DNA double-strand break repair by homologous recombination

Andrej Dudáš, Miroslav Chovanec∗
Laboratory of Molecular Genetics, Cancer Research Institute, Slovak Academy of Sciences, Vlárska 7, 833 91 Bratislava 37, Slovak Republic
Received 26 May 2003; received in revised form 29 July 2003; accepted 30 July 2003

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 methane sulfonate (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, 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...
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 [3,8,10,11,17–32].

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 helicohetorimer (exonuclease V), which has both exonuclease and helicase activities. In the presence of Mg<sup>2+</sup> 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.
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, RuvA, can be activated and act on Holliday junction. Suppression function of RuvA, 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]. Nonetheless, 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, RAD54/TID1, MRE11 and XR52 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 RH54/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/RCC2051H2, 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.

4.1. Mutant phenotypes

4. Mre11, Rad50 and Xrs2

4.1. Mutant phenotypes

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/RCC2051H2, 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 XRCC2 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 Nijmegen breakage syndrome (NBS) and A-Taxia elangiectasia-like disorder (ATLD), respectively [93–96]. These two syndromes share certain features with A-Taxia 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+/− 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 G2 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, 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 G2/M checkpoints and prone to premature senescence [105]. On the other hand, a mild phenotype at the cellular level was reported for RAD50+/−, 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 Mn2+ 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]). Rad50 possesses a conserved hinge motif in the center of the coiled-coil region, that has a sequence Cys-X-X-Cys (CXCC) [123–125].

Recently, the structure of the Mre11-Rad50 complex has been elucidated. It has been shown that the CXCC motif promotes Zn2+−dependent Rad50 dimerization, as the two CXCC motifs (each present on different Rad50 molecules) form interlocking hooks that bind one Zn2+ ion. Coiled coils extend from the Zn2+−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 (M2R2) 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 M2R2 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 archaeabacteria. The Mre11-Rad50 family of proteins involves gp47:gp46 of bacteriophage T4, SbCD-SbCC of *E. coli*, and Mre11-Rad50 of eukaryotes and archaeabacteria [116,127].
Analysis of Nbs1 has revealed three functional regions: the N-terminal region (amino acid residues 1–196), the central region (amino acid residues 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 phosphorylated 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 [144]. 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 (Brca1-associated genome surveillance complex). 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, Tel1 and Tel2 (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 l DNA 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 synopsis 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 synopsis 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

<table>
<thead>
<tr>
<th>S. cerevisiae</th>
<th>Humans</th>
<th>Biochemical activity/function</th>
<th>Interacting partners</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rad50</td>
<td>Rad50</td>
<td>ATP-dependent DNA-binding activity; ATPase activity</td>
<td>Yeast: Mei1; human: Mei1, Nbs1, Rint-1, Tel2, Brca1, Bloom’s syndrome protein, PCNA</td>
</tr>
<tr>
<td>Mre11</td>
<td>Mre11</td>
<td>3′ to 5′ dsDNA exonuclease and ssDNA endonuclease activities; DNA-binding activity; DNA annealing activity; DNA duplex unwinding and hairpin cleavage activities</td>
<td>Yeast: itself; Rad50, Xrs2, Zip2, Zip3; human: Mlh1, Tel2, Ku70, Bloom’s syndrome protein</td>
</tr>
<tr>
<td>Xrs2</td>
<td>Nbs1</td>
<td>Human: regulates DNA duplex unwinding and nuclease activities of the MRN complex; recruits the MRN complex to vicinity of DNA damage</td>
<td>Yeast: Mei1; human: Sp100, Tel1, Tel2, E2F1</td>
</tr>
<tr>
<td>Rad51</td>
<td>Rad51</td>
<td>ATP-dependent DNA-binding activity; ATP-dependent homologous pairing and DNA strand exchange activities</td>
<td>Yeast: Rad52, Rad54, Rad55, Rad51B, Rad51C, Rad51D, Xrcc3, c-Abl, p53, Brca1, Bica2, Ube2, Ube3, Ube4, Pir51, Rpl1</td>
</tr>
<tr>
<td>–</td>
<td>Rad51B</td>
<td>DNA-binding activity (enhanced in complex with Rad51C); protein kinase activity; DNA-stimulated ATPase activity</td>
<td>Human: Rad51C</td>
</tr>
<tr>
<td>–</td>
<td>Rad51C</td>
<td>DNA-binding activity (enhanced in complex with Rad51B; also exhibited in complex with Xec3); DNA-stimulated ATPase activity; ATP-independent DNA-strand exchange activity; ATP-independent and Mg2+-dependent homologous pairing activity on its own or in complex with Xec3; forms filamentous structures in complex with Xec3</td>
<td>Human: Rad51, Rad51B, Rad51D, Xec3</td>
</tr>
<tr>
<td>–</td>
<td>Rad51D</td>
<td>DNA-binding activity on its own or in complex with Xec2; DNA-stimulated ATPase activity; ATP-dependent and Mg2+-dependent homologous pairing activity in complex with Xec2; forms filamentous structures in complex with Xec2</td>
<td>Human: Rad51C, Xec2</td>
</tr>
<tr>
<td>Rad52</td>
<td>Rad52</td>
<td>DNA-binding activity; ssDNA annealing activity; weak DNA strand exchange activity; weak homologous pairing activity</td>
<td>Yeast: Rpa, Rad51, Rad52, Rad59, human: Rpa, Rad51, Rad52, Ube2, Ube3, Ube4, Xpb, Xpd, c-Abl</td>
</tr>
<tr>
<td>Rad54</td>
<td>Rad54</td>
<td>DNA-binding activity; dsDNA-dependent ATPase activity; dsDNA unwinding activity; dsDNA topology-modifying activity</td>
<td>Yeast: Rad51, Mms81; human: Rad51, Mms81</td>
</tr>
<tr>
<td>Rdh54/Tid1</td>
<td>Rdh54B</td>
<td>DNA-binding activity; dsDNA-dependent ATPase activity</td>
<td>Yeast: Rad51, Dmc1; human: Rad51*</td>
</tr>
<tr>
<td>Rad55</td>
<td>Xec2</td>
<td>Yeast: ATPase; human: DNA-binding activity on its own or in complex with Rad51D; ATP-independent and Mg2+-dependent homologous pairing activity on its own or in complex with Rad51D; forms filamentous structures in complex with Rad51D</td>
<td>Yeast: Rad51, Rad57; human: Rad51D</td>
</tr>
<tr>
<td>Rad57</td>
<td>Xec3</td>
<td>Yeast: ATPase; human: DNA-binding activity on its own or in complex with Rad51C; ATP-independent and Mg2+-dependent homologous pairing activity on its own or in complex with Rad51C; forms filamentous structures in complex with Rad51C</td>
<td>Yeast: Rad55, Zip3; human: Rad51, Rad51C</td>
</tr>
<tr>
<td>Rad59</td>
<td>–</td>
<td>DNA-binding activity; ssDNA annealing activity</td>
<td>Yeast: Rad52, Rad51*</td>
</tr>
</tbody>
</table>

For references, see text.

* 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, Zp3. Zp3 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 G2/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 G2/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 E2F 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-E2F interaction occurs near the origins of DNA replication and is basically restricted to non-stressed S phase cells. There is evidence 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 promyelocytic leukemia (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 are sensitive to IR and IR-activated apoptosis [170,171]. However, inactivation of other Rad51-like genes is tolerated [171–174] particularly, 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 rejoicing 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 [183,195]. 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] 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 G1 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 Xcc3–Rad51C and Xcc2–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 Xcc3–Rad51C and Xcc2–Rad51D complexes were shown to exhibit ATP-independent and Mg2+-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 Xcc3–Rad51C, it may represent a catalytic subunit of Xcc3–Rad51C in this process [218]. On the other hand, Xcc3 seems to be a catalytic subunit of the complex that is required for DNA-binding [218]. Unfortunately, experiments by Kurumizaka et al. [218] lacked Xcc3-only controls in the reactions, and this makes the results difficult to interpret. In the presence of ssDNA, both the Xcc3–Rad51C and Xcc2–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 Xrc3 [67, 237] and that Rad51C interacts with Rad51B, Xrc3 and weakly with Rad51 [66, 214, 237–239] (Table 1). Compared to that of Rad51C–HsRad51, the Rad51C–Rad51B and Rad51C–Xrc3 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–Xrc3 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–Xrc3 heterodimer and was shown not to be the case [218]. Furthermore, Rad51D associates with Rad51C and Xrc2 [216, 237, 239, 240] (Table 1). Therefore, there are clearly at least two complexes of the Rad51-like proteins in human cells: Rad51C–Xrc3 and Rad51B–Rad51C–Rad51D–Xrc2, 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 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 [186] (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*<sup>−/−</sup> knockout mice, the *BRCA1*<sup>−/−</sup> and *BRCA2*<sup>−/−</sup> 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 G1/S and probably G2/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/Ub1 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/Ub1 possess counterparts in *S. cerevisiae* that are highly homologous to the human proteins [274]. This suggests conservation of the SUMO-1 pathway through 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/Ub1 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/Ub1 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/Ub1 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, MMN, 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 meiotic and mitotic 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. Krejci 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 G1 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.
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 G1/early S phase, while HR acts in late S/G2 phase [346]. Consistent with this are the findings of Fukushima et al. who demonstrated that **KU70**−/− knockouts are markedly sensitive to IR in G1/early S phase, but are as resistant as wild type cells to IR in late S/G2 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 G1 phase [353]. Consistent with this, expression of the **RAD54** gene in HeLa cells is increased in late G1 phase [350] and in mouse ES cells, the amount of the Rad54 protein is higher in the S and G2 phases of the cell cycle than in the G1 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 homologue 54)/TID1 (two-hybrid interaction 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 meiosis 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]. Accord-ingly, defects caused by the rad54/tid1 K315R mutant allele are identical to those of the rdh54/tid1 null mu-tant. 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 G2/M arrest by moni-toring the extent of ssDNA produced by resection of DNA ends during DSB repair as it functions in the adaptation of yeast cells to checkpoint mediated G2/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 ex-tent of homology with Rdh54/Tid1 than with ScRad54 whereas in both the central region and C-terminal do-main, 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 localiza-tion signal that is well-conserved being present in GdRad54B and a putative S. pombe Rdh54/Tid1 ho-mologue [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 pro-tein. 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 pro-motes the co-localization of these proteins during mei-otic 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 func-tional 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 func-tion 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 52.4 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 Rad51 paralogs) [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 rad53 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 checkpoint, 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 checkpoint 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]. Lastly, 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 Veau 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.

Acknowledgements

We apologize to all colleagues whose work has not been cited due to space limitations. We thank Drs. G.P. Margison, M. Priel, J. Brozmanová and Z. Dudášová for critical reading of the manuscript. This work was supported by the VEGA Grant Agency of the Slovak Republic (grant no. 203012/23).

References

[22] G. Herrmann, T. Lindahl, P. Schär, Saccharomyces cerevisiae LIF1: a function involved in DNA double-strand break repair


Identification and purification of two distinct complexes containing the five RAD51 paralogs, Genes Dev. 15 (2001) 3296–3307.

Saccharomyces cerevisiae, DNA N-glycosylase/apurinic or apyrimidinic -glycosylase/apurinic or apyrimidinic 533–544.
brca2

BRC motifs in the human breast cancer susceptibility gene

Bartel, RAD51 interacts with the evolutionarily conserved


Lee, The BRC repeats in BRCA2 are critical for RAD51


BRCA1 with Rad51 in mitotic and meiotic cells, Cell 88

J. Feunteun, T. Ashley, D.M. Livingston, Association of

induced Rad52 nuclear foci formation by c-Abl-mediated


12752.

requires ATM and c-Abl, J. Biol. Chem. 274 (1999) 12748–

12752.

Y.Z. Li, H. Shimizu, S.L. Xiang, Y. Maru, N. Takao, K.

Gasser, M.S. Park, P. Sung, E.Y.H.P. Lee, Radiation-induced


G. Chen, S.F.F. Yuan, W. Liu, Y. Xu, K. Tejwali, B.W. Song,

F. Cong, S.P. Goell, 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.

Y.Z. Li, H. Shimizu, S.L. Xiang, Y. Maru, N. Takao, K.

Yamamoto, Arg tyrosine kinase is involved in homologous

recombinational DNA repair, Biochim. Biophys. Res.


R. Kitao, Z.M. Yuan, Regulation of ionizing radiation-

induced Rad52 nuclear foci formation by c-Abl-mediated


R. Scully, J.J. Chen, A. Plug, V.H. Xiao, D. Weaver,

J. Feunteun, T. Ashley, D.M. Livingston, Association of

BRCA1 with Rad51 in mitotic and meiotic cells, Cell 88


Lee, The BRCA repeats in BRCA2 are critical for RAD51

binding and resistance to methyl methanesulfonate treatment,


Bartell, RAD51 interacts with the evolutionarily conserved

BBC motifs in the human breast cancer susceptibility gene


L.Y. Marmetien, T. Ouchi, S.A. Aaronson, The BRCA2
gene product functionally interacts with p53 and RAD51,


T. Ludwig, D.L. Chapman, V.E. Papasimakis, A.

Eliotlasio, Targeted mutations of breast cancer suscepti-

bility gene homologs in mice: lethal phenotypes of Brca1,

Brca2, Brca1/Brca2, Brca1/p53, and Brca1/p53 nullizygous


S.K. Sharan, M. Morimoto, U. Albrecht, D.S. Lim, E.

Reigel, 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.

N. Fong, D. Marot, V. Randrianarison, N. Dallia Vencezia,

D. Picard, M. Perucchini, V. Fusarolo, P. Jorgo, Constitutive

association of BRCA1 and c-Abl and its ATM-dependent


4032.

H.W. Sturmfeder, B. Donzelmann, W. Henning, U.

Knappskidl, S. Buchhop, p53 is linked directly to

homologous recombination processes via RAD51RecA


Sturmfeder, C.C. Harris, Interaction of p53 with the human


S. Sano, C. J am, F. Janus, W. Deppert, L. Wiesmuller,

Role of heteroduplex joints in the functional interactions

between human Rad51 and wild-type p53, Oncogene 19


Zhang, D.K. Bi, B.L. Weber, W.S. Elderly, BRCA1

physically associates with p53 and stimulates its transcrip-


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–

1756b.

T. Yaeugi, P.M. Howley, Identification of the structural and

functional human homolog of the yeast abutment conjugating


R.T. Hay, Protein modification by SUMO, Trends Biochem.


O.V. Kovalevsky, A.W. Plug, T. Hauk, D.K. Gonda,

T. Ashley, D.C. Ward, C.M. Liu, D.J. Moyzis, D.J. Chen,

E. Maldonado, R. Shiekhattar, M. Sheldon, H. Cho, R.

Ogawa, N. Copeland, N.A. Jenkins, M. Lalande, F.W.

Reinberg, A human RNA polymerase II complex asso-

ciates of UBE21 with RAD52, Nucleic Acids Res. 28


O.V. Kovalevsky, E.I. Golub, Mammalian abutment-conjugating enzyme Ubc9 interacts with Rad51


298–2993.

Z.Y. Shen, J.E. Parish, T. Becraft, J.C. Comeaux, R.K.

Moyzis, D.J. Chen, K. Chinn, D. Gutierrez, J.F. Koenig,

J. Brozmanová, V. Vlˇcková, M. Černáková, L. Èernáková,

RadioSensitivity and BRCA2: the yeast BRCA2 gene in

S. cerevisiae provides cellular resistance to


[29] A.R. Ratliff, L.S. Symington, Use of a chromosomal inverted repeat to demonstrate that the RAD51 and RAD52 genes of replication lead to non-reciprocal translocations and telomere capture, Genetics 150 (1998) 1037–1047.


[337] E.L. Ivanov, N. Sugawara, J. Fishmanlobell, J.E. Haber, J. Schmuckli-Maurer, W.D. Heyer. Genome instability in rad54 mutants of Saccharo-


[342] T. Suzuki, N. Sugawara, J. Fishmanlobell, J.E. Haber, J. Schmuckli-Maurer, W.D. Heyer. Genome instability in rad54 mutants of Saccharo-


[344] T. Suzuki, N. Sugawara, J. Fishmanlobell, J.E. Haber, J. Schmuckli-Maurer, W.D. Heyer. Genome instability in rad54 mutants of Saccharo-


[346] T. Suzuki, N. Sugawara, J. Fishmanlobell, J.E. Haber, J. Schmuckli-Maurer, W.D. Heyer. Genome instability in rad54 mutants of Saccharo-


