# 1

# Introduction

## 1.1 DNA DAMAGE AND REPAIR

Since the discovery of DNA in 1869 by Friedrich Miescher our understanding of its function has grown considerably. Initially the importance of DNA might not have been appreciated but subsequent experimentation, amongst others by Fredrick Griffith (Griffith, 1928) and Oswald Avery and co-workers (Avery et al., 1944), laid the foundation for our current understanding of DNA being the carrier of genetic, hereditary information. Conservation of the genetic information is of great importance as changes, in the form of DNA mutations, have the potential to contribute to the development of disease. It can be easily perceived that genomic mutations can lead to hereditary illnesses but also cancer, a common non-inherited disease caused by genetic alterations.

One factor that contributes to mutagenesis is damage to the DNA. DNA can be damaged through various processes which can be of endogenous or exogenous origin. Examples of endogenous processes that damage DNA are spontaneous deamination of bases or depurination of DNA as well as damage inferred through reactions involving reactive cellular metabolites such as reactive oxygen species (ROS). In addition to the endogenous processes environmental agents can also damage DNA, either through exposure to certain chemical compounds or by exposure to ultraviolet (UV) or ionizing radiation (IR). Damaged DNA may be toxic for the cell as it can interfere with metabolic processes such as transcription and replication which can lead to cell death (Ljungman et al., 1999;Kaina, 2003). However, perhaps more dangerous is the conversion of DNA damage to a mutation, a process that primarily depends on DNA replication. Once a DNA lesion has been converted into a mutation all information required to restore the original DNA sequence is lost. In contrast to mutations, damaged DNA often represents a reversible situation as the damage can be removed in order to reconstitute the original DNA configuration.

Given the importance of DNA for the health of an organism it is important to realize that chemical moieties of DNA are prone to DNA damage formation either by spontaneous degradation or attacks by endogenous agents. Although the frequency at which DNA lesions are formed within a cell depends to some extend on its environment it has been estimated that a human cell is subject to approximately 10.000 depurination events per day (Lindahl and Nyberg, 1972) as well as around 10.000 ROS induced DNA adducts (Ames and Shigenaga, 1992). Despite these high lesion frequencies the intergeneration mutation rate in humans was found to be low at approximately $1.1 \times 10^{-8}$ per base pair per generation, which equates to approximately 70 *de novo* mutations per diploid genome (The 1000 Genomes Consortium, 2010;Roach et al., 2010). To maintain such high level of genome integrity cells rely on DNA damage signaling pathways as well as DNA repair mechanisms for efficient removal of DNA lesions. Several pathways have been identified which respond to different types of DNA lesions (Figure1) (Hoeijmakers, 2001).

Nucleotide excision repair (NER) is a mechanism that is capable of removing a wide range of structurally unrelated DNA adducts, including solar UV-induced lesions as well as endogenously induced oxidative DNA lesions. The autosomal recessive disorders xeroderma pigmentosum, Cockayne syndrome, UV sensitivity syndrome and trichothiodystrophy are associated with defects in NER. An overview of NER is given in chapter 2.

Base excision repair (BER) is a pathway that is responsible for removing endogenous base lesions as well as repairing similar lesions generated by environmental agents. Among the base damages that are repaired by BER are ROS induced damages as well as base deaminations and depurinations. Also single strand DNA breaks are repaired via this pathway. Two subpathways have been identified: short-patch BER which depends on polβ for resynthesizing a single nucleotide (Kubota et al., 1996) and long-patch BER which utilizes polδ or polε for repairing a two to eight nucleotide gap (Klungland and Lindahl, 1997). Defects in DNA end processing factors have been associated with the neurological diseases ataxia with oculomotor apraxia type-1 (AOA1) and spinocerebellar ataxia with axonal neuropathy (SCAN1). The BER protein uracil-DNA glycosylase (UNG) also functions in immunoglobulin development and mutation induction in the respective gene can lead to hyper-IgM syndrome (Imai et al., 2003).

Mismatch repair is a pathway that is central to ensuring the fidelity of DNA replication (Hsieh and Yamane, 2008). Its function is to correct base substitution mismatches as well as insertion-deletion mismatches that are formed as a result of replication errors. Mutations in mismatch repair genes cause Lynch syndrome, a hereditary condition that is associated with a high risk of developing colorectal cancer.

Non-homologous end joining (NHEJ) is one of two major pathways that exist for the repair of double strand DNA breaks (DSBs). It functions by detecting and tethering DNA ends, processing of damaged DNA ends and ligation (Lieber, 2008). This method of rejoining broken DNA is regarded as being error prone as it does not require a template

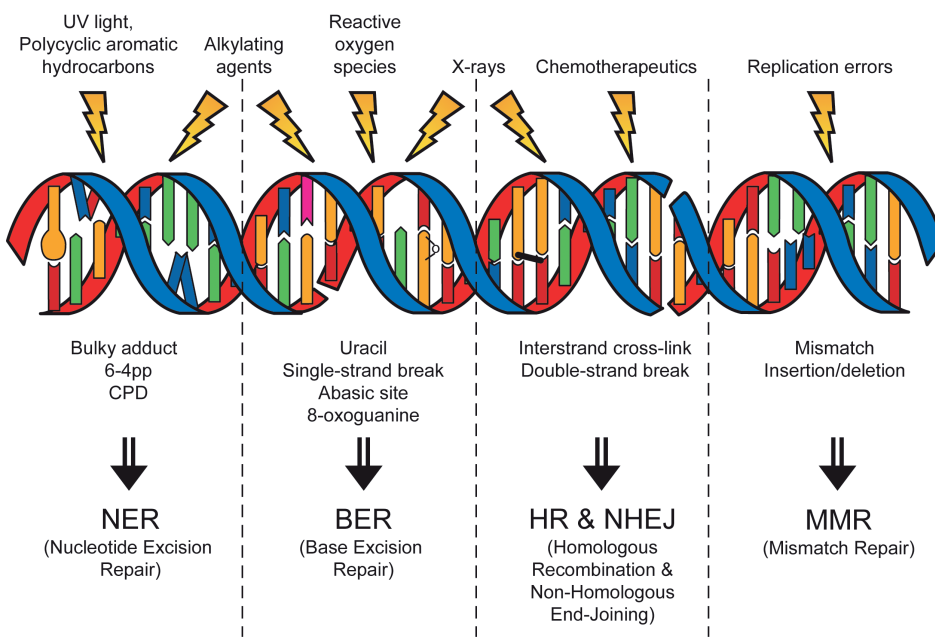


**Figure 1: Overview of damage causing agents and resulting DNA adducts along with their respective repair pathway.**

DNA for repair. As a result, small insertions or deletions can be found at the ligated break site. In mammalian cells NHEJ is the dominant pathway for repairing DSBs, in particular when cells are in the non-cycling G0/G1 state. Although DSBs can be induced by exogenous factors such as ionizing radiation, they are also created during immunoglobulin development. Consequently, defects in NHEJ can lead to radiation-sensitive severe combined immunodeficiency (RS-SCID) as well as ligase IV syndrome.

Homologous recombination (HR) is the second major pathway for repairing DSBs (Li and Heyer, 2008). Lesions that are repaired by HR include IR-induced DSBs, interstrand crosslinks and collapsed replication forks. HR repair is dependent on a sister chromatid to act as a DNA template and is therefore considered to be relatively error free. The requirement for a sister chromatid consequently restricts this form of repair to the S and G2 phases of the cell cycle where the chromatids are held in proximity by sister chromatid cohesion (Watrin and Peters, 2006). Defects in the HR genes BRCA1 and BRCA2 are linked to hereditary breast cancer.

Complementing these repair pathways are the DNA damage signaling cascades which regulate processes such as cell cycle progression and apoptosis (see also chapter 5). Although DNA repair and DNA damage signaling are distinct entities there exist proteins that function in both processes, for example ATM (Lobrich and Jeggo, 2005), the Mre11/Nbs1/Rad50 complex (Stracker and Petrini, 2011) and the cohesion complex (Yazdi et al., 2002;Kim et al., 2002b). While defects in cell cycle checkpoints are an important and easily measured consequence of defects in DNA damage signaling, the importance of the pathway is likely to reach beyond cell cycle regulation. Phosphoproteomic screens have revealed many proteins with diverse functions to be phosphorylated upon DNA damage, suggesting their functions may be modulated by DNA damage. One example being the regulation of DSB repair through the phosphorylation of Kap1 by the ATM kinase, which is a critical event for the repair of breaks in heterochromatinized DNA (Goodarzi et al., 2008).

## 1.2 DNA DAMAGE AND SIGNALING

There is overwhelming evidence that recognition of aberrant DNA structures by cellular surveillance proteins can initiate DNA damage signaling. Although damage signaling is often thought of to be synonymous with DNA damage induced checkpoint activation, it should be considered in a broader context of cellular responses to DNA damage. Most notably and in addition to checkpoint activation, DNA damage signaling can lead to induction of processes such as DNA damage repair, to changes in chromatin structure, transcriptional responses and apoptosis. To regulate these processes cells use a plethora of different post-translational modifications (PTM). Here those events most relevant for NER are described.

It has been long recognized that the checkpoint protein p53 becomes upregulated in response to DNA damaging agents such as UV light. In fact, as early as in 1984 it was reported by Maltzman and Czyzyk that p53 becomes stabilized in response to both UV and 4NQO treatment (Maltzman and Czyzyk, 1984). Moreover, these authors noted that while cycling cells were particularly efficient in their p53 response, also non-cycling cells were

able to induce p53 upon DNA damage, an effect that could be enhanced through the use of the DNA synthesis inhibitor hydroxyurea. Back then it was difficult to interpret these data, not least because p53 was thought to be an activator of replication rather than a checkpoint protein that is frequently mutated in tumours. However, ongoing research over the years has given new insights into the molecular mechanisms of both NER (chapter 4) and checkpoint activation allowing us to understand, in part, how these processes are linked.

### ATR dependent signaling

ATR is a serine/threonine protein kinase that is part of the PI(3) kinase-like kinase family to which also the ATM, DNA-PKcs, mTOR and SMG1 protein kinases belong (Durocher and Jackson, 2001;Yamashita et al., 2001;Brumbaugh et al., 2004). ATR together with ATM are considered to be the key kinases that orchestrate DNA damage signaling. Moreover there is evidence that each kinase is activated by a distinct DNA structure. For ATM it is the DNA double strand break (DSB) that is considered to be main activating DNA lesion (Lee and Paull, 2005), although other stressors can activate ATM as well (Bakkenist and Kastan, 2003;Kanu and Behrens, 2007;Soutoglou and Misteli, 2008). ATR in contrast is activated by single stranded DNA (ssDNA) containing ssDNA/dsDNA junctions (Zou and Elledge, 2003;MacDougall et al., 2007;Costanzo and Gautier, 2003).

Activation of ATR is particularly important in sensing DNA replication stress (Guo et al., 2000) and to prevent untimely entry into mitosis (Cliby et al., 1998;Nghiem et al., 2001). Detection of ssDNA and initiation of the DNA damage checkpoint is, however, a process that requires several other proteins in addition to ATR itself (figure 1). ATR forms an obligate dimer with ATRIP (Cortez et al., 2001) and it is the latter subunit of the complex that can interact with ssDNA bound RPA (Zou et al., 2003). However, recruitment of ATR to ssDNA is by itself insufficient for kinase activation (MacDougall et al., 2007) as ATR signaling was found to be dependent on the RAD9-RAD1-HUS1 complex (known as 9-1-1) (Bao et al., 2004). The 9-1-1 complex has a heterotrimeric structure bearing similarities with the homotrimeric PCNA complex (Parrilla-Castellar et al., 2004). Analogous to the loading of PCNA onto DNA by the replication factor C (RFC) complex the 9-1-1 complex is loaded onto DNA by an RFC complex containing RAD17 (Majka and Burgers, 2003). Loading of either PCNA or 9-1-1 complexes requires ssDNA/dsDNA junctions but, whereas PCNA is loaded at recessed 3' ends, 9-1-1 is preferentially loaded at 5' template junctions (Ellison and Stillman, 2003). The presence of ssDNA bound RPA is important here as well as this complex directs the loading of 9-1-1 towards the 5' primed DNA (Ellison and Stillman, 2003;Majka et al., 2006).

Both ATR-ATRIP and the 9-1-1 complex are recruited to signaling competent DNA structures independently of one another yet the presence of both complexes still does not suffice for kinase activation. To achieve activation a direct interaction between ATR and its activator TopBP1 (Kumagai et al., 2006;Mordes et al., 2008) is required. A phosphorylation site on RAD9, one of the 9-1-1 complex members, facilitates the interaction with the BRCT domains of TopBP1 and has been proposed to recruit the protein towards the ATR complex (Lee et al., 2007;Delacroix et al., 2007;Furuya et al., 2004;St Onge et al., 2003). However, other reports suggest that recruitment of TopBP1 is mediated directly via ATR-ATRIP (Choi et al., 2010;Yan and Michael, 2009;Rendtlew Danielsen et al., 2009).

Once activated, ATR can phosphorylate checkpoint proteins like H2AX, p53 and CHK1 (Tibbetts et al., 1999;Liu et al., 2000;Ward and Chen, 2001) which can initiate a cell cycle arrest. Also proteins that are crucial for ATR activation like ATRIP, TopBP1 and RAD17 are themselves targets for phosphorylation (Cimprich and Cortez, 2008). The importance of these phosphorylation events is, however, not known. Moreover, large scale phosphoproteomic screens for targets of ATR and ATM have revealed several hundreds of different proteins to be targets for these kinases after DNA damage suggesting that the DNA damage signaling response might effect many additional processes that are still unexplored (Matsuoka et al., 2007;Stokes et al., 2007).

### The MDC1 protein complex

A prominent feature of DNA damage mediated ATM/ATR activation, by virtue of H2AX phosphorylation, is the assembly of a multiprotein complex at or near the site of DNA damage in the vicinity of the kinase. Proteins that have thus far been identified as complex members include MDC1, the MRE11/RAD50/NBS1 subcomplex, 53BP1/ULP28, the BRCA1 complex (BRCA1, BARD1, BRCC36, ABRA1, RAP80), the ubiquitin ligases RNF8/HERC1 and RNF168, the sumo ligases PIAS1 and PIAS4, as well as UBC9 and UBC13 (reviewed in Panier and Durocher, 2009;Zlatanou and Stewart, 2010;Ciccia and Elledge, 2010). Complex assembly is achieved through the sequential recruitment of proteins and is regulated by post translational modifications like phosphorylation, ubiquitination and SUMOylation. Recruitment of the first factor, MDC1, is facilitated by the interaction of the MDC1 BRCT domain with phosphorylated H2AX. Subsequent ubiquitination and SUMOylation steps are then essential for full complex assembly i.e. the recruitment of 53BP1 and the BRCA1 complex. Thus far H2A type histones have been identified as targets for ubiquitin modification while BRCA1 was found to be modified by SUMO, however, it is well possible that additional proteins might be subject to these modifications.

The biological significance and functions of this multifactorial complex is perhaps not fully understood, but it is evident that proteins like BRCA1 and 53BP1 function in the DSB repair and checkpoint pathways (FitzGerald et al., 2009;Moynahan et al., 1999;Xu et al., 1999;Wang et al., 2002;Dimitrova et al., 2008). Furthermore, deficiency in H2AX, MDC1, 53BP1, RNF8 and RNF168 all result in immunodeficiency (Manis et al., 2004;Ward et al., 2004;Lou et al., 2006;Stewart et al., 2007;Ramachandran et al., 2010;Celeste et al., 2002).

### DNA damage signaling in UV-irradiated cells

In addition to the response to DSB, several components of the MDC1 complex, including MDC1, RNF8 and 53BP1 have been shown to respond to UV-induced DNA damage in an ATR dependent manner (Marteijn et al., 2009;Jowsey et al., 2007). This response is not restricted to replicating cells but also occurs in non-cycling (G0/G1) cells where it depends on functional NER (Marteijn et al., 2009). It should, however, be noted that the complex composition differs in quiescent cells when compared to replicating cells as at least one of its components (BRCA1) is expressed at very low levels in non-dividing cells (Chen et al., 1996;Choudhury et al., 2004; chapter 4). The functional importance of the UV-induced MDC1 complex in quiescent cells has yet to be established. It is clear that ATR dependent

signaling does not affect NER dependent removal of 6-4PP (Auclair et al., 2008; chapter 4) although it is very likely that repair of 6-4PP triggers this response.

Better understanding of which factors contribute to UV mediated checkpoint signaling came from studies using cells with genetic defects in various NER genes. It was demonstrated that impairment of TC-NER results in high p53 expression following treatment with either UV or inhibitors of transcription elongation. These observations led to the conclusion that most probably persistent stalling of RNA polymerase II initiated this signaling response (Ljungman and Zhang, 1996;Ljungman et al., 1999;Yamaizumi and Sugano, 1994). However, it has also been demonstrated that repair of DNA lesions by GG-NER could itself contribute to checkpoint signaling (Matsumoto et al., 2007;Marti et al., 2006). Surprisingly, even in the absence of both GG-NER and persistent RNA polymerase stalling DNA damage checkpoints are still activated through an alternative mechanism involving the endonuclease APE1 (chapter 4). It is surprising that although the DNA damage checkpoint can be induced through distinct mechanisms, they all depend on the ATR kinase to transduce the signal (chapter 4; O'Driscoll et al., 2003;Derheimer et al., 2007).

Poly(ADP-ribosylation) (PAR) is a post translational modification that plays key roles in a wide variety of processes (reviewed in Krishnakumar and Kraus, 2010) including DNA repair. The PAR modification is catalyzed by members of the poly(ADP-ribose) polymerase (PARP) family to which currently 17 proteins are ascribed. Of these the founding member, PARP1 as well as PARP2 have been implicated in DNA repair (de Murcia et al., 1997;Wang et al., 1997;Schreiber et al., 2002).The PAR modification is thought to assert its function through two different mechanisms. Firstly, the addition of ADP-ribose moieties might directly modulate the activities of the target protein through both steric as well as charge effects. Secondly, PAR structures can promote the recruitment of other proteins that contain PAR-specific binding motifs. Currently 3 such sequences have been identified: an 8 amino acid basic residue rich cluster (Gagne et al., 2008), the PAR-binding zincfinger (PBZ) (Ahel et al., 2008) and the macrodomain (Timinszky et al., 2009). A variety of DDR proteins contains PAR-binding motifs, although the significance of these motifs for the protein function in many cases has not been determined. For some of these proteins, however, PAR-dependent recruitment to sites of DNA damage has been observed, for example XRCC1 (Okano et al., 2003), CHFR (Ahel et al., 2008), APLF (Rulten et al., 2008;Kanno et al., 2007;Bekker-Jensen et al., 2007) and ALC1 (Gottschalk et al., 2009; Ahel et al., 2009; chapter 3).

Several DNA repair pathways utilize the PAR modification, most notably SSB repair and DSB repair (both microhomology mediated end joining and homologous recombination) (Wang et al., 2006;Hochegger et al., 2006), although a role in NER has also been proposed (Ghodgaonkar et al., 2008;Berger et al., 1980). The mechanisms by which PARP activity enhances NER are not fully understood but involve both TCR protein CSB as well as GGR (Flohr et al., 2003; chapter 3). The role of PARP in GGR is dependent on UV-DDB, although it is unclear whether UV-DDB directly activates PARP or whether other proteins are involved. The consequence of PARP activation and subsequent PAR synthesis is the recruitment of the chromatin remodeling protein ALC1, which is necessary for efficient repair of CPD lesions (chapter 3). The implication of PARP1 in CSB dependent repair of DNA lesions is based on

the epistatic relationship between the two proteins (Flohr et al., 2003). How PARP1 regulates CSB function or visa versa is currently unknown. CSB has a putative PAR binding site (Gagne et al., 2008); however, it is unclear if and how this contributes to the proteins function.

## 1.3 COHESINOPATHIES AND DNA DAMAGE

The term cohesinopathy is used to indicate diseases that affect the function of the cohesin complex. To date three such syndromes have been described i.e. Cornelia de Lange Syndrome (CdLS), Roberts Syndrome / SC phocomelia (RBS) and Warsaw Breakage Syndrome (WABS). Disease causing mutations have been identified for all three syndromes and are predicted to affect sister chromatid cohesion (SCC). The establishment and dissolution of SCC has been studied in some detail and it is clear that defects in this process compromise chromosome segregation. In little over a decade it has, however, become apparent that the function of cohesin reaches beyond its role in mitosis by participating in gene regulation (Rollins et al., 1999), DNA repair (Klein et al., 1999) and DNA damage signaling (Kim et al., 2002b). The underlying mechanisms for these diverse processes are not understood in detail, but it is likely that the unique ability for cohesin to shape chromatin topology has led to diversification of its function.

The cohesion complex consists of four subunits: Smc1, Smc3, Rad21 and either one of the SA1 or SA2 paralogues. Both Smc1 and Smc3 are members of a family of proteins known as the structural maintenance of chromosomes superfamily. Smc1 and Smc3 form long antiparallel intramolecular coiled coils by folding back on themselves at a central hinge domain, allowing the N- and C- termini of the protein to interact and form a functional ATPase (Losada and Hirano, 2005). The ATPase heads of Smc1 and Smc3 are linked by the Rad21-SA1/SA2 heterodimer which, combined with the interaction between the Smc1 and Smc3 hinge domains results in a ring-like structure. How cohesin rings interact with chromosomes in order to establish SCC is not exactly known. One model, known as the embrace model, sees both sister chromatids encircled by a single cohesin ring (figure 2) (Gruber et al., 2003). An alternative model, the handcuff model, envisions that both sister chromatids are individually encircled by a cohesin ring, which than are linked together via SA1/SA2 (Zhang et al., 2008b).

In addition to cohesin ring components, several accessory factors are required for cohesion regulation. Loading of cohesin onto chromatin requires the NIPBL-Scc4 complex (also referred to as Scc2-Scc4) (Ciosk et al., 2000). Although SCC can only be established during or after replication, cohesin is already bound to chromatin in G1 phase cells. It is, however, during S phase that cohesin becomes associated with chromatin in a far more stable manner (Gerlich et al., 2006). It is probable that this increased stability is the result of SCC establishment during replication, a process that requires Ctf18 (Lengronne et al., 2006;Terret et al., 2009) and the Esco1 and Esco2 paralogues (Skibbens et al., 1999;Toth et al., 1999). The latter two proteins both have acetyltransferase activity and acetylation of Smc3 is a critical event for establishing SCC (Zhang et al., 2008a;Rolef Ben-Shahar et al., 2008;Unal et al., 2008). Acetylation of Smc3 decreases the association of Wapl and Pds5 with cohesin and hence might regulate stability of the ring (Rowland et al., 2009;Terret et

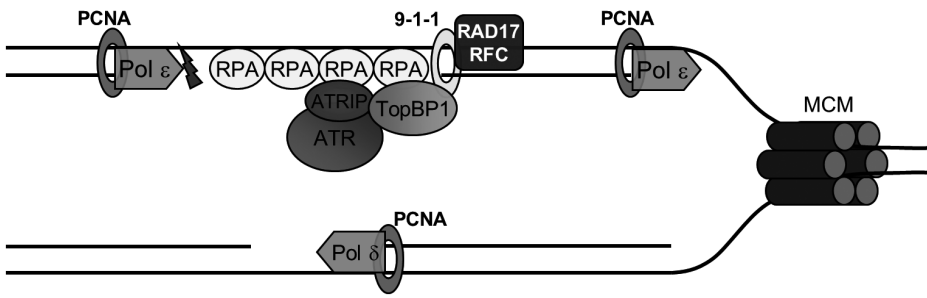


**Figure 2:Model of replication fork stalling and ATR activation**. DNA lesions can stall replicative DNA polymerases δ and ε. When the helicase becomes uncoupled from the arrested leading strand polymerase ε continuous unwinding of the parental DNA duplex by the MCM helicase creates a stretch of RPA bound ssDNA. Downstream reinitiating replication creates an 5'primer ssDNA/dsDNA junction that allows loading of the 9-1-1 complex by RAD17-RFC. Independently, the ATR-ATRIP complex is recruited to ssDNA through the interaction of ATRIP with RPA. Recruitment of TopBP1, which interacts both with RAD9 as well as ATR-ATRIP, promotes the activation of the ATR kinase activity.

al., 2009). Another, rather enigmatic, factor implicated in establishing SCC is DDX11 (ChlR1). The DDX11 homologue in *S. cerevisiae* was shown to physically and genetically interact with Eco1, the yeast Esco1/2 homologue (Skibbens, 2004;Parish et al., 2006). Depletion of DDX11 in human cells resulted in abnormal SCC, although it is unclear how the protein contributes to cohesion. The fact that it has DNA dependent helicase activity and interacts with replication associated proteins like Ctf18-RFC and Timeless would indicate it functions in cohesion establishment rather than maintenance (Farina et al., 2008;Leman et al., 2010).

Defects in various genes can cause cohesinopathy in humans. CdLS was originally found to be associated with heterozygous mutations in the NIPBL gene (Krantz et al., 2004;Tonkin et al., 2004), responsible for cohesin loading. Also the structural cohesin components SMC1 and SMC3 were found to be affected in CdLS (Musio et al., 2006;Deardorff et al., 2007). RBS on the other hand is caused by mutations in ESCO2 (Vega et al., 2005), whereas DDX11 was found to be defective in WABS (van der Lelij et al., 2010). While on the cellular level defects in SCC are evident in all three syndromes, clinical features such as growth and mental retardation, craniofacial anomalies and limb deformities indicate normal development is disrupted, possibly due to altered gene expression and cell differentiation (Dorsett, 2007). This suspected link between cohesin and gene regulation was strengthened when it was found that cohesin binds to the same genomic regions as the CCCTC-binding factor (CTCF) (Stedman et al., 2008;Wendt et al., 2008;Parelho et al., 2008;Rubio et al., 2008). CTCF has been implicated in diverse regulatory functions including transcriptional activation/repression and imprinting (Phillips and Corces, 2009). It has now emerged that cohesin shapes local chromatin topology at specific loci which is likely to modulate gene activity (Nativio et al., 2009;Hadjur et al., 2009;Hou et al., 2010;Mishiro et al., 2009) and thus would provide a basis for the observed developmental abnormalities in cohesinopathies.

Studies in *S. pombe* initially identified RAD21 as an important factor in DNA double strand break (DSB) repair (Birkenbihl and Subramani, 1992). However, subsequent studies

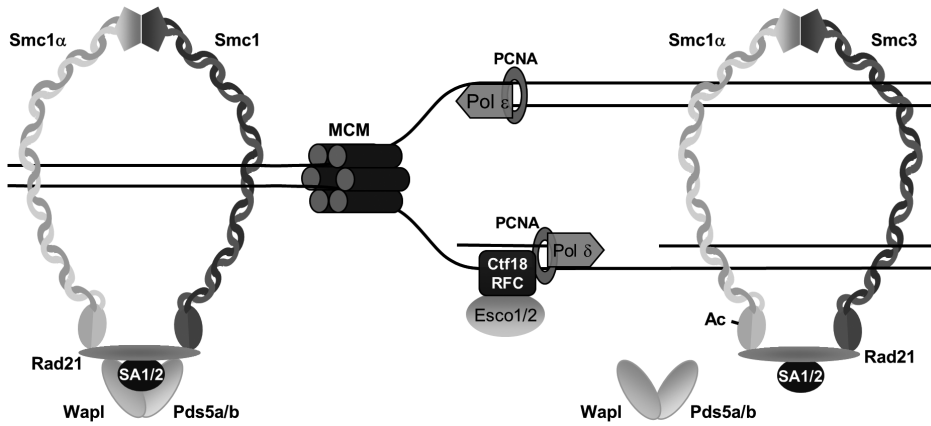


**Figure 3: Model for cohesin establishment at the replication fork**. During the cell cycle cohesion can dynamically interact with chromatin aided by NIPBL-Scc2 (not shown). Sister chromatid cohesion is established during S-phase in a replication dependent manner requiring the Ctf18-RFC complex and Esco1/2. Acetylation of Smc3 by the Esco1 or 2 proteins destabilizes the interaction of Pds5a or its paralog Pds5b with Wapl, causing a transition of cohesion to a more stable chromatin binding state.

demonstrated that other cohesin subunits are also important for this process when it became clear that establishing SSC was crucial for efficient DSB repair (Sjogren and Nasmyth, 2001). When it was found that CdLS was caused by defects in NIPBL, the question was how patient derived cells would respond to DNA damaging agents. As described in chapter 6 CdLS cells are indeed sensitive for DNA damage, in particular for the crosslinking agent mitomycin C. A similar sensitivity towards DNA damage was also observed in RBS (Van den Berg and Francke, 1993;Gordillo et al., 2008;van der Lelij et al., 2009) and WABS (van der Lelij et al., 2010) cells.

The reason why a deficiency in SCC results in sensitivity for DNA damaging agents is thought to lay in defective homologous recombination. When a DSB is created cohesin is recruited to the break site and establishes *de novo* cohesion (Kim et al., 2002a;Strom et al., 2004;Unal et al., 2004). This increased SCC is believed to bring the sister chromatids into close proximity thereby enhancing repair. Surprisingly, however, when a single DSB was introduced this not only led to increased SCC near the break site, but also on undamaged chromosomes (Strom et al., 2007;Unal et al., 2007). In yeast damage induced recruitment of cohesin near break sites is dependent on components of the DSB repair and signaling pathways such as Mre11, Mec1 and Tel1 (ATR and ATM in human) as well as H2AX phosphorylation (γH2AX). Establishing cohesion at the DNA break site also requires the acetyltransferase activity of Eco1. In undamaged cells the activity of this protein is impaired once S phase induced SCC has been established (Strom et al., 2007;Unal et al., 2007); a DSB would therefore need to activate the protein. This is achieved via phosphorylation of serine 83 of Mcd1 (Scc1) by the Chk1 kinase (Heidinger-Pauli et al., 2008). Chk1 is an effector kinase in the Mec1/Tel1 signaling pathway and is activated upon DNA damage.

Once phosphorylated, Mcd1 is acetylated by Eco1 generating a cohesin complex capable of establishing SCC (Heidinger-Pauli et al., 2009). Interestingly, as activity of the Chk1 kinase, once activated, is unlikely to be restricted to the break site it is possible that Mcd1 on other chromosomes is also targeted for phosphorylation, explaining the *trans* effect for *de novo* SCC after DNA damage.

Currently it is unclear if damage induced cohesion establishment is regulated in a similar manner in human cells. It is, however, without question that the that the ATM/ATR kinases play an important role in cohesin function as SMC1 and SMC3 are targets for damage induced phosphorylation (Yazdi et al., 2002;Kim et al., 2002b;Luo et al., 2008). Failure to phosphorylate SMC1 results in both checkpoint and repair defects (Kitagawa et al., 2004). Although the role of cohesin in checkpoint activation is independent of sister chromatid cohesion (Watrin and Peters, 2009), it is unclear whether the ability to establish damage induced cohesion is compromised when SMC1 or SMC3 cannot be phosphorylated. From phospho-proteomic screens designed to identify ATM/ATR targets it is also evident that SMC1 and SMC3 are not the only proteins in the cohesion pathway that are phosphorylated (Matsuoka et al., 2007;Stokes et al., 2007). Both CTF18 and ESCO1 were found to be modified following DNA damage, contributing to the complexity of this response.

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