necessary for triggering Mus81 endonuclease activity [374]. It might thus be speculated that the Rad54–Mus81 interaction provides a link between the early and late steps of HR.

8. Rdh54/Tid1

8.1. Mutant phenotype

Additional complexities in HR were revealed by the discovery of a *RAD54* homologue, *RDH54* (*RAD h*omologue 54)/*TID1* (*t*wo-hybrid *i*nteraction with *DMC1*) [229–231]. The *rdh54*/*tid1* mutant is not sensitive to UV light and DSB induced by HO endonuclease [230,231,339]. Furthermore, it is not sensitive to MMS at concentrations that kill the *rad54* mutant, but it is sensitive to prolonged exposure to higher concentrations of this agent. Interestingly, the *rad54 rdh54*/*tid1* double mutant is slightly more sensitive to MMS than the *rad54* single mutant and, a defect in interchromosomal gene conversion during mitosis is more severe in the *rad54 rdh54*/*tid1* double mutant than that in the *rad54* single mutant. In diploid cells, *rdh54*/*tid1* mutations cause a marked decrease in interchromosomal recombination and in growth retardation, the latter probably due to attempted but incomplete recombination [230,231]. Homozygous mutant diploid cells show very poor sporulation and reduced spore viability [230]. Based on phenotypic analysis, the *RDH54*/*TID1* gene product appears to be required for diploid-specific mitotic recombination (interchromosomal but not intrachromosomal gene conversion) and complete meiotic viability [230]. This is in accord with the results of Arbel et al., who showed that Rad54 is required for sister chromatid-based repair, whereas Rdh54/Tid1 is dispensable for this process [377].

A human homologue of Rdh54/Tid1 may be Rad54B (HsRad54B), since the phenotype of the *rdh54*/*tid1* mutant partially overlaps with that of *RAD54B*-deficient HCT116 human cells. These cells display a reduced frequency of targeted integration compared to the wild type cells. On the other hand, they are not disabled in cell growth nor are they sensitive to IR, MMS or cisplatin. The levels of

spontaneous and MMC-induced SCE are also not affected by *RAD54B* deficiency in humans [378].

8.2. Gene and protein

The *RDH54*/*TID1* gene product contains 958 amino acid residues, has a molecular weight of 108 kDa and shares 34% identity with ScRad54 [231]. Like Rad54, it belongs to the helicase-like Swi2/Snf2 protein family [354], albeit being devoid of DNA helicase activity [379]. The protein possesses Walker type nucleotide binding motifs and consistent with this it displays dsDNA-dependent ATPase activity in vitro. The ATPase activity is not retained in the lysine to arginine (K315R) substitution mutant protein that affects the highly conserved Walker type A motif, supporting the implication that the Walker type motifs are involved in ATP binding and hydrolysis. The ATPase activity has been shown to be essential for stimulating Rad51-promoted D-loop formation and modification of DNA topology in vitro [379]. Accordingly, defects caused by the *rad54*/*tid1 K315R* mutant allele are identical to those of the *rdh54*/*tid1* null mutant. Therefore, the ATPase activity of Rdh54/Tid1 is completely indispensable for its biological function.

In addition to its role in HR, Rdh54/Tid1 probably plays an important role in cell cycle regulation during DSB repair, as it functions in the adaptation of yeast cells to checkpoint mediated G_2/M arrest by monitoring the extent of ssDNA produced by resection of DNA ends during DSB repair [380].

As mentioned above, HsRad54B is thought to be a homologue of Rdh54/Tid1 in humans [69,364,378]. This is supported by the observation that the N-terminal region of HsRad54B shares a greater extent of homology with Rdh54/Tid1 than with ScRad54 whereas in both the central region and C-terminal domain, homology between Rdh54/Tid1 and HsRad54B is identical to that of between HsRad54 and HsRad54B [364]. It is worth noting that the overall similarity between the N-terminal domains of Rdh54/Tid1 and HsRad54B is not as extensive. There is, however, a specific region containing potential nuclear localization signal that is well-conserved being present in GdRad54B and a putative *S. pombe* Rdh54/Tid1 homologue [229]. It also exists in the *Caenorhabditis elegans* Swi2/Snf2 family protein [364] implying that Rdh54/Tid1 has been conserved throughout evolution.

HsRad54B consists of 910 amino acids and is a dsDNA-dependent ATPase and a DNA-binding protein. In contrast to HsRad54, the ATPase activity of HsRad54B is not stimulated by the addition of HsRad51 [364].

8.3. Interacting partners

In yeast, Rdh54/Tid1 physically interacts with both Dmc1 and Rad51 [229,379] (Table 1, Fig. 2) and promotes the co-localization of these proteins during meiotic recombination [251]. In contrast, using purified recombinant proteins neither HsRad51 nor HsDmc1 interacts with HsRad54B [364], even though HsRad51 was originally shown to co-immunoprecipitate with HsRad54B from cell extracts. HsRad54B, however, forms nuclear foci that co-localize with HsRad51 [69]. Collectively, these results indicate that (i) if there is an interaction between HsRad54B and HsRad51, it is presumably indirect; and (ii) there may be a functional difference between Rdh54/Tid1 and HsRad54B. Therefore, the question of whether HsRad54B is a functional homologue of Rdh54/Tid1 requires further characterization of HsRad54B with respect to its function in meiosis as well as to its overlapping functions with HsRad54.

9. Rad55 and Rad57

9.1. Mutant phenotype

The *rad55* and *rad57* mutants are sensitive to IR [226,227,333,381,382], MMS [383] and bleomycin [157] and this sensitivity is temperature-dependent, being more pronounced at 23 °C than at 36–37 °C [226,227,381,384]. Moreover, these mutants are cold-sensitive in inverted-repeat recombination [164] and in IR-induced Rad51 focus formation [385]. A cold-sensitivity phenotype is usually associated with proteins that are subunits of multiprotein complexes. Accordingly, both the two-hybrid system [226,227] and co-immunoprecipitation [204] have shown that Rad55 and Rad57 form a highly stable heterodimer in vivo. Furthermore, *rad55* and *rad57* mutants are defective in HO-induced mating-type switching [227,386] but not in NHEJ [340] or SSA [337].

9.2. Genes and proteins

The predicted protein encoded by the *RAD55* gene is 406 amino acids in length with a molecular weight of 46.3 kDa [387]. The Rad57 protein consists of 460 amino acid residues and has a molecular weight of 52.2 kDa [388]. Both proteins share homology with members of the RecA/Rad51 family of recombinases, although this is significantly restricted to the conserved Walker A and B motifs [204,227,387,388].

The *S. cerevisiae* Rad51, Rad55 and Rad57 proteins may possess distinct biochemical properties and fulfill highly specific roles, rather than simply providing redundant and overlapping functions [204]. Like Rad52 [206,207,316], the Rad55–Rad57 heterodimer stimulates Rad51-mediated DNA strand pairing and exchange by overcoming the inhibition imposed by Rpa [204]. The function of the Rad55–Rad57 heterodimer in Rad51–ssDNA promotion and stabilization has been further supported by identification of *srp* mutants (suppressor of *R*ad51 *p*aralogs) [382]. One of the *srp* mutants, *rad51 I345T*, partially suppresses the DNA repair defect of the *rad55* and *rad57* mutants by overcoming the inhibitory effect imposed by Rpa in the absence of Rad55–Rad57. This process, however, still required the presence of Rad52, indicating that Rad52 and Rad55–Rad57 mediators have dissimilar functions in the stimulation of Rad51-mediated DNA strand pairing and exchange [382].

On the other hand, the cold-sensitivity and recombination defects of the *rad55* or *rad57* mutants can be partially suppressed by overexpression of Rad51 [226,227] or Rad52 [226], and completely suppressed by the simultaneous overexpression of both proteins [226]. It was therefore proposed that the functions of Rad52 and Rad55–Rad57 are either partially redundant and might involve similar molecular mechanisms [226,227] or that Rad55–Rad57 might act to stabilize recombination complexes under suboptimal growth conditions [389]. Consistent with this is the observation of Gasior et al. [245,385] who showed that Rad52 and Rad55–Rad57 can substitute for one another in promoting Rad51 focus formation at 30 °C. In the absence of Rad55–Rad57, however, Rad52 is able to promote nearly normal numbers of Rad51 foci at 30 °C, but not at 20 °C. Hence, it seems that Rad55–Rad57 functions only when cells are in a cool environment and that Rad52 accounts for the cold-sensitivity of the *rad55* or *rad57* mutant because Rad52-promoted assembly of Rad51 is naturally a temperature-dependent process.

Rad55 exhibits DNA damage- and replication block-induced phosphorylation [390,391], which is dependent on Mec1, Rad53 and Dun1, the three main signal transducing kinases in *S. cerevisiae* (for reviews, see [392,393]). Since the other responses of the DNA damage check points, i.e. cell cycle arrest and DNA damage induced gene expression, were intact in *rad55* null mutants, Rad55 seems to be a terminal substrate of the DNA damage check point rather than being an intermediate member of the cascade. It would be interesting to establish if phosphorylation of Rad55 affects its ability to form a complex with Rad57 or to stimulate Rad51-mediated DNA strand exchange.

9.3. Interacting partners

Rad55 interacts with Rad51 [226,227] (Table 1, Fig. 2) and this interaction has been shown to be considerably less and more stable than that of Rad55 with Rad57 according to Sung [204] and Johnson and Symington [227], respectively. The basis of this discrepancy between the results of these two groups is unknown. Rad57 has also been reported to interact with Zip3 (Table 1, Fig. 2), a protein that plays a key role in meiosis and this interaction was described in detail in Section 4.3.

10. Rad59

10.1. Mutant phenotype

To identify components of the Rad51-independent recombination pathway, the recombination frequency between intrachromosomally inverted *ade2* repeats [289] was assessed in an MNNG-mutagenized *rad51* strain [165]. One of the mutations identified, *rad59*, very significantly reduced spontaneous and DSB-induced mitotic recombination in the *rad51* strain, but it had only slight effect on recombination in the wild type strain. As there was no defect in the *rad59* mutant cells in interchromosomal recombination, the Rad59 protein was proposed to be specifically

required for Rad51-independent intrachromosomal recombination [165].

10.2. Gene and protein

Although the *rad59* mutation leads only to moderate sensitivity to IR [165,394], the *RAD59* gene was cloned by complementation of the radiosensitive phenotype of the *rad59* mutant [165]. The predicted Rad59 protein consists of 238 amino acid residues and has a molecular weight of 26.6 kDa [165]. It has significant homology to proteins of the Rad52 family, including those of *S. cerevisiae*, *K. lactis*, chicken, mouse, human, and to the Rad22A protein from *S. pombe* [221,300–303,395]. The Rad52 family proteins are highly conserved at the N-terminus and the Rad59 protein, which is about half the length of the Rad52 family, is homologous to their conserved N-terminus. However, among the Rad52 family, Rad59 is the least conserved member. For the yeast Rad52 and Rad59 proteins, amino acid identity and similarity is 28 and 50%, respectively, being somewhat higher within the conserved N-terminus [165]. Until now, the only Rad59 homologue described for other organisms is KlRad59. The amino acid sequence of the putative KlRad59 protein shares 53% identity and 11% similarity with that of Rad59 [396].

Since the N-terminal region of the Rad52 proteins is responsible for DNA-binding [184], the Rad59 protein was assumed to possess a DNA-binding activity. Indeed, Petukhova et al. [313] demonstrated that purified Rad59 protein binds DNA, preferentially ssDNA. Furthermore, it anneals complementary DNA strands [313,332] almost as efficiently as Rad52 [332]. Davis and Symington [332] showed that Rad59 is also able to overcome the inhibitory effect of Rpa on the annealing of complementary DNA, but less effectively than Rad52, and that it moderately stimulates Rad52-promoted annealing of Rpa-coated DNA. Therefore, one of the functions of Rad59 could be to enhance of the DNA strand annealing activity of Rad52.

As mentioned above, Rad59 has several activities in common with Rad52. This is consistent with biological data showing that overexpression of Rad52 rescues the IR sensitivity of the *rad59* mutant strain. Therefore, it was suggested that Rad52 and Rad59 have overlapping roles and/or function as a complex [165]. However, overexpression of Rad59 was unable to suppress the DNA repair defect of the *rad52* strain, indicating that Rad59 cannot substitute for Rad52 in vivo. Furthermore, Rad59 was unable to substitute for Rad52 in SSA, even when overexpressed [332]. Hence, Rad52 and Rad59 may still have largely overlapping functions, even though both proteins are required for efficient SSA [397]. This concept is supported by the observation that a hypomorphic *rad52 R70K* mutant allele confers a defect in recombination very similar to that of the *rad59* mutation [394].

10.3. Interacting partners

Physical interaction between Rad52 and Rad59 (Table 1, Fig. 2) was established using the two-hybrid system and by co-immunoprecipitation of the two proteins from yeast extracts [332] as well as by HMS–PCI [86]. In addition, a heterotrimeric complex consisting of Rad51, Rad52 and Rad59 could be immunoprecipitated from yeast extracts, the Rad51–Rad59 interaction requiring Rad52 [16]. In the filtered HMS–PCI interaction dataset published by Ho et al. [86], there are many more interactions involving Rad59, the significance of which remains to be elucidated.

11. Conclusions

Over the last few years, considerable progress has been made in our understanding of early stage of HR in eukaryotes. Firstly, the structure of the Mre11-Rad50 complex has been revealed. This structure clearly suggests that the complex functions in HR by processing broken DNA ends and bridging sister chromatids [124]. Secondly, the biochemical features of the Rad51-like proteins have been described [209,212–217]. This supports the opinion that the Rad51-like proteins do not have redundant functions in the process of HR. Thirdly, the structure of the Rad52 protein has been solved: ssDNA-bound Rad52 forms the ring-like structures that are distributed along the length of the DNA. Interestingly, Rad52 can adopt two ring forms: a heptamer of full-length protein and an undecamer of the N-terminal, homologous pairing domain of the protein and possible functions of both forms have been proposed [307,310]. Fourthly, further characterization of Rad54 has led to

the integration of its function into both the synaptic and postsynaptic phases of HR. It has been shown that Rad54 dissociates Rad51 from dsDNA and this process presumably leads to turnover of Rad51 in HR [364–367]. Contrary to early stage of HR, very little is known about late stage of this process in both lower and higher eukaryotes. Although recently some Holliday junction resolution activities have been found in both yeast and mammals [398–402], these appear not to have all of the expected properties of eukaryotic Holliday junction resolvases [403]. Moreover, interconnection between HR and other cellular processes is not yet understood. Very recently two mechanisms that can regulate early stage of HR have been proposed. Firstly, inhibitory effect of yeast linker histone, Hho1, on HR in vivo has been shown by Downs et al. [404]. Secondly, suppression of Rad51-mediated DNA strand exchange by Srs2 helicase in vitro has been reported by Krejči et al. [405] and Veaute et al. [406]. Despite this substantial progress in our knowledge about HR over recent years, further investigations are required for a complete understanding of the molecular mechanisms involved in this process.

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References

[1] P.L. Olive, The role of DNA single- and double-strand breaks in cell killing by ionizing radiation, Radiat. Res. 150 (1998) S42–S51.
[2] S.S. Wallace, Enzymatic processing of radiation-induced free radical damage in DNA, Radiat. Res. 150 (1998) S60–S79.
[3] P.A. Jeggo, DNA breakage and repair, in: J.C. Hall, T. Friedmann, J.C. Dunlap, F. Giannelli (Eds.), Advances in Genetics, vol. 38, Academic Press, San Diego, 1998, pp. 185–218.
[4] L.F. Povirk, W. Wubter, W. Kohnlein, F. Hutchinson, DNA double-strand breaks and alkali-labile bonds produced by bleomycin, Nucleic Acids Res. 4 (1977) 3573–3580.
[5] E. Chlebowicz, W.J. Jachymczyk, Repair of MMS-induced DNA double-strand breaks in haploid cells of *Saccharomyces cerevisiae*, which requires the presence of a duplicate genome, Mol. Gen. Genet. 167 (1979) 279–286.
[6] L.F. Povirk, DNA damage and mutagenesis by radiomimetic DNA-cleaving agents: bleomycin, Mutat. Res. 355 (1996) 71–89.
[7] J.E. Haber, DNA recombination: the replication connection, Trends Biochem. Sci. 24 (1999) 271–275.
[8] A. Pastink, P.H.M. Lohman, Repair and consequences of double-strand breaks in DNA, Mutat. Res. Fundam. Mol. Mech. Mut. 428 (1999) 141–156.
[9] H. Flores-Rozas, R.D. Kolodner, Links between replication, recombination and genome instability in eukaryotes, Trends Biochem. Sci. 25 (2000) 196–200.
[10] A. Pastink, J.C.J. Eeken, P.H.M. Lohman, Genomic integrity and the repair of double-strand DNA breaks, Mutat. Res. Fundam. Mol. Mech. Mut. 480 (2001) 37–50.
[11] M. van den Bosch, P.H.M. Lohman, A. Pastink, DNA double-strand break repair by homologous recombination, Biol. Chem. 383 (2002) 873–892.
[12] S.P. Jackson, Detection, repair and signalling of DNA double-strand breaks, Biochem. Soc. Trans. 27 (1999) 1–13.
[13] J. Thacker, The nature of mutants induced by ionising radiation in cultured hamster cells. III. Molecular characterization of *HPRT*-deficient mutants induced by gamma-rays or alpha-particles showing that the majority have deletions of all or part of the *hprt* gene, Mutat. Res. 160 (1986) 267–275.
[14] P.A. Jeggo, Identification of genes involved in repair of DNA double-strand breaks in mammalian cells, Radiat. Res. 150 (1998) S80–S91.
[15] R. Kanaar, J.H. Hoeijmakers, D.C. van Gent, Molecular mechanisms of DNA double-strand break repair, Trends Cell. Biol. 8 (1998) 483–489.
[16] L.S. Symington, Role of *RAD52* epistasis group genes in homologous recombination and double-strand break repair, Microbiol. Mol. Biol. Rev. 66 (2002) 630–670.
[17] S.E. Critchlow, S.P. Jackson, DNA end-joining: from yeast to man, Trends Biochem. Sci. 23 (1998) 394–398.
[18] H. Feldmann, L. Driller, B. Meier, G. Mages, J. Kellermann, E.L. Winnacker, HDF2, the second subunit of the Ku homologue from *Saccharomyces cerevisiae*, J. Biol. Chem. 271 (1996) 27765–27769.
[19] S.J. Boulton, S.P. Jackson, Identification of a *Saccharomyces cerevisiae* Ku80 homologue: roles in DNA double-strand break rejoining and in telomeric maintenance, Nucleic Acids Res. 24 (1996) 4639–4648.
[20] P. Schär, G. Herrmann, G. Daly, T. Lindahl, A newly identified DNA ligase of *Saccharomyces cerevisiae* involved in *RAD52*-independent repair of DNA double-strand breaks, Genes Dev. 11 (1997) 1912–1924.
[21] S.H. Teo, S.P. Jackson, Identification of *Saccharomyces cerevisiae* DNA ligase IV: involvement in DNA double-strand break repair, EMBO J. 16 (1997) 4788–4795.
[22] G. Herrmann, T. Lindahl, P. Schär, *Saccharomyces cerevisiae* LIF1: a function involved in DNA double-strand break repair

related to mammalian XRCC4, EMBO J. 17 (1998) 4188–4198.
[23] W. Ramos, G. Liu, C.N. Giroux, A.E. Tomkinson, Biochemical and genetic characterization of the DNA ligase encoded by *Saccharomyces cerevisiae* open reading frame YOR005c, a homolog of mammalian DNA ligase IV, Nucleic Acids Res. 26 (1998) 5676–5683.
[24] S. Wilson, N. Warr, D.L. Taylor, F.Z. Watts, The role of *Schizosaccharomyces pombe* Rad32, the Mre11 homologue, and other DNA damage response proteins in non-homologous end joining and telomere length maintenance, Nucleic Acids Res. 27 (1999) 2655–2661.
[25] K.G. Manolis, E.R. Nimmo, E. Hartsuiker, A.M. Carr, P.A. Jeggo, R.C. Allshire, Novel functional requirements for non-homologous DNA end joining in *Schizosaccharomyces pombe*, EMBO J. 20 (2001) 210–221.
[26] G.R. Weller, B. Kysela, R. Roy, L.M. Tonkin, E. Scanlan, M. Della, S.K. Devine, J.P. Day, A. Wilkinson, F.D. di Fagagna, K.M. Devine, R.P. Bowater, P.A. Jeggo, S.P. Jackson, A.J. Doherty, Identification of a DNA nonhomologous end-joining complex in bacteria, Science 297 (2002) 1686–1689.
[27] Y. Tsukamoto, H. Ikeda, Double-strand break repair mediated by DNA end-joining, Genes Cells 3 (1998) 135–144.
[28] F. Pâques, J.E. Haber, Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*, Microbiol. Mol. Biol. Rev. 63 (1999) 349–404.
[29] M.R. Lieber, The biochemistry and biological significance of nonhomologous DNA end joining: an essential repair process in multicellular eukaryotes, Genes Cells 4 (1999) 77–85.
[30] P. Karran, DNA double-strand break repair in mammalian cells, Curr. Opin. Genet. Dev. 10 (2000) 144–150.
[31] K.K. Khanna, S.P. Jackson, DNA double-strand breaks: signaling, repair and the cancer connection, Nat. Genet. 27 (2001) 247–254.
[32] A. Kuzminov, DNA replication meets genetic exchange: chromosomal damage and its repair by homologous recombination, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 8461–8468.
[33] P.R. Bianco, R.B. Tracy, S.C. Kowalczykowski, DNA strand exchange proteins: a biochemical and physical comparison, Front. Biosci. 3 (1998) D570–D603.
[34] J.A. Nickoloff, M.F. Hoekstra, Double-strand break and recombinational repair in *Saccharomyces cerevisiae*, in: J.A. Nickoloff, M.F. Hoekstra (Eds.), DNA Damage and Repair: DNA Repair in Prokaryotes and Lower Eukaryotes, vol. 1, Human Press, Totowa, NJ, 1998, pp. 335–362.
[35] A.I. Roca, M.M. Cox, RecA protein: structure, function, and role in recombinational DNA repair, Prog. Nucleic Acids Res. Mol. Biol. 56 (1997) 129–223.
[36] M.M. Cox, Recombinational DNA repair in bacteria and the RecA protein, Prog. Nucleic Acids Res. Mol. Biol. 63 (1999) 311–366.
[37] S.C. Kowalczykowski, D.A. Dixon, A.K. Eggleston, S.D. Lauder, W.M. Rehrauer, Biochemistry of homologous recombination in *Escherichia coli*, Microbiol. Rev. 58 (1994) 401–465.
[38] P.R. Bianco, S.C. Kowalczykowski, The recombination hotspot Chi is recognized by the translocating RecBCD enzyme as the single-strand of DNA containing the sequence 5′-GCTGGTGG-3′, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 6706–6711.
[39] S.K. Amundsen, A.F. Taylor, G.R. Smith, The RecD subunit of the *Escherichia coli* RecBCD enzyme inhibits RecA loading, homologous recombination, and DNA repair, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 7399–7404.
[40] F.G. Harmon, S.C. Kowalczykowski, RecQ helicase, in concert with RecA and SSB proteins, initiates and disrupts DNA recombination, Genes Dev. 12 (1998) 1134–1144.
[41] D.A. Arnold, S.C. Kowalczykowski, Facilitated loading of RecA protein is essential to recombination by RecBCD enzyme, J. Biol. Chem. 275 (2000) 12261–12265.
[42] R.R. Meyer, P.S. Laine, The single-stranded DNA-binding protein of *Escherichia coli*, Microbiol. Rev. 54 (1990) 342–380.
[43] L.J. Roman, D.A. Dixon, S.C. Kowalczykowski, RecBCD-dependent joint molecule formation promoted by the *Escherichia coli* RecA and SSB proteins, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 3367–3371.
[44] K. Umezu, R.D. Kolodner, Protein interactions in genetic recombination in *Escherichia coli*. Interactions involving RecO and RecR overcome the inhibition of RecA by single-stranded DNA-binding protein, J. Biol. Chem. 269 (1994) 30005–30013.
[45] K. Morimatsu, S.C. Kowalczykowski, RecFOR proteins load RecA protein onto gapped DNA to accelerate DNA strand exchange: a universal step of recombinational repair, Mol. Cell 11 (2003) 1337–1347.
[46] B.J. Rao, C.M. Radding, RecA protein mediates homologous recognition via non-Watson–Crick bonds in base triplets, Philos. Trans. R. Soc. Lond. [Biol]. 347 (1995) 5–12.
[47] S.C. West, The RuvABC proteins and Holliday-junction processing in *Escherichia coli*, J. Bacteriol. 178 (1996) 1237–1241.
[48] M.C. Whitby, R.G. Lloyd, Targeting Holliday-junctions by the RecG branch migration protein of *Escherichia coli*, J. Biol. Chem. 273 (1998) 19729–19739.
[49] A.A. Mahdi, G.J. Sharples, T.N. Mandal, R.G. Lloyd, Holliday junction resolvases encoded by homologous *rusA* genes in *Escherichia coli* K-12 and phage 82, J. Mol. Biol. 257 (1996) 561–573.
[50] F.N. Capaldo, G. Ramsey, S.D. Barbour, Analysis of the growth of recombination-deficient strains of *Escherichia coli* K-12, J. Bacteriol. 118 (1974) 242–249.
[51] W. Siede, A.A. Friedl, I. Dianova, F. Eckardt-Schupp, E.C. Friedberg, The *Saccharomyces cerevisiae* Ku autoantigen homologue affects radiosensitivity only in the absence of homologous recombination, Genetics 142 (1996) 91–102.
[52] P. Sung, K.M. Trujillo, S. van Komen, Recombination factors of *Saccharomyces cerevisiae*, Mutat. Res. Fundam. Mol. Mech. Mut. 451 (2000) 257–275.
[53] T.D. Petes, R.E. Malone, L.S. Symington, Recombination in yeast, in: J.R. Broach, J.R. Pringle, E.W. Jones (Eds.), The Molecular and Cellular Biology of the Yeast Saccharomyces:

Genome Dynamics, Protein Synthesis, and Energetics, vol. I, Cold Spring Harbor Laboratory Press, 1991, pp. 407–521.
[54] J.C. Game, R.K. Mortimer, A genetic study of X-ray sensitive mutants in yeast, Mutat. Res. 24 (1974) 281–292.
[55] C. Morrison, S. Takeda, Genetic analysis of homologous DNA recombination in vertebrate somatic cells, Int. J. Biochem. Cell Biol. 32 (2000) 817–831.
[56] R.D. Johnson, M. Jasin, Double-strand-break-induced homologous recombination in mammalian cells, Biochem. Soc. Trans. 29 (2001) 196–201.
[57] L.H. Thompson, D. Schild, Homologous recombinational repair of DNA ensures mammalian chromosome stability, Mutat. Res. Fundam. Mol. Mech. Mut. 477 (2001) 131–153.
[58] T. Morita, Y. Yoshimura, A. Yamamoto, K. Murata, M. Mori, H. Yamamoto, A. Matsushiro, A mouse homolog of the *Escherichia coli recA* and *Saccharomyces cerevisiae RAD51* genes, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 6577–6580.
[59] A. Shinohara, H. Ogawa, Y. Matsuda, N. Ushio, K. Ikeo, T. Ogawa, Cloning of human, mouse and fission yeast recombination genes homologous to *RAD51* and *recA*, Nat. Genet. 4 (1993) 239–243.
[60] M.S. Park, Expression of human *RAD52* confers resistance to ionizing radiation in mammalian cells, J. Biol. Chem. 270 (1995) 15467–15470.
[61] J.H. Petrini, M.E. Walsh, C. DiMare, X.N. Chen, J.R. Korenberg, D.T. Weaver, Isolation and characterization of the human *MRE11* homologue, Genomics 29 (1995) 80–86.
[62] R.S. Tebbs, Y. Zhao, J.D. Tucker, J.B. Scheerer, M.J. Siciliano, M. Hwang, N. Liu, R.J. Legerski, L.H. Thompson, Correction of chromosomal instability and sensitivity to diverse mutagens by a cloned cDNA of the *XRCC3* DNA repair gene, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 6354–6358.
[63] J.S. Albala, M.P. Thelen, C. Prange, W.F. Fan, M. Christensen, L.H. Thompson, G.G. Lennon, Identification of a novel human *RAD51* homolog, *RAD51B*, Genomics 46 (1997) 476–479.
[64] C.E. Tambini, A.M. George, J.M. Rommens, L.C. Tsui, S.W. Scherer, J. Thacker, The *XRCC2* DNA repair gene: identification of a positional candidate, Genomics 41 (1997) 84–92.
[65] R. Cartwright, A.M. Dunn, P.J. Simpson, C.E. Tambini, J. Thacker, Isolation of novel human and mouse genes of the *recA*/*RAD51* recombination–repair gene family, Nucleic Acids Res. 26 (1998) 1653–1659.
[66] M.K. Dosanjh, D.W. Collins, W.F. Fan, G.G. Lennon, J.S. Albala, Z.Y. Shen, D. Schild, Isolation and characterization of *RAD51C*, a new human member of the *RAD51* family of related genes, Nucleic Acids Res. 26 (1998) 1179–1184.
[67] N. Liu, J.E. Lamerdin, R.S. Tebbs, D. Schild, J.D. Tucker, M.R. Shen, K.W. Brookman, M.J. Siciliano, C.A. Walter, W.F. Fan, L.S. Narayana, Z.Q. Zhou, A.W. Adamson, K.J. Sorensen, D.J. Chen, N.J. Jones, L.H. Thompson, *XRCC2 and XRCC3*, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages, Mol. Cell 1 (1998) 783–793.
[68] D.L. Pittman, L.R. Weinberg, J.C. Schimenti, Identification, characterization and genetic mapping of *Rad51d*, a new mouse and human *RAD51*/*RecA*-related gene, Genomics 49 (1998) 103–111.
[69] K. Tanaka, T. Hiramoto, T. Fukuda, K. Miyagawa, A novel human Rad54 homologue, Rad54B, associates with Rad51, J. Biol. Chem. 275 (2000) 26316–26321.
[70] C.S. Leasure, J. Chandler, D.J. Gilbert, D.B. Householder, R. Stephens, N.G. Copeland, N.A. Jenkins, S.K. Sharan, Sequence, chromosomal location and expression analysis of the murine homologue of human *RAD51L2*/*RAD51C*, Gene 271 (2001) 59–67.
[71] D.S. Lim, P. Hasty, A mutation in mouse *rad51* results in an early embryonic lethal that is suppressed by a mutation in *p53*, Mol. Cell Biol. 16 (1996) 7133–7143.
[72] T. Tsuzuki, Y. Fujii, K. Sakumi, Y. Tominaga, K. Nakao, M. Sekiguchi, A. Matsushiro, Y. Yoshimura, T. Morita, Targeted disruption of the *Rad51* gene leads to lethality in embryonic mice, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 6236–6240.
[73] J. Essers, R.W. Hendriks, S.M.A. Swagemakers, C. Troelstra, J. Dewit, D. Bootsma, J.H.J. Hoeijmakers, R. Kanaar, Disruption of mouse *RAD54* reduces ionizing radiation resistance and homologous recombination, Cell 89 (1997) 195–204.
[74] T. Rijkers, J. van den Ouweland, B. Morolli, A.G. Rolink, W.M. Baarends, P.P.H. van Sloun, P.H.M. Lohman, A. Pastink, Targeted inactivation of mouse *RAD52* reduces homologous recombination but not resistance to ionizing radiation, Mol. Cell Biol. 18 (1998) 6423–6429.
[75] G.B. Luo, M.S. Yao, C.F. Bender, M. Mills, A.R. Bladl, A. Bradley, J.H.J. Petrini, Disruption of *mRad50* causes embryonic stem cell lethality, abnormal embryonic development, and sensitivity to ionizing radiation, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 7376–7381.
[76] Z.G. Shu, S. Smith, L.J. Wang, M.C. Rice, E.B. Kmiec, Disruption of *muREC2*/*RAD51L1* in mice results in early embryonic lethality which can be partially rescued in a $p53^{-/-}$ background, Mol. Cell Biol. 19 (1999) 8686–8693.
[77] B. Deans, C.S. Griffin, M. Maconochie, J. Thacker, *Xrcc2* is required for genetic stability, embryonic neurogenesis and viability in mice, EMBO J. 19 (2000) 6675–6685.
[78] D.L. Pittman, J.C. Schimenti, Midgestation lethality in mice deficient for the RecA-related gene, *Rad51d*/*Rad51l3*, Genesis 26 (2000) 167–173.
[79] G.B. Luo, M.S. Yao, C.F. Bender, M. Mills, A.R. Bladl, A. Bradley, J.H.J. Petrini, Disruption of *mRad50* causes embryonic stem cell lethality, abnormal embryonic development, and sensitivity to ionizing radiation, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 7376–7381.
[80] L.H. Thompson, D. Schild, The contribution of homologous recombination in preserving genome integrity in mammalian cells, Biochimie 81 (1999) 87–105.
[81] K. Johzuka, H. Ogawa, Interaction of Mre11 and Rad50: two proteins required for DNA repair and meiosis-specific double-strand break formation in *Saccharomyces cerevisiae*, Genetics 139 (1995) 1521–1532.
[82] H. Ogawa, K. Johzuka, T. Nakagawa, S.H. Leem, A.H. Hagihara, Functions of the yeast meiotic recombination genes, *MRE11* and *MRE2*, Adv. Biophys. 31 (1995) 67–76.

[83] D.A. Bressan, H.A. Olivares, B.E. Nelms, J.H. Petrini, Alteration of N-terminal phosphoesterase signature motifs inactivates *Saccharomyces cerevisiae* Mre11, Genetics 150 (1998) 591–600.

[84] T. Usui, T. Ohta, H. Oshiumi, J. Tomizawa, H. Ogawa, T. Ogawa, Complex formation and functional versatility of Mre11 of budding yeast in recombination, Cell 95 (1998) 705–716.

[85] M. Chamankhah, W. Xiao, Formation of the yeast Mre11–Rad50–Xrs2 complex is correlated with DNA repair and telomere maintenance, Nucleic Acids Res. 27 (1999) 2072–2079.

[86] Y. Ho, A. Gruhler, A. Heilbut, G.D. Bader, L. Moore, S.L. Adams, A. Millar, P. Taylor, K. Bennett, K. Boutilier, L. Yang, C. Wolting, I. Donaldson, S. Schandorff, J. Shewnarane, M. Vo, J. Taggart, M. Goudreault, B. Muskat, C. Alfarano, D. Dewar, Z. Lin, K. Michalickova, A.R. Willems, H. Sassi, P.A. Nielsen, K.J. Rasmussen, J.R. Andersen, L.E. Johansen, L.H. Hansen, H. Jespersen, A. Podtelejnikov, E. Nielsen, J. Crawford, V. Poulsen, B.D. Sorensen, J. Matthiesen, R.C. Hendrickson, F. Gleeson, T. Pawson, M.F. Moran, D. Durocher, M. Mann, C.W. Hogue, D. Figeys, M. Tyers, Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry, Nature 415 (2002) 180–183.

[87] G.M. Dolganov, R.S. Maser, A. Novikov, L. Tosto, S. Chong, D.A. Bressan, J.H.J. Petrini, Human Rad50 is physically associated with human Mre11: identification of a conserved multiprotein complex implicated in recombinational DNA repair, Mol. Cell Biol. 16 (1996) 4832–4841.

[88] R.S. Maser, K.J. Monsen, B.E. Nelms, J.H. Petrini, hMre11 and hRad50 nuclear foci are induced during the normal cellular response to DNA double-strand breaks, Mol. Cell Biol. 17 (1997) 6087–6096.

[89] A. Desai-Mehta, K.M. Cerosaletti, P. Concannon, Distinct functional domains of nibrin mediate Mre11 binding, focus formation, and nuclear localization, Mol. Cell Biol. 21 (2001) 2184–2191.

[90] Y. Xiao, D.T. Weaver, Conditional gene targeted deletion by Cre recombinase demonstrates the requirement for the double-strand break repair Mre11 protein in murine embryonic stem cells, Nucleic Acids Res. 25 (1997) 2985–2991.

[91] Y. Yamaguchi-Iwai, E. Sonoda, M.S. Sasaki, C. Morrison, T. Haraguchi, Y. Hiraoka, Y.M. Yamashita, T. Yagi, M. Takata, C. Price, N. Kakazu, S. Takeda, Mre11 is essential for the maintenance of chromosomal DNA in vertebrate cells, EMBO J. 18 (1999) 6619–6629.

[92] J. Zhu, S. Petersen, L. Tessarollo, A. Nussenzweig, Targeted disruption of the Nijmegen breakage syndrome gene *NBS1* leads to early embryonic lethality in mice, Curr. Biol. 11 (2001) 105–109.

[93] J.P. Carney, R.S. Maser, H. Olivares, E.M. Davis, M. Lebeau, J.R. Yates, L. Hays, W.F. Morgan, J.H.J. Petrini, The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response, Cell 93 (1998) 477–486.

[94] S. Matsuura, H. Tauchi, A. Nakamura, N. Kondo, S. Sakamoto, S. Endo, D. Smeets, B. Solder, B.H. Belohradsky, K. Der, V.M. Oshimura, M. Isomura, Y. Nakamura, K. Komatsu, Positional cloning of the gene for Nijmegen breakage syndrome, Nat. Genet. 19 (1998) 179–181.

[95] R. Varon, C. Vissinga, M. Platzer, K.M. Cerosaletti, K.H. Chrzanowska, K. Saar, G. Beckmann, E. Seemanova, P.R. Cooper, N.J. Nowak, M. Stumm, C.M.R. Weemaes, R.A. Gatti, R.K. Wilson, M. Digweed, A. Rosenthal, K. Sperling, P. Concannon, A. Reis, Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome, Cell 93 (1998) 467–476.

[96] G.S. Stewart, R.S. Maser, T. Stankovic, D.A. Bressan, M.I. Kaplan, N.G.J. Jaspers, A. Raams, P.J. Byrd, J.H.J. Petrini, A.M.R. Taylor, The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder, Cell 99 (1999) 577–587.

[97] C.F. Bender, M.L. Sikes, R. Sullivan, L.E. Huye, M.M. Le Beau, D.B. Roth, O.K. Mirzoeva, E.M. Oltz, J.H.J. Petrini, Cancer predisposition and hematopoietic failure in *Rad50*$^{S/S}$ mice, Genes Dev. 16 (2002) 2237–2251.

[98] Y. Shiloh, Ataxia-telangiectasia and the Nijmegen breakage syndrome: related disorders but genes apart, Annu. Rev. Genet. 31 (1997) 635–662.

[99] M. Digweed, A. Reis, K. Sperling, Nijmegen breakage syndrome: consequences of defective DNA double-strand break repair, Bioessays 21 (1999) 649–656.

[100] A.M. Taylor, Chromosome instability syndromes, Best. Pract. Res. Clin. Haematol. 14 (2001) 631–644.

[101] H. Tauchi, S. Matsuura, J. Kobayashi, S. Sakamoto, K. Komatsu, Nijmegen breakage syndrome gene, *NBS1*, and molecular links to factors for genome stability, Oncogene 21 (2002) 8967–8980.

[102] L.H. Thompson, D. Schild, Recombinational DNA repair and human disease, Mut. Res. Fundam. Mol. Mech. Mut. 509 (2002) 49–78.

[103] H. Tauchi, J. Kobayashi, K. Morishima, D.C. van Gent, T. Shiraishi, N.S. Verkaik, D. van Heems, E. Ito, A. Nakamura, E. Sonodo, M. Takata, S. Takeda, S. Matsuura, K. Komatsu, Nbs1 is essential for DNA repair by homologous recombination in higher vertebrate cells, Nature 420 (2002) 93–98.

[104] B.R. Williams, O.K. Mirzoeva, W.F. Morgan, J.Y. Lin, W. Dunnick, J.H.J. Petrini, A murine model of Nijmegen breakage syndrome, Curr. Biol. 12 (2002) 648–653.

[105] J. Kang, R.T. Bronson, Y. Xu, Targeted disruption of NBS1 reveals its roles in mouse development and DNA repair, EMBO J. 21 (2002) 1447–1455.

[106] E. Alani, R. Padmore, N. Kleckner, Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination, Cell 61 (1990) 419–436.

[107] M. de Jager, R. Kanaar, Genome instability and *Rad50*^{S}: subtle yet severe, Genes Dev. 16 (2002) 2173–2178.

[108] M. Kupiec, G. Simchen, Cloning and mapping of the *RAD50* gene of *Saccharomyces cerevisiae*, Mol. Gen. Genet. 193 (1984) 525–531.

[109] E. Alani, S. Subbiah, N. Kleckner, The yeast *RAD50* gene encodes a predicted 153-kD protein containing a purine nucleotide-binding domain and two large heptad-repeat regions, Genetics 122 (1989) 47–57.
[110] E.L. Ivanov, N. Sugawara, C.I. White, F. Fabre, J.E. Haber, Mutations in *XRS2 and RAD50* delay but do not prevent mating-type switching in *Saccharomyces cerevisiae*, Mol. Cell Biol. 14 (1994) 3414–3425.
[111] S.A. Kozhin, O.V. Chepurnaya, V.G. Korolev, The *RAD58* (*XRS4*) gene: map position on the right arm of chromosome XIII, Yeast 11 (1995) 1211–1213.
[112] K.K. Kim, B.A. Shin, K.H. Seo, P.N. Kim, J.T. Koh, J.H. Kim, B.R. Park, Molecular cloning and characterization of splice variants of human *RAD50* gene, Gene 235 (1999) 59–67.
[113] J.H. Petrini, D.A. Bressan, M.S. Yao, The *RAD52* epistasis group in mammalian double-strand break repair, Semin. Immunol. 9 (1997) 181–188.
[114] M. Furuse, Y. Nagase, H. Tsubouchi, K. Murakami-murofushi, T. Shibata, K. Ohta, Distinct roles of two separable in vitro activities of yeast Mre11 in mitotic and meiotic recombination, EMBO J. 17 (1998) 6412–6425.
[115] D. D'Amours, S.P. Jackson, The Mre11 complex: at the crossroads of DNA repair and checkpoint signalling, Nat. Rev. Mol. Cell Biol. 3 (2002) 317–327.
[116] G.J. Sharples, D.R. Leach, Structural and functional similarities between the SbcCD proteins of *Escherichia coli* and the RAD50 and MRE11 (RAD32) recombination and repair proteins of yeast, Mol. Microbiol. 17 (1995) 1215–1217.
[117] T.T. Paull, M. Gellert, The 3′ to 5′ exonuclease activity of Mre11 facilitates repair of DNA double-strand breaks, Mol. Cell 1 (1998) 969–979.
[118] K.M. Trujillo, S.S.F. Yuan, E.Y.H.P. Lee, P. Sung, Nuclease activities in a complex of human recombination and DNA repair factors Rad50, Mre11, and p95, J. Biol. Chem. 273 (1998) 21447–21450.
[119] K.M. Trujillo, P. Sung, DNA structure-specific nuclease activities in the *Saccharomyces cerevisiae* Rad50–Mre11 complex, J. Biol. Chem. 276 (2001) 35458–35464.
[120] T.T. Paull, M. Gellert, Nbs1 potentiates ATP-driven DNA unwinding and endonuclease cleavage by the Mre11/Rad50 complex, Genes Dev. 13 (1999) 1276–1288.
[121] M. de Jager, M.L.G. Dronkert, M. Modesti, C.E.M.T. Beerens, R. Kanaar, D.C. van Gent, DNA-binding and strand-annealing activities of human Mre11: implications for its roles in DNA double-strand break repair pathways, Nucleic Acids Res. 29 (2001) 1317–1325.
[122] T. Hirano, The ABCs of SMC proteins: two-armed ATPases for chromosome condensation, cohesion, and repair, Genes Dev. 16 (2002) 399–414.
[123] K.P. Hopfner, A. Karcher, D.S. Shin, L. Craig, L.M. Arthur, J.P. Carney, J.A. Tainer, Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double-strand break repair and the ABC-ATPase superfamily, Cell 101 (2000) 789–800.
[124] K.P. Hopfner, A. Karcher, L. Craig, T.T. Woo, J.P. Carney, J.A. Tainer, Structural biochemistry and interaction architecture of the DNA double-strand break repair Mre11 nuclease and Rad50-ATPase, Cell 105 (2001) 473–485.
[125] K.P. Hopfner, L. Craig, G. Moncalian, R.A. Zinkel, T. Usui, B.A.L. Owen, A. Karcher, B. Henderson, J.L. Bodmer, C.T. McMurray, J.P. Carney, J.H.J. Petrini, J.A. Tainer, The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair, Nature 418 (2002) 562–566.
[126] K.P. Hopfner, C.D. Putnam, J.A. Tainer, DNA double-strand break repair from head to tail, Curr. Opin. Struct. Biol. 12 (2002) 115–122.
[127] J.C. Connelly, D.R.F. Leach, Tethering on the brink: the evolutionarily conserved Mre11–Rad50 complex, Trends Biochem. Sci. 27 (2002) 410–418.
[128] C. Featherstone, S.P. Jackson, DNA repair: the Nijmegen breakage syndrome protein, Curr. Biol. 8 (1998) R622–R625.
[129] H. Tauchi, J. Kobayashi, K. Morishima, S. Matsuura, A. Nakamura, T. Shiraishi, E. Ito, D. Masnada, D. Delia, K. Komatsu, The forkhead-associated domain of NBS1 is essential for nuclear foci formation after irradiation but not essential for hRAD50 center dot hMRE11 center dot NBS1 complex DNA repair activity, J. Biol. Chem. 276 (2001) 12–15.
[130] S. Zhao, W. Renthal, E.Y. Lee, Functional analysis of FHA and BRCT domains of NBS1 in chromatin association and DNA damage responses, Nucleic Acids Res. 30 (2002) 4815–4822.
[131] K.M. Cerosaletti, P. Concannon, The Nibrin FHA and BRCT domains are both required for nuclear focus formation and phosphorylation, J. Biol. Chem., 2003, in press.
[132] M. Gatei, D. Young, K.M. Cerosaletti, A. Desai-Mehta, K. Spring, S. Kozlov, M.F. Lavin, R.A. Gatti, P. Concannon, K. Khanna, ATM-dependent phosphorylation of nibrin in response to radiation exposure, Nat. Genet. 25 (2000) 115–119.
[133] D.S. Lim, S.T. Kim, B. Xu, R.S. Maser, J.Y. Lin, J.H.J. Petrini, M.B. Kastan, ATM phosphorylates p95/nbs1 in an S-phase checkpoint pathway, Nature 404 (2000) 613–617.
[134] S. Zhao, Y.C. Weng, S.S.F. Yuan, Y.T. Lin, H.C. Hsu, S.C.J. Lin, E. Gerbino, M.H. Song, M.Z. Zdzienicka, R.A. Gatti, J.W. Shay, Y. Ziv, Y. Shiloh, E.Y.H.P. Lee, Functional link between ataxia-telangiectasia and Nijmegen breakage syndrome gene products, Nature 405 (2000) 473–477.
[135] X.H. Wu, V. Ranganathan, D.S. Weisman, W.F. Heine, D.N. Ciccone, T.B. Oneill, K.E. Crick, K.A. Pierce, W.S. Lane, G. Rathbum, D.M. Livingston, D.T. Weaver, ATM phosphorylation of Nijmegen breakage syndrome protein is required in a DNA damage response, Nature 405 (2000) 477–482.
[136] Z.W. Dong, Q. Zhong, P.L. Chen, The Nijmegen breakage syndrome protein is essential for Mre11 phosphorylation upon DNA damage, J. Biol. Chem. 274 (1999) 19513–19516.
[137] O.K. Mirzoeva, J.H.J. Petrini, DNA damage-dependent nuclear dynamics of the Mre11 complex, Mol. Cell Biol. 21 (2001) 281–288.
[138] J. Kobayashi, H. Tauchi, S. Sakamoto, A. Nakamura, K. Morishima, S. Matsuura, T. Kobayashi, K. Tamai, K.

Tanimoto, K. Komatsu, NBS1 localizes to gamma-H2AX foci through interaction with the FHA/BRCT domain, Curr. Biol. 12 (2002) 1846–1851.
[139] W. Goedecke, M. Eijpe, H.H. Offenberg, M. van Aalderen, C. Heyting, Mre11 and Ku70 interact in somatic cells, but are differentially expressed in early meiosis, Nat. Genet. 23 (1999) 194–198.
[140] Q. Zhong, C.F. Chen, S. Li, Y.M. Chen, C.C. Wang, J. Xiao, P.L. Chen, Z.D. Sharp, W.H. Lee, Association of BRCA1 with the hRad50–hMre11–p95 complex and the DNA damage response, Science 285 (1999) 747–750.
[141] Y. Wang, D. Cortez, P. Yazdi, N. Neff, S.J. Elledge, J. Qin, BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures, Genes Dev. 14 (2000) 927–939.
[142] C.T. Her, A.T. Vo, X.L. Wu, Evidence for a direct association of hMRE11 with the human mismatch repair protein hMLH1, DNA Repair 1 (2002) 719–729.
[143] D.B. Lombard, L. Guarente, Nijmegen breakage syndrome disease protein and MRE11 at PML nuclear bodies and meiotic telomeres, Cancer Res. 60 (2000) 2331–2334.
[144] X.D. Zhu, B. Kuster, M. Mann, J.H.J. Petrini, T. de Lange, Cell-cycle-regulated association of RAD50/MRE11/NBS1 with TRF2 and human telomeres, Nat. Genet. 25 (2000) 347–352.
[145] G.K. Wu, W.H. Lee, P.L. Chen, NBS1 and TRF1 colocalize at promyelocytic leukemia bodies during late S/G_2 phases in immortalized telomerase-negative cells—implication of NBS1 in alternative lengthening of telomeres, J. Biol. Chem. 275 (2000) 30618–30622.
[146] J.D. Griffith, L. Comeau, S. Rosenfield, R.M. Stansel, A. Bianchi, H. Moss, T. de Lange, Mammalian telomeres end in a large duplex loop, Cell 97 (1999) 503–514.
[147] P.R. Chua, G.S. Roeder, Zip2, a meiosis-specific protein required for the initiation of chromosome synapsis, Cell 93 (1998) 349–359.
[148] M. Sym, J.A. Engebrecht, G.S. Roeder, ZIP1 is a synaptonemal complex protein required for meiotic chromosome synapsis, Cell 72 (1993) 365–378.
[149] J.E. Haber, Meiosis: avoiding inappropriate relationships, Curr. Biol. 8 (1998) R832–R835.
[150] S. Agarwal, G.S. Roeder, Zip3 provides a link between recombination enzymes and synaptonemal complex proteins, Cell 102 (2000) 245–255.
[151] J. Xiao, C.C. Liu, P.L. Chen, W.H. Lee, RINT-1, a novel Rad50-interacting protein, participates in radiation-induced G_2/M checkpoint control, J. Biol. Chem. 276 (2001) 6105–6111.
[152] R.S. Maser, O.K. Mirzoeva, J. Wells, H. Olivares, B.R. Williams, R.A. Zinkel, P.J. Farnham, J.H.J. Petrini, Mre11 complex and DNA replication: linkage to E2F and sites of DNA synthesist, Mol. Cell Biol. 21 (2001) 6006–6016.
[153] K. Naka, K. Ikeda, N. Motoyama, Recruitment of NBS1 into PML oncogenic domains via interaction with SP100 protein, Biochem. Biophys. Res. Commun. 299 (2002) 863–871.
[154] V. Fogal, M. Gostissa, P. Sandy, P. Zacchi, T. Sternsdorf, K. Jensen, P.P. Pandolfi, H. Will, C. Schneider, G. Del Sal, Regulation of p53 activity in nuclear bodies by a specific PML isoform, EMBO J. 19 (2000) 6185–6195.
[155] M. Pearson, R. Carbone, C. Sebastiani, M. Cioce, M. Fagioli, S. Saito, Y. Higashimoto, E. Appella, S. Minucci, P.P. Pandolfi, P.G. Pelicci, PML regulates p53 acetylation and premature senescence induced by oncogenic Ras, Nature 406 (2000) 207–210.
[156] H. Abe, M. Wada, K. Kohno, M. Kuwano, Altered drug sensitivities to anticancer agents in radiation-sensitive DNA repair deficient yeast mutants, Anticancer Res. 14 (1994) 1807–1810.
[157] C.W. Moore, Responses of radiation-sensitive mutants of *Saccharomyces cerevisiae* to lethal effects of bleomycin, Mutat. Res. 51 (1978) 165–180.
[158] J.A. Simon, P. Szankasi, D.K. Nguyen, C. Ludlow, H.M. Dunstan, C.J. Roberts, E.L. Jensen, L.H. Hartwell, S.H. Friend, Differential toxicities of anticancer agents among DNA repair and checkpoint mutants of *Saccharomyces cerevisiae*, Cancer Res. 60 (2000) 328–333.
[159] M. Slaninová, V. Vlčková, J. Brozmanová, M.A. Morais, J.A.P. Henriques, Biological consequences of *E. coli* RecA protein expression in the repair defective *pso4-1* and *rad51:URA3* mutants of *S.cerevisiae* after treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, Neoplasma 43 (1996) 315–319.
[160] J.W. Donovan, G.T. Milne, D.T. Weaver, Homotypic and heterotypic protein associations control Rad51 function in double-strand break repair, Genes Dev. 8 (1994) 2552–2562.
[161] M.A. de Morais Jr., E.J. Vicente, J. Brozmanová, A.C.G. Schenberg, J.A.P. Henriques, Further characterization of the yeast *pso4-1* mutant: interaction with *rad51* and *rad52* mutants after photoinduced psoralen lesions, Curr. Genet. 29 (1996) 211–218.
[162] M.A. Morais, V. Vlčková, I. Fridrichová, M. Slaninová, J. Brozmanová, J.A.P. Henriques, Effect of bacterial *recA* expression on DNA repair in the *rad51* and *rad52* mutants of *Saccharomyces cerevisiae*, Genet. Mol. Biol. 21 (1998) 3–9.
[163] J.P. McDonald, R. Rothstein, Unrepaired heteroduplex DNA in *Saccharomyces cerevisiae* is decreased in *RAD1 RAD52*-independent recombination, Genetics 137 (1994) 393–405.
[164] A.J. Rattray, L.S. Symington, Multiple pathways for homologous recombination in *Saccharomyces cerevisiae*, Genetics 139 (1995) 45–56.
[165] Y. Bai, L.S. Symington, A Rad52 homolog is required for *RAD51*-independent mitotic recombination in *Saccharomyces cerevisiae*, Genes Dev. 10 (1996) 2025–2037.
[166] M. Fasullo, P. Giallanza, Z. Dong, C. Cera, T. Bennett, *Saccharomyces cerevisiae rad51* mutants are defective in DNA damage-associated sister chromatid exchanges but exhibit increased rates of homology-directed translocations, Genetics 158 (2001) 959–972.
[167] A. Shinohara, A. Ogawa, T. Ogawa, Rad51 protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein, Cell 69 (1992) 457–470.
[168] G. Basile, M. Aker, R.K. Mortimer, Nucleotide sequence and transcriptional regulation of the yeast recombinational repair gene *RAD51*, Mol. Cell Biol. 12 (1992) 3235–3246.

[169] L. Cao, E. Alani, N. Kleckner, A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*, Cell 61 (1990) 1089–1101.
[170] E. Sonoda, M.S. Sasaki, J.M. Buerstedde, O. Bezzubova, A. Shinohara, H. Ogawa, M. Takata, Y. Yamaguchiiwai, S. Takeda, Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death, EMBO J. 17 (1998) 598–608.
[171] M. Takata, M.S. Sasaki, E. Sonoda, T. Fukushima, C. Morrison, J.S. Albala, S.M.A. Swagemakers, R. Kanaar, L.H. Thompson, S. Takeda, The Rad51 paralog Rad51B promotes homologous recombinational repair, Mol. Cell Biol. 20 (2000) 6476–6482.
[172] A. Fujimori, S. Tachiiri, E. Sonoda, L.H. Thompson, P.K. Dhar, M. Hiraoka, S. Takeda, Y. Zhang, M. Reth, M. Takata, Rad52 partially substitutes for the Rad51 paralog XRCC3 in maintaining chromosomal integrity in vertebrate cells, EMBO J. 20 (2001) 5513–5520.
[173] E. Sonoda, M. Takata, Y.M. Yamashita, C. Morrison, S. Takeda, Homologous DNA recombination in vertebrate cells, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 8388–8394.
[174] M. Takata, M.S. Sasaki, S. Tachiiri, T. Fukushima, E. Sonoda, D. Schild, L.H. Thompson, S. Takeda, Chromosome instability and defective recombinational repair in knockout mutants of the five Rad51 paralogs, Mol. Cell Biol. 21 (2001) 2858–2866.
[175] K. Caldecott, P. Jeggo, Cross-sensitivity of gamma-ray-sensitive hamster mutants to cross-linking agents, Mutat. Res. 255 (1991) 111–121.
[176] R.D. Johnson, N. Liu, M. Jasin, Mammalian XRCC2 promotes the repair of DNA double-strand breaks by homologous recombination, Nature 401 (1999) 397–399.
[177] A.J. Pierce, R.D. Johnson, L.H. Thompson, M. Jasin, XRCC3 promotes homology-directed repair of DNA damage in mammalian cells, Genes Dev. 13 (1999) 2633–2638.
[178] M.A. Brenneman, A.E. Weiss, J.A. Nickoloff, D.J. Chen, *XRCC3* is required for efficient repair of chromosome breaks by homologous recombination, Mutat. Res. DNA Repair 459 (2000) 89–97.
[179] X. Cui, M. Brenneman, J. Meyne, M. Oshimura, E.H. Goodwin, D.J. Chen, The *XRCC2* and *XRCC3* repair genes are required for chromosome stability in mammalian cells, Mutat. Res. DNA Repair 434 (1999) 75–88.
[180] M.A. Brenneman, B.M. Wagener, C.A. Miller, C. Allen, J.A. Nickoloff, *XRCC3* controls the fidelity of homologous recombination: roles for *XRCC3* in late stages of recombination, Mol. Cell 10 (2002) 387–395.
[181] A. Aboussekhra, R. Chanet, A. Adjiri, F. Fabre, Semidominant suppressors of Srs2 helicase mutations of *Saccharomyces cerevisiae* map in the *RAD51* Gene, whose sequence predicts a protein with similarities to procaryotic RecA proteins, Mol. Cell Biol. 12 (1992) 3224–3234.
[182] P. Baumann, S.C. West, Role of the human RAD51 protein in homologous recombination and double-stranded break repair, Trends Biochem. Sci. 23 (1998) 247–251.
[183] A. Aboussekhra, R. Chanet, Z. Zgaga, C. Cassier-Chauvat, M. Heude, F. Fabre, *RADH*, a gene of *Saccharomyces cerevisiae* encoding a putative DNA helicase involved in DNA repair. Characteristics of *radH* mutants and sequence of the gene, Nucleic Acids Res. 17 (1989) 7211–7219.
[184] T. Ogawa, A. Shinohara, A. Nabetani, T. Ikeya, X. Yu, E.H. Egelman, H. Ogawa, RecA-like recombination proteins in eukaryotes: functions and structures of *RAD51* genes, Cold Spring Harb. Sym. Quant. Biol. 68 (1993) 567–576.
[185] F.E. Benson, A. Stasiak, S.C. West, Purification and characterization of the human Rad51 protein, an analogue of *E. coli* RecA, EMBO J. 13 (1994) 5764–5771.
[186] Y. Yoshimura, T. Morita, A. Yamamoto, A. Matsushiro, Cloning and sequence of the human RecA-like gene cDNA, Nucleic Acids Res. 21 (1993) 1665.
[187] R. Cartwright, C.E. Tambini, P.J. Simpson, J. Thacker, The *XRCC2* DNA repair gene from human and mouse encodes a novel member of the *recA*/*RAD51* family, Nucleic Acids Res. 26 (1998) 3084–3089.
[188] O. Bezzubova, A. Shinohara, R.G. Mueller, H. Ogawa, J.M. Buerstedde, A chicken *RAD51* homologue is expressed at high levels in lymphoid and reproductive organs, Nucleic Acids Res. 21 (1993) 1577–1580.
[189] T. Ogawa, X. Yu, A. Shinohara, E.H. Egelman, Similarity of the yeast RAD51 filament to the bacterial RecA filament, Science 259 (1993) 1896–1899.
[190] P. Sung, Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein, Science 265 (1994) 1241–1243.
[191] P. Sung, D.L. Robberson, DNA strand exchange mediated by a Rad51–ssDNA nucleoprotein filament with polarity opposite to that of RecA, Cell 82 (1995) 453–461.
[192] E.A. Namsaraev, P. Berg, Branch migration during Rad51-promoted strand exchange proceeds in either direction, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 10477–10481.
[193] E.A. Namsaraev, P. Berg, Rad51 uses one mechanism to drive DNA strand exchange in both directions, J. Biol. Chem. 275 (2000) 3970–3976.
[194] A.V. Mazin, E. Zaitseva, P. Sung, S.C. Kowalczykowski, Tailed duplex DNA is the preferred substrate for Rad51 protein-mediated homologous pairing, EMBO J. 19 (2000) 1148–1156.
[195] R.C. Gupta, L.R. Bazemore, E.I. Golub, C.M. Radding, Activities of human recombination protein Rad51, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 463–468.
[196] H. Aihara, Y. Ito, H. Kurumizaka, S. Yokoyama, T. Shibata, The N-terminal domain of the human Rad51 protein binds DNA: structure and a DNA binding surface as revealed by NMR, J. Mol. Biol. 290 (1999) 495–504.
[197] X. Yu, S.A. Jacobs, S.C. West, T. Ogawa, E.H. Egelman, Domain structure and dynamics in the helical filaments formed by RecA and Rad51 on DNA, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 8419–8424.
[198] P. Baumann, F.E. Benson, S.C. West, Human Rad51 protein promotes ATP-dependent homologous pairing and strand transfer reactions in vitro, Cell 87 (1996) 757–766.
[199] P. Baumann, S.C. West, The human Rad51 protein: polarity of strand transfer and stimulation by hRP-A, EMBO J. 16 (1997) 5198–5206.

[200] S. Sigurdsson, K. Trujillo, B.W. Song, S. Stratton, P. Sung, Basis for avid homologous DNA strand exchange by human Rad51 and RPA, J. Biol. Chem. 276 (2001) 8798–8806.
[201] H.K. Kim, K. Morimatsu, B. Norden, M. Ardhammar, M. Takahashi, ADP stabilizes the human Rad51-single-stranded DNA complex and promotes its DNA annealing activity, Genes Cells 7 (2002) 1125–1134.
[202] T. Sugiyama, E.M. Zaitseva, S.C. Kowalczykowski, A single-stranded DNA-binding protein is needed for efficient presynaptic complex formation by the *Saccharomyces cerevisiae* Rad51 protein, J. Biol. Chem. 272 (1997) 7940–7945.
[203] E. Namsaraev, P. Berg, Characterization of strand exchange activity of yeast Rad51 protein, Mol. Cell Biol. 17 (1997) 5359–5368.
[204] P. Sung, Yeast Rad55 and Rad57 proteins form a heterodimer that functions with replication protein A to promote DNA strand exchange by Rad51 recombinase, Genes Dev. 11 (1997) 1111–1121.
[205] T. Sugiyama, S.C. Kowalczykowski, Rad52 protein associates with replication protein A (RPA)-single-stranded DNA to accelerate Rad51-mediated displacement of RPA and presynaptic complex formation, J. Biol. Chem. 277 (2002) 31663–31672.
[206] P. Sung, Function of yeast Rad52 protein as a mediator between replication protein A and the Rad51 recombinase, J. Biol. Chem. 272 (1997) 28194–28197.
[207] J.H. New, T. Sugiyama, E. Zaitseva, S.C. Kowalczykowski, Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein A, Nature 391 (1998) 407–410.
[208] R.C. Gupta, E.I. Golub, M.S. Wold, C.M. Radding, Polarity of DNA strand exchange promoted by recombination proteins of the RecA family, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 9843–9848.
[209] H. Kurumizaka, H. Aihara, W. Kagawa, T. Shibata, S. Yokoyama, Human Rad51 amino acid residues required for Rad52 binding, J. Mol. Biol. 291 (1999) 537–548.
[210] F.E. Benson, P. Baumann, S.C. West, Synergistic actions of Rad51 and Rad52 in recombination and DNA repair, Nature 391 (1998) 401–404.
[211] S. Sigurdsson, S. van Komen, W. Bussen, D. Schild, J.S. Albala, P. Sung, Mediator function of the human Rad51B–Rad51C complex in Rad51/RPA-catalyzed DNA strand exchange, Genes Dev. 15 (2001) 3308–3318.
[212] P.A. Havre, M. Rice, R. Ramos, E.B. Kmiec, HsRec2/Rad51L1, a protein influencing cell cycle progression, has protein kinase activity, Exp. Cell Res. 254 (2000) 33–44.
[213] P.A. Havre, M.C. Rice, M. Noe, E.B. Kmiec, The human *REC2*/*RAD51B* gene acts as a DNA damage sensor by inducing G_1 delay and hypersensitivity to ultraviolet irradiation, Cancer Res. 58 (1998) 4733–4739.
[214] Y.C. Lio, A.V. Mazin, S.C. Kowalczykowski, D.J. Chen, Complex formation by the human Rad51B and Rad51C DNA repair proteins and their activities in vitro, J. Biol. Chem. 278 (2003) 2469–2478.
[215] H. Yokoyama, H. Kurumizaka, S. Ikawa, S. Yokoyama, T. Shibata, Holliday junction binding activity of the human Rad51B protein, J. Biol. Chem. 278 (2003) 2767–2772.
[216] J.P. Braybrooke, K.G. Spink, J. Thacker, I.D. Hickson, The RAD51 family member, RAD51L3, is a DNA-stimulated ATPase that forms a complex with XRCC2, J. Biol. Chem. 275 (2000) 29100–29106.
[217] J.Y. Masson, A.Z. Stasiak, A. Stasiak, F.E. Benson, S.C. West, Complex formation by the human RAD51C and XRCC3 recombination repair proteins, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 8440–8446.
[218] H. Kurumizaka, S. Ikawa, M. Nakada, K. Eda, W. Kagawa, M. Takata, S. Takeda, S. Yokoyama, T. Shibata, Homologous-pairing activity of the human DNA-repair proteins Xrcc3–Rad51C, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 5538–5543.
[219] H. Kurumizaka, S. Ikawa, M. Nakada, R. Enomoto, W. Kagawa, T. Kinebuchi, M. Yamazoe, S. Yokoyama, T. Shibata, Homologous pairing and ring and filament structure formation activities of the human Xrcc2–Rad51D complex, J. Biol. Chem. 277 (2002) 14315–14320.
[220] G. Ira, J.E. Haber, Characterization of *RAD51*-independent break-induced replication that acts preferentially with short homologous sequences, Mol. Cell Biol. 22 (2002) 6384–6392.
[221] G.T. Milne, D.T. Weaver, Dominant negative alleles of *RAD52* reveal a DNA repair/recombination complex including *RAD51* and *RAD52*, Genes Dev. 7 (1993) 1755–1765.
[222] A.C. Gavin, M. Bosche, R. Krause, P. Grandi, M. Marzioch, A. Bauer, J. Schultz, J.M. Rick, A.M. Michon, C.M. Cruciat, M. Remor, C. Hofert, M. Schelder, M. Brajenovic, H. Ruffner, A. Merino, K. Klein, M. Hudak, D. Dickson, T. Rudi, V. Gnau, A. Bauch, S. Bastuck, B. Huhse, C. Leutwein, M.A. Heurtier, R.R. Copley, A. Edelmann, E. Querfurth, V. Rybin, G. Drewes, M. Raida, T. Bouwmeester, P. Bork, B. Seraphin, B. Kuster, G. Neubauer, G. Superti-Furga, Functional organization of the yeast proteome by systematic analysis of protein complexes, Nature 415 (2002) 141–147.
[223] H. Jiang, Y.Q. Xie, P. Houston, K. Stemkehale, U.H. Mortensen, R. Rothstein, T. Kodadek, Direct association between the yeast Rad51 and Rad54 recombination proteins, J. Biol. Chem. 271 (1996) 33181–33186.
[224] B. Clever, H. Interthal, J. Schmucklimaurer, J. King, M. Sigrist, W.D. Heyer, Recombinational repair in yeast: functional interactions between Rad51 and Rad54 proteins, EMBO J. 16 (1997) 2535–2544.
[225] G. Petukhova, S. Stratton, P. Sung, Catalysis of homologous DNA pairing by yeast Rad51 and Rad54 proteins, Nature 393 (1998) 91–94.
[226] S.L. Hays, A.A. Firmenich, P. Berg, Complex formation in yeast double-strand break repair: participation of Rad51, Rad52, Rad55, and Rad57 proteins, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 6925–6929.
[227] R.D. Johnson, L.S. Symington, Functional differences and interactions among the putative RecA homologs Rad51, Rad55, and Rad57, Mol. Cell Biol. 15 (1995) 4843–4850.
[228] P. Uetz, L. Giot, G. Cagney, T.A. Mansfield, R.S. Judson, J.R. Knight, D. Lockshon, V. Narayan, M. Srinivasan, P. Pochart, A. Qureshi-Emili, Y. Li, B. Godwin, D. Conover,

T. Kalbfleisch, G. Vijayadamodar, M. Yang, M. Johnston, S. Fields, J.M. Rothberg, A comprehensive analysis of protein–protein interactions in *Saccharomyces cerevisiae*, Nature 403 (2000) 623–627.

[229] M.E. Dresser, D.J. Ewing, M.N. Conrad, A.M. Dominguez, R. Barstead, H. Jiang, T. Kodadek, *DMC1* functions in a *Saccharomyces cerevisiae* meiotic pathway that is largely independent of the *RAD51* pathway, Genetics 147 (1997) 533–544.

[230] H.L. Klein, *RDH54*, a *RAD54* homologue in *Saccharomyces cerevisiae*, is required for mitotic diploid-specific recombination and repair and for meiosis, Genetics 147 (1997) 1533–1543.

[231] M. Shinohara, E. Shitayamaguchi, J.M. Buerstedde, H. Shinagawa, H. Ogawa, A. Shinohara, Characterization of the roles of the *Saccharomyces cerevisiae RAD54* gene and a homologue of *RAD54*, *RDH54*/*TID1*, in mitosis and meiosis, Genetics 147 (1997) 1545–1556.

[232] L. Wu, S.L. Davies, N.C. Levitt, I.D. Hickson, Potential role for the BLM helicase in recombinational repair via a conserved interaction with RAD51, J. Biol. Chem. 276 (2001) 19375–19381.

[233] P.M. Watt, I.D. Hickson, R.H. Borts, E.J. Louis, *SGS1*, a homologue of the Bloom's and Werner's syndrome genes, is required for maintenance of genome stability in *Saccharomyces cerevisiae*, Genetics 144 (1996) 935–945.

[234] Z.Y. Shen, K.G. Cloud, D.J. Chen, M.S. Park, Specific interactions between the human RAD51 and RAD52 proteins, J. Biol. Chem. 271 (1996) 148–152.

[235] E.I. Golub, O.V. Kovalenko, R.C. Gupta, D.C. Ward, C.M. Radding, Interaction of human recombination proteins Rad51 and Rad54, Nucleic Acids Res. 25 (1997) 4106–4110.

[236] S. Sakamoto, K. Nishikawa, S.J. Heo, M. Coto, Y. Furuichi, A. Shimamoto, Werner helicase relocates into nuclear foci in response to DNA damaging agents and co-localizes with RPA and Rad51, Genes Cells 6 (2001) 421–430.

[237] N. Liu, D. Schild, M.P. Thelen, L.H. Thompson, Involvement of Rad51C in two distinct protein complexes of Rad51 paralogs in human cells, Nucleic Acids Res. 30 (2002) 1009–1015.

[238] K.A. Miller, D.M. Yoshikawa, I.R. McConnell, R. Clark, D. Schild, J.S. Albala, RAD51C interacts with RAD51B and is central to a larger protein complex in vivo exclusive of RAD51, J. Biol. Chem. 277 (2002) 8406–8411.

[239] C. Wiese, D.W. Collins, J.S. Albala, L.H. Thompson, A. Kronenberg, D. Schild, Interactions involving the Rad51 paralogs Rad51C and XRCC3 in human cells, Nucleic Acids Res. 30 (2002) 1001–1008.

[240] D. Schild, Y.C. Lio, D.W. Collins, T. Tsomondo, D.J. Chen, Evidence for simultaneous protein interactions between human Rad51 paralogs, J. Biol. Chem. 275 (2000) 16443–16449.

[241] J.Y. Masson, M.C. Tarsounas, A.Z. Stasiak, A. Stasiak, R. Shah, M.J. McIlwraith, F.E. Benson, S.C. West, Identification and purification of two distinct complexes containing the five RAD51 paralogs, Genes Dev. 15 (2001) 3296–3307.

[242] C.A. French, J.Y. Masson, C.S. Griffin, P. O'Regan, S.C. West, J. Thacker, Role of mammalian RAD51L2 (RAD51C) in recombination and genetic stability, J. Biol. Chem. 277 (2002) 19322–19330.

[243] J. Essers, A.B. Houtsmuller, L. van Veelen, C. Paulusma, A.L. Nigg, A. Pastink, W. Vermeulen, J.H.J. Hoeijmakers, R. Kanaar, Nuclear dynamics of *RAD52* group homologous recombination proteins in response to DNA damage, EMBO J. 21 (2002) 2030–2037.

[244] S.L. Hays, A.A. Firmenich, P. Massey, R. Banerjee, P. Berg, Studies of the interaction between Rad52 protein and the yeast single-stranded DNA binding protein RPA, Mol. Cell Biol. 18 (1998) 4400–4406.

[245] S.L. Gasior, A.K. Wong, Y. Kora, A. Shinohara, D.K. Bishop, Rad52 associates with RPA and functions with Rad55 and Rad57 to assemble meiotic recombination complexes, Genes Dev. 12 (1998) 2208–2221.

[246] L. Krejči, J. Damborský, B. Thomsen, M. Duno, C. Bendixen, Molecular dissection of interactions between Rad51 and members of the recombination–repair group, Mol. Cell Biol. 21 (2001) 966–976.

[247] K.M. Kramer, D. Fesquet, A.L. Johnson, L.H. Johnston, Budding yeast *RSI1*/*APC2*, a novel gene necessary for initiation of anaphase, encodes an APC subunit, EMBO J. 17 (1998) 498–506.

[248] I.I. Ouspenski, S.J. Elledge, B.R. Brinkley, New yeast genes important for chromosome integrity and segregation identified by dosage effects on genome stability, Nucleic Acids Res. 27 (1999) 3001–3008.

[249] D.K. Bishop, D. Park, L. Xu, N. Kleckner, *DMC1*: a meiosis-specific yeast homolog of *E.coli recA* required for recombination, synaptonemal complex formation and cycle progression, Cell 69 (1992) 439–456.

[250] D.K. Bishop, RecA homologs Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis, Cell 79 (1994) 1081–1092.

[251] M. Shinohara, S.L. Gasior, D.K. Bishop, A. Shinohara, Tid1/Rdh54 promotes colocalization of Rad51 and Dmc1 during meiotic recombination, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 10814–10819.

[252] J.Y. Masson, A.A. Davies, N. Hajibagheri, E. van Dyck, F.E. Benson, A.Z. Stasiak, A. Stasiak, S.C. West, The meiosis-specific recombinase hDmc1 forms ring structures and interacts with hRad51, EMBO J. 18 (1999) 6552–6560.

[253] M. Tarsounas, T. Morita, R.E. Pearlman, P.B. Moens, RAD51 and DMC1 form mixed complexes associated with mouse meiotic chromosome cores and synaptonemal complexes, J. Cell Biol. 147 (1999) 207–219.

[254] B.D. Harfe, S. Jinks-Robertson, Mismatch repair proteins and mitotic genome stability, Mutat. Res. Fundam. Mol. Mech. Mut. 451 (2000) 151–167.

[255] T.A. Prolla, D.M. Christie, R.M. Liskay, Dual requirement in yeast DNA mismatch repair for *MLH1* and *PMS1*, two homologs of the bacterial *mutL* gene, Mol. Cell Biol. 14 (1994) 407–415.

[256] L. Gellon, M. Werner, S. Boiteux, Ntg2p, a *Saccharomyces cerevisiae* DNA *N*-glycosylase/apurinic or apyrimidinic

lyase involved in base excision repair of oxidative DNA damage, interacts with the DNA mismatch repair protein Mlh1p. Identification of a Mlh1p binding motif, J. Biol. Chem. 277 (2002) 29963–29972.
[257] S. Kharbanda, R. Ren, P. Pandey, T.D. Shafman, S.M. Feller, R.R. Weichselbaum, D.W. Kufe, Activation of the c-Abl tyrosine kinase in the stress response to DNA-damaging agents, Nature 376 (1995) 785–788.
[258] Z.M. Yuan, Y.Y. Huang, T. Ishiko, S. Nakada, T. Utsugisawa, S. Kharbanda, R. Wang, P. Sung, A. Shinohara, R. Weichselbaum, D. Kufe, Regulation of Rad51 function by c-Abl in response to DNA damage, J. Biol. Chem. 273 (1998) 3799–3802.
[259] G. Chen, S.S.F. Yuan, W. Liu, Y. Xu, K. Trujillo, B.W. Song, F. Cong, S.P. Goff, Y. Wu, R. Arlinghaus, D. Baltimore, P.J. Gasser, M.S. Park, P. Sung, E.Y.H.P. Lee, Radiation-induced assembly of Rad51 and Rad52 recombination complex requires ATM and c-Abl, J. Biol. Chem. 274 (1999) 12748–12752.
[260] Y.Z. Li, H. Shimizu, S.L. Xiang, Y. Maru, N. Takao, K. Yamamoto, Arg tyrosine kinase is involved in homologous recombinational DNA repair, Biochem. Biophys. Res. Commun. 299 (2002) 697–702.
[261] H. Kitao, Z.M. Yuan, Regulation of ionizing radiation-induced Rad52 nuclear foci formation by c-Abl-mediated phosphorylation, J. Biol. Chem. 277 (2002) 48944–48948.
[262] R. Scully, J.J. Chen, A. Plug, Y.H. Xiao, D. Weaver, J. Feunteun, T. Ashley, D.M. Livingston, Association of BRCA1 with Rad51 in mitotic and meiotic cells, Cell 88 (1997) 265–275.
[263] P.L. Chen, C.F. Chen, Y.M. Chen, J. Xiao, Z.D. Sharp, W.H. Lee, The BRC repeats in BRCA2 are critical for RAD51 binding and resistance to methyl methanesulfonate treatment, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 5287–5292.
[264] A.K.C. Wong, R. Pero, P.A. Ormonde, S.V. Tavtigian, P.L. Bartel, RAD51 interacts with the evolutionarily conserved BRC motifs in the human breast cancer susceptibility gene *brca2*, J. Biol. Chem. 272 (1997) 31941–31944.
[265] L.Y. Marmorstein, T. Ouchi, S.A. Aaronson, The *BRCA2* gene product functionally interacts with p53 and RAD51, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 13869–13874.
[266] T. Ludwig, D.L. Chapman, V.E. Papaioannou, A. Efstratiadis, Targeted mutations of breast cancer susceptibility gene homologs in mice: lethal phenotypes of Brca1, Brca2, Brca1/Brca2, Brca1/p53, and Brca2/p53 nullizygous embryos, Genes Dev. 11 (1997) 1226–1241.
[267] S.K. Sharan, M. Morimatsu, U. Albrecht, D.S. Lim, E. Regel, C. Dinh, A. Sands, G. Eichele, P. Hasty, A. Bradley, Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2, Nature 386 (1997) 804–810.
[268] N. Foray, D. Marot, V. Randrianarison, N. Dalla Venezia, D. Picard, M. Perricaudet, V. Favaudon, P. Jeggo, Constitutive association of BRCA1 and c-Abl and its ATM-dependent disruption after irradiation, Mol. Cell Biol. 22 (2002) 4020–4032.
[269] H.W. Sturzbecher, B. Donzelmann, W. Henning, U. Knippschild, S. Buchhop, p53 is linked directly to homologous recombination processes via RAD51/RecA protein interaction, EMBO J. 15 (1996) 1992–2002.
[270] S. Buchhop, M.K. Gibson, X.W. Wang, P. Wagner, H.W. Sturzbecher, C.C. Harris, Interaction of p53 with the human Rad51 protein, Nucleic Acids Res. 25 (1997) 3868–3874.
[271] S. Susse, C. Janz, F. Janus, W. Deppert, L. Wiesmuller, Role of heteroduplex joints in the functional interactions between human Rad51 and wild-type p53, Oncogene 19 (2000) 4500–4512.
[272] H.B. Zhang, K. Somasundaram, Y. Peng, H. Tian, H.X. Zhang, D.K. Bi, B.L. Weber, W.S. Eldeiry, BRCA1 physically associates with p53 and stimulates its transcriptional activity, Oncogene 16 (1998) 1713–1721.
[273] J.J. Chen, D. Silver, S. Cantor, D.M. Livingston, R. Scully, BRCA1, BRCA2, and Rad51 operate in a common DNA damage response pathway, Cancer Res. 59 (1999) 1752S–1756S.
[274] T. Yasugi, P.M. Howley, Identification of the structural and functional human homolog of the yeast ubiquitin conjugating enzyme UBC9, Nucleic Acids Res. 24 (1996) 2005–2010.
[275] R.T. Hay, Protein modification by SUMO, Trends Biochem. Sci. 26 (2001) 332–333.
[276] O.V. Kovalenko, A.W. Plug, T. Haaf, D.K. Gonda, T. Ashley, D.C. Ward, C.M. Radding, E.I. Golub, Mammalian ubiquitin-conjugating enzyme Ubc9 interacts with Rad51 recombination protein and localizes in synaptonemal complexes, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 2958–2963.
[277] Z.Y. Shen, P.E. Pardingtonpurtymun, J.C. Comeaux, R.K. Moyzis, D.J. Chen, Associations of UBE21 with RAD52, UBL1, p53, and RAD51 proteins in a yeast two-hybrid system, Genomics 37 (1996) 183–186.
[278] W.H. Li, B. Hesabi, A. Babbo, C. Pacione, J.M. Liu, D.J. Chen, J.A. Nickoloff, Z.Y. Shen, Regulation of double-strand break-induced mammalian homologous recombination by UBL1, a RAD51-interacting protein, Nucleic Acids Res. 28 (2000) 1145–1153.
[279] O.V. Kovalenko, E.I. Golub, P. Brayward, D.C. Ward, C.M. Radding, A novel nucleic acid-binding protein that interacts with human Rad51 recombinase, Nucleic Acids Res. 25 (1997) 4946–4953.
[280] R. Mizuta, J.M. Lasalle, H.L. Cheng, A. Shinohara, H. Ogawa, N. Copeland, N.A. Jenkins, M. Lalande, F.W. Alt, RAB22 and RAB163/mouse BRCA2: proteins that specifically interact with the RAD51 protein, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 6927–6932.
[281] E. Maldonado, R. Shiekhattar, M. Sheldon, H. Cho, R. Drapkin, P. Rickert, E. Lees, C.W. Anderson, S. Linn, D. Reinberg, A human RNA polymerase II complex associated with SRB and DNA-repair proteins, Nature 381 (1996) 86–89.
[282] K.S.Y. Ho, Induction of DNA double-strand breaks by X-rays in a radiosensitive strain of the yeast *Saccharomyces cerevisiae*, Mutat. Res. 30 (1975) 327–334.
[283] J. Brozmanová, V. Vlčková, M. Černáková, L. Èernáková, M. Škorvaga, G.P. Margison, Expression of the *E. coli ada* gene in *S. cerevisiae* provides cellular resistance to

N-methyl-*N*(-nitro-*N*-nitrosoguanidine) in *rad6* but not in *rad52* mutants, Nucleic Acids Res. 22 (1994) 5717–5722.
[284] J.A. Dolling, D.R. Boreham, D.L. Brown, G.P. Raaphorst, R.E.J. Mitchel, Cisplatin-modification of DNA repair and ionizing radiation lethality in yeast, *Saccharomyces cerevisiae*, Mutat. Res. DNA Repair 433 (1999) 127–136.
[285] S.T. Durant, M.M. Morris, M. Illand, H.J. McKay, C. McCormick, G.L. Hirst, R.H. Borts, R. Brown, Dependence on *RAD52* and *RAD1* for anticancer drug resistance mediated by inactivation of mismatch repair genes, Curr. Biol. 9 (1999) 51–54.
[286] T.J. O'Brien, J.L. Fornsaglio, S. Ceryak, S.R. Patierno, Effects of hexavalent chromium on the survival and cell cycle distribution of DNA repair-deficient *S. cerevisiae*, DNA Repair (Amsterdam) 1 (2002) 617–627.
[287] N. Sugawara, J.E. Haber, Characterization of double-strand break-induced recombination: homology requirements and single-stranded DNA formation, Mol. Cell Biol. 12 (1992) 563–575.
[288] C.I. White, J.E. Haber, Intermediates of recombination during mating type switching in *Saccharomyces cerevisiae*, EMBO J. 9 (1990) 663–673.
[289] A.J. Rattray, L.S. Symington, Use of a chromosomal inverted repeat to demonstrate that the *RAD51* and *RAD52* genes of *Saccharomyces cerevisiae* have different roles in mitotic recombination, Genetics 138 (1994) 587–595.
[290] G. Bosco, J.E. Haber, Chromosome break-induced DNA replication leads to nonreciprocal translocations and telomere capture, Genetics 150 (1998) 1037–1047.
[291] H. Zou, R. Rothstein, Holliday-junctions accumulate in replication mutants via a RecA homolog-independent mechanism, Cell 90 (1997) 87–96.
[292] M.A. Resnick, J.N. Kasimos, J.C. Game, R.J. Braun, R.M. Roth, Changes in DNA during meiosis in a repair-deficient mutant (rad 52) of yeast, Science 212 (1981) 543–545.
[293] J.E. Haber, DNA repair: gatekeepers of recombination, Nature 398 (1999) 665–666.
[294] Y. Yamaguchiiwai, E. Sonoda, J.M. Buerstedde, O. Bezzubova, C. Morrison, M. Takata, A. Shinohara, S. Takeda, Homologous recombination, but not DNA repair, is reduced in vertebrate cells deficient in *RAD52*, Mol. Cell Biol. 18 (1998) 6430–6435.
[295] K. Suto, A. Nagata, H. Murakami, H. Okayama, A double-strand break repair component is essential for S phase completion in fission yeast cell cycling, Mol. Biol. Cell. 10 (1999) 3331–3343.
[296] M. van den Bosch, K. Vreeken, J.B.M. Zonneveld, J.A. Brandsma, M. Lombaerts, J.M. Murray, P.H.M. Lohman, A. Pastink, Characterization of *RAD52* homologs in the fission yeast *Schizosaccharomyces pombe*, Mutat. Res. DNA Repair 461 (2001) 311–323.
[297] K. Adzuma, T. Ogawa, H. Ogawa, Primary structure of the *RAD52* gene in *Saccharomyces cerevisiae*, Mol. Cell Biol. 4 (1984) 2735–2744.
[298] U.H. Mortensen, N. Erdeniz, Q. Feng, R. Rothstein, A molecular genetic dissection of the evolutionarily conserved N-terminus of yeast Rad52, Genetics 161 (2002) 549–562.
[299] A. Shinohara, M. Shinohara, T. Ohta, S. Matsuda, T. Ogawa, Rad52 forms ring structures and co-operates with RPA in single-strand DNA annealing, Genes Cells 3 (1998) 145–156.
[300] C. Bendixen, I. Sunjevaric, R. Bauchwitz, R. Rothstein, Identification of a mouse homologue of the *Saccharomyces cerevisiae* recombination and repair gene, *RAD52*, Genomics 23 (1994) 300–303.
[301] D.F.R. Muris, O. Bezzubova, J.M. Buerstedde, K. Vreeken, A.S. Balajee, C.J. Osgood, C. Troelstra, J.H.J. Hoeijmakers, K. Ostermann, H. Schmidt, A.T. Natarajan, J.C.J. Eeken, P.H.M. Lohman, A. Pastink, Cloning of human and mouse genes homologous to *RAD52*, a yeast gene involved in DNA repair and recombination, Mutat. Res. 315 (1994) 295–305.
[302] Z.Y. Shen, K. Denison, R. Lobb, J.M. Gatewood, D.J. Chen, The human and mouse homologs of the yeast *RAD52* gene: cDNA cloning, sequence analysis, assignment to human chromosome 12p12.2-p13, and mRNA expression in mouse tissues, Genomics 25 (1995) 199–206.
[303] O.Y. Bezzubova, H. Schmidt, K. Ostermann, W.D. Heyer, J.M. Buerstedde, Identification of a chicken RAD52 homologue suggests conservation of the RAD52 recombination pathway throughout the evolution of higher eukaryotes, Nucleic Acids Res. 21 (1993) 5945–5949.
[304] U.H. Mortensen, C. Bendixen, I. Sunjevaric, R. Rothstein, DNA strand annealing is promoted by the yeast Rad52 protein, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 10729–10734.
[305] E. van Dyck, N.M.A. Hajibagheri, A. Stasiak, S.C. West, Visualisation of human Rad52 protein and its complexes with hRad51 and DNA, J. Mol. Biol. 284 (1998) 1027–1038.
[306] T. Sugiyama, J.H. New, S.C. Kowalczykowski, DNA annealing by Rad52 protein is stimulated by specific interaction with the complex of replication protein A and single-stranded DNA, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 6049–6054.
[307] W. Kagawa, H. Kurumizaka, S. Ikawa, S. Yokoyama, T. Shibata, Homologous pairing promoted by the human Rad52 protein, J. Biol. Chem. 276 (2001) 35201–35208.
[308] W. Ranatunga, D. Jackson, J.A. Lloyd, A.L. Forget, K.L. Knight, G.E.O. Borgstahl, Human RAD52 exhibits two modes of self-association, J. Biol. Chem. 276 (2001) 15876–15880.
[309] W. Kagawa, H. Kurumizaka, R. Ishitani, S. Fukai, O. Nureki, T. Shibata, S. Yokoyama, Crystal structure of the homologous-pairing domain from the human Rad52 recombinase in the undecameric form, Mol. Cell 10 (2002) 359–371.
[310] J.A. Lloyd, A.L. Forget, K.L. Knight, Correlation of biochemical properties with the oligomeric state of human Rad52 protein, J. Biol. Chem. 277 (2002) 46172–46178.
[311] A.Z. Stasiak, E. Larquet, A. Stasiak, S. Muller, A. Engel, E. van Dyck, S.C. West, E.H. Egelman, The human Rad52 protein exists as a heptameric ring, Curr. Biol. 10 (2000) 337–340.
[312] M.R. Singleton, L.M. Wentzell, Y.L. Liu, S.C. West, D.B. Wigley, Structure of the single-strand annealing domain of

human RAD52 protein, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 13492–13497.
[313] G. Petukhova, S.A. Stratton, P. Sung, Single-strand DNA binding and annealing activities in the yeast recombination factor Rad59, J. Biol. Chem. 274 (1999) 33839–33842.
[314] K. Kito, H. Wada, E.T.H. Yeh, T. Kamitani, Identification of novel isoforms of human RAD52, Biochim. Biophys. Acta 1489 (1999) 303–314.
[315] P. Sung, Function of yeast Rad52 protein as a mediator between replication protein A and the Rad51 recombinase, J. Biol. Chem. 272 (1997) 28194–28197.
[316] A. Shinohara, T. Ogawa, Stimulation by Rad52 of yeast Rad51-mediated recombination, Nature 391 (1998) 404–407.
[317] J.H. New, S.C. Kowalczykowski, Rad52 protein has a second stimulatory role in DNA strand exchange that complements replication protein—a function, J. Biol. Chem. 277 (2002) 26171–26176.
[318] Z.Y. Shen, S.R. Peterson, J.C. Comeaux, D. Zastrow, R.K. Moyzis, E.M. Bradbury, D.J. Chen, Self-association of human RAD52 protein, Mutat. Res. DNA Repair 364 (1996) 81–89.
[319] L. Krejèi, B. Thomsen, M. Duno, O. Westergaard, C. Bendixen, Homomeric interaction of the mouse Rad52 protein, Mol. Biol. Rep. 27 (2000) 55–59.
[320] E.N. Asleson, D.M. Livingston, Investigation of the stability of yeast *rad52* mutant proteins uncovers post-translational and transcriptional regulation of Rad52p, Genetics 163 (2003) 91–101.
[321] L. Krejèi, B.W. Song, W. Bussen, R. Rothstein, U.H. Mortensen, P. Sung, Interaction with Rad51 is indispensable for recombination mediator function of Rad52, J. Biol. Chem. 277 (2002) 40132–40141.
[322] J.M. Liu, X.B. Meng, Z.Y. Shen, Association of human RAD52 protein with transcription factors, Biochem. Biophys. Res. Commun. 297 (2002) 1191–1196.
[323] J.A. Freedman, S. Jinks-Robertson, Genetic requirements for spontaneous and transcription-stimulated mitotic recombination in *Saccharomyces cerevisiae*, Genetics 162 (2002) 15–27.
[324] S. Gonzalez-Barrera, M. Garcia-Rubio, A. Aguilera, Transcription and double-strand breaks induce similar mitotic recombination events in *Saccharomyces cerevisiae*, Genetics 162 (2002) 603–614.
[325] G.M. Cole, D. Schild, R.K. Mortimer, Two DNA repair and recombination genes in *Saccharomyces cerevisiae*, *RAD52* and *RAD54*, are induced during meiosis, Mol. Cell Biol. (1989) 3101–3104.
[326] G.M. Cole, D. Schild, S.T. Lovett, R.K. Mortimer, Regulation of *RAD54*- and *RAD52-lacZ* gene fusion in *Saccharomyces cerevisiae* in response to DNA damage, Mol. Cell Biol. 7 (1987) 1078–1084.
[327] M. Lisby, R. Rothstein, U.H. Mortensen, Rad52 forms DNA repair and recombination centers during S phase, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 8276–8282.
[328] Y.L. Liu, N. Maizels, Coordinated response of mammalian Rad51 and Rad52 to DNA damage, EMBO Rep. 1 (2000) 85–90.
[329] A.A. Firmenich, M. Eliasarnanz, P. Berg, A novel allele of *Saccharomyces cerevisiae RFA1* that is deficient in recombination and repair and suppressible by *RAD52*, Mol. Cell Biol. 15 (1995) 1620–1631.
[330] D. Jackson, K. Dhar, J.K. Wahl, M.S. Wold, G.E.O. Borgstahl, Analysis of the human replication protein A:Rad52 complex: evidence for crosstalk between RPA32, RPA70, Rad52 and DNA, J. Mol. Biol. 321 (2002) 133–148.
[331] Z.Y. Shen, P.E. Pardingtonpurtymun, J.C. Comeaux, R.K. Moyzis, D.J. Chen, UBL1, a human ubiquitin-like protein associating with human RAD51/RAD52 proteins, Genomics 36 (1996) 271–279.
[332] A.P. Davis, L.S. Symington, The yeast recombinational repair protein Rad59 interacts with Rad52 and stimulates single-strand annealing, Genetics 159 (2001) 515–525.
[333] K.S. Ho, R.K. Mortimer, X-ray-induced lethality and chromosome breakage and repair in a radiosensitive strain of yeast, Basic Life Sci. 5 (1975) 545–547.
[334] G.M. Cole, R.K. Mortimer, Failure to induce a DNA repair gene, *RAD54*, in *Saccharomyces cerevisiae* does not affect repair or recombination phenotypes, Mol. Cell Biol. 9 (1989) 3314–3322.
[335] C.R. Contopoulou, V.E. Cook, R.K. Mortimer, Analysis of DNA double-strand breakage and repair using orthogonal field alternation gel electrophoresis, Yeast 3 (1987) 71–76.
[336] J.R. Perera, A.V. Glasunov, V.M. Glaser, A.V. Boreiko, Repair of double-strand breaks in plasmid DNA in the yeast *Saccharomyces cerevisiae*, Mol. Gen. Genet. 213 (1988) 421–424.
[337] E.L. Ivanov, N. Sugawara, J. Fishmanlobell, J.E. Haber, Genetic requirements for the single-strand annealing pathway of double-strand break repair in *Saccharomyces cerevisiae*, Genetics 142 (1996) 693–704.
[338] J. Schmuckli-Maurer, M. Rolfsmeier, H. Nguyen, W.D. Heyer, Genome instability in *rad54* mutants of *Saccharomyces cerevisiae*, Nucleic Acids Res. 31 (2003) 1013–1023.
[339] L. Signon, A. Malkova, M.L. Naylor, H. Klein, J.E. Haber, Genetic requirements for *RAD51*- and *RAD54*-independent break-induced replication repair of a chromosomal double-strand break, Mol. Cell Biol. 21 (2001) 2048–2056.
[340] Y. Tsukamoto, J. Kato, H. Ikeda, Effects of mutations of *RAD50*, *RAD51*, *RAD52*, and related genes on illegitimate recombination in *Saccharomyces cerevisiae*, Genetics 142 (1996) 383–391.
[341] J. Schmuckli-Maurer, W.D. Heyer, Meiotic recombination in *RAD54* mutants of *Saccharomyces cerevisiae*, Chromosoma 109 (2000) 86–93.
[342] T. Saeki, I. Machida, S. Nakai, Genetic control of diploid recovery after gamma-irradiation in the yeast *Saccharomyces cerevisiae*, Mutat. Res. 73 (1980) 251–265.
[343] M.L.G. Dronkert, H.B. Beverloo, R.D. Johnson, J.H.J. Hoeijmakers, M. Jasin, R. Kanaar, Mouse *RAD54* affects DNA double-strand break repair and sister chromatid exchange, Mol. Cell Biol. 20 (2000) 3147–3156.
[344] O. Bezzubova, A. Silbergleit, Y. Yamaguchi-Iwai, S. Takeda, J.M. Buerstedde, Reduced X-ray resistance and homologous recombination frequencies in a RAD54$^{-/-}$ mutant of the chicken DT40 cell line, Cell 89 (1997) 185–193.

[345] E. Sonoda, M.S. Sasaki, C. Morrison, Y. Yamaguchi-Iwai, M. Takata, S. Takeda, Sister chromatid exchanges are mediated by homologous recombination in vertebrate cells, Mol. Cell Biol. 19 (1999) 5166–5169.
[346] M. Takata, M.S. Sasaki, E. Sonoda, C. Morrison, M. Hashimoto, H. Utsumi, Y. Yamaguchiiwai, A. Shinohara, S. Takeda, Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells, EMBO J. 17 (1998) 5497–5508.
[347] T. Fukushima, M. Takata, C. Morrison, R. Araki, A. Fujimori, M. Abe, K. Tatsumi, M. Jasin, P.K. Dhar, E. Sonoda, T. Chiba, S. Takeda, Genetic analysis of the DNA-dependent protein kinase reveals an inhibitory role of Ku in late S-G_2 phase DNA double-strand break repair, J. Biol. Chem. 276 (2001) 44413–44418.
[348] H.C. Wang, Z.C. Zeng, T.A. Bui, E. Sonoda, M. Takata, S. Takeda, G. Iliakis, Efficient rejoining of radiation-induced DNA double-strand breaks in vertebrate cells deficient in genes of the *RAD52* epistasis group, Oncogene 20 (2001) 2212–2224.
[349] H.S. Emery, D. Schild, D.E. Kellogg, R.K. Mortimer, Sequence of *RAD54*, a *Saccharomyces cerevisiae* gene involved in recombination and repair, Gene 104 (1991) 103–106.
[350] R. Kanaar, C. Troelstra, S.M.A. Swagemakers, J. Essers, B. Smit, J.H. Franssen, A. Pastink, O.Y. Bezzubova, J.M. Buerstedde, B. Clever, W.D. Heyer, J.H.J. Hoeijmakers, Human and mouse homologs of the *Saccharomyces cerevisiae RAD54* DNA repair gene: evidence for functional conservation, Curr. Biol. 6 (1996) 828–838.
[351] D. Rasio, Y. Murakumo, D. Robbins, T. Roth, A. Silver, M. Negrini, C. Schmidt, J. Burczak, R. Fishel, C.M. Croce, Characterization of the human homologue of *RAD54*: a gene located on chromosome 1p32 at a region of high loss of heterozygosity in breast tumors, Cancer Res. 57 (1997) 2378–2383.
[352] D. Averbeck, S. Averbeck, Induction of the genes *RAD54 and RNR2* by various DNA damaging agents in *Saccharomyces cerevisiae*, Mut. Res. 315 (1994) 123–138.
[353] L.H. Johnston, A.L. Johnson, The DNA repair genes *RAD54* and *UNG1* are cell cycle regulated in budding yeast but MCB promoter elements have no essential role in the DNA damage response, Nucleic Acids Res. 23 (1995) 2147–2152.
[354] J.A. Eisen, K.S. Sweder, P.C. Hanawalt, Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions, Nucleic Acids Res. 23 (1995) 2715–2723.
[355] C.L. Peterson, J.W. Tamkun, The SWI–SNF complex: a chromatin remodeling machine? Trends Biochem. Sci. 20 (1995) 143–146.
[356] B. Clever, J. Schmucklimaurer, M. Sigirst, B.J. Glassner, W.D. Heyer, Specific negative effects resulting from elevated levels of the recombinational repair protein Rad54p in *Saccharomyces cerevisiae*, Yeast 15 (1999) 721–740.
[357] S.M.A. Swagemakers, J. Essers, J. Dewit, J.H.J. Hoeijmakers, R. Kanaar, The human Rad54 recombinational DNA repair protein is a double-stranded DNA-dependent ATPase, J. Biol. Chem. 273 (1998) 28292–28297.
[358] S. Sigurdsson, S. van Komen, G. Petukhova, P. Sung, Homologous DNA pairing by human recombination factors Rad51 and Rad54, J. Biol. Chem. 277 (2002) 42790–42794.
[359] G. Petukhova, S. van Komen, S. Vergano, H. Klein, P. Sung, Yeast Rad54 promotes Rad51-dependent homologous DNA pairing via ATP hydrolysis-driven change in DNA double helix conformation, J. Biol. Chem. 274 (1999) 29453–29462.
[360] J.A. Solinger, G. Lutz, T. Sugiyama, S.C. Kowalczykowski, W.D. Heyer, Rad54 protein stimulates heteroduplex DNA formation in the synaptic phase of DNA strand exchange via specific interactions with the presynaptic Rad51 nucleoprotein filament, J. Mol. Biol. 307 (2001) 1207–1221.
[361] P.M. Kim, K.S. Paffett, J.A. Solinger, W.D. Heyer, J.A. Nickoloff, Spontaneous and double-strand break-induced recombination, and gene conversion tract lengths, are differentially affected by overexpression of wild-type or ATPase-defective yeast Rad54, Nucleic Acids Res. 30 (2002) 2727–2735.
[362] S. van Komen, G. Petukhova, S. Sigurdsson, S. Stratton, P. Sung, Superhelicity-driven homologous DNA pairing by yeast recombination factors Rad51 and Rad54, Mol. Cell 6 (2000) 563–572.
[363] A.V. Mazin, C.J. Bornarth, J.A. Solinger, W.D. Heyer, S.C. Kowalczykowski, Rad54 protein is targeted to pairing loci by the Rad51 nucleoprotein filament, Mol. Cell 6 (2000) 583–592.
[364] K. Tanaka, W. Kagawa, T. Kinebuchi, H. Kurumizaka, K. Miyagawa, Human Rad54B is a double-stranded DNA-dependent ATPase and has biochemical properties different from its structural homolog in yeast, Tid1/Rdh54, Nucleic Acids Res. 30 (2002) 1346–1353.
[365] J.A. Solinger, W.D. Heyer, Rad54 protein stimulates the postsynaptic phase of Rad51 protein-mediated DNA strand exchange, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 8447–8453.
[366] A. Alexeev, A. Mazin, S.C. Kowalczykowski, Rad54 protein possesses chromatin-remodeling activity stimulated by the Rad51–ssDNA nucleoprotein filament, Nat. Struct. Biol. 10 (2003) 182–186.
[367] A.V. Mazin, A.A. Alexeev, S.C. Kowalczykowski, A Novel function of Rad54 protein. Stabilization of the Rad51 nucleoprotein filament, J. Biol. Chem. 278 (2003) 14029–14036.
[368] K. Kiianitsa, J.A. Solinger, W.D. Heyer, Rad54 protein exerts diverse modes of ATPase activity on duplex DNA partially and fully covered with Rad51 protein, J. Biol. Chem. 277 (2002) 46205–46215.
[369] J.A. Solinger, K. Kiianitsa, W.D. Heyer, Rad54, a Swi2/Snf2-like recombinational repair protein, disassembles Rad51:dsDNA filaments, Mol. Cell 10 (2002) 1175–1188.
[370] T.L. Tan, J. Essers, E. Citterio, S.M. Swagemakers, J. de Wit, F.E. Benson, J.H. Hoeijmakers, R. Kanaar, Mouse Rad54 affects DNA conformation and DNA-damage-induced Rad51 foci formation, Curr. Biol. 9 (1999) 325–328.
[371] H. Interthal, W.D. Heyer, *MUS81* encodes a novel Helix-hairpin-Helix protein involved in the response to UV- and methylation-induced DNA damage in *Saccharomyces cerevisiae*, Mol. Gen. Genet. 263 (2000) 812–827.

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

[373] A. Constantinou, X.B. Chen, C.H. McGowan, S.C. West, Holliday junction resolution in human cells: two junction endonucleases with distinct substrate specificities, EMBO J. 21 (2002) 5577–5585.

[374] M. Ogrunc, A. Sancar, Identification and characterization of human MUS81-MMS4 structure specific endonuclease, J. Biol. Chem., 2003, in press.

[375] M.C. Whitby, F. Osman, J. Dixon, Cleavage of model replication forks by fission yeast Mus81–Eme1 and budding yeast Mus81–Mms4, J. Biol. Chem. 278 (2003) 6928–6935.

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

[377] A. Arbel, D. Zenvirth, G. Simchen, Sister chromatid-based DNA repair is mediated by *RAD54*, not by *DMC1* or *TID1*, EMBO J. 18 (1999) 2648–2658.

[378] K. Miyagawa, T. Tsuruga, A. Kinomura, K. Usui, M. Katsura, S. Tashiro, H. Mishima, K. Tanaka, A role for *RAD54B* in homologous recombination in human cells, EMBO J. 21 (2002) 175–180.

[379] G. Petukhova, P. Sung, H. Klein, Promotion of Rad51-dependent D-loop formation by yeast recombination factor Rdh54/Tid1, Genes Dev. 14 (2000) 2206–2215.

[380] S.E. Lee, A. Pellicioli, A. Malkova, M. Foiani, J.E. Haber, The *Saccharomyces* recombination protein Tid1p is required for adaptation from G2/M arrest induced by a double-strand break, Curr. Biol. 11 (2001) 1053–1057.

[381] S.T. Lovett, R.K. Mortimer, Characterization of null mutants of the *RAD55* gene of *Saccharomyces cerevisiae*: effects of temperature, osmotic strength and mating type, Genetics 116 (1987) 547–553.

[382] G.S. Fortin, L.S. Symington, Mutations in yeast Rad51 that partially bypass the requirement for Rad55 and Rad57 in DNA repair by increasing the stability of Rad51–DNA complexes, EMBO J. 21 (2002) 3160–3170.

[383] L. Prakash, S. Prakash, Isolation and characterization of MMS-sensitive mutants of *Saccharomyces cerevisiae*, Genetics 86 (1977) 33–55.

[384] K.S. Ho, R.K. Mortimer, Two mutations which confer temperature-sensitive radiation sensitivity in the yeast *Saccharomyces cerevisiae*, Mutat. Res. 33 (1975) 157–164.

[385] S.L. Gasior, H. Olivares, U. Ear, D.M. Hari, R. Weichselbaum, D.K. Bishop, Assembly of RecA-like recombinases: distinct roles for mediator proteins in mitosis and meiosis, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 8411–8418.

[386] N. Sugawara, E.L. Ivanov, J. Fishman-Lobell, B.L. Ray, X. Wu, J.E. Haber, DNA structure-dependent requirements for yeast *RAD* genes in gene conversion, Nature 373 (1995) 84–86.

[387] S.T. Lovett, Sequence of the *RAD55* gene of *Saccharomyces cerevisiae*: similarity of RAD55 to prokaryotic RecA and other RecA-like proteins, Gene 142 (1994) 103–106.

[388] J.A. Kans, R.K. Mortimer, Nucleotide sequence of the *RAD57* gene of *Saccharomyces cerevisiae*, Gene 105 (1991) 139–140.

[389] J.C. Game, The *Saccharomyces* repair genes at the end of the century, Mutat. Res. Fundam. Mol. Mech. Mut. 451 (2000) 277–293.

[390] V.I. Bashkirov, J.S. King, E.V. Bashkirova, J. Schmuckli-maurer, W.D. Heyer, DNA repair protein Rad55 is a terminal substrate of the DNA damage checkpoints, Mol. Cell Biol. 20 (2000) 4393–4404.

[391] V.I. Bashkirov, E.V. Bashkirova, E. Haghnazari, W.D. Heyer, Direct kinase-to-kinase signaling mediated by the FHA phosphoprotein recognition domain of the Dun1 DNA damage checkpoint kinase, Mol. Cell Biol. 23 (2003) 1441–1452.

[392] T. Weinert, DNA damage and checkpoint pathways: molecular anatomy and interactions with repair, Cell 94 (1998) 555–558.

[393] B.B.S. Zhou, S.J. Elledge, The DNA damage response: putting checkpoints in perspective, Nature 408 (2000) 433–439.

[394] Y. Bai, A.P. Davis, L.S. Symington, A novel allele of *RAD52* that causes severe DNA repair and recombination deficiencies only in the absence of *RAD51* or *RAD59*, Genetics 153 (1999) 1117–1130.

[395] K. Ostermann, A. Lorentz, H. Schmidt, The fission yeast *rad22* gene, having a function in mating-type switching and repair of DNA damages, encodes a protein homolog to Rad52 of *Saccharomyces cerevisiae*, Nucleic Acids Res. 21 (1993) 5940–5944.

[396] M. van den Bosch, J.B. Zonneveld, P.H. Lohman, A. Pastink, Isolation and characterization of the *RAD59* homologue of *Kluyveromyces lactis*, Curr. Genet. 39 (2001) 305–310.

[397] N. Sugawara, G. Ira, J.E. Haber, DNA length dependence of the single-strand annealing pathway and the role of *Saccharomyces cerevisiae RAD59* in double-strand break repair, Mol. Cell Biol. 20 (2000) 5300–5309.

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

[399] A. Ciccia, A. Constantinou, S.C. West, Identification and characterization of the human mus81–eme1 endonuclease, J. Biol. Chem. 278 (2003) 25172–25178.

[400] A. Constantinou, X.B. Chen, C.H. McGowan, S.C. West, Holliday junction resolution in human cells: two junction endonucleases with distinct substrate specificities, EMBO J. 21 (2002) 5577–5585.

[401] A. Constantinou, A.A. Davies, S.C. West, Branch migration and Holliday junction resolution catalyzed by activities from mammalian cells, Cell 104 (2001) 259–268.

[402] C.L. Doe, J.S. Ahn, J. Dixon, M.C. Whitby, Mus81–Eme1 and Rqh1 involvement in processing stalled and collapsed

replication forks, J. Biol. Chem. 277 (2002) 32753–32759.
[403] J.E. Haber, W.D. Heyer, The fuss about Mus81, Cell 107 (2001) 551–554.
[404] J.A. Downs, E. Kosmidou, A. Morgan, S.P. Jackson, Suppression of homologous recombination by the *Saccharomyces cerevisiae* linker histone, Mol. Cell 11 (2003) 1685–1692.
[405] L. Krejči, S. van Komen, Y. Li, J. Villemain, M.S. Reddy, H. Klein, T. Ellenberger, P. Sung, DNA helicase Srs2 disrupts the Rad51 presynaptic filament, Nature 423 (2003) 305–309.
[406] X. Veaute, J. Jeusset, C. Soustelle, S.C. Kowalczykowski, E. Le Cam, F. Fabre, The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments, Nature 423 (2003) 309–312.