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Posttranslational Modifications of Rad51 Protein and Its Direct Partners: Role and Effect on Homologous Recombination – Mediated DNA Repair

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1. Introduction

Double-strand breaks (DSB) are probably the most deleterious form of DNA alteration in a cell. They may arise from ionizing radiation, free radicals, chemicals, or during replication of single-strand breaks. There are two distinct and complementary mechanisms for DSB repair: non-homologous end-joining (NHEJ) and homologous recombination (HR). Both repair pathways are important for the elimination of DSBs in eukaryotes.

Although the mechanisms of the cellular choice between these two pathways remain unclear, there is evidence that it depends on the cell cycle, as well as on mechanisms such as posttranslational modifications. When an intact DNA copy is available, HR is preferred and it is mainly active during late S and G2 phases of the cell cycle, while NHEJ is predominant during G0 and early S phases. The NHEJ pathway is characterised by a phosphorylation cascade where the first step is the activation of DNA-PKc protein which comprises a catalytic subunit and which is essential to complete the repair process. In contrast to NHEJ, the role of posttranslational modifications of proteins involved in the HR pathway is not clearly defined. Rad51 is a central protein in HR repair and its activity is based on pairing and strand exchange between homologous DNAs. The molecular regulation of Rad51 levels and activity has not been completely established. However, the kinase-induced phosphorylation of this protein modulates its recombinase activity by changing its interface and recognition sites and probably its intracellular distribution. Indeed, Rad51 associates with its paralogues and with other partner proteins, such as Rad52, Rad54, BRCA2 tumour suppressor, BLM helicase (Fig.1). Rad51 forms distinct subnuclear complexes called foci, which represent the functional units in DNA repair by HR. This accumulation of repair proteins to sites of double-strand break repair is closely dependant on protein-protein interactions which can be regulated by posttranslational modification processes including tyrosine, serine and threonine phosphorylations. This underlines the high complexity of HR regulation in mammalian cells.

Regulation of Rad51 recombinase activity and its interactions following DNA damage are poorly understood. In this chapter we have summarized the posttranslational modifications

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of Rad51 and of the proteins interacting physically with Rad51 during HR repair. We then attempt to relate the impact of these modifications on HR DNA repair and on the intracellular distribution of DNA repair proteins.

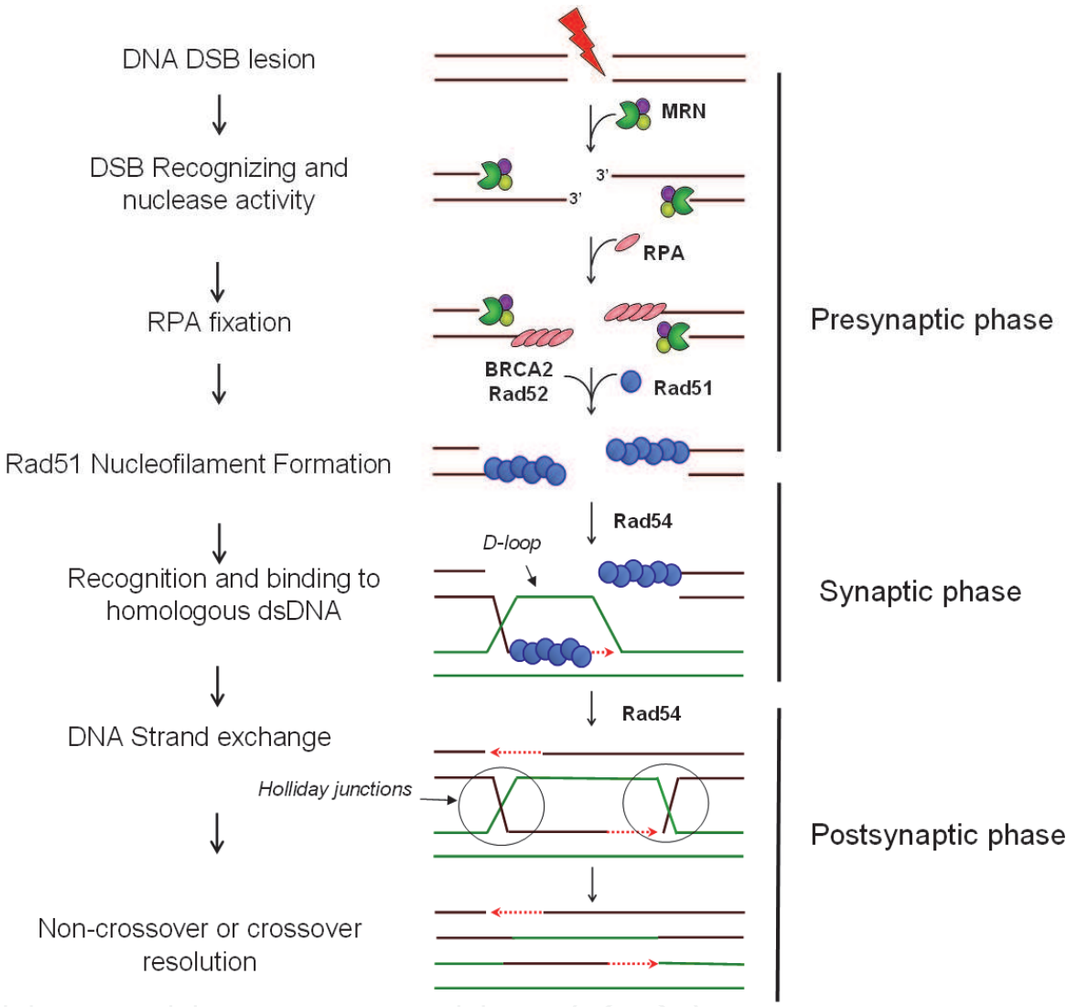

Fig. 1. Schematic representation of the mechanism of DNA DSB repair by homologous recombination.

2. Post-translational modifications of Rad51

2.1 Tyrosine phosphorylation of Rad51 by the c-Abl family of tyrosine kinases

Several studies have shown that Rad51 can be phosphorylated on tyrosine but until recently there were discrepancies on the exact site of phosphorylation. Three studies had shown the phosphorylation of Tyrosine 315 (Y315) and only one the phosphorylation of Tyrosine 54 (Y54). A recent publication demonstrated that both of these tyrosines can be phosphorylated. The kinases which phosphorylate Rad51 belong to the c-Abl family which has two members, c-Abl and Arg. The oncogenic fusion tyrosine kinase BCR/Abl has also been shown to phosphorylate Rad51. However, other tyrosine kinases can also phosphorylate Rad51 at a different site than Tyrosine 315 in MEF cAbl-/- cells (Chen et al., 1999b).

2.1.1 Phosphorylation on Tyrosine 54

The first study showing that Rad51 can be phosphorylated was published in 1998 by Yuan and colleagues. Using co-immunoprecipitation, the authors observed that human Rad51 (hRad51) binds to c-Abl in cells. This association was unaffected by irradiation of the cells and was not dependent on DNA binding. Pull-down assays were performed with a GST-c-Abl fusion protein or a GST-c-Abl SH3 domain fusion peptide. These were incubated with cell lysates or purified hRad51. The results confirmed the association between hRad51 and c-Abl *in vitro* and showed that the binding is direct and is mediated by the SH3 domain of c-Abl.

In vitro phosphorylation assays with purified c-Abl and hRad51 demonstrated that hRad51 is a substrate for this kinase. Immunoprecipitation of Rad51 was performed with lysates from irradiated cells overexpressing hRad51 and c-Abl. The analyses of the immunoprecipitated protein with an anti-phosphoTyrosine antibody confirmed the phosphorylation of Rad51 *in vivo*. The *in vivo* and *in vitro* phosphorylated hRad51 proteins were then purified and analyzed by mass spectroscopy. The detected peaks indicated that the phosphorylation is located on Tyrosine 54 on both *in vivo* and *in vitro* phosphorylated Rad51 (Chen et al., 1999a; Chen et al., 1999b; Chen et al., 1999c; Dong et al., 1999; Yuan et al., 1999; Zhong et al., 1999).

2.1.2 Phosphorylation on Tyrosine 315 by c-Abl

Two years after Yuan and colleagues published their study, another group demonstrated that Rad51 can be phosphorylated. However Chen and colleagues did not observe the phosphorylation of Tyrosine 54 but detected the phosphorylation of another tyrosine residue, in position 315.

The authors used GST pull-down assays and immunoprecipitation to show that Rad51 forms a complex with c-Abl and ATM in cells. The association between the three proteins was independent of irradiation and DNA binding. The level of phosphorylation of Rad51 after irradiation of cells was investigated. The analyses of immunoprecipitated Rad51 with an anti-phosphoTyrosine antibody showed that the level of phosphorylation increases after irradiation. Rad51 was a direct substrate for c-Abl and the phosphorylation was dependent on both c-Abl and ATM. In order to determine which tyrosine residue was phosphorylated, the authors co-expressed c-Abl and wild type or mutated Rad51 in cells. Different tyrosine to phenylalanine Rad51 mutants were performed. Phenylalanine is an amino acid that cannot be phosphorylated. Thus, a signal would no longer be detected by the anti-phosphoTyrosine antibody when the phosphorylated residue is mutated. The mutation of Y315 to phenylalanine abolished Rad51 phosphorylation, indicating that c-Abl phosphorylates Rad51 on this residue (Yuan et al., 1998).

2.1.3 Phosphorylation on Tyrosine 315 by BCR/Abl

Rad51 can also be phosphorylated by the oncogenic fusion tyrosine kinase BCR/Abl. BCR/Abl is expressed in most cases of chronic myeloid leukemia and in some cases of acute myeloid leukemia and possesses constitutive kinase activity.

Slupianek and colleagues suggested that Rad51 and BCR/Abl interact physically since a portion of Rad51 co-localizes with the fusion tyrosine kinase in the cytoplasm of BCR/Abl overexpressing cells. This interaction was confirmed by the co-immunoprecipitation of the two proteins.

Rad51 was immunoprecipitated from cells overexpressing BCR/Abl and its phosphorylation state was examined with an anti-phosphoTyrosine antibody. The interaction between

BCR/Abl and Rad51 resulted in the constitutive phosphorylation of Rad51 on tyrosine. Rad51 was also phosphorylated by c-Abl after treatment of cells with cisplatin and mitomycin C. In order to determine the position of phosphorylation, the authors transiently co-expressed BCR/Abl and wild type or mutated Rad51 in cells. Tyrosine to phenylalanine mutations were performed at Tyrosine 54 or Tyrosine 315. The analysis of the Rad51 immunoprecipitates with an anti-phosphoTyrosine antibody revealed the phosphorylation of the wild type and the Y54F Rad51 protein. A substantial reduction in the phosphorylation level of Rad51 was observed when Y315 was mutated to phenylalanine, indicating that the majority of the phosphorylation of Rad51 occurred on Y315. To further confirm the phosphorylation of the Y315 residue, Slupianek and colleagues prepared an antiserum using a phosphorylated Y315 peptide. Western blots were then performed with lysates from cells overexpressiong Rad51 alone or with BCR/Abl. The antiserum did not recognize Rad51 when the protein was overexpressed in cells alone. In contrast, in cells co-expressing BCR/Abl a strong signal was observed. This confirms that the fusion tyrosine kinase BCR/Abl phosphorylates Rad51 on Tyrosine 315 (Slupianek et al., 2001).

2.1.4 Phosphorylation by Arg

The only other member of the c-Abl family, the kinase Arg, also phosphorylates Rad51. Arg shares considerable structural and sequence homology with c-Abl in the N-terminal SH3 and SH2 domains, as well as in the tyrosine kinase domain (Kruh et al., 1990). Co-immunoprecipitation of Rad51 from cells overexpressing Rad51 and Arg indicated that Arg can interact with Rad51 *in vivo*. An anti-phosphoTyrosine antibody showed that Rad51 is phosphorylated by Arg and this phosphorylation seemed to be more effective than the phosphorylation by c-Abl. However, the position of phosphorylation was not determined (Li et al., 2002).

2.1.5 Phosphorylation of both Tyrosine 54 and Tyrosine 315 by c-Abl

The study conducted by Popova and colleagues has allowed to reconcile the discrepancies on which tyrosine residue is phosphorylated in Rad51. The authors purified specific anti-phosphoTyrosine antibodies for each site of phosphorylation. These antibodies were used to analyze the phosphorylation state of Rad51 by immunoblotting of lysates from cells overexpressing Rad51 and c-Abl. The ability of these specific antibodies to detect distinctively the phosphorylation of the two tyrosine residues has allowed to observe the phosphorylation of both Y54 and Y315 in the same experiment. This confirmed that both Tyrosine 54 and 315 can be phosphorylated (Popova et al., 2009).

In all previous studies the phosphorylation of only one site was observed, either Y54 or Y315. The fact that Yuan and colleagues observed only the phosphorylation of Y54 and did not detect the phosphorylation of Y315 could be due to the technique they used. In their study, the *in vitro* or *in vivo* phosphorylated Rad51 protein, as well as the unphosphorylated protein were digested by trypsin. The obtained fragments were then analyzed by mass spectroscopy and the spectra of the unphosphorylated and the phosphorylated proteins were compared. The lack of a phosphorylation peak in the fragment containing Y315 could be explained by its biophysical characteristics. Following trypsin digestion, the peptide containing Tyrosine 54 is 17 amino acids long and has a pHi of 4,83. On the contrary, the peptide containing Tyrosine 315 is 28 amino acids long and its pHi is 4,03. Thus, the Y315 peptide is longer and more negatively charged compared to the Y54 peptide which could interfere with its detection by mass spectroscopy (Raggiaschi et al., 2005).

Another possible explanation could be the proximity of the digestion and the phosphorylation sites. The presence of phosphorylation near a digestion site may decrease its digestion efficiency (Benore-Parsons et al., 1989; Kjeldsen et al., 2007). Thus the phosphorylated protein would be partially digested resulting in a longer phospho-peptide. A corresponding peptide would not be obtained from the digestion of the unphosphorylated protein. A phosphorylation peak would not be observed in these conditions. In the amino acid sequence of Rad51, only one residue separates the trypsin digestion site from Tyrosine 315. Due to the proximity of the two sites, Rad51 would rather be digested at arginine 310 than on lysine 313. This would result in the generation of a phosphopeptide which would be 3 amino acids longer than the corresponding peptide from the unphosphorylated protein. Consequently, the phosphorylation of Rad51 on Y315 would not be detected by mass spectroscopy.

2.1.6 Model of sequential phosphorylation

Popova and co-authors have established a possible mechanism by which Rad51 is phosphorylated by c-Abl. They co-expressed c-Abl and wild type or mutated hRad51 in cells. In the amino acid sequence of hRad51, Tyrosine 54 or Tyrosine 315 were mutated to phenylalanine, thus rendering the residue at this position nonphosphorylatable. Western blot analysis of the cell lysates, revealed with their specific anti-phosphoTyrosine antibodies, showed a relationship between the phosphorylation of Y54 and Y315. When residue 315 was mutated to phenylalanine and nonphosphorylatable, Tyrosine 54 was no longer phosphorylated. On the contrary, the mutation of residue 54 had no effect on the phosphorylation of Tyrosine 315. The authors hypothesized that the phosphorylation of Tyrosine 315 is needed for the phosphorylation of Tyrosine 54.

The c-Abl kinase possesses a SH3 and a SH2 domain in its N-terminal region. The SH3 domain recognizes and binds preferentially to proline rich regions containing the sequence PXXP. The SH2 domain recognizes pYXXP sequences. hRad51 has two PXXP motifs in its amino acid sequence – between amino acids 283 and 286, and between amino acids 318 and 321. When Tyrosine 315 is phosphorylated, a pYXXP motif is revealed between amino acids 315 and 318. This motif might be recognized by the SH2 domain of c-Abl.

According to this model of sequential phosphorylation, c-Abl recognizes a PXXP motif in the sequence of Rad51 through its SH3 domain and phosphorylates Tyrosine 315. The phosphorylation of this residue reveals the pYXXP binding motif which is recognized by the SH2 domain of c-Abl. This allows the phosphorylation of Tyrosine 54.

To confirm this model, GST pull-down assays were performed. A GST- c-Abl SH2 domain peptide was incubated with lysates from cells overexpressing Rad51 and c-Abl. The results showed that hRad51 binds to the SH2 domain of c-Abl and that this interaction takes place when Rad51 is phosphorylated on Tyrosine 315. Therefore a model of sequential phosphorylation of Rad51, where the phosphorylation of Tyrosine 315 by c-Abl reveals a novel binding site for the kinase thus allowing the phosphorylation of Tyrosine 54, is highly plausible.

2.2 Role of Rad51 phosphorylation

Even though the process of phosphorylation seems to be of considerable importance in the regulation of Rad51 activity, its exact roles and consequences have not been elucidated yet. Moreover, the existing data is contradictory.

In their study, Yuan and colleagues investigated the possible effect of Y54 phosphorylation on Rad51 activity. Strand exchange assays showed that phosphorylation of S. cerevisiae

Rad51 (ScRad51) results in the inhibition of dsDNA conversion to joint molecules and nicked circular dsDNA. An inhibition of the binding of phospho-ScRad51 and phospho-hRad51 to ssDNA was also observed. Because Rad51 exerts its activity by binding to and forming nucleofilaments with ssDNA, the authors concluded that by inhibiting the binding to ssDNA, phosphorylation inhibits Rad51 function (Yuan et al., 1998).

In the search of a possible role for Y315 phosphorylation, Chen and colleagues investigated if the phosphorylation impacts the interaction between Rad51 and Rad52. Rad52 is a protein needed in the presynaptic stage of homologous recombination (Fig. 1). Binding assays with purified *in vitro* phosphorylated Rad51 and Rad52, as well as co-immunoprecipitation of Rad51 and Rad52 from irradiated cells were performed. The results indicated that phosphorylation enhances the interaction between these two proteins *in vitro* and *in vivo*. The authors hypothesized that this irradiation-induced phosphorylation of Rad51 on tyrosine residues and the concomitant increase in association with Rad52 may lead to increased DNA repair efficiency (Chen et al., 1999b). *In vitro* studies with different Y315 mutants suggest that the phosphorylation of this residue is important for the binding of Rad51 to dsDNA and for nucleofilament formation (Takizawa et al., 2004). Moreover, Y315 is located near the polymerisation site of the protein, a region which is essential for the filament formation of Rad51 on DSBs, (Conilleau et al., 2004).

Slupianek and colleagues analyzed the role of Rad51 phosphorylation in the resistance of cells to DNA damaging agents. The resistance of BCR/Abl expressing cells to cisplatin and mitomycin C was decreased upon overexpression of nonphosphorylatable Rad51 Y315F. The mutation of Y54 had no effect on resistance. These results link the phosphorylation of Y315 to the resistance to DNA cross-linking agents and suggest that it has an important impact on DNA repair (Slupianek et al., 2001).

Recently, the same team reported an implication of Y315 phosphorylation in the regulation of BCR/Abl-Rad51 interaction. BCR/Abl-mediated phosphorylation of Y315 appears to be important for the dissociation of Rad51 from BCR/Abl in chronic myeloid leukemia cells (Slupianek et al., 2009). The authors studied the intracellular localization of wild type and mutated Rad51 in response to DSBs induced by genotoxic treatment. The nonphosphorylatable Rad51 Y315F mutant remained mostly in the cytoplasm, while the wild-type protein accumulated in the nucleus in BCR/Abl-positive cells. This indicates that phospho-Y315 stimulates abundant nuclear localization of Rad51 on DSBs.

2.3 Phosphorylation on Threonine 309 by Chk1

Rad51 can also be phosphorylated on threonine. Sorensen and colleagues observed that a Chk1 signal is necessary for efficient homologous recombination. The inhibition of this kinase decreased the level of homologous recombination and of DNA DSB repair. The inhibition of Chk1 also impaired the formation of Rad51 foci which was not due to decreased Rad51 levels. The interaction of Rad51 with chromatin was dependent on Chk1 activity. Using immunoprecipitation, Sorensen and colleagues showed that Chk1 and Rad51 can interact physically in cells. Chk1 phosphorylates Rad51 on Threonine 309 which is located in a Chk1 consensus phosphorylation site. Cells transfected with a nonphosphorylatable Rad51 mutant were more sensitive to hydroxyurea which confirms that Chk1 signaling is required for homologous recombination repair (Sorensen et al., 2005).

2.4 Sumoylation – Ubiquitination of Rad51

Yeast two-hybrid assays have shown that Rad51 can interact with HsUbc9, later named UBE21. HsUbc9/UBE21 is the human homologue of *S. cerevisiae* UBC9 and *S. pombe* Hus5 ubiquitin conjugating enzymes (Kovalenko et al., 1996; Shen et al., 1996). In mammalian cells the downregulation of Ubc9 was associated with defects in cytokinesis and an increased number of apoptotic cells. Furthermore, its gene inactivation is lethal in mouse embryos (Moschos and Mo, 2006). Nuclear depletion of Ubc9 disrupts the intracellular trafficking of Rad51 and thus inhibits the formation of Rad51 nuclear foci following DNA damage (Saitoh et al., 2002).

Rad51 also interacts with UBL1 (ubiquitin like 1), also called PIC1, GMP1, SUMO-1 and Sentrin (Shen et al., 1996). The yeast homologue of UBL1, SMT3, inhibits a centrosome protein involved in centrosome segregation (Shen et al., 1996). UBL1 interacts with HsUBC9/UBE21 (Shen et al., 1996). Studies have shown that HsUbc9/UBE21 is a UBL1-conjugating enzyme, rather than an ubiquitin-conjugating enzyme. Immunoprecipitation essays in HeLa cells and GST pull-down essays have shown that the interaction between Rad51 and Ubl1 is mediated by Rad52 and/or Ubc9. This suggests that Ubc9 can conjugate UBL1 to Rad51. The overexpression of UBL1 in mammalian cells decreases DSB-induced HR and resistance to IR (Li et al., 2000).

3. Rad51-interacting proteins involved in the nuclear translocation of Rad51 and in the HR process

The number and size of Rad51 nuclear foci is a hallmark of the cellular response to genotoxic stress. These nuclear foci characterize the formation of Rad51 filaments. Indeed Rad51 is recruited to sites of DNA DSBs in response to damage where it promotes DNA strand invasion and strand exchange. Impaired formation of Rad51 foci in response to DNA damage has been demonstrated in hamster or chicken cells defective in the Rad51 paralogs XRCC2, XRCC3, Rad51B, Rad51C, and in mammalian BRCA1 or BRCA2-defective cells (Chen et al., 1999c; Takata et al., 2001; Yuan et al., 1999).

The foci formation requires the translocation of Rad51 into the nucleus after DSB induction by genotoxic stress or stalled replication forks (Haaf et al., 1995).) This process is often accompanied by posttranslational modifications of Rad51 partners which cooperate to achieve the fidelity of DNA repair. Several works have shown that these modifications can modulate protein interactions involving Rad51 and can affect Rad51 foci formation.

3.1 Nuclear translocation of Rad51

The first stage of DNA DSB repair by HR requires the delivery of Rad51 at the sites of DNA damage. Since Rad51 does not have a Nuclear Localisation Signal (NLS) sequence, its nuclear entry likely requires the interaction with other proteins containing functional NLS sequences (Gildemeister et al., 2009). BRCA1 and BRCA2 proteins have both been described as primordial recombination mediators for the nuclear translocation of Rad51.

3.1.1 Involvement of BRCA1/Akt1

Several studies have demonstrated that the overexpression of Rad51 results in its cytoplasmic accumulation (Mladenov et al., 2006) but genotoxic stress triggers the translocation of Rad51 from the cytoplasm to the nucleus (Gildemeister et al., 2009). Plo and

colleagues have reported that the nuclear translocation of Rad51 was impaired by AKT1 which repressed HR (Plo et al., 2008). In tumour cells with high levels of active AKT1, BRCA1 and Rad51 are retained in the cytoplasm. However, BRCA1 phosphorylation by AKT1 was not required for this retention. Interestingly, 77% of tumours containing high levels of AKT1 exhibited also cytoplasmic retention of Rad51 (Plo et al., 2008). This shows that AKT1 activation strongly favors the cytoplasmic localization of both BRCA1 and Rad51 proteins.

3.1.2 BRCA2-mediated nuclear translocation of Rad51

Like BRCA1, BRCA2 is a tumour suppressor implicated in familial breast cancer. BRCA2 protein contains six highly conserved BRC repeats which are involved in the interaction between BRCA2 and Rad51 (Marmorstein et al., 1998; Mizuta et al., 1997; Wong et al., 1997). It has been proposed that the BRCA2 protein is directly involved in the regulation of the nucleofilament formation and in the nuclear transport of Rad51 (Davies et al., 2001).

Medova and colleagues have demonstrated that the inhibition of the MET receptor tyrosine kinase by a small inhibitor molecule impairs the formation of the Rad51-BRCA2 complex. By targeting MET, the authors have shown the incapacity of tumour cells to repair DNA DSBs through homologous recombination. This was due to the impaired translocation of Rad51 into the nucleus (Medova et al.).

The pancreatic adenocarcinoma cell line CAPAN-1 is the best characterized BRCA2 defective human cell line (Jasin, 2002). CAPAN-1 cells have indeed lost a wild-type BRCA2 allele and presents a 6174delT mutation on the other allele. This mutation causes the premature C-terminal truncation of the protein. This results in the deletion of the BRCA2 domains for DNA repair and the nuclear localization signals (Holt et al., 2008). Rad51 exhibits impaired nuclear translocation in CAPAN-1 cells. Therefore it has been proposed that Rad51 requires BRCA2 for its nuclear translocation and that C-terminally truncated BRCA2 retains Rad51 in the cytoplasm.

Another group has however observed a DNA damage-induced increase in nuclear Rad51 in the BRCA2-defective cell line CAPAN-1. Moreover, chromatin-associated Rad51 levels were found to be increased (2-fold) following IR exposure (Gildemeister et al., 2009).

To analyze a possible BRCA2-independent mechanism for Rad51 nuclear transport, the authors studied two other Rad51-interacting proteins, Rad51C and Xrcc3. Both of these proteins contain a functional NLS. In contrast to Xrcc3, subcellular distribution of Rad51C was affected by DNA damage since nuclear Rad51C was significantly increased following IR exposure. Furthermore, the depletion of Rad51C in HeLa and CAPAN-1 cells by RNA interference resulted in lower levels of nuclear Rad51. These results provide an important overview of the cellular regulation of Rad51 nuclear entry. This data underlines the potential role for Rad51C in the nuclear translocation of Rad51, which suggests a BRCA2-independent mechanism for Rad51 nuclear entry both before and after DNA damage. Other studies have also demonstrated that an interaction between Rad51 and BRCA2 is not required for nuclear transport of Rad51 but it may prevent the formation of Rad51 filaments in the cytoplasm.

3.2 Recruitment of Rad51 at the damage site – Presynaptic phase of HR

Following damage, DSB are recognized by the MRN complex (MRE11-Rad51-NSB1 complex). MRN binds to and resects the extremities of the DSB through its nuclease activity.

This results in the generation of 3' single-stranded DNA (ssDNA). RPA (Replication Protein A) binds to the 3' overhangs and thus protects them from further resection. This protein also removes secondary structures present on the ssDNA which allows efficient Rad51 nucleofilament formation (McIlwraith et al., 2000).
During the presynaptic phase Rad51 is loaded on the ssDNA ends with the help of BRCA2 (Huen et al., 2010). Rad51 recognizes and binds to the BRC repeats and the TR2 domain of BRCA2 (Fig.2). The Oligonucleotide Binding Folds (OB Folds) in the C-terminal region of the protein are also required for the recruitment of Rad51 (O'Donovan and Livingston, 2010; Wong et al., 1997).
The interaction of BRCA2 with two other proteins, BRCA1 and the bridging factor PALB2, is necessary for its role in the presynaptic phase of HR. These proteins along with other factors form a macro-complex named BRCC whose role in DNA repair has been described elsewhere (Dong et al., 2003).
In addition to its linking function between BRCA1 and BRCA2, PALB2 also interacts with a domain in Rad51 which is comprised between amino acids 184 and 257 (Fig.3) (Buisson et al., 2010). Thus, PALB2 cooperates with BRCA2 to stimulate Rad51 filament assembly during HR. The stimulation of the filament assembly by PALB2 is also mediated by its interaction with another co-factor, Rad51AP1 (Dray et al., 2010).

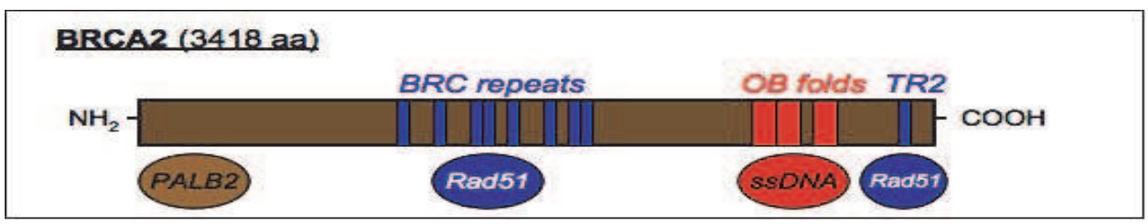

Fig. 2. Domain organization of BRCA2. Schematic drawing indicating the interaction sites with Rad51, PALB2 and DNA.

According to these data, BRCA2 plays an essential role in recruiting and loading Rad51 on sites of DSB and in initiating the HR process.
In order for the Rad51 presynaptic filament to assemble, Rad52 has to displace RPA from the ssDNA (Sugiyama and Kowalczykowski, 2002). RPA is a single-stranded DNA binding protein composed of three subunits, with sizes of respectively 70, 32 and 14 kDa (Wold, 1997). It has previously been shown by co-immunoprecipitation experiments that each of the three subunits of RPA interacts with Rad51, and that the RPA-Rad51 interaction is regulated by the 70kDa subunit (Golub et al., 1998). The co-localization of Rad51 and RPA foci in response to ionizing radiation was observed in a mice fibroblast model and suggests a possible *in vivo* interaction between the two proteins. Furthermore, a recent study has shown that depletion of RPA in mammalian cells leads to the impairment of Rad51 foci formation following DSB induced by hydroxyurea treatment. This confirms the importance of RPA in the presynaptic assembly of Rad51 (Sleeth et al., 2007).
Because RPA binding on ssDNA may prevent Rad51 access to DSB, the presynaptic filament formation needs to be time-regulated by the mediator Rad52. Rad52 is a key member of the RAD52 epistasis group, which includes Rad51, and whose function in HR has been previously described (Symington, 2002). The human Rad52 (hRad52) protein contains 418 amino acids. It has a highly conserved region in its N-terminus, and possesses a

ssDNA/dsDNA binding region and a RPA binding site (Kagawa et al., 2002; Park et al., 1996). Shen and colleagues have demonstrated both *in vitro* and *in vivo* that hRad52 physically interacts with hRad51. The Rad51 binding domain on Rad52 has been identified between residues 291 to 330 (Fig.3) located in the C-terminal region of the protein (Shen et al., 1996).

Furthermore, five amino acid residues of hRad51 have been shown to participate in the Rad51-Rad52 interaction. These residues are located in the C-terminal region of hRad51 (Kurumizaka et al., 1999). Interestingly, the Rad52 binding site on Rad51 is not the same in *Homo Sapiens* and *Saccahromyces cerevisiae,* suggesting that this interaction is not conserved among species.

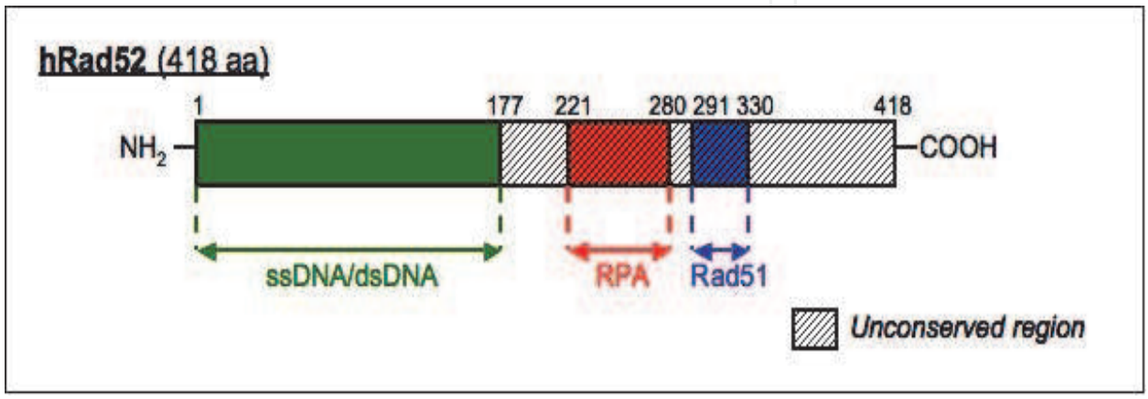

Fig. 3. Human Rad52 (hRad52) domains involved in HR.

The capacity to bind RPA and DNA confers to Rad52 the ability to displace RPA from the ssDNA and thus helps the formation of the Rad51 presynaptic filament (Plate et al., 2008; San Filippo et al., 2008).

The posttranslational modifications of RPA and Rad52 could modulate the formation of the presynaptic filament. Indeed, RPA is phosphorylated on one of its three subunits in a DNA damage-dependent manner and the resulting hyperphosphorylated RPA proteins directly interact with Rad51 (Binz et al., 2004; Wu et al., 2005). More recently, Shi and colleagues demonstrated by mutating the phosphorylation site of RPA that this posttranslational modification is required for Rad51 assembly (Shi et al., 2010). The importance of RPA phosphorylation during the presynaptic phase of HR was confirmed by Deng and colleagues who proposed a model in which RPA phosphorylation promotes Rad52 function and thus prepares DSB to be processed by Rad51 (Deng et al., 2009).

Phosphorylation of the Rad52 mediator in a c-Abl dependant manner has also been described in response to ionizing treatment (Kitao and Yuan, 2002). There is no evidence for the direct effect of Rad52 phosphorylation on Rad51 assembly. However, anterior studies have shown that the phosphorylation of Rad51 by c-Abl has an impact on the interaction between Rad51 and Rad52 (Chen et al., 1999b).

Another important posttranslational modification which plays a role in this stage of the HR process is SUMOylation. SUMOylation is already known to regulate the properties and stability of different proteins (Hay, 2005). It has recently been shown that the 70 kDa subunit of RPA can be SUMOylated and this process may regulate Rad51 presynaptic filament formation (Dou et al., 2010).

3.3 Regulation of Rad51 nucleofilament stability and enhancement of the strand exchange activity - Synaptic phase

Once the Rad51 nucleofilament is assembled, it has to be stabilized before Rad51 strand exchange activity may occur. This is mainly achieved by the Rad54 protein, which interacts both *in vitro* and *in vivo* with Rad51 during the synaptic phase of HR (Golub et al., 1997; Mazin et al., 2010). This protein-protein interaction is mediated by the Rad54 N-terminal region. It can occur either with the free Rad51 protein or with the assembled nucelofilament (Mazin et al., 2003; Raschle et al., 2004). Furthermore, using mouse embryonic stem cells, Tan and colleagues have demonstrated that Rad54 is required for Rad51 IR-induced foci formation (Tan et al., 1999). Rad54 functions in an ATP-independent manner to stabilize the Rad51 nucleofilament (Wolner and Peterson, 2005). However, it can also disrupt the assembled Rad51 complex (Li et al., 2007; Solinger et al., 2002). Thus, Rad54 modulates the stability of the Rad51 filament.

Another important consequence of the Rad51-Rad54 interaction is that Rad54 stimulates the recombinase and strand exchange activities of Rad51 (Mazina and Mazin, 2004; Sigurdsson et al., 2002). An additional protein interacting with Rad51 in the mature synaptic filament has been discovered. First identified as Pir51 (for Protein interacting with Rad51), this cofactor was later renamed Rad51AP1 (Rad51 Associated Protein 1). This protein was first characterized for its DNA crosslink repair activity (Henson et al., 2006; Kovalenko et al., 1997). Modesti and colleagues proposed a model in which Rad51AP1 could stimulate the formation of the D-loop by Rad51, which is the final step of the synaptic phase (Modesti et al., 2007).

To this day, the potential effect of Rad54 posttranslational modifications on Rad51 activity during this late stage of HR has not been demonstrated. Recent results obtained in yeast show that Rad54 phosphorylation leads to a reduction in Rad51-Rad54 complexes (Niu et al., 2009). It is not excluded that a similar mechanism could exist in superior eukaryotes.

3.4 Post-synaptic phase of HR – Resolution of Holliday junction

Following the synaptic phase, D-loops can be eliminated by different subpathways, each requiring different proteins. Here we will present only the pathways involving double Holliday junctions (dHJ) (Bzymek et al., 2010). Double HJ are structural intermediates which are resolved by specific endonucleases and result in either crossover or non-crossover products. The dHJ intermediates can also be resolved by helicases (RecQ helicase family) combined with topoisomerase action. In human cells, this pathway combines BLM helicase and topoisomerase IIIa, both of which catalyze dHJ dissolution (Wu and Hickson, 2003). Interestingly, BLM helicase is phosphorylated by different kinases, such as Chk1, at different stages of the cell cycle or in response to DNA damage. BLM can interact with 53PB1, a signal transducer, and with Topoisomerase IIIa during the presynaptic and the postsynaptic phases of HR respectively. It has been shown that BLM and 53BP1 can interact physically with Rad51 and regulate HR by modulating the assembly of Rad51 filaments. The *in vivo* phosphorylation of both BLM and 53BP1 affects negatively Rad51 foci formation (Tripathi et al., 2007). Concerning Topoisomerase IIIa, Rao and colleagues suggested that the BLM phosphorylation on T99 results in its dissociation from topoisomerase IIIa, thereby modulating the resolution of dHJ (Rao et al., 2005).

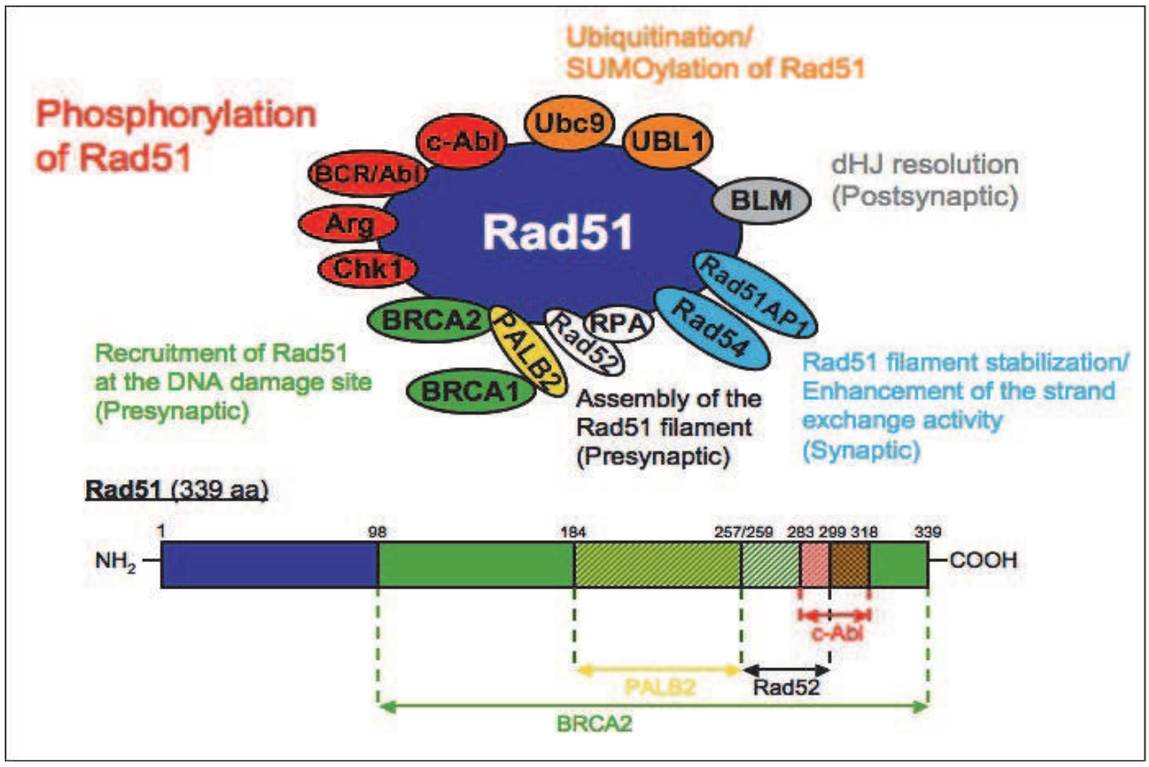

Fig. 4. Schematic representation of Rad51 interactions with its direct partners involved in its posttranslational modification and the steps of HR (top). Localization of binding sites in the hRad51 sequence (bottom).

4. Conclusion

In all living organisms HR is strictly regulated in time and in space to maintain the stability of the genome. Rad51 is the central protein in the HR process. The regulation of HR involves many protein interactions (Fig. 4) which are strongly dependent on posttranslational modifications. Indeed, almost all key mediator proteins of HR are subject to phosphorylation by specific kinases, thereby modulating some stage of this process (e.g. the nucleofilament formation). Hence, these posttranslational reactions underline the complexity of the regulation of HR. Despite of the several studies on the mechanism of Rad51 phosphorylation, its biochemical role in the HR reaction remains unclear.
The impact of phosphorylation on the interactions of Rad51 with its partners still needs to be determined. In order to better understand the regulation of HR, the future challenge will be to identify the complete interaction network of Rad51, the motor protein of HR.

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6. References

Benore-Parsons, M., Seidah, N.G., & Wennogle, L.P. (1989). Substrate phosphorylation can inhibit proteolysis by trypsin-like enzymes. Arch Biochem Biophys *272*, 274-280.

Binz, S.K., Sheehan, A.M., & Wold, M.S. (2004). Replication protein A phosphorylation and the cellular response to DNA damage. DNA Repair (Amst) *3*, 1015-1024.

Buisson, R., Dion-Cote, A.M., Coulombe, Y., Launay, H., Cai, H., Stasiak, A.Z., Stasiak, A., Xia, B., & Masson, J.Y. (2010). Cooperation of breast cancer proteins PALB2 and piccolo BRCA2 in stimulating homologous recombination. Nat Struct Mol Biol *17*, 1247-1254.

Bzymek, M., Thayer, N.H., Oh, S.D., Kleckner, N., & Hunter, N. (2010). Double Holliday junctions are intermediates of DNA break repair. Nature *464*, 937-941.

Chen, C.F., Chen, P.L., Zhong, Q., Sharp, Z.D., & Lee, W.H. (1999a). Expression of BRC repeats in breast cancer cells disrupts the BRCA2-Rad51 complex and leads to radiation hypersensitivity and loss of G(2)/M checkpoint control. J Biol Chem *274*, 32931-32935.

Chen, G., Yuan, S.S., Liu, W., Xu, Y., Trujillo, K., Song, B., Cong, F., Goff, S.P., Wu, Y., Arlinghaus, R., *et al.* (1999b). Radiation-induced assembly of Rad51 and Rad52 recombination complex requires ATM and c-Abl. J Biol Chem *274*, 12748-12752.

Chen, J.J., Silver, D., Cantor, S., Livingston, D.M., & Scully, R. (1999c). BRCA1, BRCA2, & Rad51 operate in a common DNA damage response pathway. Cancer Res *59*, 1752s-1756s.

Conilleau, S., Takizawa, Y., Tachiwana, H., Fleury, F., Kurumizaka, H., & Takahashi, M. (2004). Location of tyrosine 315, a target for phosphorylation by cAbl tyrosine kinase, at the edge of the subunit-subunit interface of the human Rad51 filament. J Mol Biol *339*, 797-804.

Davies, A.A., Masson, J.Y., McIlwraith, M.J., Stasiak, A.Z., Stasiak, A., Venkitaraman, A.R., & West, S.C. (2001). Role of BRCA2 in control of the RAD51 recombination and DNA repair protein. Mol Cell *7*, 273-282.

Deng, X., Prakash, A., Dhar, K., Baia, G.S., Kolar, C., Oakley, G.G., & Borgstahl, G.E. (2009). Human replication protein A-Rad52-single-stranded DNA complex: stoichiometry and evidence for strand transfer regulation by phosphorylation. Biochemistry *48*, 6633-6643.

Dong, Y., Hakimi, M.A., Chen, X., Kumaraswamy, E., Cooch, N.S., Godwin, A.K., & Shiekhattar, R. (2003). Regulation of BRCC, a holoenzyme complex containing BRCA1 and BRCA2, by a signalosome-like subunit and its role in DNA repair. Mol Cell *12*, 1087-1099.

Dong, Z., Zhong, Q., & Chen, P.L. (1999). The Nijmegen breakage syndrome protein is essential for Mre11 phosphorylation upon DNA damage. J Biol Chem *274*, 19513-19516.

Dou, H., Huang, C., Singh, M., Carpenter, P.B., & Yeh, E.T. (2010). Regulation of DNA repair through deSUMOylation and SUMOylation of replication protein A complex. Mol Cell *39*, 333-345.

Dray, E., Etchin, J., Wiese, C., Saro, D., Williams, G.J., Hammel, M., Yu, X., Galkin, V.E., Liu, D., Tsai, M.S., *et al.* (2010). Enhancement of RAD51 recombinase activity by the tumor suppressor PALB2. Nat Struct Mol Biol *17*, 1255-1259.

Gildemeister, O.S., Sage, J.M., & Knight, K.L. (2009). Cellular redistribution of Rad51 in response to DNA damage: novel role for Rad51C. J Biol Chem *284*, 31945-31952.

Golub, E.I., Gupta, R.C., Haaf, T., Wold, M.S., & Radding, C.M. (1998). Interaction of human rad51 recombination protein with single-stranded DNA binding protein, RPA. Nucleic Acids Res *26*, 5388-5393.

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

Haaf, T., Golub, E.I., Reddy, G., Radding, C.M., and Ward, D.C. (1995). Nuclear foci of mammalian Rad51 recombination protein in somatic cells after DNA damage and its localization in synaptonemal complexes. Proc Natl Acad Sci U S A *92*, 2298-2302.

Hay, R.T. (2005). SUMO: a history of modification. Mol Cell *18*, 1-12.

Henson, S.E., Tsai, S.C., Malone, C.S., Soghomonian, S.V., Ouyang, Y., Wall, R., Marahrens, Y., & Teitell, M.A. (2006). Pir51, a Rad51-interacting protein with high expression in aggressive lymphoma, controls mitomycin C sensitivity and prevents chromosomal breaks. Mutat Res *601*, 113-124.

Holt, J.T., Toole, W.P., Patel, V.R., Hwang, H., & Brown, E.T. (2008). Restoration of CAPAN-1 cells with functional BRCA2 provides insight into the DNA repair activity of individuals who are heterozygous for BRCA2 mutations. Cancer Genet Cytogenet *186*, 85-94.

Huen, M.S., Sy, S.M., & Chen, J. (2010). BRCA1 and its toolbox for the maintenance of genome integrity. Nat Rev Mol Cell Biol *11*, 138-148.

Jasin, M. (2002). Homologous repair of DNA damage and tumorigenesis: the BRCA connection. Oncogene *21*, 8981-8993.

Kagawa, W., Kurumizaka, H., Ishitani, R., Fukai, S., Nureki, O., Shibata, T., & Yokoyama, S. (2002). Crystal structure of the homologous-pairing domain from the human Rad52 recombinase in the undecameric form. Mol Cell *10*, 359-371.

Kitao, H., & Yuan, Z.M. (2002). Regulation of ionizing radiation-induced Rad52 nuclear foci formation by c-Abl-mediated phosphorylation. J Biol Chem *277*, 48944-48948.

Kjeldsen, F., Savitski, M.M., Nielsen, M.L., Shi, L., & Zubarev, R.A. (2007). On studying protein phosphorylation patterns using bottom-up LC-MS/MS: the case of human alpha-casein. Analyst *132*, 768-776.

Kovalenko, O.V., Golub, E.I., Bray-Ward, P., Ward, D.C., & Radding, C.M. (1997). A novel nucleic acid-binding protein that interacts with human rad51 recombinase. Nucleic Acids Res *25*, 4946-4953.

Kovalenko, O.V., Plug, A.W., Haaf, T., Gonda, D.K., Ashley, T., Ward, D.C., Radding, C.M., & Golub, E.I. (1996). Mammalian ubiquitin-conjugating enzyme Ubc9 interacts with Rad51 recombination protein and localizes in synaptonemal complexes. Proc Natl Acad Sci U S A *93*, 2958-2963.

Kruh, G.D., Perego, R., Miki, T., & Aaronson, S.A. (1990). The complete coding sequence of arg defines the Abelson subfamily of cytoplasmic tyrosine kinases. Proc Natl Acad Sci U S A *87*, 5802-5806.

Kurumizaka, H., Aihara, H., Kagawa, W., Shibata, T., & Yokoyama, S. (1999). Human Rad51 amino acid residues required for Rad52 binding. J Mol Biol *291*, 537-548.

Li, W., Hesabi, B., Babbo, A., Pacione, C., Liu, J., Chen, D.J., Nickoloff, J.A., & Shen, Z. (2000). Regulation of double-strand break-induced mammalian homologous recombination by UBL1, a RAD51-interacting protein. Nucleic Acids Res *28*, 1145-1153.

Li, X., Zhang, X.P., Solinger, J.A., Kiianitsa, K., Yu, X., Egelman, E.H., & Heyer, W.D. (2007). Rad51 and Rad54 ATPase activities are both required to modulate Rad51-dsDNA filament dynamics. Nucleic Acids Res *35*, 4124-4140.

Li, Y., Shimizu, H., Xiang, S.L., Maru, Y., Takao, N., & Yamamoto, K. (2002). Arg tyrosine kinase is involved in homologous recombinational DNA repair. Biochem Biophys Res Commun *299*, 697-702.

Marmorstein, L.Y., Ouchi, T., & Aaronson, S.A. (1998). The BRCA2 gene product functionally interacts with p53 and RAD51. Proc Natl Acad Sci U S A *95*, 13869-13874.

Mazin, A.V., Alexeev, A.A., & Kowalczykowski, S.C. (2003). A novel function of Rad54 protein. Stabilization of the Rad51 nucleoprotein filament. J Biol Chem *278*, 14029-14036.

Mazin, A.V., Mazina, O.M., Bugreev, D.V., & Rossi, M.J. (2010). Rad54, the motor of homologous recombination. DNA Repair (Amst) *9*, 286-302.

Mazina, O.M., & Mazin, A.V. (2004). Human Rad54 protein stimulates DNA strand exchange activity of hRad51 protein in the presence of Ca2+. J Biol Chem *279*, 52042-52051.

McIlwraith, M.J., Van Dyck, E., Masson, J.Y., Stasiak, A.Z., Stasiak, A., & West, S.C. (2000). Reconstitution of the strand invasion step of double-strand break repair using human Rad51 Rad52 and RPA proteins. J Mol Biol *304*, 151-164.

Medova, M., Aebersold, D.M., & Zimmer, Y. MET inhibition in tumor cells by PHA665752 impairs homologous recombination repair of DNA double strand breaks. Int J Cancer.

Mizuta, R., LaSalle, J.M., Cheng, H.L., Shinohara, A., Ogawa, H., Copeland, N., Jenkins, N.A., Lalande, M., & Alt, F.W. (1997). RAB22 and RAB163/mouse BRCA2: proteins that specifically interact with the RAD51 protein. Proc Natl Acad Sci U S A *94*, 6927-6932.

Mladenov, E., Anachkova, B., & Tsaneva, I. (2006). Sub-nuclear localization of Rad51 in response to DNA damage. Genes Cells *11*, 513-524.

Modesti, M., Budzowska, M., Baldeyron, C., Demmers, J.A., Ghirlando, R., & Kanaar, R. (2007). RAD51AP1 is a structure-specific DNA binding protein that stimulates joint molecule formation during RAD51-mediated homologous recombination. Mol Cell *28*, 468-481.

Moschos, S.J., & Mo, Y.Y. (2006). Role of SUMO/Ubc9 in DNA damage repair and tumorigenesis. J Mol Histol *37*, 309-319.

Niu, H., Wan, L., Busygina, V., Kwon, Y., Allen, J.A., Li, X., Kunz, R.C., Kubota, K., Wang, B., Sung, P., *et al.* (2009). Regulation of meiotic recombination via Mek1-mediated Rad54 phosphorylation. Mol Cell *36*, 393-404.

O'Donovan, P.J., & Livingston, D.M. (2010). BRCA1 and BRCA2: breast/ovarian cancer susceptibility gene products and participants in DNA double-strand break repair. Carcinogenesis *31*, 961-967.

Park, M.S., Ludwig, D.L., Stigger, E., & Lee, S.H. (1996). Physical interaction between human RAD52 and RPA is required for homologous recombination in mammalian cells. J Biol Chem *271*, 18996-19000.

Plate, I., Hallwyl, S.C., Shi, I., Krejci, L., Muller, C., Albertsen, L., Sung, P., & Mortensen, U.H. (2008). Interaction with RPA is necessary for Rad52 repair center formation and for its mediator activity. J Biol Chem *283*, 29077-29085.

Plo, I., Laulier, C., Gauthier, L., Lebrun, F., Calvo, F., & Lopez, B.S. (2008). AKT1 inhibits homologous recombination by inducing cytoplasmic retention of BRCA1 and RAD51. Cancer Res *68*, 9404-9412.

Popova, M., Shimizu, H., Yamamoto, K., Lebechec, M., Takahashi, M., & Fleury, F. (2009). Detection of c-Abl kinase-promoted phosphorylation of Rad51 by specific antibodies reveals that Y54 phosphorylation is dependent on that of Y315. FEBS Lett *583*, 1867-1872.

Raggiaschi, R., Gotta, S., & Terstappen, G.C. (2005). Phosphoproteome analysis. Biosci Rep *25*, 33-44.

Rao, V.A., Fan, A.M., Meng, L., Doe, C.F., North, P.S., Hickson, I.D., & Pommier, Y. (2005). Phosphorylation of BLM, dissociation from topoisomerase IIIalpha, and colocalization with gamma-H2AX after topoisomerase I-induced replication damage. Mol Cell Biol *25*, 8925-8937.

Raschle, M., Van Komen, S., Chi, P., Ellenberger, T., & Sung, P. (2004). Multiple interactions with the Rad51 recombinase govern the homologous recombination function of Rad54. J Biol Chem *279*, 51973-51980.

Saitoh, H., Pizzi, M.D., & Wang, J. (2002). Perturbation of SUMOlation enzyme Ubc9 by distinct domain within nucleoporin RanBP2/Nup358. J Biol Chem *277*, 4755-4763.

San Filippo, J., Sung, P., & Klein, H. (2008). Mechanism of eukaryotic homologous recombination. Annu Rev Biochem *77*, 229-257.

Shen, Z., Cloud, K.G., Chen, D.J., & Park, M.S. (1996). Specific interactions between the human RAD51 and RAD52 proteins. J Biol Chem *271*, 148-152.

Shi, W., Feng, Z., Zhang, J., Gonzalez-Suarez, I., Vanderwaal, R.P., Wu, X., Powell, S.N., Roti Roti, J.L., & Gonzalo, S. (2010). The role of RPA2 phosphorylation in homologous recombination in response to replication arrest. Carcinogenesis *31*, 994-1002.

Sigurdsson, S., Van Komen, S., Petukhova, G., & Sung, P. (2002). Homologous DNA pairing by human recombination factors Rad51 and Rad54. J Biol Chem *277*, 42790-42794.

Sleeth, K.M., Sorensen, C.S., Issaeva, N., Dziegielewski, J., Bartek, J., & Helleday, T. (2007). RPA mediates recombination repair during replication stress and is displaced from DNA by checkpoint signalling in human cells. J Mol Biol *373*, 38-47.

Slupianek, A., Dasgupta, Y., Ren, S., Cramer, K., & Skorski, T. (2009). Targeting BCR/ABL-RAD51 Interaction to Prevent Unfaithful Homeologous Recombination Repair In 51st ASH Annual Meeting and Exposition.

Slupianek, A., Schmutte, C., Tombline, G., Nieborowska-Skorska, M., Hoser, G., Nowicki, M.O., Pierce, A.J., Fishel, R., & Skorski, T. (2001). BCR/ABL regulates mammalian RecA homologs, resulting in drug resistance. Mol Cell *8*, 795-806.

Solinger, J.A., Kiianitsa, K., & Heyer, W.D. (2002). Rad54, a Swi2/Snf2-like recombinational repair protein, disassembles Rad51:dsDNA filaments. Mol Cell *10*, 1175-1188.

Sorensen, C.S., Hansen, L.T., Dziegielewski, J., Syljuasen, R.G., Lundin, C., Bartek, J., & Helleday, T. (2005). The cell-cycle checkpoint kinase Chk1 is required for mammalian homologous recombination repair. Nat Cell Biol *7*, 195-201.

Sugiyama, T., & Kowalczykowski, S.C. (2002). Rad52 protein associates with replication protein A (RPA)-single-stranded DNA to accelerate Rad51-mediated displacement of RPA and presynaptic complex formation. J Biol Chem *277*, 31663-31672.

Symington, L.S. (2002). Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. Microbiol Mol Biol Rev *66*, 630-670, table of contents.

Takata, M., Sasaki, M.S., Tachiiri, S., Fukushima, T., Sonoda, E., Schild, D., Thompson, L.H., & Takeda, S. (2001). Chromosome instability and defective recombinational repair in knockout mutants of the five Rad51 paralogs. Mol Cell Biol *21*, 2858-2866.

Takizawa, Y., Kinebuchi, T., Kagawa, W., Yokoyama, S., Shibata, T., & Kurumizaka, H. (2004). Mutational analyses of the human Rad51-Tyr315 residue, a site for phosphorylation in leukaemia cells. Genes Cells *9*, 781-790.

Tan, T.L., Essers, J., Citterio, E., Swagemakers, S.M., de Wit, J., Benson, F.E., Hoeijmakers, J.H., & Kanaar, R. (1999). Mouse Rad54 affects DNA conformation and DNA-damage-induced Rad51 foci formation. Curr Biol *9*, 325-328.

Tripathi, V., Nagarjuna, T., & Sengupta, S. (2007). BLM helicase-dependent and -independent roles of 53BP1 during replication stress-mediated homologous recombination. J Cell Biol *178*, 9-14.

Wold, M.S. (1997). Replication protein A: a heterotrimeric, single-stranded DNA-binding protein required for eukaryotic DNA metabolism. Annu Rev Biochem *66*, 61-92.

Wolner, B., & Peterson, C.L. (2005). ATP-dependent and ATP-independent roles for the Rad54 chromatin remodeling enzyme during recombinational repair of a DNA double strand break. J Biol Chem *280*, 10855-10860.

Wong, A.K., Pero, R., Ormonde, P.A., Tavtigian, S.V., & Bartel, P.L. (1997). RAD51 interacts with the evolutionarily conserved BRC motifs in the human breast cancer susceptibility gene brca2. J Biol Chem *272*, 31941-31944.

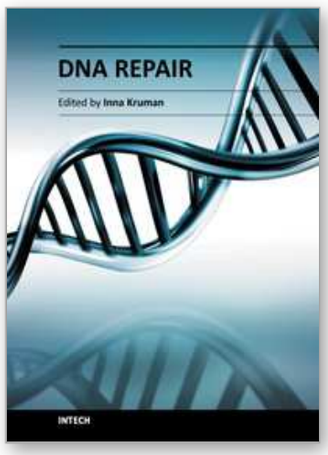
Wu, L., & Hickson, I.D. (2003). The Bloom's syndrome helicase suppresses crossing over during homologous recombination. Nature *426*, 870-874.

Wu, X., Yang, Z., Liu, Y., & Zou, Y. (2005). Preferential localization of hyperphosphorylated replication protein A to double-strand break repair and checkpoint complexes upon DNA damage. Biochem J *391*, 473-480.

Yuan, S.S., Lee, S.Y., Chen, G., Song, M., Tomlinson, G.E., & Lee, E.Y. (1999). BRCA2 is required for ionizing radiation-induced assembly of Rad51 complex in vivo. Cancer Res *59*, 3547-3551.

Yuan, Z.M., Huang, Y., Ishiko, T., Nakada, S., Utsugisawa, T., Kharbanda, S., Wang, R., Sung, P., Shinohara, A., Weichselbaum, R., & Kufe, D. (1998). Regulation of Rad51 function by c-Abl in response to DNA damage. J Biol Chem *273*, 3799-3802.

Zhong, Q., Chen, C.F., Li, S., Chen, Y., Wang, C.C., Xiao, J., Chen, P.L., Sharp, Z.D., & Lee, W.H. (1999). Association of BRCA1 with the hRad50-hMre11-p95 complex and the DNA damage response. Science *285*, 747-750.



DNA Repair

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The book consists of 31 chapters, divided into six parts. Each chapter is written by one or several experts in the corresponding area. The scope of the book varies from the DNA damage response and DNA repair mechanisms to evolutionary aspects of DNA repair, providing a snapshot of current understanding of the DNA repair processes. A collection of articles presented by active and laboratory-based investigators provides a clear understanding of the recent advances in the field of DNA repair.

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