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Double Strand Break Signaling in Health and Diseases

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1. Introduction

Living organisms are constantly subjected to DNA damage whether it originates from intrinsic physiological processes or extrinsic stressors. Of the various types of DNA damage, double strand breaks (DSBs) are the most dangerous since if left unrepaired they can lead to either cell death or genomic instability. For this reason cells have evolved an arsenal of signaling and repair proteins involved in DNA double strand break sensing and repair as well as downstream physiological responses such as apoptosis or cell cycle arrest. DSBs can be generated by reactive oxygen species which are produced during normal metabolism. DSB formation and repair also occurs during the tightly regulated physiological processes of gametogenesis, V(D)J recombination of T- and B-cell receptors and antibody diversification during class switch recombination (CSR). In addition, DSBs can be caused by external agents such as ionizing radiation or chemotherapeutic agents such as etoposide. The importance of DSB signaling and repair is underscored by the many human diseases and syndromes caused by the mutation of genes coding for DNA damage response (DDR) proteins. The study of knockout mouse models for DDR genes has also furthered our understanding of the role of these proteins in DSB repair and in normal physiology. In this chapter, DSB signaling and repair are reviewed. In addition, an overview of the human diseases associated with mutations of DSB signaling and repair proteins is given. Finally, the impact of mouse models on our understanding of DSB signaling and repair and its physiological roles is discussed.

2. Signaling at DNA double strand breaks

2.1 Sensing the DNA double strand breaks

When DSBs are generated they are initially recognized by either the Ku70/Ku80 heterodimer, the Mre11-Rad50-NBS1 (MRN) complex or members of the PARP (PARP1/2) family of proteins (Ciccia & Elledge, 2010). The role of these protein sensors is to bind to and tether the DNA ends, thereby preventing further breakage as well as to recruit additional proteins that are required for DSB signaling and repair. The first group of proteins to be recruited to DSBs after initial sensing of the breaks belongs to the phosphatidylinositol-3-kinase-like protein kinases family. These include ATM (ataxia-telangiectasia mutated), ATR (ATM and Rad3-related) and the catalytic subunit of DNA-PK known as DNA-PKcs. While ATM and DNA-PK respond only to DSBs, ATR also responds to single strand DNA breaks.

Ku70/80 recruits DNA-PKcs to DSBs where it promotes DNA repair by non-homologous end joining whereas both PARP1/2 and the MRN complex lead to the recruitment of ATM which promotes homologous recombination. The MRN complex is recruited to DSBs in both a PARP1/2 dependent and independent manner (Ciccia & Elledge, 2010). ATM is a central component of the cellular response to DSBs and is predicted to have several hundred downstream targets many of which play a role in the DNA damage response (Matsuoka et al, 2007). Under normal conditions ATM is in a homodimeric form. Following DNA damage, it becomes autophosphorylated at Ser1981 and dissociates into its monomeric form and binds the damaged DNA (Bakkenist & Kastan, 2003). There, it leads to the phosphorylation of many downstream targets involved in the DSB response. The phosphorylation of ATM at Ser1981 and its initial binding to DSBs depend upon the MRN complex. MRN consists of three different proteins Mre11, NBS1 and Rad50. Mre11 is a DNA nuclease that interacts with both Rad50 and NBS1 as well as with other Mre11 molecules to form dimers. When paired with the other components of the MRN complex, Mre11 can have both double strand DNA exonuclease activity and single strand DNA endonuclease activity (D'Amours & Jackson, 2002). In addition, Mre11 has two DNA binding sites and intrinsic DNA binding activity. Rad50 is a protein that bears homology to the structural maintenance of chromosome (SMC) family. It is an ATPase and is needed for tethering of DNA ends together during the process of DNA repair. NBS1 has a fork-head-associated (FHA) domain and two BRCT (BRCA1-tandem repeats) domains at its N-terminus which are used to recognize phospho-threonine and phospho-serine residues respectively in Ser-X-Thr motifs. These domains allow RAD50 to interact with several DNA damage signaling proteins following DSB formation. NBS1 also contains a nuclear localization signal (NLS) that allows the translocation of the MRN complex into the nucleus following DNA damage (Lamarche et al, 2010). NBS1 interacts with ATM thereby leading to its recruitment to DSBs. There, ATM is involved in one of the very early response to the formation of DSBs, mainly the phosphorylation of histone variant H2AX on Ser139 to form γ-H2AX (Figure 1). This phosphorylation can extend over a megabase of DNA from the site of DSBs (Modesti & Kanaar, 2001). The formation of γ-H2AX at the sites of DSBs is key for the recruitment of many effector proteins to the break sites including the regulators of cell cycle checkpoint and DNA repair 53BP1, BRCA1 and Rad51 (Bohgaki et al, 2010). The accumulation of γ-H2AX and other DNA damage signaling and repair proteins at the sites of ionizing-radiation induced breaks leads to the formation of microscopically distinct foci known as IR-induced nuclear foci (IRIFs) which can be used experimentally to study IR-induced DNA damage signaling and repair. The ability of H2AX to recruit DNA damage proteins under normal physiological conditions is hampered by its constitutive phosphorylation at Tyr142 by William's syndrome transcription factor (WSTF). This phosphorylation suppresses the ability of H2AX to recruit downstream signaling and effectors of the DNA damage response to the breaks. However, following DNA damage Tyr142 residue is dephosphorylated by the EYA protein phosphatases (Cook et al, 2009). γ-H2AX recruits MDC1, a mediator of the DNA damage response that functions as an adaptor to recruit downstream effector proteins to the break sites. MDC1 has two BRCT domains at its C-terminus and one FHA domain at its N-terminus that allow it to recognize and interact with other DNA damage response proteins (Stewart et al, 2003). The BRCT domains of MDC1 can recognize phosphorylation sites and were shown to mediate MDC1 binding to γ-H2AX.
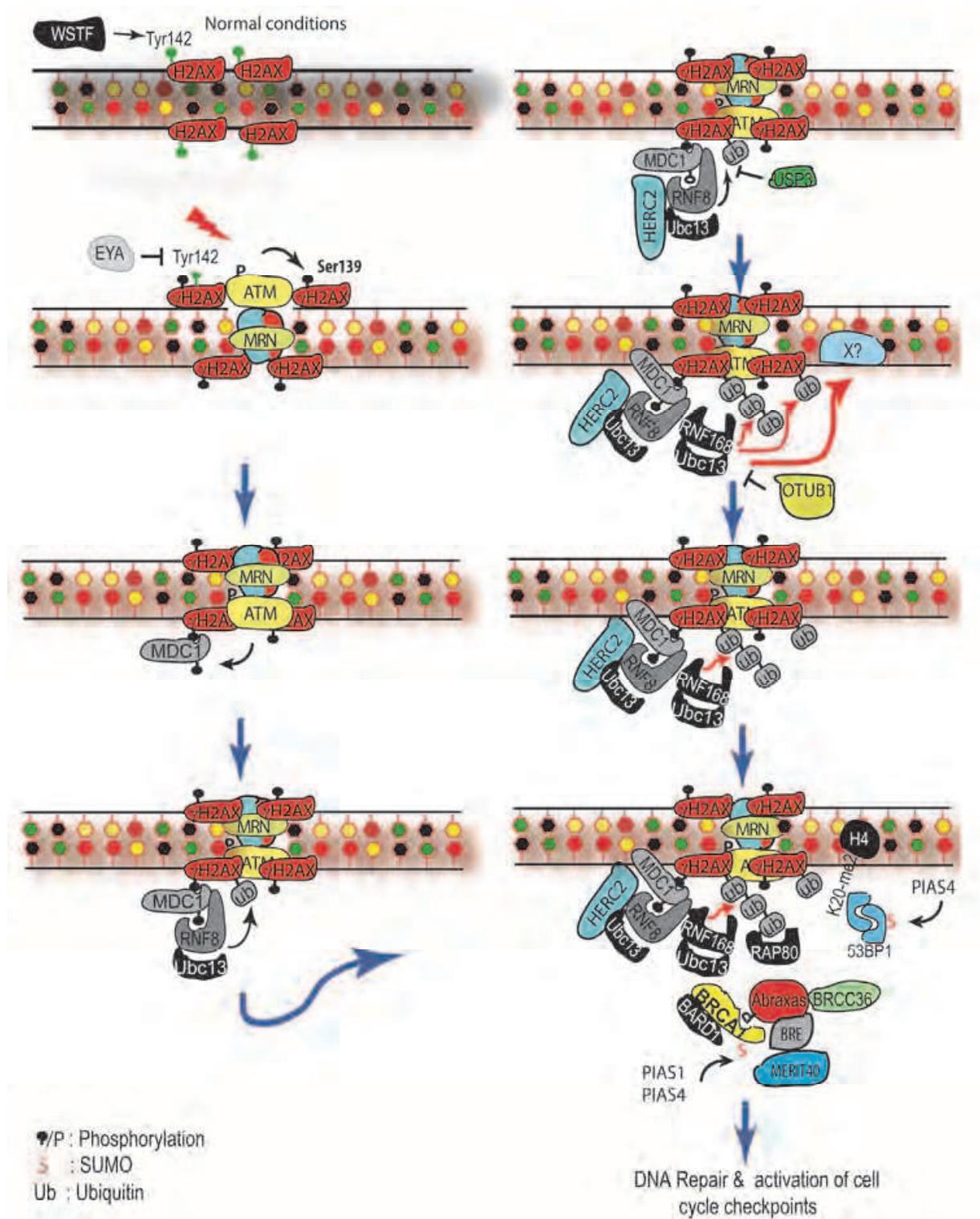
Fig. 1. DNA double strand break signaling

This interaction occurs specifically between the BRCT domain of MDC1 and the Ser139 phosphoylation site of γ-H2AX located at the C-terminus of this protein. MDC1 also interacts with ATM and is phosphorylated by it at its FHA domain (Goldberg et al, 2003; Stewart et al, 2003). Four potential consensus TQFX sites for ATM phosphorylation have been found and two sites, T719 and T752, were confirmed to be phosphorylated by ATM

(Kolas et al, 2007; Matsuoka et al, 2007). MDC1 is thought to be dispensable for the initial binding of ATM to the DSBs and early H2AX phosphorylation but important for ATM and γ-H2AX retention at the damaged DNA sites. In fact, MDC1 acts in a positive feedback loop with γ-H2AX and ATM to amplify the signal at the breaks (Lou et al, 2006). MDC1 was also found to be important for the recruitment of other DNA damage response proteins such as 53BP1 and NBS1 to the break sites. Recent studies have provided us with greater insight about the mechanisms by which MDC1 leads to the recruitment of DNA damage proteins to the DSBs and have highlighted the role of posttranslational modifications such as ubiquitylation and sumoylation in the amplification of the DNA damage response.

2.2 Role of ubiquitylation in the DNA double strand break response

The important role ubiquitylation plays in DNA damage signaling and DNA repair has been recently highlighted (Bohgaki et al, 2010; Panier & Durocher, 2009). Ubiquitin is a small polypeptide of about 8 KDa that can be covalently attached through an isopeptide bond to substrate proteins. This requires the activity of three different enzymes, a ubiquitin-activating enzyme (E1), a ubiquitin conjugating enzyme (E2) and a ubiquitin ligase (E3). The combination of E2 and E3 enzymes used to ubiquitylate target proteins differ from one to the other thereby conferring substrate specificity to the enzyme. Ubiquitin chain attachment can occur through several lysine residues on the ubiquitin polypeptide. The best studied ones are lysine 48 (K48) and lysine 63 (K63). While K48-linked ubiquitin most often leads to proteasomal degradation, K63-linked ubiquitylation is involved in cellular signaling including DSB signaling and DNA repair. Using an siRNA screen for proteins whose absence lead to an impairment of 53BP1 foci formation it was found that knock-down of MDC1 and the E3 ubiquitin ligase RNF8 (Ring Finger protein 8) strongly inhibited 53BP1 recruitment to DSBs (Kolas et al, 2007). RNF8 has a RING domain which is required for its E3 ligase activity and is located at its C-terminus and an FHA domain located at its N-terminus. The FHA domain of RNF8 can recognize phosphorylated MDC1 thereby allowing binding of RNF8 to MDC1. Therefore, MDC1 phosphorylation is required for formation of RNF8 IRIFs. Furthermore, it was found that RNF8 partners up with the E2 ligase UBC13 to mediate K63-linked ubiquitylation of H2A, H2AX and H2B (Huen et al, 2007; Kolas et al, 2007; Mailand et al, 2007). This initial ubiquitylation of chromatin components leads to the recruitment of another ubiquitin ligase RNF168 (Doil et al, 2009; Stewart et al, 2009). RNF168 contains two motifs interacting with ubiquitin (MIUs) and is thought to interact with ubiquitylated chromatin components through its MIUs. At the DNA breaks, RNF168, in collaboration with the E2 ligase UBC13, adds K63-linked polybiquitin chains to H2A and H2AX. This ultimately results in the subsequent recruitment of other DDR proteins to the break sites. For example, RNF168 is required for the formation of 53BP1 IRIFs and the recruitment of BRCA1 to DSB site is severely reduced in the absence of this E3 ligase (Bohgaki et al, 2011; Doil et al, 2009; Stewart et al, 2009). HERC2 is a recently identified protein that leads to binding and stabilization of the RNF8-UBC13 complex to mediate K63-linked histone ubiquitylation (Bekker-Jensen et al, 2010). Additionally, HERC2 was also found to stabilize RNF168 and to promote recruitment of DSB signaling proteins including 53BP1 and BRCA1 to the break sites. The mechanisms through which chromatin component ubiquitylation leads to the recruitment of 53BP1 and BRCA1 are only partially understood. BRCA1 interacts with the receptor-associated protein 80 (RAP80). RAP80 recruitment to DSBs is dependent on γ-H2AX, MDC1 and RNF8 (Kim et al, 2007; Kolas et al, 2007; Mailand

et al, 2007). RAP80 contains two ubiquitin interacting motifs (UIMs) which are capable of specifically recognizing K-63 linked polybiquitin chains (Kim et al, 2007). The interaction of RAP80 and BRCA1 is mediated by another protein named Abraxas (Mailand et al, 2007; Wang et al, 2007). The RAP80-Abraxas-BRCA1 complex is thought to bind DSB sites through the recognition of RNF8- and RNF168-ubiquitylated chromatin by the RAP80 UIM domains (Wang et al, 2007). The way 53BP1 is recruited to the break sites is less well understood. 53BP1 recruitment to DSBs is mediated by the binding of its two TUDOR domains to di-methylated histone H4 at lysine (K) 20. Histone methylation is not increased from its constitutive level following ionizing radiation   suggesting that changes in chromatin structure following DNA damage might be responsible for the uncovering of the methylated histones and the binding of 53BP1. It is therefore plausible that ubiquitylation of H2A and H2B by RNF8 and RNF168 triggers conformational changes in the chromatin that lead to a better accessibility of 53BP1 to the methylated histones (Mailand et al, 2007). However, this hypothesis still remains to be tested. The ubiquitylation of chromatin components leads to the rapid amplification of the DNA damage response. However, once the DNA is repaired, a rapid deubiquitination of histone H2A, H2B and H2AX should occur to return cells to their steady-state levels. This is accomplished by ubiquitin isopeptidases known as deubiquitinating enzymes or DUBs. Several DUBs have been described to negatively regulate DSB signaling. Ubiquitin-specific protease 3 (USP3) was shown to deubiquitylate H2A and H2B (Nicassio et al, 2007) and to negatively regulate the activity of RNF8 (Doil et al, 2009). BRCC36 binds to RAP80 and is in the BRCA1 complex that also includes Abraxas. BRCC36 along with RAP80 can antagonize the activity of RNF8-UBC13 by acting as a deubiquitinating enzyme for γ-H2AX. It also leads to decreased ubiquitylation signal at DSB sites (Shao et al, 2009). Recently, OTUB1 (OTU domain, ubiquitin aldehyde binding 1) was identified as an RNF168 DUB. OTUB1 does not use its catalytic activity to deubiquitylate RNF168 but rather acts by binding to and inhibiting UBC13, the RNF168 E2 conjugating enzyme (Nakada et al, 2010).

2.3 Role of SUMOylation in DNA double strand break signaling

SUMOylation is a posttranslational modification that involves the covalent linkage of a small ubiquitin-like modifier (SUMO) polypeptide to a target protein (Al-Hakim et al, 2010; Ciccia & Elledge, 2010). The process of SUMOylation is similar to that of ubiquitylation in that it also requires a SUMO-specific E1 (SAE1/SAE2), an E2 conjugating enzyme (Ubc9) and substrate-specific E3 ligases. Three types of SUMO molecules have been identified, SUMO1, SUMO2 and SUMO3. SUMO2 and SUMO3 have strong homology and share the same function and are therefore usually referred to as SUMO2/3. Both SUMO1 and SUMO2/3 are recruited to DSBs and this recruitment is dependent on the presence of RNF8 and RNF168. The E3 ligases PIAS1 and PIAS4 are needed for the accumulation of SUMO molecules to the break sites. PIAS1 is involved in the accumulation of SUMO2/3 but not SUMO1 at DSBs whereas PIAS4 is involved in the accumulation of all three SUMO moieties. PIAS1 and PIAS4 were both found to be important for accumulation of RNF168 and ubiquitylated H2A at DSB sites. Furthermore, PIAS1 and PIAS4 SUMOylate BRCA1 which is thought to increase its ubiquitin E3 ligase activity and the ubiquitylation of H2A by BRCA1 which would further amplify the DNA damage signal (Morris et al, 2009). SUMOylation of 53BP1 by PIAS1 and PIAS4 was also demonstrated and shown to be required for the efficient recruitment of 53BP1 to DSBs (Galanty et al, 2009). In summary,

complex posttranslation modifications such as ubiquitylation, SUMOylation, phosphorylation and methylation, at the sites of DSBs lead to recruitment of proteins that are involved in DNA repair and the regulation of cell cycle checkpoints following DNA damage as will be discussed in the next section.

2.4 Cellular responses following DNA double strand breaks

Following DNA damage, cells are faced with different options. Either to undergo cell cycle arrest and repair the damaged DNA, to undergo senescence or to die by apoptosis. There are three checkpoints put in place to arrest the cell cycle following DNA damage. The G1/S checkpoint, the intra-S checkpoint and the G2/M checkpoint (Warmerdam & Kanaar, 2010). These checkpoints are carefully regulated by ATM and its downstream effectors. ATM phosphorylates and activates the protein kinase Chk2 and the tumor suppressor p53 which then act to enforce cell cycle arrest or apoptosis. Chk2 activation requires phosphorylation by ATM at Thr68 and autophosphorylation at multiple other residues. Chk2 phosphorylation of the cdc25 phosphatases which are needed for cell cycle progression lead to either their degradation (in the case of cdc25A) or their export from the nucleus (for cdc25B and cdc25C) thereby preventing interaction with their respective cdk/cyclin substrates (Donzelli & Draetta, 2003). Chk2 also phosphorylates p53 at Ser20 whereas ATM phosphorylates it at Ser15. This results in the accumulation of p53 and its activation as a transcription factor which transactivates many genes whose products are involved in cell cycle arrest such as p21, GADD45 and 14-3-3σ. p53 also transactivates several proapoptotic genes such as Noxa, PUMA and Bax, thus triggering apoptotic cell death of the damaged cells. The mechanisms that lead to p53-dependent cell cycle arrest versus p53-dependent apoptosis remain poorly understood but are likely to depend on the multiple posttranslational modifications of p53 (Vousden, 2006) .

3. DNA repair

Cells have evolved different pathways to repair DSBs. Currently, homologous recombination (HR) and non-homologous end joining (NHEJ) are recognized as the two major pathways for DSB repair (Kass & Jasin, 2010). The presence of undamaged sister chromatid is required for the error-free HR-mediated DSB repair, whereas NHEJ repair can occur in the absence of a homologous template sequence and is therefore considered to be more error-prone. Alternative NHEJ is another form of DNA repair that does not necessitate the presence of classical NHEJ DNA repair proteins and is characterized by sequence deletion and the introduction of microhomologies within the repaired DNA (Kotnis et al, 2009).

3.1 Non–homologous end joining

NHEJ is the most commonly used pathway for the repair of DSBs. Since it does not require the presence of a homologous sequence on a sister chromatid, it can occur throughout the cell cycle but particularly during G0, G1 and early S-phase (Kass & Jasin, 2010).

The first step of NHEJ is the recognition of the DSBs by the Ku70/Ku80 heterodimer which consists of the Ku70 and Ku80 subunit (Figure 2). The Ku70/Ku80 complex then slides inwards, away from the edge of the DSBs to allow binding of two molecules of the catalytic subunit of DNA-PK, DNA-PKcs. When DNA-PKcs binds to DNA and Ku70/Ku80 it is known as DNA-PK. The two DNA-PKcs molecules bind to each other, thereby bringing
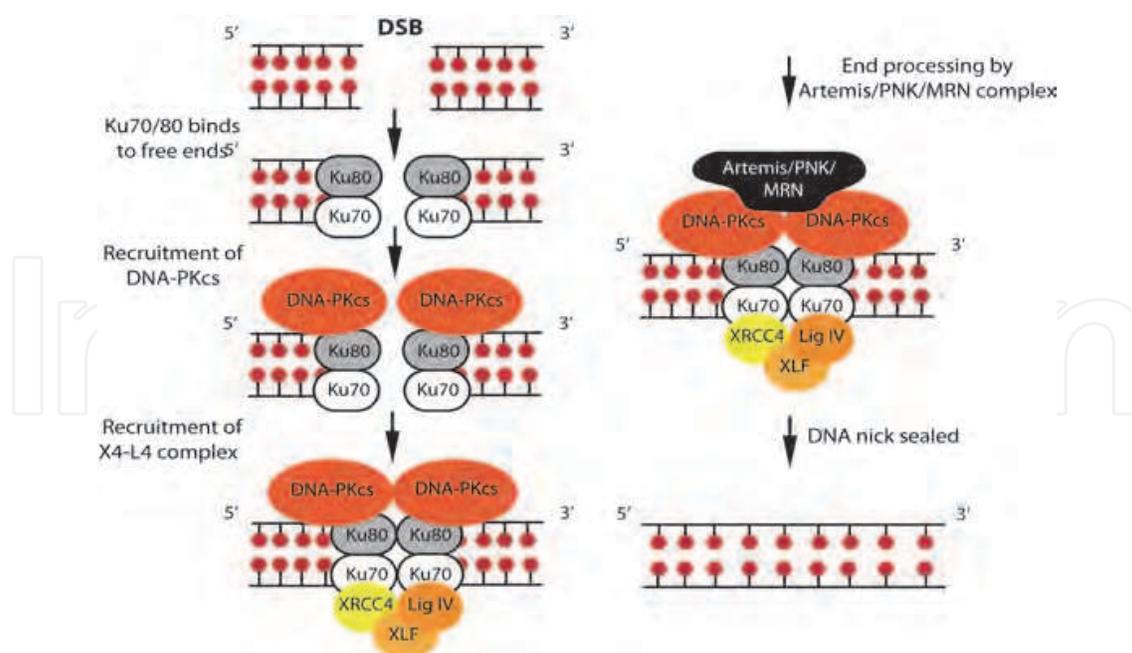
Fig. 2. Repair of DNA double strand breaks by non-homologous end joining

together the DNA ends in a process called synapsis. Binding of Ku70/Ku80 and DNA-PKcs to the DNA ends in this manner protects them against nuclease degradation in the cell. DNA-PKcs is a serine-threonine kinase whose activity is stimulated by binding to double stranded DNA and the Ku heterodimer. Phosphorylation of DNA-PKcs is needed to bring the proteins involved in DNA end processing such as Artemis, the DNA polymerase family members polymerase μ and polymerase λ, and the Polynucleotide kinase (PNK) to the break sites. End processing of the DNA at DSBs allows the removal of DNA lesions that interfere in the ligation process (Mahaney et al, 2009). Artemis is thought to be recruited to DSBs by binding to autophosphorylated DNA-PKcs. While Artemis has inherent 5'→3' exonuclease activity, in the presence of DNA-PK and ATP it also acquires endonuclease activity. DNA-PK promotes the endonucleolytic activity of Artemis versus its exonucleolytic activity and decreases the speed of nucleotide removal. This is important to limit the amount of trimming done at the DNA ends to the minimum. DNA polymerases μ and λ are brought to the DSB sites via their interactions with either Ku70/Ku80 or the XRCC4/DNA ligase complex. Their role at the DSBs involves filling in gaps to allow ligation. DNA polymerase μ is less dependent than DNA polymerase λ on the presence of a template. PNK was also shown to play a role in NHEJ. It associates with XRCC4 and is dependent on its presence for its activity. It can phosphorylate 5'-OH terminal groups and dephosphorylate 3'-ends to restore normal DNA ends that can be ligated by the XRCC4/DNA ligase complex (Chappell et al, 2002). Aprataxin and PNK–like factor (APLF) is an endo-exonuclease that was shown to be required for full NHEJ activity. It can interact with other NHEJ factors such as Ku and XRCC4 and is phosphorylated by ATM in a DNA damage–dependent manner (Macrae et al, 2008). XRCC4 is thought to act as a scaffolding protein that brings many factors involved in NHEJ to the break sites. XRCC4 and DNA ligase IV, along with XLF (also known as Cernunnos) form a complex known as X4-L4. DNA ligase activity is thought to be stimulated by its binding to XRCC4 and to XLF. XRCC4 and XLF can bind to Ku proteins and to DNA. The X4-L4 complex is capable of ligating one strand of DNA at a time which would allow for concomitant processing and ligation of the ends (Hartlerode & Scully, 2009).

3.2 Alternative non-homologous end joining

When one or more classical NHEJ factors (Ku, DNA-PKcs, XRCC4, DNA ligase IV) are missing, DSBs can be repaired through the alternative NHEJ (Alt-NHEJ) pathway. This type of DNA repair relies on the presence of microhomologies at the terminal ends of the DNA breaks. It was found that Alt-NHEJ can occur in the absence of DNA ligase IV suggesting that one of the two remaining ligases (LigI or LigIII in eukaryotes) can function in DSB end ligation. End joining in the absence of LigIV requires 2-3 nucleotides of homology to stabilize the DNA at broken ends whereas no microhomology is needed in normal cells for NHEJ to occur. Furthermore it was found that a 4 nucleotide long microhomology at the break ends greatly decreases the requirement for Ku70, probably because of the increased stabilization of the DNA ends. Alt-NHEJ is stimulated by the presence of CtIP (CtBP-interacting protein) and suppressed by the classical NHEJ factors Ku and XRCC4-LigIV (Bennardo et al, 2008; Simsek & Jasin, 2010). It was found that Alt-NHEJ promotes chromosomal translocations which might explain the increase of hematological cancer incidence in the absence of one or more classical NHEJ factors (Simsek & Jasin, 2010).

3.3 Homologous recombination

Homologous recombination requires the presence of homologous sequences for the accurate repair of DSBs. It usually occurs in the late S phase or G2 phase when a sister chromatid is available to be used as a template for repair (Ciccia & Elledge, 2010; Kass & Jasin, 2010). The first step of HR is the generation of 3'- ssDNA (single stranded DNA) overhangs with 3'-hydroxyl ends which subsequently invade a homologous duplex DNA sequence (Figure 3). Initial processing of the DNA ends requires the MRN complex along with CtIP. Further processing is done by Exo1 in association with the helicase Bloom syndrome protein BLM (Nimonkar et al, 2008). The 3'–ssDNA overhangs generated by this process are then be bound by RPA (replication protein A) which is needed to melt the secondary structures of the DNA and protect the DNA ends before the binding of other HR proteins can occur. RPA is required for the recruitment of HR factors such as the DNA-dependent ATPase Rad51 to the break sites (Sleeth et al, 2007).
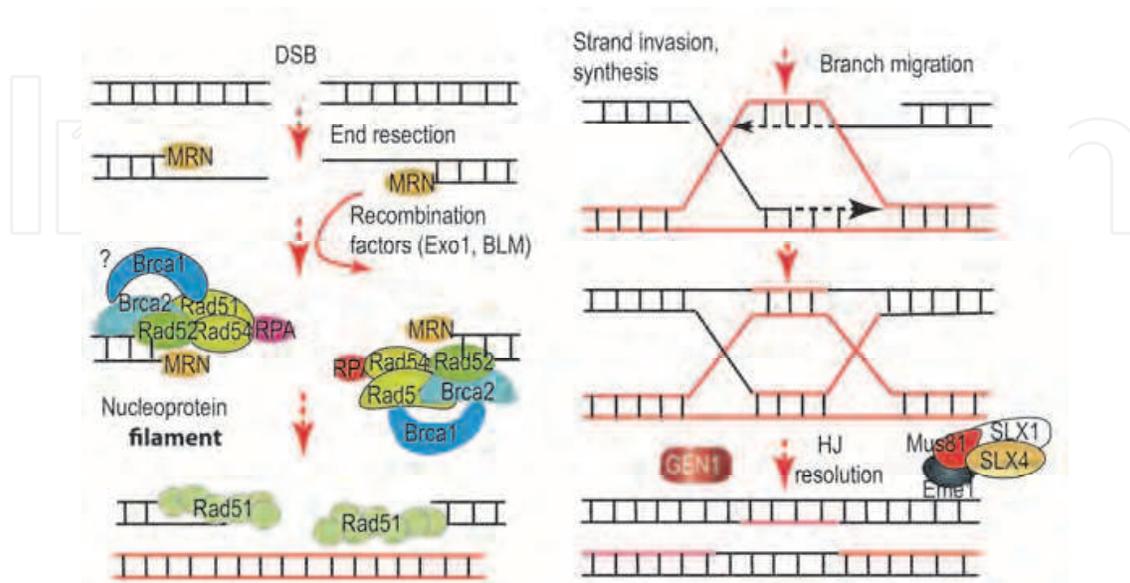

Fig. 3. Double strand break repair by homologous recombination

Rad51, a key HR player, belongs to the Rad52 epistasis group in yeast which is involved in recombinational DNA repair. Rad51 competes with RPA for binding to the DNA. Since it has lower affinity to DNA than RPA, other factors are needed to displace RPA and lead to the formation of Rad51-DNA nucleofilaments. This step requires the presence of the breast cancer susceptibility protein 2 (BRCA2). BRCA2 binds to Rad51 through its BRC repeats and its carboxy-terminus. BRCA2 facilitates the binding of Rad51 to the DNA by promoting RPA displacement and decreasing its ability to bind double stranded DNA. In addition, it inhibits the ATPase activity of Rad51, thereby stabilizing the Rad51-ssDNA complexes (Jensen et al, 2010). Once it binds to the ssDNA, Rad51 catalyzes invasion of a homologous duplex DNA sequence, thereby forming a displacement loop (D-loop). This process is known as synapsis and requires the presence of the Rad54 motor protein. Rad54, a member of the Rad52 epistasis group, binds to the Rad51-DNA nucleofilament, stabilizes it and therefore enhances D-loop formation. However, following synapsis, Rad54 promotes dissociation of Rad51 from double stranded DNA (dsDNA) which allows synthesis of the DNA strand. Rad54-mediated dissociation of Rad51 from dsDNA also allows for rapid Rad51 turnover (Heyer et al, 2010). After the D-loop is formed, DNA repair can occur in three distinct pathways: break-induced replication (BIR), synthesis-dependent strand annealing (SDSA) and double Holliday junctions (dHJ) (Heyer et al, 2010). BIR occurs when only one DNA strand is available to invade duplex DNA. This could occur during replication fork collapse or the uncapping of telomeres. This leads to the formation of a *bona fide* replication fork at this site. DNA synthesis at this site occurs using the regular DNA polymerases (Llorente et al, 2008). During SDSA the invading strand is elongated by DNA synthesis. The D-loop is then reversed, allowing the newly synthesized end to anneal to the resected opposite end of the break. The remaining gaps in the DNA are then filled by DNA synthesis and ligation. SDSA results in non-crossover recombination and is the main pathway used in somatic cells (Sung & Klein, 2006). Finally dHJ mediated recombination involves invasion of the second DNA break into the D loop, DNA synthesis and ligation to join the two invading DNA ends and then dHJ resolution in either a crossover or a non-crossover manner (Sung & Klein, 2006). BLM helicase in conjunction with topoisomerase IIIa leads to the dissolution of HJs to form non-crossover products. The Mus81-Eme1 endonuclease complex, SLX1-SLX4 complex or the human 5'-flap endonuclease GEN1 are capable of resolving dHJ to form both crossover and non-crossover products (Ciccia & Elledge, 2010; Hartlerode & Scully, 2009; Rass et al, 2010).

3.4 Choosing between homologous recombination and non-homologous end joining

The choice of the DNA repair pathway following DSB formation is dependent on many factors. One of the important determinants that allow the cells to choose whether the damaged DNA should be repaired by HR or NHEJ is the cell cycle phase in which it is in. NHEJ can occur throughout the whole cell cycle and is the pathway of choice during the G1 phase whereas DSBs in cells in late S and G2 phases are most likely to be repaired by HR. DNA resection to form 3'ssDNA overhangs is a tightly regulated step because if it occurs it actually commits the cells to repair their DNA using HR versus NHEJ. Binding of CtIP to DSBs promotes resection of the break ends. CtIP levels are regulated in a cell cycle-dependent manner and are elevated during the S, G2 and M phases of the cell cycle but are low during the G1 phase. In addition, CtIP activity is regulated through posttranslational modifications. CtIP is phosphorylated by CDK2 (cyclin dependent kinase 2) during the S

and G2 phases of the cell cycle at two different sites S327 and T847. Phosphorylation of CtIP at S327 allows it to interact with the BRCT domain of BRCA1 and with the MRN complex. This then results in the ubiquitylation of CtIP by BRCA1 (Yu et al, 2006). Posttranslational modification of CtIP by phosphorylation and ubiquitination as well as its interaction with BRCA1 were found to be necessary for it binding to DNA and its role in DNA resection. CtIP is also predicted to be a target of ATM phosphorylation and ATM is required for end resection.

There are many lines of evidence showing competition between the NHEJ and HR pathways for DSB repair. In the absence of the NHEJ factors Ku and XRCC4-LigIV there is an increase in end resection and HR. Conversely, mutations in resection factors such as CtIP result in increased NHEJ (Kass & Jasin, 2010). Recently, 53BP1, a protein involved in cell cycle checkpoint and DNA repair has been implicated in the regulation of the switch between NHEJ and HR repair pathways. Some studies have shown that 53BP1 is important for NHEJ. In addition, 53BP1 is thought to bind to the DSB sites and inhibit DNA resection. Loss of 53BP1 in Brca1-null cells results in increased HR activity in these cells suggesting a model whereby 53BP1 inhibition of end resection is overcome by BRCA1, thereby leading to HR-mediated DNA repair (Bouwman et al, 2010; Bunting et al, 2010) . The mechanisms by which BRCA1 can counteract 53BP1 function and promote HR are still unknown but promise to be the focus of intense research in the future.

4. Double strand break repair in normal physiological processes

4.1 Meotic recombination

Meosis is a specialized type of cell division occurring during gametogenesis. It allows the production of haploid cells, containing one copy of the genetic material, from diploid cells containing two copies of the genetic material (Kumar et al, 2010). Meotic recombination is a crucial process during gametogenesis. It occurs during the prophase of the first meiotic division (prophase I) and allows the formation of physical links called chiasmata between two homologous chromosomes. This ensures proper alignment and segregation of the homologous chromosomes during the later phases of meiosis I. DNA recombination during meiosis also allows the exchange of genetic material between homologous chromosomes. This is required to ensure genetic diversity of the organisms and for introduction of mutations needed for the evolution of species. Meiotic recombination requires a highly regulated generation of DSBs followed by their repair through HR. The first step of meiotic recombination is the generation of DSBs during the leptotene stage of prophase I. This stage occurs directly following S phase when DNA replication has occurred. Spo11, a highly conserved type II-like topoisomerase, generates the DSBs needed to initiate meiotic recombination through a transesterification reaction (Keeney et al, 1997). Spo11 binds to both strands of the DNA as a homodimer. Once the DSB is generated, the DNA on which Spo11 is attached is cleaved, thereby releasing a Spo11-oligonucleotide complex. In addition to Spo11, several other proteins were identified as been involved in DSB formation although their mechanism of action is still poorly understood. One of them, Mei4 (MEIosis-specific 4), is needed for DSB formation in both yeast and mammals (Kumar et al., 2010). Mei4 binds to another protein, Rec114 (RECombination 114) which is also essential for DSB formation in yeast. Although the role of Rec114 in mammals is still unknown, its interaction with Mei4 makes it plausible that they function together in DSB generation in mammals (Kumar et al, 2010).

DSBs do not occur evenly throughout the genome but arise more frequently in specific areas called "hotspots". In yeast and mouse it was found that these hotspots are marked by the trimethylation of histone 3 at Lys4 (H3K4Me3) (Borde et al, 2009). Recently, a histone methyltransferase, PRDM9 (PR domain containing 9), was identified as a protein that can bind to and activate hotspots in mammalian genomes (Baudat et al, 2010). After end processing to form 3'-ssDNA, the resected DNA is bound by Rad51 and Dmc1 (Disrupted meiotic cDNA1), a meiosis-specific recombinase. In the yeast *Saccharomyces cerevisiae,* recruitment of Dmc1 to resected DNA ends is dependent upon the two proteins Mei5 and Sae3. Mei5 and Sae3 (Sporulation in the Absence of Spo Eleven 3) form a complex that allows Dmc1 binding to the single stranded DNA and enhances its recombinase activity (Ferrari et al, 2009). The resected ends can then invade double stranded DNA and form a D-loop. Two proteins, Hop2 and Mnd1 which exist in cells as a heterodimeric complex help in the stabilization of the Rad51 and Dmc1 nucleofilaments and increase their ability to invade homologous duplex DNA. Hop2 and Mnd1 function both in yeast and higher eukaryotes, including mammals (Petukhova et al, 2005; Tsubouchi & Roeder, 2002). In contrast to HR occurring in somatic cells which use sister chromatids as a template, the use of a non-sister homologous chromatid is favored during meiotic recombination (Shinohara et al, 1992). The Holliday junctions that form following strand invasion are then resolved to form crossover and non-crossover products. In contrast to what happens during HR in somatic cells, meiotic recombination generates a much higher proportion of crossover products as compared to non-crossover products (Andersen & Sekelsky, 2010). In both *S. cerevisiae* and higher eukaryotes the Msh4-Msh5 (MutS homolog 4-5) complex plays an important role in the resolution of HJs and in crossover formation. It was also suggested that GEN1 could play a role in HJ resolution during human meiotic recombination (Lorenz et al, 2009), however further investigation is needed to prove this hypothesis.

4.2 V(D)J recombination

V(D)J recombination is a crucial process in lymphocyte development through which diverse B and T cell receptors can be generated (Soulas-Sprauel et al, 2007). It involves the assembly of a variable (V), diversity (D) and joining (J) exons to form a B cell or T cell antigen receptor. The multiple combinations that can be obtained by joining together different V, D and J segments are the underlying mechanism for antigen receptor diversity and the ability of the immune system to respond to different types of pathogens. RAG1 and RAG2 (recombination activating genes 1 and 2) are two lymphocyte-specific recombinases that introduce DSBs at specific recombination signal (RS) sequences that border every V, D and J gene segment (Dudley et al, 2005). RS sequences consist of a conserved heptamer and a conserved nonamer separated by a nonconserved 12 bp or 23 bp spacer sequence. In the heavy chain immunoglobulin gene (IgH) for example, RS sequences that surround D sequences have a 12 bp spacer whereas those surrounding the V and J segments have 23 bp. Since recombination can occur only between RS sequences containing a 12 bp spacer and one containing a 23 bp spacer this ensures productive joining of V, D and J sequences (Dudley et al, 2005). RAG mediated cutting of the DNA generates hairpin loops at the joining DNA ends. These hairpin loops are cleaved by the endonuclease Artemis which is recruited and activated by the DNA-PKcs and Ku70/80 complex. The XRCC4/DNAligase IV/XLF complex then religates the joining ends (Soulas-Sprauel et al, 2007).

4.3 Class switch recombination

Class switch recombination (CSR) is a specialized mechanism occurring in B cells that allows the cells to switch from expressing immunoglobulin (Ig) M (IgM) to IgG, IgA or IgE (Stavnezer et al, 2008). This involves replacement of the IgH constant region Cμ encoded in IgM with more downstream constant regions such as Cγ, Cε and Cα which code for IgG, IgE and IgA heavy chain constant regions respectively. This replacement of IgH constant regions is mediated through deletion of parts of the chromosome and subsequent recombination. CSR occurs within or nearby specific sequences called the switch regions that precede every constant region. CSR is initiated upon antigenic stimulation of mature B cells and requires at least two rounds of cell division and the presence of a B cell-specific enzyme activation induced cytidine deaminase (AID) that converts deoxycytosine (dC) to deoxyuracil (dU) in the donor and receiver S regions (Muramatsu et al, 2000). The dU is then removed through the base excision repair pathway. The enzyme that cleaves dU in S regions is the uracil DNA glycosylase UNG. The phosphate backbone of the resulting abasic site is then cleaved by the apurinic/apyrimidinic endonucleases APE1 and APE2, two proteins which were found to be essential for CSR, to form a single strand DNA break (Guikema et al, 2007). In order for CSR to occur, DSBs must be created in the donor and acceptor S regions and the single stranded breaks generated by APE1 and 2 are not enough. When single stranded breaks are generated close enough to each other they might lead to the formation of DSBs. Otherwise, DSBs must be generated by mismatch repair (MMR) proteins. It is hypothesized that the MMR heterodimer Msh2-Msh6 (MutS homolog 2-6) recognizes the mismatched U:G pair and binds to it. Mlh1-Pms2 (MutL homolog 1-PostMeiotic Segregation 2) then binds to Msh2-Msh6 to form a heterotetramer capable of recruiting the exonuclease Exo1. Exo1 cuts the DNA between two single stranded breaks thereby resulting in a DSB (Stavnezer et al, 2008).

DSBs in the S regions are religated using the NHEJ repair machinery. However NHEJ factors are not the only proteins involved in CSR. Studies of mouse knockout models have implicated Atm, Mdc-1, γ-H2ax and 53bp1 in the CSR process (Kotnis et al, 2009). More recently, the E3 ligases Rnf8 and Rnf168 have also been implicated in the process of CSR (Bohgaki et al, 2011; Li et al, 2010; Santos et al, 2010; Stewart et al, 2007). Interestingly, in B cells of mice that are deficient in classical NHEJ repair proteins, sequencing of switch junctions have shown a pattern of increased microhomology suggesting that CSR could be occurring through alternative NHEJ pathways in these cells (Kotnis et al, 2009). Of all of the DSB repair proteins, loss of 53bp1 seems to have the most severe effect on CSR with a 90% decrease in CSR in 53bp1-null B cells (Kotnis et al, 2009). There have been several roles proposed for 53BP1 in CSR. One of them is that that 53BP1 inhibits intraswitch religation of DSBs and promotes synapsis between DSBs occurring in distal switch sequences. In addition, 53BP1 inhibits DSB resection which is needed for alternative NHEJ, thereby leading to increased DSB repair through the classical NHEJ pathway and to increased CSR (Bothmer et al, 2010). Despite many advances in the field of CSR and in the function of factors required for this process, further investigation is still required to determine the precise role of each DSB repair protein in CSR.

5. DSB signaling and repair defects: Human diseases and mouse models

The study of rare hereditary diseases and knockout mouse models in which DSB signaling and repair genes are mutated has greatly increased our understanding of the DNA damage

signaling and repair pathways and the underlying mechanisms regulating them (Bohgaki et al, 2010; Hakem, 2008). In this section, a summary of the human syndromes and mouse phenotypes associated with the loss of the major DSB signaling and repair proteins is provided.

5.1 ATM

Mutations in the *ATM* gene result in the devastating disease ataxia-telangiectasia (A-T). A-T is an autosomal recessive disorder. Clinical symptoms of A-T include cerebellar ataxia, oculocutaneous telangiectasias, immunodeficiency, growth retardation, lack of gonadal development, insulin resistance and increased susceptibility to lymphoid cancers (Lavin, 2008). The ataxia manifests itself at an early age when the child starts to walk and worsens with time as the patients usually becomes wheel-chair bound by the end of the first decade of their life. Ataxia in A-T patients is caused by degeneration of Purkinje and granular cells in the cerebellum. A-T patients are immunodeficient, have reduced thymus size and lower serum levels of IgG, IgA and IgE. One of the striking features of A-T is a severe sensitivity to ionizing radiation. This has been observed in both A-T patients who were undergoing radiotherapy and in A-T cells grown in culture. Some patients with A-T develop insulin resistance and diabetes. Although this particular symptom was initially hard to explain through the DNA damage signaling functions of ATM, it has recently become clear that ATM is also a key player in insulin signaling and that it can protect against metabolic disorders (Halaby et al, 2008; Matsuoka et al, 2007). About one third of A-T patients develop lymphoid malignancies.

The phenotype of Atm-deficient mice closely resembles what has been observed in A-T patients. The mice are growth-deficient, sterile, immunodeficient and display increased radiosensitivity. In addition, most mice succumb to thymic lymphoma by 6 months of age (Barlow et al, 1996). Furthermore, it has been reported that *Atm⁻/⁻* mice have CSR defects (Kotnis et al, 2009). Interestingly, *Atm⁻/⁻* mice do not recapitulate entirely the severe neurodegeneration observed in A-T patients. Mild ataxia has been reported in *Atm⁻/⁻* mice, however there were no signs of degeneration in the cerebellum such as those observed in A-T patients (Barlow et al, 1996). This suggests that although ATM functions are mostly conserved from mice to humans, it might not be the case in neuronal cells of these organisms.

5.2 The MRN complex
5.2.1 MRE11

Hypomorphic mutations in the human *MRE11* gene lead to A-T like disorder (ATLD). ATLD is a very rare disorder that shares many similarities with A-T disease (Stewart et al, 1999). ATLD patients display progressive cerebellar ataxia and their cells are radiosensitive. However, in contrast with A-T patients, ATLD patients display normal Ig levels, lack of telangiectasia occurrence and they do not have increased susceptibility for cancer development (Taylor et al, 2004).

Straight knockout mice for *Mre11* are embryonic lethal, suggesting that Mre11 is necessary for embryonic development (Xiao & Weaver, 1997). An ATLD mouse model was developed in which a hypomorphic Mre11 is expressed (*Mre11^ATLD1/ATLD1* mice). These mice appear to have normal growth; however mouse embryonic fibroblasts derived from these mice have increased radiosensitivity, blunted intra-S and G2/M checkpoints and higher levels of genomic instability (Theunissen et al, 2003). However, *Mre11^ATLD1/ATLD1* mice did not develop

lymphomas. Interestingly, female $Mre11^{ATLD1/ATLD1}$ mice have a severely reduced fertility due to an inability of developing embryos to proliferate properly.

5.2.2 NBS1

NBS1 hypomorphic mutations are the underlying cause for the Nijmegen Breakage syndrome (NBS) (Carney et al, 1998). NBS patients display stunted growth, microencephaly, immunodeficiency, increased radiosensitivity and increased cancer incidence (Digweed & Sperling, 2004). NBS patients have decreased IgA and IgG level which shows that NBS1 plays an important in CSR (van Engelen et al, 2001). Some female patients also showed ovarian failure and amenorrhea. Lymphomas are the most common malignancies observed in NBS patients although other cancers such as medulloblastomas were also diagnosed in these patients (Digweed & Sperling, 2004).

As with *Mre11*, straight knockout mice of *Nbs1* are not viable (Zhu et al, 2001). Mice with conditional deletion of Nbs1 in B cells had increased genomic instability in B cells and CSR defects (Reina-San-Martin et al, 2005). However and interestingly so, mice with hypomorphic mutation of *Nbs1* that mimics a mutation observed in NBS1 patients display a generally milder phenotype than what is observed in NBS patients (Williams et al, 2002). Mouse embryonic fibroblasts derived from these mice show increased sensitivity to DNA damaging compounds, defects in cell cycle checkpoints and increased chromosomal instability. However, these mice with homozygous *Nbs1* hypomorph mutation do not show immunodeficiency, increased susceptibility to tumor development or female sterility.

5.2.3 RAD50

Recently, a human disorder caused by mutations in the *RAD50* gene was characterized (Waltes et al, 2009). This disorder was described as being NBS-like since the only known patient with this disorder shares similar features with NBS patients. Clinical features of the RAD50 (NBS-like) disorder patient include microencephaly, growth retardation and slight ataxia. The patient has a normal immune system and did not develop any tumors by the age of 23. Cells derived from the patient displayed radiosensitivity, G1/S and G2/M checkpoint defects, radioresistant DNA synthesis and increased genomic instability.

Rad50-null mutant mice are not viable and die early during embryonic development. Viable *Rad50* hypomorphic mice were generated (Bender et al, 2002). These mice have strong growth defects and most die from anemia caused by hematopoietic stem cell failure. Mutant mice that survive develop lymphomas and leukemia and males exhibit degeneration in the testes. Mouse embryonic fibroblasts with the *Rad50* hypomorphic mutation are not sensitive to radiation or DNA damaging agents and do not perform radioresistant DNA synthesis.

5.3 RNF168

RNF168 was recently identified as the gene mutated in the RIDDLE (radiosensitivity, immunodeficiency, dysmorphic features and learning difficulties) syndrome (Stewart et al, 2009; Stewart et al, 2007). The RIDDLE syndrome was identified in only one patient to date and is characterized by immunodeficiency with decreased IgG levels but slightly increased IgA and IgM levels. The RIDDLE patient also displayed a mild decrease in motor and learning abilities, shorter stature and facial dysmorphism. Fibroblasts derived from the RIDDLE patient showed increased radiosensitivity. Interestingly, another patient with

RNF168 gene mutation was recently identified (Devgan et al, 2011). This patient also displayed short stature, cellular radiosensitivity and low serum IgA. In addition, this patient was reported to also display A-T like symptoms including ataxia, ocular telangiectasias, microencephaly, and immunodeficiency with very low IgA levels. Both patients with RNF168 deficiency did not develop tumors.

Rnf168 null mice were recently generated (Bohgaki et al, 2011). These mice display normal growth and development and do not develop malignancies. They do however exhibit immunodeficiency mainly characterized by defects in CSR. Cells deficient for Rnf168 display increased radiosensitivity and genomic instability and elevated cancer risk was observed in mice lacking both Rnf168 and p53.

5.4 RNF8

Human syndromes caused by mutations of the *RNF8* gene have yet to be identified. However, Rnf8 mouse knockout models have been generated and have produced interesting phenotypes that are worth discussing here. *Rnf8⁻/⁻* mice are viable and are born in normal mendelian ratio (Li et al, 2010; Santos et al, 2010). They have growth defects, male sterility, increased radiosensitivity and immunodeficiency. *Rnf8⁻/⁻* mice display reduced CSR and increased genomic instability. A broad spectrum of tumors including lymphomas, sarcomas and breast tumors developed in *Rnf8⁻/⁻* mice (Li et al, 2010). It would be interesting to determine whether mutations of *RNF8* gene lead to human genetic disorders or if RNF8 loss correlates with cancer development in humans.

5.5 BRCA1 and BRCA2

BRCA1 and *BRCA2* are two breast and ovarian cancer susceptibility genes (O'Donovan & Livingston, 2010). Germline mutation of one *BRCA1* allele results in an up to 80% cumulative risk of breast cancer and a 30-40% risk of ovarian cancer by 70 years of age. Carriers of a *BRCA2* mutation have a 50% cumulative risk of breast cancer and a 10-15% risk of developing ovarian cancer by age 70. In addition, germline *BRCA2* mutations have been implicated in familial prostate cancer (McKinnon & Caldecott, 2007). Tumors in *BRCA1* or *BRCA2* mutation carriers most often lose the second *BRCA1* or *BRCA2* allele through loss of heterozygocity. Recently, it has been discovered that BRCA1 and BRCA2-negative tumors are very sensitive to a class of compounds known as PARP inhibitors. PARP inhibitors leave unrepaired single strand breaks in the DNA. If these unrepaired breaks meet a replication fork either fork collapse or DSBs. In the absence of BRCA1 and BRCA2 DSBs are left unrepaired, thereby leading to cell death. In this way PARP inhibitors are able to specifically target and kill *BRCA1* and *BRCA2*-null cells, making these inhibitors powerful therapeutic candidates for BRCA1 and BRCA2 negative breast and ovarian tumors. This treatment strategy might also work for tumors with somatic inactivation of BRCA1 and BRCA2 (Carden et al, 2010).

Brca1 and *Brca2* knockout mice are embryonic lethal, reflecting the essential requirement for these two proteins (Hakem et al, 1996; Suzuki et al, 1997). Brca1-deficient cells displayed radiosensitivity and increased chromosomal abnormalities (Mak et al, 2000; McPherson et al, 2004). Females carrying *Brca1* targeted mutations in mammary tissue exhibit a long latency time before development of mammary tumors, however this latency time is reduced considerably in the absence of tumor suppressor proteins such as p53 and Chk2 (McPherson et al, 2004; Xu et al, 1999). Interestingly, it was recently shown that loss of 53bp1 in Brca1-null mammary epithelium prevented mammary tumor development (Bunting et al, 2010), suggesting that 53bp1 could eventually be targeted to treated Brca1-deficient breast cancer.

Similar to what was observed with *Brca1* conditional mutant mice, loss of Brca2 expression in mouse mammary epithelium leads to increased incidence of mammary tumors, the latency of which is shortened in the absence of a *p53* allele (Jonkers et al, 2001). Recently, study of a conditional knockout of Brca2 in mouse prostate epithelium showed that loss of Brca2 leads to increased prostate cancer incidence which is accelerated in the absence of p53 (Francis et al, 2010).

5.6 DNA ligase IV

Hypomorphic mutations in the *DNA ligase IV* gene in humans give rise to the ligase IV (Lig4) syndrome. Lig4 syndrome is an autosomal recessive disorder characterized by microencephaly, growth retardation, mental retardation, decreased red and white blood cell count, immunodeficiency and increased cancer susceptibility (O'Driscoll et al, 2001). Cells from Lig4 patients display increased radiosensitivity and are defective in NHEJ DSB repair, but they have normal cell cycle checkpoints (O'Driscoll et al, 2001).

Knocking out *DNA ligase IV* in mice results in late embryonic lethality with massive neuronal apoptosis and lymphocyte development arrest due to lack of V(D)J recombination (Frank et al, 1998). Mice with hypomorphic mutation of *DNA ligase IV* were obtained through a mutagenesis screen. These mice have growth defects, are immunodeficient and have hematopoietic stem cell exhaustion with age (Nijnik et al, 2007).

5.7 Artemis

Artemis is the gene mutated in radiosensitive-severe combined immunodeficiency (RS-SCID) (Moshous et al, 2001). RS-SCID is characterized by normal development but increased radiosensitivity and a complete absence of mature B and T cells that can be attributable to defects in V(D)J recombination. While null mutations of *Artemis* give rise to RD-SCID, hypomorphic mutations lead to a plethora of less severe immunodeficiency syndromes that are characterized by increased incidence of Esptein-Barr virus-induced lymphomas (Moshous et al, 2003).

Artemis knockout mice are viable and display normal growth. B cell development in these mice is arrested at early progenitor stages. In contrast to what is observed in humans some T cells are able to undergo V(D)J recombination and mature normally leading to a "leaky" SCID phenotype (Rooney et al, 2002). Artemis-deficient cells display increased radiosensitivity and genomic instability. Recently, Artemis-null mice were generated in a different genetic background (Xiao et al, 2009). These mice exhibit complete arrest of B and T lymphocyte development and do not present a leaky phenotype and thus recapitulate more closely RS-SCID symptoms.

5.8 DNA-PKcs

The first *DNA-PKcs* human gene mutation was recently identified in a patient presenting classical symptoms of RS-SCID (van der Burg et al, 2009). This patient did not have mature B or T cells but had normal natural killer cell numbers. Cells from the patient were unable to properly repair DNA double strand breaks and were deficient in NHEJ.

DNA-PKcs was initially identified as the gene inactivated in the classical scid mice. Later on, knockout mice for *DNA-PKcs* recapitulated the phenotypes of the scid mice (Gao et al, 1998). *DNA-PKcs-/-* mice exhibited no growth defects but they displayed arrested B and T cell development at early progenitor stages, impaired V(D)J recombination and increased cellular radiosensitivity.

5.9 NHEJ1

NHEJ1 (*Cernunnos-XLF*) gene mutations were identified in patients with immunodeficiency and microencephaly (Buck et al, 2006). These patients are characterized by bird-like features, microencephaly, progressive loss of B and T cells and growth retardation. They have low levels of circulating IgA and IgG. Cells derived from these patients are radiosensitive but display normal cell cycle checkpoint.

A mouse model in which *Nhej1* is deleted has been generated (Li et al, 2008). These mice display a much milder phenotype than what is seen in humans. *Nhej1*-null mice are viable, born at the mendelian ratio and have normal growth and development. Although B and T cell numbers are reduced in *Nhej1*-deficient mice, a normal development of immune cells is observed and V(D)J recombination was not impaired in Nhej1-null lymphocytes. On the other hand, B cells from Nhej1-deficient mice had defective CSR. Concomitant loss of Nhej1 and p53 resulted in the rapid occurrence of thymic lymphomas and medulloblastomas.

5.10 Ku70 and Ku80

Mutations in *Ku70* or *Ku80* genes have not yet been described in humans. However knockout mice have been generated for these two proteins. Similar to what has been observed for knockout mice of other NHEJ factors described above, Ku70 and Ku80 deficient mice display immunodeficiency with arrested B and T cell development and defective V(D)J recombination (Gu et al, 1997; Nussenzweig et al, 1996). Interestingly though, *Ku70$^{-/-}$* and *Ku80$^{-/-}$* mice displayed significant growth defects and reduced size compared to wild-type mice.

6. Conclusion

DNA double strand breaks are constantly generated in our cells either through external stressors such as radiation or through internal programmed events that are needed for normal physiological processes such as gametogenesis, V(D)J recombination and class switch recombination. The importance of quickly detecting and repairing these breaks is underscored by the plethora of human syndromes caused by mutation of genes coding for DSB signaling and repair proteins. These syndromes share many similarities which include neurological defects, growth defects, immunodeficiency, radiosensitivity, sterility and increased cancer incidence. Although many of these symptoms have been recapitulated in knockout mouse models of DSB response proteins, some discrepancies between human syndromes and mouse models are sometimes observed which highlight differential role or redundancy between DSB response proteins in humans and mice. Nevertheless, study of these models have provided great insight into the physiological functions of DSB response proteins and have led to rapid discoveries in this field. Finally, these studies resulted in a better understanding of the etiology of certain diseases such as cancer and provided potential new ways of treating these diseases.

7. Acknowledgements

8. References

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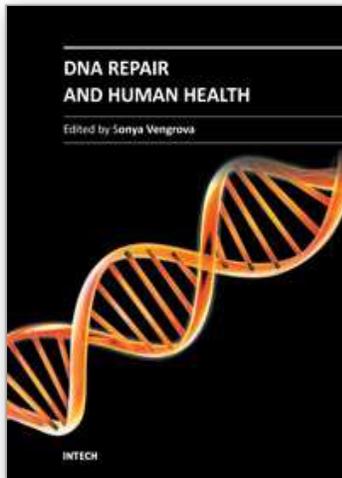
DNA Repair and Human Health

Edited by Dr. Sonya Vengrova

Over the past decades, great advances have been made in understanding the cellular DNA repair pathways. At the same time, a wealth of descriptive knowledge of human diseases has been accumulated. Now, the basic research of the mechanisms of DNA repair is merging with clinical research, placing the action of the DNA repair pathways in the context of the whole organism. Such integrative approach enables understanding of the disease mechanisms and is invaluable in improving diagnostics and prevention, as well as designing better therapies. This book highlights the central role of DNA repair in human health and well-being. The reviews presented here, contain detailed descriptions of DNA repair pathways, as well as analysis of a large body of evidence addressing links between DNA damage repair and human health. They will be of interest to a broad audience, from molecular biologists working on DNA repair in any model system, to medical researchers.

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