CHAPTER 1

CHROMATIN DYNAMICS DURING THE CELLULAR RESPONSE TO DNA DOUBLE-STRAND BREAKS

INTRODUCTION

The cells in our body are constantly exposed to a multitude of agents causing various types of DNA damage. DNA double stand breaks (DSBs) are among the most toxic types of damage because, if left unrepaired, they can give rise to e.g. deletions or translocations that may lead to cell death or even cancer. The cell has developed a number of intricate mechanisms to deal with different types of DNA damage, which is collectively called the DNA damage response (DDR). The importance of the DDR is illustrated by several genetic disorders, including Ataxia Telangiectasia, Xeroderma Pigmentosum and Nijmegen breakage syndrome, caused by inactivating mutations of DDR genes. Patients that suffer from these syndromes have a high prevalence of cancer due to the inability to properly repair DNA damage.

When cells encounter a DSB, cell cycle progression can be arrested at one of the cell cycle checkpoints. In this way, damaged cells are prevented to progress into the next cell cycle phase and are allowed time to repair the damage. Cell cycle progression can be stopped at the G1/S boundary before DNA replication, at the intra-S checkpoint during replication, or at the G2/M boundary before cell division. In the absence of checkpoints, unrepaired damage can result in mutations or deletions that either will be passed on to the next generation or lead to cell death (Warmerdam and Kanaar, 2010).

There are two major pathways to repair DSBs: Homologous Recombination (HR) and non-homologous end-joining (NHEJ). NHEJ represents fast but error prone repair of DSBs, whereas HR represents slow but error free repair (Figure 1,2).

HR requires a homologous sequence such as the sister chromatid as a template for repair. The repair process is initiated by the detection and binding of DSBs by the MRN complex which consists of MRE11, RAD50, NBS1. The MRN complex helps to process DSB which results in 3' hydroxyl single stranded DNA overhangs. Additionally, CtIP which forms a complex with BRCA1 promotes processing of DSB ends by the MRN complex. The single stranded DNA overhangs are rapidly bound by RPA which is eventually replaced by the central HR protein RAD51 involving BRCA2. RAD51 promotes strand invasion of the homologous sequence, DNA synthesis and exchange of the copied genetic information followed by ligation to complete repair (Figure 1).

NHEJ starts with detection of the DSB by the Ku70/80 heterodimer. Together with the DNA-PKcs kinase and the DNA, Ku forms the DNA-PK complex. DNA-PK is thought to mainly phosphorylate itself. Subsequently, the endonuclease Artemis is recruited to a subset of breaks where it associates with DNA-PK to resect the DNA (Riballo et al., 2004). After gap filling by DNA polymerases μ and λ, the XRCC4-ligase IV complex in association with XLF/Cernunnos carries out the final ligation of the DNA ends (Mahaney et al., 2009) (Figure 2).

NHEJ functions throughout the cell cycle, whereas HR only takes place in S and G2 phase when the sister chromatid is present as a template for repair. It is not entirely clear what determines the choice between NHEJ and HR in G2 phase. One factor that

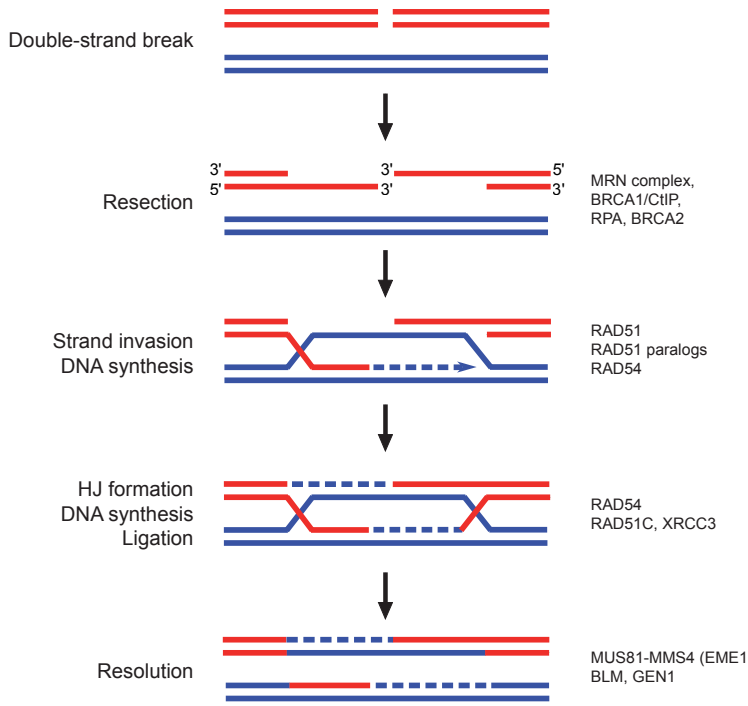

Figure 1. Model for DSB repair by Homologous Recombination. 5′–3′ resection of a broken end creates 3′ ssDNA tails that are rapidly coated by RPA. RPA is replaced by Rad51 to form a ssDNA-Rad51 nucleoprotein filament, which can initiate pairing with and strand invasion of a homologous duplex DNA. The 3′ end of the invading strand is extended by DNA synthesis using this duplex DNA as a template. The invading and extended strand is displaced and pairs with the other 3′ single stranded tail, allowing DNA synthesis to complete repair. The proteins involved in te distinct steps of HR are indicated.

might play a role in this choice is the complexity of the break: simple breaks that do not require (much) resection are repaired by NHEJ and complex breaks that require extensive resection are repaired by HR (Shibata et al., 2011). Another distinction can be made between the chromatin environments of DSBs: In G2 phase euchromatic breaks are repaired with fast kinetics by NHEJ and heterochromatic breaks are repaired with slow kinetics by HR (Beucher et al., 2009).

Upon detection of a DSB, a complex signaling cascade is set into motion that starts with the recruitment of the PIKK kinase ATM by the MRN complex. ATM phosphorylates histone H2AX forming γH2AX which is considered the major landmark of DSBs. γH2AX interacts with MDC1, an important checkpoint protein that acts as a binding platform for many proteins such as the E3 ubiquitin ligase RNF8 which, together with RNF168, ubiquitylates H2A-type histones at DSBs (Stucki et al., 2005; Huen et al., 2007; Kolas et al., 2007; Mailand et al., 2007) (Doil et al., 2009; Stewart et al., 2009). These ubiquitin chains are detected by the RAP80 subunit of

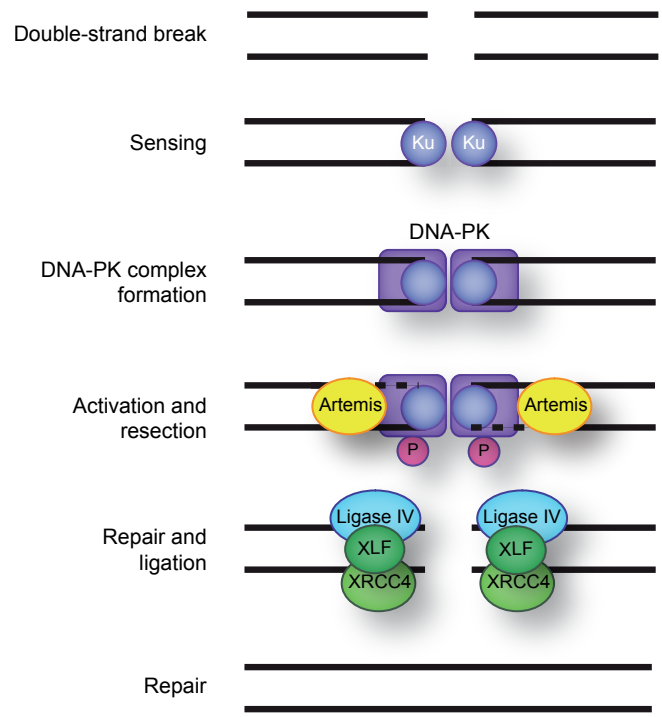

Figure 2. Model for DSB repair by Non-homologous End-joining. The DSB is detected and bound by the Ku70/80 heterodimer. Once bound to the DSB, Ku80 recruits DNA-PKcs. Following this, the DNA ends are processed by Artemis and/or MRN. Either before or after end processing, DNA-PKcs undergoes autophosphorylation, resulting in a conformational change that opens the central DNA binding cavity, releasing autophosphorylated DNA-PKcs from the DNA. Finally, the XRCC4/DNA ligase IV complex ligates the DNA ends in a reaction that is stimulated by XLF.

the Abraxas complex that contains BRCA1, BRCC36, Abraxas and MERIT40 (Figure 4) (Wang et al., 2007). Accumulation of 53BP1 at the break site is also dependent on RNF8/RNF168 ubiquitylation (Doil et al., 2009; Huen et al., 2007; Mailand et al., 2007) However, 53BP1 binds H4K20me2 at DSBs which is regulated by a different mechanism (Botuyan et al., 2006). It is currently unclear how RNF8 and RNF168 affect 53BP1 assembly at DSBs.

Genomic DNA is wrapped around histone proteins and tightly packaged into a multidimensional structure called chromatin. Even though chromatin is packed tightly, it can still be damaged. Consequentially, the DNA repair and signaling machinery has to overcome this chromatin barrier to access the lesion. This is facilitated by two classes of enzymes that modify chromatin structure. The first class consists of chromatin remodelers that use the energy from ATP hydrolysis to change the position or composition of nucleosomes along the DNA. The second class consists of proteins that change histone tail residues through posttranslational

modifications (PTM). Chromatin remodelers can either slide nucleosomes along DNA, evict nucleosomes from chromatin or exchange histones or histone dimers from nucleosomes (Clapier and Cairns, 2009). Chromatin remodelers of the SWI2/SNF2 family share a catalytic ATP binding helicase domain. This superfamily is divided into four subfamilies, namely SWI/SNF, CHD, ISWI and INO80, that can be distinguished by the presence of specific functional domains outside the ATPase domain (Figure 3) (Clapier and Cairns, 2009). Members of the SWI/SNF subfamily contain a bromodomain that binds acetylated histone tails (Kasten et al., 2011). The CHD family consists of 9 members that all contain a tandem chromodomain, which has affinity for methylated histones (Figure 3)(Sims and Wade, 2011). Furthermore, ISWI family proteins have a HAND SANT and SLIDE domain involved in DNA binding in the context of nucleosomes (Figure 3) (Yadon and Tsukiyama, 2011). Finally, the INO80 family does not have specific histone binding motifs, but contains an insertion in the ATPase domain (Figure 3) (Bao and Shen, 2011).

The main post translational modifications involved in the DDR are phosphorylation, acetylation, methylation, ubiquitylation poly(ADP-ribosyl)ation and SUMOylation. These histone modifications are collectively called the 'histone code' which is regulated by 'writers' that install histone modifications, 'readers' that are able to bind histone marks and 'erasers' that remove modifications from histones. Work from the last decade illustrates the importance of chromatin remodelers and histone modifiers in the DDR. In this chapter the advances in understanding the functioning of the DSB response in the context of chromatin will be discussed.

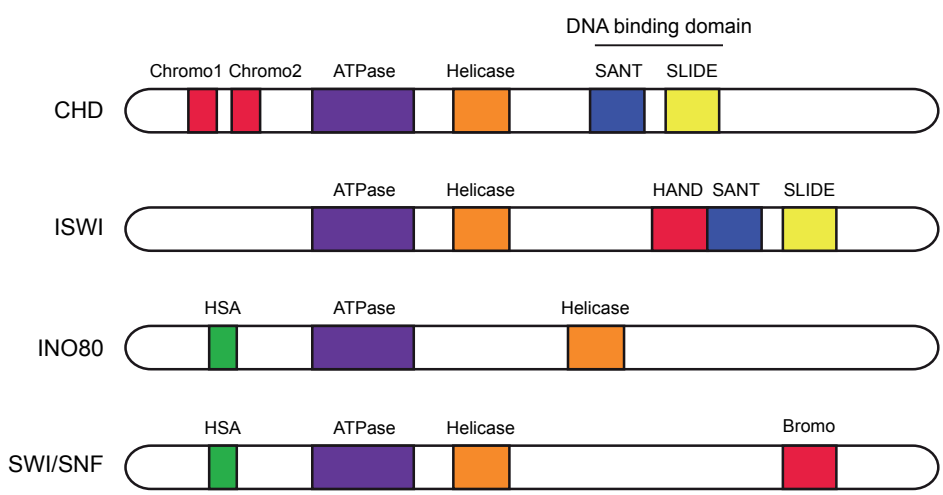

Figure 3. Schematic overview of the SWI2/SNF2 superfamily of chromatin remodelers. SNF2 family members share the highly conserved ATPase domain and helicase domain. In addition, the presence of other domains determines their classification into the four subfamilies (CHD, ISWI, INO80 and SWI/SNF).

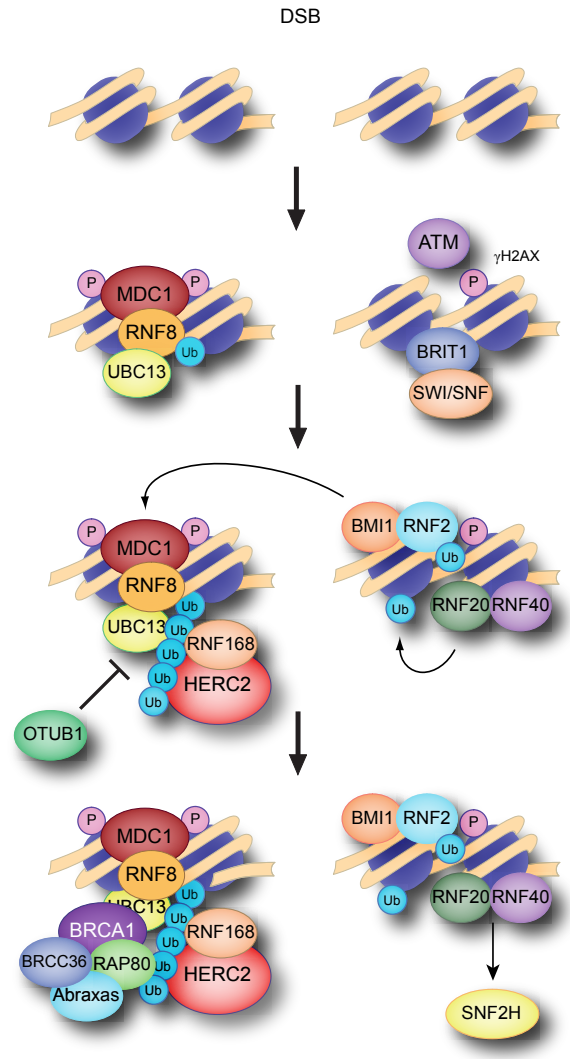

Figure 4. The DSB signaling pathway. DSBs are sensed by the MRN (MRE11-RAD50-NBS1) complex, which recruits the ATM kinase to these lesions. ATM-mediated phosphorylation of H2AX allows for accumulation of MDC1. BRIT1 facilitates accumulation of phosphorylated ATM, H2AX phosphorylation and SWI/SNF binding to γH2AX.
MDC1 generates binding sites for RNF8. Simultaneously, ATM phosphorylates HERC2, which stimulates its interaction with RNF8 and enables the formation of a MDC1-RNF8-HERC2 complex at sites of DNA damage. HERC2 stabilizes the interaction between RNF8 and Ubc13 to promote RNF8/Ubc13-mediated K63-linked poly-ubiquitylation of H2A-type histones. This in turn serves as a binding site for the MIU domains of RNF168. RNF168 augments poly-ubiquitylation of H2A type histones at sites of DNA damage in a Ubc13-dependent manner, which allows for recruitment of DNA repair factors such as the BRCA1 A complex. The accumulation of the BRCA1 A complex is mediated by its binding partner RAP80, which contains UIM domains that bind with high efficiency to K63-linked poly-ubiquitin chains on H2A-type histones. P: γH2AX ; Ub: Ubiquitin.

PHOSPHORYLATION

One of the most important PTMs leading to activation of DDR proteins is phosphorylation. There are three kinases of the phosphatidylinositol-3 kinase (PIKK) family responsible for activating proteins involved in the DSB response by phosphorylation on SQ/TQ motifs. Proteins that have a forkhead associated (FHA) domain or BRCA1 C terminal (BRCT) domain recognize and bind phospho-groups on phosphorylated proteins. Phosphorylation of H2AX at ser 139 (forming γH2AX) is considered the major hallmark of DSB recognition. H2AX makes up about 2-10% of the H2A pool in mammalian cells (Mannironi et al., 1994). H2AX differs from H2A by the presence of a C-terminal SQY motif which can be phosphorylated by the PIKK family members ATM, ATR and DNA-PK. Yeast H2A contains the same motif and can be phosphorylated by the ATM and ATR orthologs Mec1 and Tel1. ATM is the primary kinase responsible for phosphorylation of H2AX at DSBs (Burma et al., 2001). However, ATM functions redundantly with DNA-PK because cells deficient in either ATM or DNA-PK have normal γH2AX formation at DSBs (Stiff et al., 2004). Additionally, ATR phosphorylates H2AX in response to UV damage and replication stress (Ward and Chen, 2001).

The rapid phosphorylation of H2AX by ATM at DSB sites is followed by spreading of the γH2AX signal along large stretches of chromatin (0.5-2 Mb) flanking the DSB (Rogakou et al., 1999). At defined DSBs in yeast γH2A levels peak between 3 and 5 kb from the break site whereas γH2A is nearly absent at sites 1-2 kb away of the DSB (Savic et al., 2009; Shroff et al., 2004; Unal et al., 2004). A similar pattern can be found in human cells where γH2AX levels peak at sites between 20-300 kb away from the DSB, while levels are lower close to the DSB (Savic et al., 2009).

H2AX knock out mice are viable but show increased chromosomal aberrations in M-phase, enhanced sensitivity to IR and a G2/M checkpoint defect (Bassing et al., 2002; Celeste et al., 2002; Fernandez-Capetillo et al., 2002). Moreover, H2AX is dispensable for the initial DSB signaling steps but is necessary for the retention of signaling factors such as NBS1 and 53BP1 at the site of damage (Celeste et al., 2003). However, RAD51 foci formation is not affected in H2AX knock out mice (Fernandez-Capetillo et al., 2002). This suggests that H2AX is dispensable for DSB repair but important for the maintenance of genome stability. It has been reported that γH2AX mainly forms in euchromatic DNA (Cowell et al., 2007; Kim et al., 2007). This could indicate that heterochromatin first needs to be opened to facilitate γH2AX dependent signaling of DSBs in these regions. Indeed, the initial remodeling of damaged chromatin takes place independent of H2AX (Kruhlak et al., 2006).

A tight balance exists between phosphorylation and dephosphorylation of γH2AX. A number of phosphatases are involved in the regulation of γH2AX. In yeast, only one member of the PP2a phosphatase family, namely PPH3, is involved in maintenance of the G2/M checkpoint by dephosphorylation of γH2A (Keogh et al., 2006). In mammals, PP2ACα, PP2ACβ, PP4C, PP6C and WIP1 all have been found to dephosphorylate γH2AX (Keogh et al., 2006; Chowdhury et al., 2008; Douglas et al., 2010; Nakada et

al., 2008; Macurek et al., 2010; Cha et al., 2010; Moon et al., 2010). Depletion of these phosphatases impairs γH2AX removal and DSB repair and increases IR sensitivity of cells. However, other targets of these phosphatases have also been found within the DDR, e.g. PP4C dephosphorylates RPA which contributes to its role in HR (Lee et al., 2010a). Furthermore, WIP1 dephosphorylates p53 and MDMX, which also contributes to its role in the DDR (Lindqvist et al., 2009; Zhang et al., 2009). It would be interesting to further explore how these pleiotropic phosphatases contribute to the regulation of the DDR in response to damage.

ACETYLATION

Histone acetylation marks have been associated with a transcriptionally active or relaxed state of chromatin. It has recently become clear that acetylation of histone marks by histone acetyl transferases (HATs) is important to provide access for the DNA signaling and repair machinery to DSBs. Several HATs and histone deacetylases (HDACs) accumulate at DSBs and a number of acetylated histone marks have been associated with DSB signaling and repair.

For example, IR dependent acetylation of H2AX at K5 by the HAT Tip60 is required for ubiquitylation of H2AX at K119 which is required for proper DSB signaling (Ikura et al., 2007). However, Tip60 not only acetylates H2AX but also acetylates histone H4 at DSB sites in association with its cofactor TRRAP (Murr et al., 2006). Similar to Tip60, which accumulates at IRIF, the HATs CBP and p300 are recruited to laser induced damage and site-specific DSB sites monitored by ChIP (Ogiwara et al., 2011). CBP and p300 constitutively acetylate H2AX at K36 independent of H2AX s139 phosphorylation (Jiang et al., 2010). Although this H2AXK36ac is not increased in response to IR, it is required for a proper DSB response. Indeed, reconstitution with a non-acetylatable form of H2AX (H2AXK36A) severely impaired cellular survival in response to IR (Jiang et al., 2010). In addition, P300 and CBP also acetylate H3K18 and H4 (K5/K8/K12/K16) at DSBs (Ogiwara et al., 2011). It has been suggested that CBP and p300 facilitate SWI/SNF chromatin remodeling to provide access to the damage site for the NHEJ factors Ku70/80 (Ogiwara et al., 2011).

Another histone mark that may be required for the DSB response is H3K56ac. H3K56 is globally acetylated by CBP/p300 and GCN5 (Das et al., 2009; Vempati et al., 2010). Ogiwara et al. showed that H3K56ac was slightly enriched at sites of specific DSBs which was independent of CBP or p300 (Ogiwara et al., 2011). In contrast, the Jackson lab showed that H3K56ac is slightly reduced at site-specific DSBs (Miller et al., 2010; Tjeertes et al., 2009). Additionally, they observed a biphasic response of H4K16ac to DNA damage: After an initial reduction from laser induced damage sites (5 min), this modification was enriched at a later time point (2h) (Miller et al., 2010). Global deacetylation of both H3K56 and H4K16 was dependent on the joint action of HDAC1 and HDAC2. They found that the recruitment of HDAC1/2 to DNA DSB containing laser tracks facilitates removal of Ku and Artemis from the damage site thereby regulating NHEJ (Miller et al., 2010).

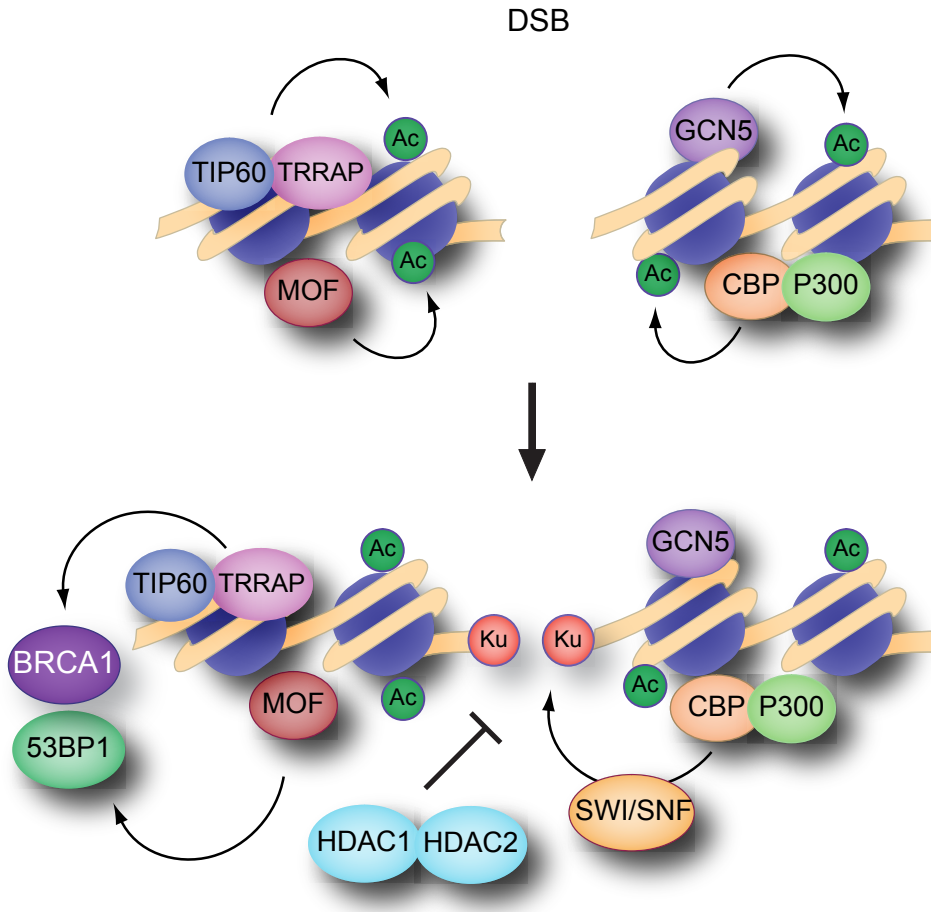

Figure 5. Histone acetylation in the DSB response. The TIP60/TRRAP, CBP/p300, GCN5 and MOF histone acetyltransferases acetylate histones at DSB sites to promote a proper DSB response. TIP60/TRAPP are required for BRCA1 and 53BP1 accumulation at DSBs. CBP/p300 mediate SWI/SNF dependent accumulation of Ku at DSBs. Acetylation of H4K16 by MOF promotes efficient MDC1 recruitment to DSBs and the subsequent accumulation of BRCA1 and 53BP1. HDAC1 and HDAC2 transiently reverse H3K56ac en H4K16ac and promote NHEJ, presumably by regulating Ku70/80 retention at the DSB site. Ac: acetylation.

Global acetylation of H4K16 in mammalian cells is facilitated by the HAT MOF (Li et al., 2010; Taipale et al., 2005). H4K16ac could potentially serve as a platform to facilitate proper DSB signaling. Indeed, MOF knockdown delayed γH2AX IRIF formation up to 20 min after IR (Sharma et al., 2010) whereas γH2AX IRIF formation in MOF depleted cells was normal between 1-2h after IR compared to control cells (Li et al., 2010; Sharma et al., 2010). Depletion of MOF resulted in defective MDC1 binding to γH2AX and impaired 53BP1 and BRCA1 IRIF formation (Li et al., 2010; Sharma et al., 2010). This defect in

DSB signaling results in impaired HR and impaired G2/M checkpoint arrest (Li et al., 2010; Sharma et al., 2010; Taipale et al., 2005). Future research will have to unravel how deacetylation and acetylation of histone marks are coordinated within the DSB response.

METHYLATION

Histone methylation is regulated by histone methyltransferases that generally have a SET domain which catalyzes the transfer of methyl groups to specific lysine or arginines residues. Proteins containing either a chromo domain or a tudor domain are able to bind methylated histones.

An example of a histone modification that is enriched at DSB sites is H3K36me2, which is installed by the methyltransferase Metnase and removed by JHDM1a (Fnu et al., 2011). H3K36me2 is required for both Ku70 and NBS1 accumulation at DSBs which promotes DSB repair by NHEJ (Fnu et al., 2011).

The best studied protein that is recruited to DNA damage sites due to the methyl binding properties of its tandem tudor domains is 53BP1 (Botuyan et al., 2006). In yeast, H3K79 methylation by Dot1 is required for mobilization of the 53BP1 paralog Rad9 (Botuyan et al., 2006; Grenon et al., 2007; Wysocki et al., 2005). It was first reported that 53BP1 would be recruited to DSBs through binding of H3K79me2 (Huyen et al., 2004) However, it was later found that DOT1-/- cells which did not express H3K79me2 did not show any defect in the accumulation of 53BP1 in IRIFs (Fitzgerald et al., 2011). Moreover, binding studies revealed that 53BP1 has high binding affinity for H4K20me2 instead of H3K79me2 (Botuyan et al., 2006; Huyen et al., 2004). Also, H3K79me2 is not upregulated at site-specific DSBs monitored by ChIP in humans cells (Fnu et al., 2011) but H4K20me2 is upregulated at ISce-I DSBs and laser-induced sites of damage (Pei et al., 2011).

Two proteins were found that are required for H4K20me2: The monomethylase PR-SET7/SET8 and the histone methyl transferase MMSET that facilitates H4K20 dimethylation (Botuyan et al., 2006; Oda et al., 2010; Pei et al., 2011). PR-SET7/SET8 accumulates at laser-induced damage sites and MMSET accumulates at site-specific DSBs (Pei et al., 2011). (Botuyan et al., 2006; Oda et al., 2010). Even though it was reported that PR-SET7/SET8 is recruited to sites of DNA damage (Oda et al., 2010), it is also likely that the constitutive monomethylation of H4K20 is required for subsequent dimethylation. It is well established that 53BP1 recruitment requires RNF8 and RNF168 dependent ubiquitylation at DSBs (Doil et al., 2009; Huen et al., 2007; Mailand et al., 2007). However it is not clear how the binding of 53BP1 to H4K20me2 is affected by RNF8/RNF168 dependent ubiquitylation. MMSET interacts with MDC1 in a DNA damage dependent way but this was independent of RNF8 (Pei et al., 2011).

Another factor that is required for 53BP1 recruitment is the E3 ubiquitin ligase BBAP that constitutively catalyzes monoubiquitylation of histone H4K91 in vitro and in vivo (Yan et al., 2009). Depletion of BBAP results in a decrease of PR-SET7 chromatin binding which reduces H4K20me and H4K20me2 (Yan et al., 2009). However, it is not clear how BBAP affects PR-SET7/SET8 retention at chromatin.

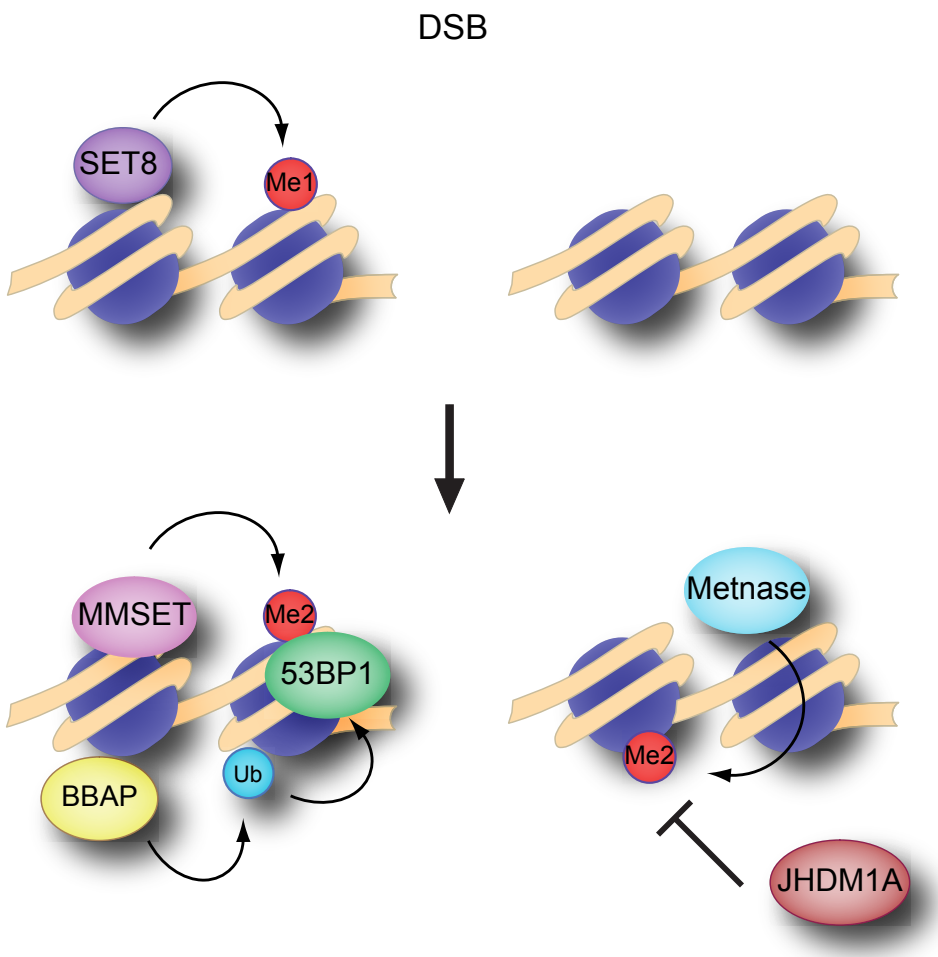

Figure 6. Histone methylation during the DSB response. Metnase dimethylates H3K36, a histone mark that promotes NHEJ and can be reversed by the histone demethylase JHDM1. SET8 monomethylates H4K20, whereas MMSET dimethylates H4K20, generating binding sites for 53BP1. Additionally, BBAP monoubiquitylates histone H4K91, which is required for SET8 chromatin retention and as such may affect 53BP1 binding at DSBs. Me: methylation; Me2: di-methylation.

UBIQUITYLATION

Ubiquitin is a highly conserved 76 amino acid protein which can 'label' proteins in a controlled manner. Ubiquitylation requires the cascade of ubiquitin-activating (E1), ubiquitin-conjugating (E2) and ubiquitin-ligating (E3) enzymes. In most cases this results in the addition of a ubiquitin to the ε-amino group of a lysine residue (Dikic et al., 2009; Haglund and Dikic, 2005; Hershko and Ciechanover, 1998). There are two distinct classes of E3 ligases: HECT-domain E3 ligases, which form a covalent intermediate with their substrate before ligating the ubiquitin to it and RING finger

domain containing E3 ligases, which do not have enzymatic activity but specifically recruit the target protein to the E2 ligase for direct attachment of ubiquitin (Ardley and Robinson, 2005; Deshaies and Joazeiro, 2009).

Ubiquitin has 7 lysine residues (K6, K11, K27, K29, K33, K48 and K63) any of which can potentially serve as a site of attachment for chain assembly. The various types of ubiquitylation events can alter the fate of target proteins in different ways. Mono-ubiquitylation of proteins can affect both transcription and chromatin remodeling. Polyubiquitylation at K48 targets proteins for proteolysis while polyubiquitylation at K63 is required for DNA repair or provides a scaffold for the nucleation of various signaling processes (Panier and Durocher, 2009).

Ubiquitylation of histones plays an important role in the development of the DSB response. Interestingly, until recently, no K48 linked ubiquitin chains have been detected in DSB containing laser tracks, which could be due to its transient nature (Doil et al., 2009; Sobhian et al., 2007). However, a recent report suggested that K48 ubiquitin chains are indeed accumulating at sites of laser-induced damage (Meerang et al., 2011). It is not known which ubiquitin ligases catalyze K48 ubiquitylation at DSB sites, but it might be the E3 ubiquitin ligase RNF8 together with the E2 ligase UBCH8 (Lok et al., 2011) However, it is not known whether UBCH8 is specifically recruited to DSBs. The only E2 ligase that is known to be recruited to DSBs is UBC13, which exclusively catalyzes K63 ubiquitylation (Kolas et al., 2007; Zhao et al., 2007). UBC13 functions together with the E3 ligases RNF8 and RNF168 to ubiquitylate H2A and H2AX at DSBs (Beucher et al., 2009; Doil et al., 2009; Huen et al., 2007; Kolas et al., 2007; Mailand et al., 2007; Pinato et al., 2009; Stewart et al., 2009). RNF8 is recruited to DSBs through interaction of its FHA domain with phosphorylated MDC1 (Huen et al., 2007; Kolas et al., 2007; Mailand et al., 2007). Next, the RING E3 ligase RNF168 binds the K63 ubiquitin chains generated by RNF8 by means of its tandem motif interacting with ubiquitin (MIU) (Doil et al., 2009; Stewart et al., 2009). RNF168 also interacts with UBC13 and is thought to stabilize and/or amplify the ubiquitin signal (Doil et al., 2009; Stewart et al., 2009).

Another regulatory level in the ubiquitylation cascade is provided by the E3 ubiquitin ligase, HERC2. It is thought that HERC2 stabilizes the interaction between RNF8 and UBC13, thereby reducing the interaction of competing E2 ligases with RNF8, allowing only K63-linked polyubiquitylation to take place (Bekker-Jensen et al., 2010). Additionally, knockdown of HERC2 resulted in reduction of over all RNF168 protein levels, yet it is not known how HERC2 affects RNF168 expression and whether these two proteins interact (Bekker-Jensen et al., 2010).

The K63 ubiquitin chains created by RNF8/RNF168 are binding substrates for RAP80. RAP80 has tandem ubiquitin interacting motifs (UIM) which are spaced in such a way that they can only bind K63 ubiquitin chains (Sato et al., 2009). RAP80 is part of the Abraxas complex together with BRCA1, BRCC36, Abraxas and MERIT40 (NBA1) (Wang et al., 2007). BRCA1 is an important player in HR, but very little is known about its exact function in these pathways. The E3 ligase activity of BRCA1 is not essential

for its function in HR and it is not known what its substrates are (Reid et al., 2008). It is thought that RAP80 binding to BRCA1 limits CtIP-BRCA1 complex formation thus restricting end-resection (Coleman and Greenberg, 2011; Hu et al., 2011).

An alternative scenario for the ubiquitylation of H2A and H2AX in response to DSBs has recently emerged. H2A and H2AX are monoubiquitylated at K119 and K120 by the E3 ligases BMI1 and RNF2 (RING1b) (Ismail et al., 2010; Pan et al., 2011; Wu et al., 2011; Facchino et al., 2010; Ginjala et al., 2011). These proteins form a dimer which is the stable core of the polycomb repressive complex 1 (PRC1). PRC1 is recruited to DSB-containing laser tracks and IRIF (Chou et al., 2010; Facchino et al., 2010; Ginjala et al., 2011; Ismail et al., 2010; Pan et al., 2011; Wu et al., 2011). It has been suggested that H2AX mono-ubiquitylation by BMI1/RNF2 precedes H2AX di-ubiquitylation by RNF8 (Ismail et al., 2010). H2AX mono-ubiquitylation by BMI1/RNF2 is required for phospho-ATM accumulation at DSBs, ATM dependent H2AX phosphorylation and the initial recruitment of MDC1 to DSBs (Pan et al., 2011; Wu et al., 2011). However, it is thought that in the absence of ATM, H2AX phosphorylation can be resumed by DNA-PK, resulting in delayed but normal MDC1 accumulation. Further studies will be required to investigate the regulation of H2A ubiquitylation by RNF8 and BMI1/RNF2 in the DSB response.

A histone modification that has also been implicated in the DSB response is ATM dependent H2B ubiquitylation by the RNF20/RNF40 E3 ubiquitin ligase heterodimer (Moyal et al., 2011; Nakamura et al., 2011). RNF20 facilitates both NHEJ and HR, yet it remains to be established whether this is solely due to its role in H2B ubiquitylation (Nakamura et al., 2011). Interestingly, it was reported that RNF20 was also required for recruitment of the chromatin remodeler SNF2H to DSBs (discussed below) (Nakamura et al., 2011).

Another dimension of ubiquitin regulation is provided by the deubiquitylating enzyme OTUB1 which regulates RNF168 mediated K63 ubiquitylation through its direct binding to UBC13, preventing the binding between RNF168 and UBC13 (Nakada et al., 2010). However, this function in the DSB response is independent of its catalytic activity (Nakada et al., 2010).

SUMOYLATION

Small Ubiquitin-like modifier (SUMO) is similar to ubiquitin and can be covalently linked to proteins to modify their function. Ligation of SUMO, or SUMOylation is organized in a very similar way to ubiquitylation (Kerscher, 2007). Most SUMO modified proteins contain a Ψ-K-x-D/E motif in which the K (lysine) can be covalently bound by SUMO. SUMO-proteases are very efficient in the removal of SUMO from target proteins.

The E1 SUMO activating enzyme SAE1, the E2 SUMO conjugating enzyme UBC9, the SUMO E3 ligases PIAS1 and PIAS4, SUMO1 and SUMO2/3 all accumulate at DNA damage sites (Galanty et al., 2009; Morris et al., 2009). PIAS1 catalyzes SUMO 2/3 and PIAS4 enables both SUMO1 and SUMO2/3 accumulation in DNA

damage-containing laser tracks (Galanty et al., 2009). It has become clear that crosstalk exists between ubiquitylation and SUMOylation within the DSB response. PIAS4 depleted cells showed a decrease in ubiquitylation and failed to recruit RNF168, but not RNF8 to damage sites (Galanty et al., 2009; Morris et al., 2009). Although both PIAS1 and PIAS4 facilitate RAP80/BRCA1 accumulation and BRCA1 K6 ubiquitin ligase activity, only PIAS4 is required for proper 53BP1 accumulation at DSB sites (Morris et al., 2009; Galanty et al., 2009). Both proteins are required for NHEJ and HR. Interestingly, RNF8 and RNF168 are not required for accumulation of PIAS1 and PIAS4 at laser tracks but are necessary for accumulation of conjugated SUMO1 and SUMO2/3. This indicates that PIAS acts in parallel to RNF8 but has an overlapping effect on the DDR (Galanty et al., 2009).

POLY(ADP-RIBOSYL)ATION

Poly(ADP-ribosyl)ation ((PAR)ylation) is another modification that has been implicated in the DDR. The synthesis of poly(ADP-ribose) (PAR) is facilitated by members of the poly(ADP-ribose) polymerase (PARP) family, which consists of 18 members (Ame et al., 2004). PARP-1 carries out the bulk of PARylation, but PARP-2 and PARP-3 are also important catalysts of PARylation. Moreover, PARP1 itself is the main PAR acceptor (Rouleau et al., 2004) and can be PARylated in vitro at lysine residues K498, K521 and K524 (Altmeyer et al., 2009). In contrast to PARP1 and PARP2, PARP3 is considered a mono(ADP-ribosyl)ase that can activate PARP1 in the absence of DNA (Loseva et al., 2010). Poly(ADP-ribosyl)ation of proteins has important regulatory properties but binding to PAR can also relocate proteins. Three types of PAR binding motifs have been described, namely the macrodomain, the PBZ zinc finger motif and a 10 amino acid consensus sequence found in a number of DNA repair and checkpoint proteins (Ahel et al., 2008; Gagne et al., 2003; Lagueux et al., 1994; Pleschke et al., 2000; Timinszky et al., 2009).

Upon DNA damage, PARP-1 rapidly binds DNA strand breaks and catalyzes PARylation of itself and that of other substrates such as histone tail residues (Mortusewicz et al., 2007; Poirier et al., 1982). PARP-1 binds single strand breaks and is required for recruitment of the single strand break repair protein XRCC1 to break sites (El Khamisy et al., 2003; Masson et al., 1998). It is thought that binding of PARP-1 to single strand breaks protects these lesions from processing until PARP-1 dissociates from the break by the accumulated negative charge induced by the presence of PAR polymers (Satoh and Lindahl, 1992).

The histone tail residues H2AK13, H2BK30, H3K27, H3K37 and H4K16 were also identified as substrates for PARP1-mediated ADP-ribosylation (Messner et al., 2010). It is known that poly(ADP-ribosyl)ation of nucleosomes induces a more relaxed chromatin state (Lagueux et al., 1994; Poirier et al., 1982). An attractive model would be that DNA damage induced chromatin relaxation is supported by the action of chromatin remodelers or histone chaperones.

Indeed, it was reported that the chromatin remodeler ALC1 accumulates at DNA damage containing laser tracks and binds to DNA damage induced poly(ADP-ribose) by means of its macrodomain (Ahel et al., 2009; Gottschalk et al., 2009). ALC1 ATPase activity requires the H4 N terminal tail which includes H4K16 (Ahel et al., 2009). However, it remains to be investigated whether poly(ADP-ribosyl)ated H4 targets ALC1 to the damage site.

Furthermore, it was shown that macrodomain containing histone variants bind poly(ADP-ribose) chains at DNA damage sites (Timinszky et al., 2009). MacroH2A1.1 incorporation at sites of damage is facilitated by the histone chaperone APLF (Mehrotra et al., 2011). APLF contains tandem PBZ domains, which facilitate its binding to PAR (Ahel et al., 2008). APLF accumulates at DSB sites where it interacts with Ku and XRCC4 and promotes the retention of XRCC4 (Bekker-Jensen et al., 2007; Iles et al., 2007; Kanno et al., 2007; Mehrotra et al., 2011; Rulten et al., 2011; Ahel et al., 2008). APLF functions in the same pathway as PARP3, facilitating proper DNA ligation by XRCC4/Ligase4 at chromosomal DSBs (Rulten et al., 2011). However, PARP3 may have functional synergy with PARP1 in the DNA damage response because PARP1$^{-/-}$/PARP3$^{-/-}$ mice are more sensitive to IR than the single mutants (Boehler et al., 2011). This suggests that PARP3 also functions separately from PARP1 and might have distinct targets for mono(ADP-ribosylation).

Another chromatin remodeler that is recruited to DSBs is CHD4, which is the ATPase subunit of the NuRD complex. Accumulation of CHD4 at laser induced damage was partially dependent on PARP (Polo et al., 2010). Furthermore, CHD4 is able to bind PAR in vitro although the protein does not have any known PAR binding domains (Polo et al., 2010). Further research will have to clarify the exact mechanism of regulation of DSB response proteins by PARP. Furthermore, we will need to establish how these chromatin remodeling factors collaborate to change the chromatin environment at DSB sites.

CHROMATIN REMODELERS

CHD

The CHD family of chromatin remodelers can be distinguished by their tandem chromodomains that bind methylated histone tail residues. CHD3 and CHD4 are mutually exclusive catalytic subunits of the NuRD complex, which combines histone deacetylation through HDAC1 and 2 with chromatin remodeling (Lai and Wade, 2011). Interestingly, it was found that the expression of several subunits of the NuRD complex (e.g. RBBP4 and RBBP7) is reduced in cells from Hutchinson-Gilford Progeria Syndrome (HGPS) patients and normally aged cells (Pegoraro et al., 2009). The reduced expression levels of these subunits coincided with loss of heterochromatic structures and increased levels of γH2AX, which is a marker of DNA damage. This suggested that NuRD prevents DNA damage accumulation by preserving higher-order chromatin structures and may act to maintain genome stability. This was further supported by

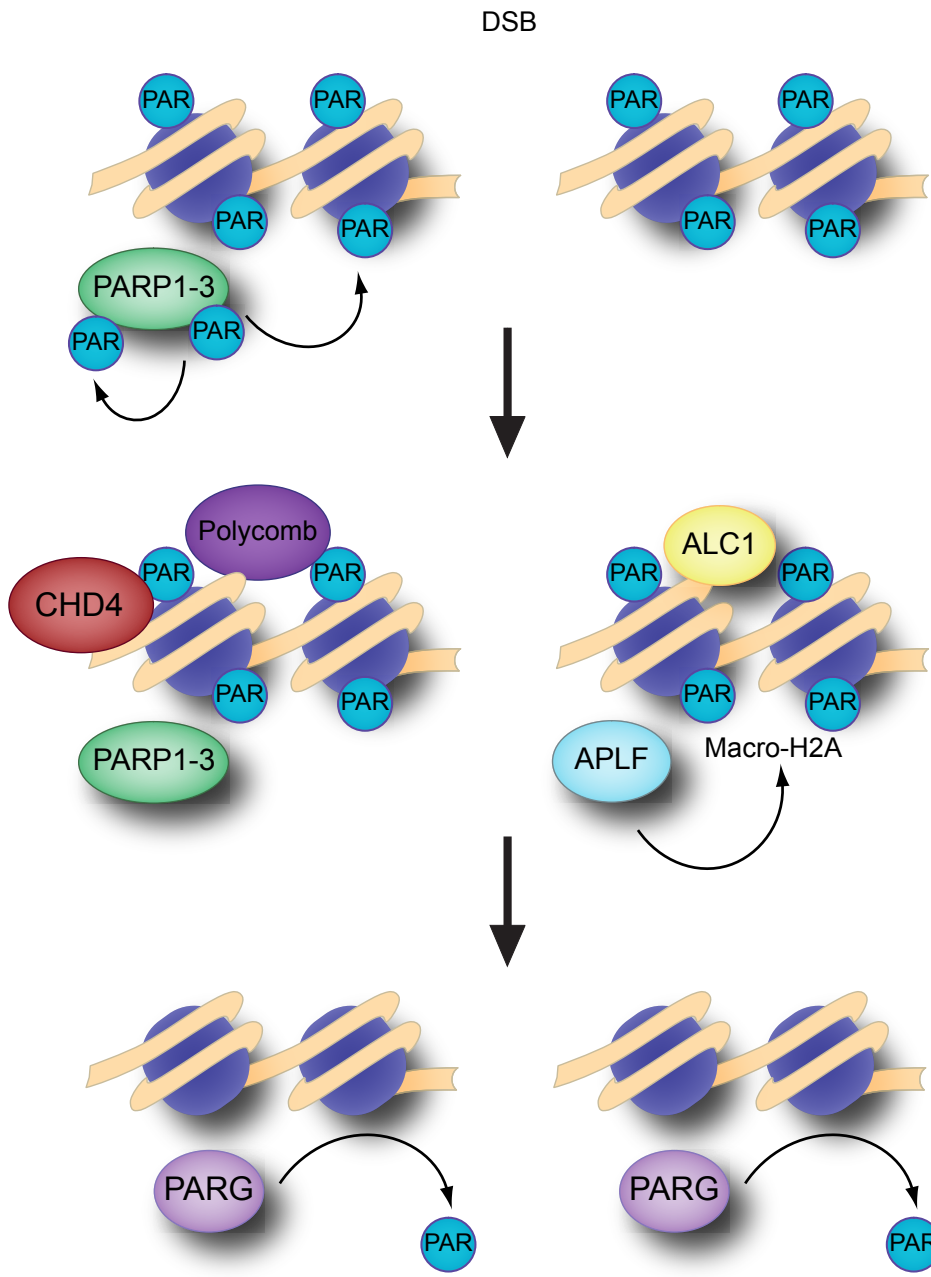

Figure 7. Poly(ADP-ribosyl)ation and the DSB response. PARP1, PARP2 and PARP3 accumulate at DSB sites and ribosylate substrates such as PARP1 (main PAR acceptor) and histone tails in response to damage. The accumulation of ALC1, APLF and CHD4 at DSBs is dependent on poly(ADP-ribose) (PAR). The histone chaperone APLF facilitates incorporation of macroH2A at DSB sites. PARylation is reversed by poly(ADP-ribose) glycohydrolase (PARG). PAR: poly(ADP-ribose)

the finding that several NuRD subunits were found to associate with ATR, one of the main kinases involved in the DDR (Schmidt and Schreiber, 1999). Moreover, many of the complex partners, such as CHD3 CHD4, MTA1, MTA2, HDAC1, HDAC2 and MBD3 accumulate at DSB containing damage (Chou et al., 2010; Goodarzi et al., 2011; Larsen et al., 2010; Polo et al., 2010; Smeenk et al., 2010). Although both CHD3 and CHD4 are involved in protection of cells against ionizing radiation (Larsen et al., 2010) they encompass distinct functions in the DDR.

CHD4 is required for proper IR induced ubiquitylation by RNF8/RNF168 and the subsequent recruitment of BRCA1 (Larsen et al., 2010; Smeenk et al., 2010). CHD4 is also required for initiation of the IR induced G2/M checkpoint (Smeenk et al., 2010). The phosphorylation of CHD4 at Ser-1349 by ATM is not required for its recruitment to laser induced damage (Polo et al., 2010; Matsuoka et al., 2007; Urquhart et al., 2011).

CHD3 functions as an inhibitory factor for DSB repair in heterochromatin. The release of CHD3 from SUMOylated KAP-1 as a result of ATM mediated KAP-1 phosphorylation induces relaxation of heterochromatin, which promotes repair of heterochromatic DSBs (Goodarzi et al., 2011). Indeed, depletion of CHD3 from cells induced global chromatin decondensation, whereas depletion of CHD4 did not (Goodarzi et al., 2011). It is tempting to speculate that CHD4 might have a more general role in the DSB response whereas CHD3 would have a role that is more specific for repair of heterochromatic DSBs. However, more experiments are needed to elucidate different and shared functions of CHD3 and CHD4 in the DSB response.

Another protein that is associated with the CHD family is Amplified in Liver Cancer (ALC1) or CHD1L which does not contain the tandem chromodomains, but has a C-terminal macrodomain that interacts with poly(ADP-ribose) (Ahel et al., 2009; Gottschalk et al., 2009). ALC1 is recruited to DNA damage in a PARP dependent fashion (Gottschalk et al., 2009; Ahel et al., 2009). However, how ALC1 operates during the DSB response remains largely elusive.

SWI/SNF

The SWI/SNF complex consists of an ATPase subunit - either BRM (SMARCA2) or BRG1 (SMARCA4) - and 8-10 BRM/BRG1 associated factors (BAFs) (Reisman et al., 2009). BRG1 is the ATPase subunit of several other complexes such as N-CoR, indicating that BRG1 has additional functions outside of SWI/SNF (Underhill et al., 2000). SWI/SNF has recently been implicated in the DDR: It is involved in a positive feedback loop in which γH2AX first triggers acetylation of H3 by recruiting the HAT GCN5 (Lee et al., 2010b; Park et al., 2006). SWI/SNF then binds to γH2AX containing nucleosomes through interaction of the BRG1 bromodomain with acetylated histone H3 (Lee et al., 2010b). This interaction increases chromatin accessibility and facilitates expansion of the γH2AX signal along the break site (Lee et al., 2010b). Other HATs that facilitate accumulation of SWI/SNF at DSBs are CBP, p300 and Tip60 (Ogiwara et al., 2011). It is thought that CBP and p300 are required for BRM accumulation at DSB-containing laser tracks which in turn facilitates the accumulation of Ku70 at DSB

sites (Ogiwara et al., 2011). In this way CBP/p300 and SWI/SNF share a functional interaction required for proper NHEJ.

Recently, the DDR responsive protein BRIT1/MCPH1 was found to associate with some of the core subunits of SWI/SNF through its N-terminal BRCT domain (Peng et al., 2009). It was suggested that BRIT1/MCPH1 is required for recruitment and retention of SWI/SNF at DSBs. Cells depleted of BRIT1/MCPH1 do not accumulate NBS1, MDC1, phosphorylated ATM and 53BP1 at IRIF, which might be caused by the lack of binding by SWI/SNF to γH2AX (Rai et al., 2006; Wood et al., 2007). Subsequently, BRIT1/MCPH1 depleted cells show a G2/M checkpoint defect and a DSB repair defect in both HR and NHEJ (Lin et al., 2005; Peng et al., 2009; Rai et al., 2006; Wood et al., 2007). Mutations in MCPH1 are associated with primary microcephaly (Jackson et al., 2002), but patients do not show dramatic sensitivity to DNA damaging agents or cancer predisposition (Wood et al., 2008). Interestingly, MCPH1 patient cells show premature chromosome condensation (PCC) which correlates with the association of BRIT1/MCPH1 to condensin II (Wood et al., 2008). This could explain the finding that depletion of BRIT1/MCPH1 induces increased chromatin relaxation after treatment with neocarzinostatin, which is suggested to be the result of loss of SWI/SNF (Peng et al., 2009).

ISWI

SNF2H (SMARCA5) is the ATPase subunit of a number of chromatin remodeling complexes (Bozhenok et al., 2002; Collins et al., 2002; LeRoy et al., 2000; Strohner et al., 2001). So far three complexes have been implicated in the DNA DSB response, namely hACF, CHRAC, and WICH, containing WSTF and SNF2H (Cook et al., 2009; Lan et al., 2010; Sanchez-Molina et al., 2011; Xiao et al., 2009). hACF and CHRAC both contain ACF1 and SNF2H and CHRAC additionally contains the subunits CHRAC15 and CHRAC17 (Collins et al., 2002; Poot et al., 2000). SNF2H is recruited to site-specific DSBs and laser-induced damage (Erdel et al., 2010; Lan et al., 2010; Nakamura et al., 2011; Sanchez-Molina et al., 2011). Similar to SNF2H, ACF1 accumulates at sites of laser damage, which is dependent on its N-terminal part containing the WAC, DTT and BAZ domains the latter of which are involved in DNA binding and the interaction with SNF2H (Lan et al., 2010; Sanchez-Molina et al., 2011). However, the recruitment of ACF1 was only partially dependent on SNF2H (Sanchez-Molina et al., 2011) suggesting that there might be another factor required for its accumulation. On the other hand, it was suggested that SNF2H accumulation at laser tracks is dependent on its C-terminal ACF1 interacting domain. However, there is no direct evidence that ACF1 promotes SNF2H recruitment to DSBs (Lan et al., 2010). In fact, RNF20 was also suggested to be required for the accumulation of SNF2H to DSBs (Nakamura et al., 2011). It remains to be established whether RNF20 is also required for ACF1 accumulation at DSBs.

All individual subunits of the CHRAC complex are required for both NHEJ and HR (Lan et al., 2010). Interestingly, ACF1, but not SNF2H is required for the accumulation of Ku in laser tracks, (Lan et al., 2010). However, it was found that ACF1 promotes

the G2/M checkpoint in response to IR (Sanchez-Molina et al., 2011). Given that it was previously observed that Ku is involved in the abrogation of the G2/M checkpoint (Lee et al., 1998; Nakamura et al., 2011; Wang et al., 2002) this opposing data might suggest that ACF1 is involved in different layers of regulation of the DSB response.

WSTF is another SNF2H associated protein implicated in the DSB response. WSTF forms a heterodimer together with SNF2H, called the WICH complex (Bozhenok et al., 2002). In addition to WICH, WSTF is part of the WINAC chromatin remodeling complex that contains BAF, FACT and CAF-1, indicating that WSTF also functions independent of SNF2H (Kitagawa et al., 2003). The kinase WSTF constitutively phosphorylates tyr142 of H2AX, a histone mark that can be removed by the phosphatase EYA1/3 upon induction of DNA damage (Xiao et al., 2009; Krishnan et al., 2009; Cook et al., 2009). Cells depleted of WSTF and cells that express a γH2AX construct containing a non-phosphorylatable Y142A mutation showed reduced expression levels of γH2AX, MDC1 and components of the MRN complex at IRIF (Xiao et al., 2009; Cook et al., 2009). This suggests that phosphorylation of tyr142 of H2AX is required for maintenance of the γH2AX signal. Interestingly, it had previously been shown that tyr142 is required for binding of MDC1 to γH2AX (Stucki et al., 2005). In this regard, another explanation for the defect in γH2AX maintenance in WSTF depleted cells could be that MDC1 needs this modification in order to bind γH2AX and recruit additional ATM for maintenance of the γH2AX signal (Stucki, 2009).

INO80

INO80 is a multisubunit complex that shares a number of subunits with other complexes such as the SWR1 complex and also contains some unique subunits such as ARP5, ARP8 and INO80. In yeast, the role of the INO80 complex in resection and repair has been well established (van Attikum et al., 2004; Morrison et al., 2004). Yeast INO80 is recruited to DSBs where it removes histones and facilitates resection and the subsequent repair of DSBs. However, the direct function of hINO80 in the DSB response is less well known. INO80 is recruited to sites of laser damage in an ARP8 dependent manner (Kashiwaba et al., 2010). Furthermore, it was suggested that both INO80 and ARP5, were required for γH2AX accumulation in chromatin suggesting that INO80 is required to open up the chromatin to enable γH2AX phosphorylation (Kitayama et al., 2009). Interestingly, the polycomb transcription factor YY1 forms a complex with several INO80 subunits. Functional assays indicated that YY1 and INO80 play a role in HR. It was suggested that in vitro YY1 preferentially binds Holliday junction recombination intermediates (Wu et al., 2007) which further supports a role for YY1 and INO80 in HR.

NuA4

The conserved NuA4 complex is a large complex that contains the HAT Tip60, the ATPase p400 and HAT cofactor TRRAP which is an enzymatically inactive member of the PIKK family of kinases (Doyon and Cote, 2004). Tip60 and p400 are recruited to

DSBs generated by both designer zinc finger nucleases and genotoxic agents (Xu et al., 2010). Acetylation of histones by Tip60 is required for proper DSB repair (Gottschalk et al., 2009; Ikura et al., 2000). Histone acetylation by Tip60 is increased at DSBs, which correlates with a decrease in nucleosome stability in response to DNA damage (Xu et al., 2010). This might reflect a switch in chromatin conformation to a more open, flexible structure. In line with this, it was shown that depletion of either p400 or TRRAP increased nucleosome stability in response to DNA damage. Additionally, p400 was found to be required for RNF8/RNF168 dependent ubiquitylation and the consequential accumulation of BRCA1 at DSBs (Xu et al., 2010). Interestingly, the recruitment of p400 to DSBs was dependent on MDC1, but not on RNF8 which suggests that p400 might interact with MDC1 to facilitate RNF8-dependent chromatin ubiquitylation (Xu et al., 2010). Further experiments are necessary to elucidate how p400 mediated chromatin remodeling in combination with acetylation by Tip60 can influence RNF8/RNF168 dependent ubiquitylation.

HISTONE CHAPERONES

Histone chaperone proteins bind histones from the moment of their translation and are involved in active shuttling of histone into nucleosomes. They shield histones from unwanted interactions to allow rapid incorporation into nucleosomes (Ransom et al., 2010). The histone chaperone FACT is a heterodimer that consists of the HMG protein hSSRP1 and hSPT16 (Orphanides et al., 1998). FACT is involved in the exchange of histone H2A with H2AX and FACT-mediated dissociation of H2AX from nucleosomes is mediated by phosphorylation of H2AX by DNA-PK (Heo et al., 2008). Yeast FACT associates with RPA and human FACT has been found to associate with PARP1, KU70/80, DNA-PK, Tip60 and PP2c (Heo et al., 2008; Huang et al., 2006; VanDemark et al., 2006). hSPT16 is PARylated by PARP1 which correlates with the dissociation of FACT from chromatin in response to DNA damage. This indicates that PARylation of FACT might down regulate FACT-mediated H2AX exchange (Huang et al., 2006). Additionally, hSSRP1 interacts with RAD54 and negatively regulates RAD54 branch migration of Holliday Junctions in vitro (Kumari et al., 2009) indicating that it might play a role in HR. Similar to FACT, the histone chaperone CAF-1 associates with Ku and DNA-PK (Hoek et al., 2011). Recently, it was shown that the CAF-1 subunit p150CAF-1 is required for HP1α and KAP-1 accumulation at DNA damage sites (Baldeyron et al., 2011). Furthermore, p150CAF1 promotes cell survival in response to IR and DNA repair by HR.

DSB REPAIR IN HETEROCHROMATIN

It has become clear that repair of DSBs in heterochromatin is different from repair in more relaxed euchromatic regions. Heterochromatic DSBs are repaired with slower kinetics compared to euchromatic lesions (Goodarzi et al., 2008; Kruhlak et al., 2006). It was

reported that ATM is specifically required for DSB repair in heterochromatin but dispensable for DSB repair in euchromatin (Goodarzi et al., 2008). ATM phosphorylates KAP-1 in response to DSBs which induces transient chromatin decondensation (Ziv et al., 2006). This chromatin decondensation is facilitated by inhibiting the repressive action of CHD3 on heterochromatin (Goodarzi et al., 2011). CHD3 interacts with constitutively SUMOylated KAP-1 and this interaction can be disrupted by the ATM-mediated phosphorylation of KAP-1 (pKAP-1) (Goodarzi et al., 2011). It was shown that heterochromatic pKAP-1 foci formation was dependent on MDC1, RNF8, RNF168 and 53BP1. Presumably 53BP1 spatially concentrates the MRN complex at DSB sites and in this way enhances ATM activity (Noon et al., 2010). Additionally, heterochromatic factors HDAC1/HDAC2 and HP1 are also required for proper heterochromatic repair (Goodarzi et al., 2008). HDAC1 and HDAC2 promote heterochromatin formation by removal of heterochromatin-inhibiting histone H3 acetylation marks (Knoepfler and Eisenman, 1999). All three HP1 proteins are recruited to sites of DNA damage. HP1-α and HP1-β, and HP1-γ accumulate at laser induced DNA damage but have different recruitment kinetics in heterochromatin and euchromatin (Baldeyron et al., 2011). Irradiation in heterochromatic domains of mouse embryonic fibroblasts (MEFs) induces transient dissociation of HP1α and HP1β directly after irradiation, whereas laser irradiation across the whole nucleus induces fast de novo accumulation at the damage site (Ayoub et al., 2008; Baldeyron et al., 2011; Luijsterburg et al., 2009). Phosphorylation of HP1-β at T51 by Casein Kinase 2 upon irradiation is thought to inhibit the interaction between the HP1β chromodomain and H3K9me2 in vitro (Ayoub et al., 2008). This might induce transient dissociation of HP1-β from damaged heterochromatic DNA facilitating γH2AX dependent signaling (Ayoub et al., 2008). On the other hand, the de novo accumulation of HP1 at DNA damage sites was found to be independent of its H3K9me2 binding properties (Dinant and Luijsterburg, 2009; Luijsterburg et al., 2009) which suggests that HP1 has additional roles in the DDR. Additionally, it was found that the histone chaperone CAF-1 is required for HP1α and KAP-1 accumulation at DNA damage sites (Baldeyron et al., 2011) possibly via the major CAF-1 subunit p150CAF-1 with the chromoshadow domain of HP1 (Baldeyron et al., 2011). Although p150CAF-1 depletion abolished RAD51 recruitment to laser induced damage sites, it did not induce a significant defect in HR (Baldeyron et al., 2011). An explanation for this discrepancy could be that RAD51 accumulation is only delayed by p150CAF1 depletion, since RAD51 was only monitored 5 min after laser damage. Furthermore, it needs to be established whether CAF-1 is only needed to deposit HP1 at sites of damage or whether there are alternate functions underlying the role of CAF-1 in the DDR.

DISEASE AND THERAPY

In the last decade, several studies identified chromatin remodelers and chromatin modifiers that are involved in the DDR, underscoring the importance of epigenetic regulation of the DDR. Most definitely, in the coming time more proteins will be found that help modulate the DDR. Future research will teach us how all these chromatin

modifiers collaborate to facilitate proper DSB signaling and repair. Possibly, the DSB cascade requires stepwise reorganization of the chromatin environment.

The gain of knowledge about the epigenetic regulation of the DDR will open doors for development of novel targeted epigenetic cancer therapies. PARP inhibitors are being used as a therapy to specifically attack BRCA1/2 deficient (breast) cancer cells in patients (Bryant et al., 2005; Farmer et al., 2005). PARP-1 binds to SSBs and is involved in base exision repair (BER) (El Khamisy et al., 2003; Masson et al., 1998). PARP inhibitors inhibit BER by trapping a SSB intermediate product (Strom et al., 2011). Additionally, PARP inhibitors may trap PARP at DNA lesions that might be converted into more toxic lesions during replication. Cells deficient in HR show synthetic lethality with PARP inhibitors. Currently, several PARP inhibitors are involved in phase II clinical trials (Fong et al., 2010; Plummer et al., 2008).

Additionally several studies have been done to investigate whether HAT, HDAC, histone demethylase and histone methyltransferase inhibitors would be suitable to use for cancer therapy (Biancotto et al., 2010). Since, in general, these drugs have a pleiotropic effect, the therapeutic mechanism would not necessarily be through inhibition of the DDR. The downside of the pleiotropic effect would be the potential occurrence of side effects, e.g. killing of healthy cells. Ideally, one would strive for the development of compounds that selectively inhibit the action of one chromatin modifier in cancer cells, similar to the PARP inhibitor. To develop these specific inhibitors, it is important to increase the knowledge of the spatio-temporal coordination of the DDR.