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Role of recA/RAD51 family proteins in mammals.

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Abstract

DNA damage causes chromosomal instability leading to oncogenesis, apoptosis, and severe failure of cell functions. The DNA repair system includes base excision repair, nucleotide excision repair, mismatch repair, translesion replication, non-homologous end-joining, and recombinational repair. Homologous recombination performs the recombinational repair. The RAD51 gene is an ortholog of Esherichia coli recA, and the gene product Rad51 protein plays a central role in the homologous recombination. In mammals, 7 recA-like genes have been identified: RAD51, RAD51L1/B, RAD51L2/C, RAD51L3/D, XRCC2, XRCC3, and DMC1. These genes, with the exception of meiosis-specific DMC1, are essential for development in mammals. Disruption of the RAD51 gene leads to cell death, whereas RAD51L1/B, RAD51L2/C, RAD51L3/D, XRCC2, and XRCC3 genes (RAD51 paralogs) are not essential for viability of cells, but these gene-deficient cells exhibit a similar defective phenotype. Yeast two-hybrid analysis, co-immunoprecipitation, mutation analysis, and domain mapping of Rad51 and Rad51 paralogs have revealed protein-protein interactions among these gene products. Recent investigations have shown that Rad51 paralogs play a role not only in an early step, but also in a late step of homologous recombination. In addition, identification of alternative transcripts of some RAD51 paralogs may reflect the complexity of the homologous recombination system.

KEYWORDS: RAD51, RAD51 paralogs, recA, recombination, DNA repair

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Review

Role of *recA/RAD51* Family Proteins in Mammals

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DNA damage causes chromosomal instability leading to oncogenesis, apoptosis, and severe failure of cell functions. The DNA repair system includes base excision repair, nucleotide excision repair, mismatch repair, translesion replication, non-homologous end-joining, and recombinational repair. Homologous recombination performs the recombinational repair. The *RAD51* gene is an ortholog of *Esherichia coli recA*, and the gene product Rad51 protein plays a central role in the homologous recombination. In mammals, 7 *recA-like* genes have been identified: *RAD51, RAD51L1/B, RAD51L2/C, RAD51L3/D, XRCC2, XRCC3*, and *DMC1*. These genes, with the exception of meiosis-specific *DMC1*, are essential for development in mammals. Disruption of the *RAD51* gene leads to cell death, whereas *RAD51L1/B, RAD51L2/C, RAD51L3/D, XRCC2*, and *XRCC3* genes (*RAD51* paralogs) are not essential for viability of cells, but these gene-deficient cells exhibit a similar defective phenotype. Yeast two-hybrid analysis, co-immunoprecipitation, mutation analysis, and domain mapping of Rad51 and Rad51 paralogs have revealed protein-protein interactions among these gene products. Recent investigations have shown that Rad51 paralogs play a role not only in an early step, but also in a late step of homologous recombination. In addition, identification of alternative transcripts of some *RAD51* paralogs may reflect the complexity of the homologous recombination system.

Key words: *RAD51*, *RAD51* paralogs, recA, recombination, DNA repair

Every cell is under constant attack not only from exogenous mutagenic chemicals and radiation but also from endogenous free radicals, causing DNA damage [1]. The accumulation of DNA damage leads to oncogenesis, cell death, and severe dysfunction of cells. The DNA repair system is crucially important for survival of living cells. The repair system includes base excision repair, nucleotide excision repair, mismatch repair, translesion replication, non-homologous end-joining, and recombinational repair. RecA/Rad51 family proteins are involved in the recombinational repair [2-5]. In the bacteria *Esherichia coli* (*E. coli*), RecA protein plays a central role in the process of homologous recombination [6]. In the budding yeast *Saccharomyces cerevisiae* (*S. cerevisiae*), 4 *recA*-like homologs, *RAD51, RAD55, RAD57*, and *DMC1*, have been identified [2]. The *RAD51* gene is an ortholog of *recA*. Heterodimeric complex of Rad55 and Rad57 proteins promotes DNA strand exchange by Rad51 with replication protein A (RPA). *DMC1* is a meiosis-specific gene. In the fission yeast *Schizosaccharomyces pombe* (*S. pombe*), 5 *recA*-like homologs, *rhp51*$^+$, *rhp55*$^+$, *rhp57*$^+$, *rlp1*$^+$, and

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Table I *recA/RAD51* family genes in bacteria, yeast and mammals

	Bacteria[a]	Budding[b] yeast	Fission[c] yeast	Mammals[d]
Ortholog	*recA*	*RAD51*	*rhp51*$^+$	*RAD51*
Paralogs		*RAD55*	*rhp55*$^+$	*RAD51L1/B/REC2/R51H2*
		RAD57	*rhp57*$^+$	*RAD51L2/C/R51H4*
		DMC1	*rlp1*$^+$	*RAD51L3/D/R51H3/TRAD*
			dmc1$^+$	*XRCC2*
				XRCC3
				DMC1/LIM15

[a] *Escherichia coli*; [b] *Saccharomyces cerevisiae*; [c] *Schizosaccharomyces pombe*; [d] human and mouse

dmc1$^+$, are found [7]. In higher eukaryotes (mammals), 7 *recA*-like genes, *RAD51, RAD51L1/B, RAD51L2/C, RAD51L3/D/TRAD, XRCC2, XRCC3*, and *DMC1*, have been identified [8–17]. *RAD51* is an ortholog of *recA* that plays a central role in homologous recombination [18]. *RAD51L1/B, RAD51L2/C, RAD51L3/D/TRAD, XRCC2*, and *XRCC3* are referred to as paralogs of *RAD51*, meaning that they are presumably derived from a common ancestral gene and have acquired more specialized roles than those of prokaryotes and lower eukaryotes. *DMC1* is a meiosis-specific gene as it is in yeast. These *recA/RAD51* family genes are summarized in Table 1.

In addition, *recA/RAD51* family genes of mammals, in contrast to prokaryotes and lower eukaryotes, express alternative transcripts [19–21]. *E. coli recA* and its ortholog *RAD51* are conserved in all organisms from bacteria to mammals, suggesting that they are essential for homologous recombination in living cells. The number of *RAD51* paralogs and their alternatve variants appears to increase as the size of genomic DNA increases in an organism.

In this review, we focus on updating the current body of knowledge on the *recA/RAD51* family and discuss potential roles of *RAD51* paralogs and their alternative variants.

Principles of homologous recombination

1. RecA and SOS response. DNA damage induces a complex series of more than 20 enzymes possessing DNA repair activities, so-called the SOS response [22]. *E. coli* RecA protein is a 352-amino acid protein, comprised of 3 domains, an N-terminal domain, a core domain, and a C-terminal domain [8]. The core domain conserved among the RecA/Rad51 family proteins includes 2 nucleotide binding consensus sequences, Walker A and B motifs, which are involved in ATP binding and hydrolysis activities. The DNA repair mechanism of double-strand breaks (DSBs) by homologous recombination has been unraveled (Fig. 1). RecBCD protein complex, which has both helicase and exonuclease activity, is recruited at the site of DBS [22]. The complex unwinds double-strand DNA and digests it from the 5'-ends, generating 3'-OH single strand (ss) DNA tails. RecA protein binds to the ssDNA tail and forms a helical nucleoprotein filament. RecF, RecO, RecR, and SSB proteins are involved in the filament formation [23]. Crystal structure analysis shows that DNA in the nucleoprotein filament is extended approximately 1.5-fold compared with common B-form DNA [24]. The filament interacts with double-strand DNA, searching for a homologous DNA sequence, followed by homologous pairing. The nucleoprotein filament also catalyzes the autoproteolysis of LexA repressor protein, leading to the induction of many enzymes involved in DNA repair (SOS response) [25].

2. RuvA, RuvB, RuvC, and Holliday junction. After homologous pairing between a single-strand DNA and a double-strand DNA (D-loop), the DNA sequence lost at the DSB site is synthesized using the paired DNA strand as a template and the 3'-OH ssDNA tail as a primer (Fig. 1). The crossover structure as a homologous recombination intermediate, called Holliday junction, can move along the duplex DNA by branch migration [26, 27]. The Holliday junction is resolved, generating 2 nicks followed by ligation (Fig. 2).

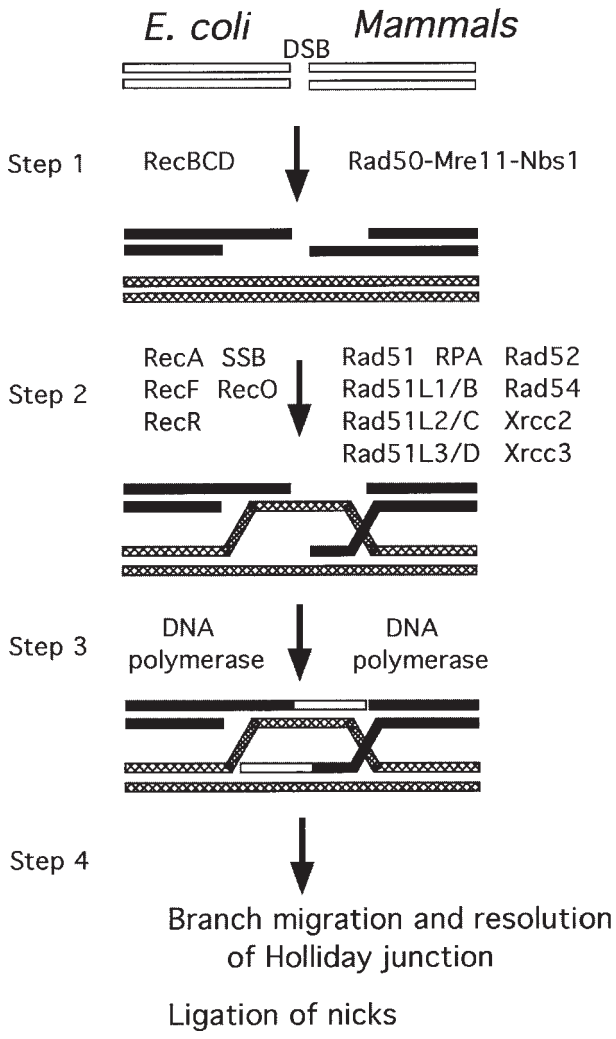

Fig. 1 Molecular mechanism of homologous recombination in double-strand break (DSB) repair. In Step 1, double-strand DNA is unwound with helicase activity and the DSB ends are deleted with 5' to 3' exonuclease activity to form 3'-single-strand DNA tails. In Step 2, RecA or Rad51 forms helical nucleoprotein filaments on DNA with assistance of associated proteins, searching for a homologous sequence. In Step 3, after homologous pairing, DNA polymerases synthesize new DNA, and the crossover structure, designated the Holliday junction, is formed, followed by branch migration and resolution of Holliday junction in the next step (to Fig. 2). The proteins involved in the steps in *E. coli* and mammals are listed on the left and right side, respectively.

Nicks in other strands release 2 recombinant DNA strands and nicks in the same strands release 2 parental DNA strands (Fig. 2). The RuvA-RuvB protein complex mediates branch migration [27]. The tetramer of RuvA protein specifically binds to the Holliday junction.

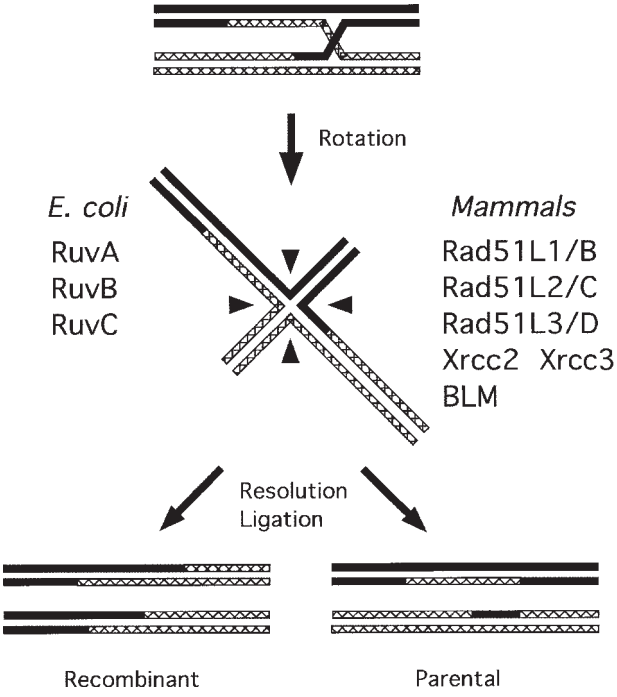

Fig. 2 Resolution of Holliday junction. In the late step of homologous recombination, the Holliday junction is resolved, generating 2 nicks followed by ligation. Nicks in other strands (vertical arrowheads) release 2 recombinant DNA strands (left) and nicks in the same strands (horizontal arrowheads) release 2 parental DNA strands (right). The proteins involved in the steps in *E. coli* and mammals are listed on the left and right side, respectively.

The hexamer of RuvB helicase protein binds to the junction by interacting with RuvA protein. RuvC resolvase protein acts as a Holliday junction-specific endonuclease, separating 2 double-strand DNAs as described above [26].

Mammalian *recA/RAD51* family

1. RAD51. The human and mouse *RAD51* gene have been cloned using yeast *RAD51* as a probe, based on their sequence similarity [8, 9]. Yeast Rad51 has approximately 80% identity with human and mouse Rad51, while bacterial RecA has approximately 30% identity with human and mouse Rad51 [8]. Human Rad51 is a 339-amino acid protein that, in contrast to RecA, includes a long N-terminal domain and a short C-terminal domain [8]. Mammalian Rad51 has a core domain structure with 2 nucleotide binding motifs, Walker A and B, also conserved in bacteria and yeast,

which have ATP binding and hydrolysis activities [28]. Like RecA, yeast and human Rad51 were found to form a helical nucleoprotein filament and to possess homologous pairing and strand exchange activities [29]. In yeast, Rad52 and the heterodimeric complex of Rad55 and Rad57 were shown to promote Rad51-mediated strand exchange [30, 31]. In human, Rad52, Rad54, and Rad51 paralogs (Rad51L1/B, Rad51L2/C, Rad51L3/D, Xrcc2, and Xrcc3) facilitated homologous recombination mediated by Rad51 [32-34].

2. RAD51 paralogs and their alternative variants. Five *RAD51* paralogs, *RAD51L1/B*, *RAD51L2/C*, *RAD51L3/D*, *XRCC2*, and *XRCC3*, have been identified in mammals [11–17]. The paralogs share approximately 20–30% amino acid sequence identity with Rad51 and about 30% identity with each other [3]. All paralogs have the core domain structure with 2 nucleotide binding consensus motifs, Walker A and B [11–17].

RAD51L1/B, *RAD51L2/C*, and *RAD51L3/D* have been cloned based on their sequence homology with *RAD51/recA*, and *XRCC2* and *XRCC3* have been cloned by functional complementation in the Chinese hamster cell lines irs1 and irs1SF, respectively [11–17]. The irs1 cell line is defective in *XRCC2*, the irs1SF cell line in *XRCC3* [35]. Both cells are highly sensitive to DNA cross-liking agents, such as mitomycin C [35]. The phenotypes of irs1 and irs1SF were complemented with *XRCC2* and *XRCC3*, respectively [35].

In addition, alternative transcript variants of *RAD51*, *DMC1*, and *RAD51* paralogs have been identified experimentally, or in the DNA database, GENBANK/EMBL/DDBJ [20, 21]. Human *RAD51* and *DMC1* have 2 variants [21]. Human *RAD51L1/B* and *RAD51L2/C* have 3 variants [11, 12, 16]. Surprisingly, 9 variants of *RAD51L3/D/TRAD* transcript including partially identified fragments have been reported in human [20]. We have also identified 8 variants of the mouse *RAD51L3/D/TRAD* [36].

Phenotypes in *recA/RAD51* family mutants

In yeast, each mutant of *RAD51*, *RAD55*, *RAD57*, or *DMC1* is viable, as *recA* is not essential for survival of bacteria. In contrast, knockout mice missing the *RAD51* gene are mortal in early embryogenesis [37, 38]. Conditional gene targeting of *RAD51* in Chicken DT40 cell lines demonstrated that the mutant cells were arrested at the G2/M phase of the cell cycle before death and carried isochromatid-type chromosomal breaks [39]. These findings indicate that *RAD51* is essential for the proliferation and survival of vertebrate cells.

Gene targeted mice in three of five *RAD51* paralogs, *RAD51L1/B*, *RAD51L3/D/TRAD*, and *XRCC2*, have been made, and each of the mice was found to be mortal during embryogenesis [40–42]. Chicken DT40 cell lines deficient in each of *RAD51L1/B*, *RAD51L2/C*, *RAD51L3/D/TRAD*, *XRCC2*, or *XRCC3* showed that all mutants were viable and exhibited very similar phenotypes [43, 44]. Spontaneous chromosomal aberrations, high sensitivity to cross-linking agents, mild sensitivity to gamma rays, and significantly attenuated Rad51 focus formation after gamma ray exposure were observed in the mutant cells, consistent with the phenotype of *XRCC2*-defective irs1 and *XRCC3*-defective irs1SF hamster cells [35]. Hamster cell lines defective in *RAD51L2/C* show a phenotype similar to those of the *XRCC2*- and *XRCC3*-deficient cells [45, 46]. These observations suggest that Rad51 paralogs are crucial for development but not for viability of cells, and participate in Rad51-mediated homologous recombination.

Protein-protein interaction of RecA/Rad51 family proteins

Extensive investigations of mammalian Rad51 have revealed that Rad51 interacts with many proteins, including Rad52, RPA, Rad54, Xrcc3, Rad51L2/C, Brca2, and c-Abl. Like RecA, Rad51 forms a helical nucleoprotein filament by homopolymerization, which is a basic key step in homologous recombination [29]. Rad52 is involved in the Rad51-dependent and independent DNA recombination [47–49]. In the Rad51-dependent pathway, Rad52 was shown to promote Rad51 in displacing RPA, which is a trimeric ssDNA binding protein complex, from ssDNA to form nucleoprotein filaments during the early phase of strand exchange [47, 48].

Rad54 and Rad54B belong to the Swi2/Snf2 family, which possesses DNA-dependent/stimulated ATPase activity related to DNA/RNA helicase [50, 51]. These proteins facilitate Rad51-mediated DNA strand exchange [50, 51]. In yeast, Rad54-Rad51-DNA nucleoprotein filaments contained equimolar amounts of each protein [52]. The binding of Rad54 protein was found to stabilize the Rad51 nucleoprotein filament formed on either single-stranded DNA or double-stranded DNA

[52].

BRCA1 and *BRCA2* are tumor suppressor genes [53, 54]. Mutations in the *BRCA1* gene are identified in ~70% of patients with familial inherited cancer of breast and ovary and ~20% of patients with familial breast cancer [55]. *BRCA2* mutations are associated with ~85 % of breast cancer and ~15% of ovarian cancer [55]. Brca2 protein includes 8 motifs of approximately 30-amino acid long designated BRC repeats, 6 of which bind to Rad51 [56, 57]. c-Abl is a non-receptor nuclear tyrosine kinase involved in apoptosis and growth arrest, and is activated by ionizing radiation and DNA-damaging agents [58, 59]. Rad51 was found to interact with c-Abl kinase [60].

p53 is a multifunctional tumor suppressor involved in cell cycle arrest, DNA repair, and apoptotic cell death. It was demonstrated that p53 had a direct interaction with Rad51 and inhibited Rad51-mediated DNA strand exchange and ssDNA-dependent ATPase activity of Rad51 [61–63].

UBL1, a ubiquitin-like protein, was shown to form a complex with Rad51 and Rad52 in human cells [64]. Pir51 possessing ssDNA, dsDNA, and RNA binding activities [65], and UBE2I, a human homolog of yeast small ubiquitin-like modifier SUMO-conjugating E2 enzyme UBC9 involved in S- and M-phase cyclin degradation and mitotic control [66, 67], were also found to interact Rad51 using yeast two-hybrid analysis.

Yeast two-hybrid and three-hybrid systems and co-immunoprecipitation have revealed the protein-protein interactions among the RecA/Rad51 family proteins [68]. Rad51 interacted with both Rad51L2/C and Xrcc3 [32, 68, 69]. Rad51l2/c interacted with Rad51L1/B [33, 68, 70], Rad51L3/D [68], and Xrcc3 [69, 71]. Rad51L3/D interacted with Rad51L2/C and Xrcc2 [72]. In addition, a larger complex of Rad51L1/C, Rad51L2/C, Rad51L3/D, and Xrcc2 were identified *in vitro* and *in vivo* [32, 73]. Unlike Rad51 and RecA, these paralogs showed no reflexive interaction (*i.e.*, with themselves, respectively) [68]. BLM protein, the gene product responsible for Bloom's syndrome, is a helicase, belonging to the RecQ helicase family, which interacts with Rad51L3/D through the N-terminal domain of BLM [74]. Rad51L3/D-Xrcc2 complex stimulates disruption of Holliday junction mediated by BLM helicase [74].

Function of Rad51 paralogs

The 5 Rad51 paralogs, as described above, were shown to form heterologous complexes between the paralogs and to exhibit ATPase activity [32–34, 69, 72]. Although phenotype analyses of the paralogs indicated that the proteins were essential components in homologous recombination, their exact roles in the homologous recombination have not been thoroughly elucidated. Human Rad51L1/B-Rad51L2/C complex was found to bind to single-strand (ss) DNA and to exhibit ssDNA-stimulated ATPase activity [33, 34, 70]. The complex mediated Rad51/RPA-catalysed DNA strand exchange, via partial alleviation of competitive RPA activity for Rad51-DNA nucleoprotein formation [34]. Other observations support the presynaptic or early roles of the Rad51 paralogs in homologous recombination [32, 73].

Another investigation showed that purified human Rad51L1/B bound to single strand DNA as well as double strand DNA in the presence of ATP and either Mg^{2+} or Mn^{2+} and hydrolysed ATP in a DNA-dependent manner [75]. In addition, the Rad51L1/B protein specifically bound to the synthetic Holliday junction [75]. Extracts from *RAD51L2/C*- or *XRCC3*-defective hamster cells reduced the level of Holliday junction resolvase activity [76]. Extracts from human HeLa cell lines showed branch migration and resolution activity of Holliday junction [76]. Depletion of Rad51L2/C from the extracts caused a loss of both branch migration and resolution activity of Holliday junction, and the activities were complemented by Rad51L1/B-Rad51L2/C, Rad51L2/C-Xrcc3, or Rad51L1/B-Rad51L2/C-Rad51L3/D-Xrcc2 complexes containing Rad51L2/C but not Rad51L3/D-Xrcc2 heterodimer [76]. Resolution of Holliday junction mediated by BLM, a helicase belonging to the RecQ family described above, were stimulated by Rad51L3/D-Xrcc2 complex via interaction with Rad51L3/D [74]. These results suggest that Rad51L1/B, Rad51L2/C, Xrcc3, Rad51L3/D, and Xrcc2 are involved in Holliday junction processing, a late step in homologous recombination.

Kurumizaka *et al.* demonstrated that each complex of human Rad51L2/C-Xrcc3 or Rad51L3/D-Xrcc2 had homologous pairing activity [77, 78]. Lio *et al.* showed that Rad51L2/C displayed an ATP-independent DNA strand exchange activity [33].

In contrast to the other Rad51 paralogs, Rad51L1/B,

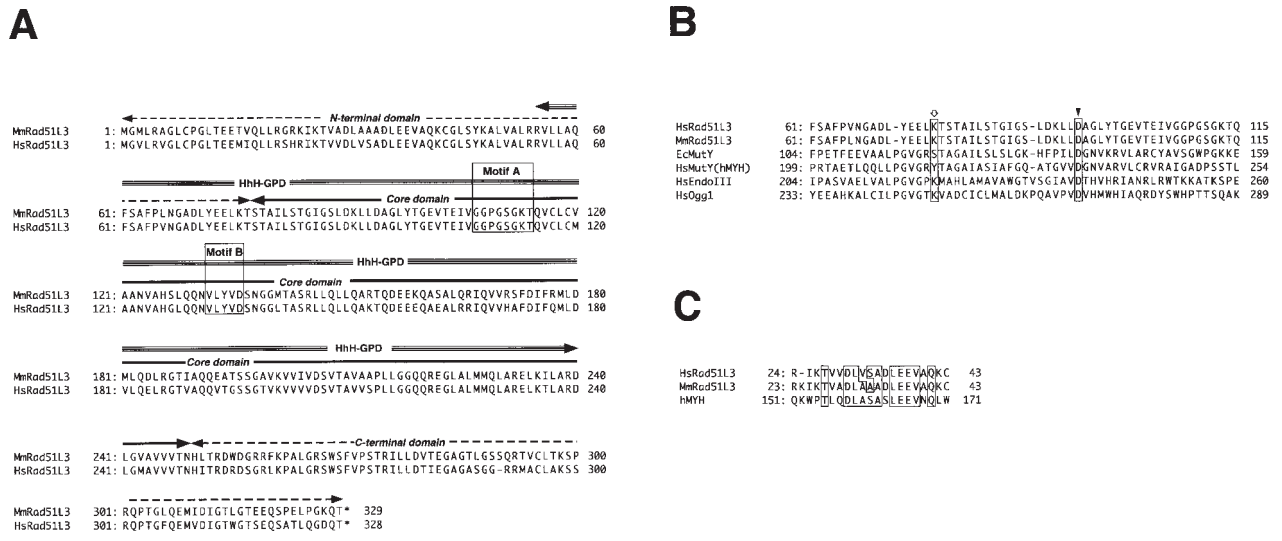

Fig. 3 Domain structure of Rad51L3/D. **A**, Human (Hs) and mouse (Mm) Rad51L3/D include a HhH-GPD motif, which is conserved in the base excision DNA repair superfamily. **B**, Multiple amino acid sequence alignment of human/mouse Rad51L3/D and base excision DNA repair superfamily members. Lysine (K), indicated by an open arrow in the boxed amino acid residues, is a catalytic residue conserved among base excision DNA repair proteins that have β-lyase activity, such as human homolog of *E. coli* endonuclease III (NTH1) (HsEndoIII) and human Ogg1 (HsOgg1). Aspartate (D), indicated by an arrowhead in the boxed amino acid residues, is a catalytically essential residue conserved among base excision DNA repair proteins that have glycosylase activity, such as *E. coli* MutY (EcMutY), human MutY homolog (hMYH) (HsMutY), HsEndoIII, and HsOgg1. Both of the invariant lysine and aspartate residues are conserved in human and mouse Rad51L3/D. **C**, Similarity of short stretch amino acid sequence between human/mouse Rad51L3/D and hMYH. Ten of fifteen amino acid residues (boxed) are identical.

alternatively named Rec2, is unique in its exhibition of protein kinase activity [79]. Human Rad51L1/B was induced by both ionizing and UV radiation, and its overexpression causes a delay in G1 [79]. This protein phosphorylates kemptide, an artificial substrate containing one serine residue, as well as myelin basic protein, p53, cyclin E, and cdk2, but not a peptide substrate containing tyrosine only [79]. Thus, the Rad51L1/B paralog may be involved in the cell cycle. Furthermore, unique Rad51L1/B-histone interactions have been reported by Coleman *et al*. [80].

A search in the protein-domain motif database [81, 82] revealed that human and mouse Rad51L3/D include the HhH-GPD domain (helix-hairpin-helix and glycine/proline rich loop followed by an invariant aspartate), conserved in the base excision DNA repair protein superfamily, including endonuclease III, MutY, MBD4, DNA-3-methyladenine glycosylase II, 8-xoguanine DNA glycosylase and AlkA family members (Fig. 3A) [83, 84], although the bioinformatical findings have not been demonstrated experimentally. Detailed *in silico* analysis revealed several putative features of the Rad51L3/D/Trad protein (unpublished). Alignment of the active site sequence in these superfamily members showed that human and mouse Rad51L3/D/Trad had some degree of similarity with those of the superfamily members (Fig. 3B). Asp138 of *E. coli*, Asp233 of human MutY homolog (hMYH), Asp239 of human endonuclease III homolog (NTH1), and Asp268 of human Ogg1 are the invariant, catalytically essential aspartate residues conserved in the base excision DNA repair superfamily [83, 84]. Human and mouse Rad51L3/D paralogs have the invariant aspartate residue Asp94. Lys220 of NTH1 and Lys249 of human Ogg1 are the lysine residues at the active site conserved in the subfamily members of the base excision DNA repair proteins that have β-lyase activity [83, 84]. Additionally, a highly homologous short sequence between human/mouse Rad51L3/D and hMYH was found (Fig. 3C). These observations suggest the possibility that mammalian Rad51L3/D might exhibit both glycosylase and β-lyase activities, although this remains to be demonstrated experimentally.

Concluding remarks

Many investigations support that *recA/RAD51* family proteins play a crucial role in homologous recombination. In particular, Rad51 protein is a central molecule in the homologous recombination machinery. Studies of phenotypes in mice and cell lines defective in the *recA/RAD51* family genes show that the genes are essential for development and cell proliferation in mammals. The number of *RAD51* paralogs have increased in evolution from bacteria to mammals depending on the size of genomic DNA in the organisms. These observations suggest that homologous recombination in mammals may be involved in more complicated functions than it is in single cell organisms, including cell differentiation. Although recent works have unraveled differential roles of the Rad51 paralogs, their roles have yet to be clearly established. In addition, identification of alternative transcript variants of some *RAD51* paralogs may reflect the complexity of the homologous recombination system. We expect that this review will contribute to an understanding of the homologous recombinational DNA repair system and to elucidation of the complex mechanism (s) underlying homologous recombination.

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