Chromatin remodeling in the UV-induced DNA damage response

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Chromatin remodeling in the UV-induced DNA damage response

Remodellering van chromatine
in de UV-geïnduceerde DNA-schade respons

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Aşkım da değişebilir gerçeklerim de
Pırıl pırıl dalgalı bir denize karşı
Yangelmişim diz boyu sulara
Hepinize iyi niyetle gülümsüyorum
Hiçbirinizle dövüşemem
Siz ne derseniz deyiniz
Benim bir gizli bildiğim var
Sizin alınız al inandım
Sizin morunuz mor inandım
Ben tam kendime göre
Ben tam dünyaya göre
Ama sizin adınız ne
Benim dengemi bozmayınız

Turgut Uyar

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Scope of the Thesis

DNA damage interferes with transcription and replication, causing cell death, chromosomal aberrations or mutations, eventually leading to aging and tumorigenesis. The integrity of DNA is protected by a network of DNA repair and associated signalling pathways, collectively called the DNA damage response (DDR). Chromatin poses a barrier for DNA repair and as such plays a critical role in controlling DDR efficiency. Chromatin is modified to regulate access of repair proteins to DNA and also needs to be restored to its original configuration afterwards. Chromatin also serves as an optimal regulation platform for DNA repair by mediating signalling events, providing docking sites for signaling proteins and controlling their activity. The work that we describe in this thesis is focused on the role of chromatin remodelling in DDR, specifically the Nucleotide Excision Repair (NER) pathway.

To provide the necessary background on this emerging field, Chapter I summarizes the current state-of-art of chromatin modifications and ATP-dependent chromatin remodeling associated with the DDR. Chapter II describes the mammalian ISWI family of ATP-dependent chromatin remodeling complexes and its versatile roles in the DDR. We describe various essential functions through which ISWI complexes regulate the DDR and highlight how targeting of ISWI complexes to sites of DNA repair improves understanding of how chromatin remodeling complexes identify and associate with substrate nucleosomes *in vivo*.

In Chapter III, we identify a novel function for two distinct mammalian ISWI ATP-dependent chromatin remodeling complexes in resolving lesion-stalled transcription. Human ISWI isoform SMARCA5 and its binding partners ACF1 and WSTF are rapidly recruited to UV-C induced DNA damage to specifically facilitate CSB binding and to promote transcription recovery. SMARCA5 recruitment to UV-C damage depends on transcription and histone modifications and requires functional ATPase and SLIDE domains. Intriguingly, after initial recruitment to UV damage, SMARCA5 relocalizes away from the center of DNA damage which requires its HAND domain. This peripheral relocalization may be indicative of actual chromatin remodeling. Our data support a model in which SMARCA5 targeting to DNA damage-stalled transcription sites is controlled by an ATP-hydrolysis dependent scanning and proofreading mechanism, highlighting how ISWI chromatin remodelers identify and bind nucleosomes containing damaged DNA.

After analysing the role of ISWI in the UV-DDR, Chapter IV aims to identify the proteins with which SMARCA5 interacts in mammalian cells to have a deeper understanding of the mechanisms underlying SMARCA5 function in the UV-DDR. This chapter focuses on two proteomics experiments which give detailed insight in the interactome of SMARCA5, both before and after UV irradiation, and describes a characterization of the pathways in which SMARCA5 functions upon UV damage. Functional classification of interacting proteins revealed associations with transcription and translation machinery, RNA processing and export pathways, and chromatin reorganisation. Thus, our proteomics based data indicate that SMARCA5 is part of complex networks of proteins, which modulate transcription, translation and chromatin reorganization in response to UV.

Chapter V discusses the function of SWI/SNF, another ATP-dependent chromatin remodeling family, in the UV-DDR. Several subunits of SWI/SNF complexes were identified in a *C. elegans* screen for genes that protect against UV irradiation. Functional analysis in human cells confirms that the SWI/SNF BRM and BRG1 ATPases and several subunits are also essential for UV survival in mammals. Our results show that BRG1 interacts with NER-initiation factor DDB2 and regulates its mobility after UV irradiation. BRM interacts with DDB2 in a UV dependent manner. Both BRG1 and BRM are recruited to local UV-C damage and also appear to be essential for transcription recovery after UV. These data suggest that BRM and BRG1 function to regulate the initial steps of Global Genome NER by interacting with DDB2 and possibly also TC-NER.

Finally, Chapter VI concludes all results and discusses the future direction of chromatin remodeling involvement in the UV-DDR.

Chapter I

Introduction

1) DNA damage

DNA is continuously damaged by various endogenous and environmental factors. Damage in DNA interferes with transcription and replication, causing aging and mutations and/or chromosomal aberrations which result in tumorigenesis (Hoeijmakers, 2009).

There are various endogenous physiological processes that lead to DNA aberrations in organisms, such as DNA replication, abortive topoisomerase I and topoisomerase II activities, hydrolytic reactions and methylations. Moreover, reactive oxygen compounds which are produced as byproducts of oxidative respiration or through redox events (Jackson & Bartek, 2009), or reactive oxygen and nitrogen compounds that are produced by the immune system at sites of inflammation and infections (Kawanishi et al, 2006) can attack DNA, leading to adducts that impair base-pairing, cause base loss, DNA single-strand breaks and/or interfere with DNA replication and transcription (Jackson & Bartek, 2009).

Ultraviolet (UV) light is one of the most toxic environmental DNA damaging agents. UVC has the shortest wavelength (<290 nm) and is the most harmful part of the solar UV spectrum for DNA. UVC is fortunately absorbed by the ozone layer and even ordinary air. However, UVA (320-400 nm) and UVB (290-320 nm) are not completely blocked by the atmosphere and residual UVA and UVB in strong sunlight can still induce large amounts of DNA lesions in the cell (Ikehata & Ono, 2011). UVA creates oxygen radicals which predominantly and indirectly produces oxidative DNA damage. In contrast, UVB is directly absorbed by DNA and induces the chemical conjugation between two adjacent pyrimidines: cyclobutane pyrimidine dimers (CPDs) and 6-4 pyrimidine-pyrimidone photoproducts (6-4PPs). Another very toxic environmental DNA damaging agent is ionizing radiation (IR) causing different forms of damage, the most toxic of which are double strand breaks (DSBs). There are different sources of IR including the sun, naturally-occurring radioactive compounds or the radioisotopes used during cancer radiotherapy. Other important DNA damaging agents are various industrial chemicals like vinyl chloride and hydrogen peroxide and environmental chemicals such as polycyclic aromatic hydrocarbons found in smoke, soot and tar, creating a huge diversity of DNA adducts including ethenobases, oxidized bases, alkylated phosphotriesters and DNA crosslinks. These chemicals trigger various cancers, most notably those of the lung, oral cavity and adjacent tissues (Wogan et al, 2004). Cancer-causing DNA-damaging chemicals are also found in different foods, such as aflatoxins found in contaminated peanuts and heterocyclic amines in over-cooked meat.

2) DNA damage response mechanisms

To protect against the adverse effects of DNA damage, organisms are equipped with diverse complementary mechanisms of DNA repair, DNA damage tolerance processes to allow replication over damaged DNA and associated DNA damage signaling processes to induce cell cycle arrest, collectively called the DNA damage response (DDR) (Fig.1) (Hoeijmakers, 2009; Jackson & Bartek, 2009).

DNA damage is repaired by different repair mechanisms depending on the type of lesion, location in the genome and cell cycle phase. The major DNA repair mechanisms are mismatch repair (MMR), base-excision repair (BER), interstrand crosslink (ICL) repair, double-strand break (DSB) repair and nucleotide excision repair (NER). In addition to DNA re-

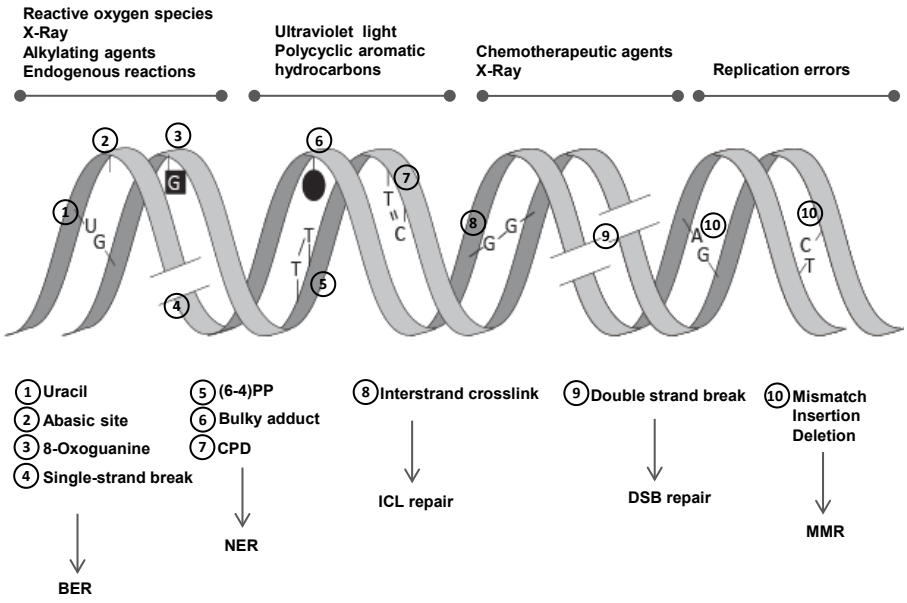

Figure 1. DNA damage and DNA repair mechanisms.
DNA is continuously damaged by various endogenous and exogenous sources in many different ways. Each class of DNA damage is repaired by a specialized DNA repair mechanism, including Base Excision Repair (BER), Nucleotide Excision Repair (NER), Interstrand Crosslink (ICL) Repair, Double Strand Break (DSB) repair and MisMatch Repair (MMR). See text for details.

pair, the presence of DNA damage is transduced by the DNA damage signaling response, which leads to cell cycle arrest or apoptosis. Since the discovery of DNA repair half a century ago, the basal mechanisms that underlie the DDR have been elucidated. In recent years, however, it has become apparent that there is extensive regulation of the DDR on both the transcriptional and posttranscriptional level and crosstalk with other cellular systems, including chromatin. In this chapter, the different DDR mechanisms will first be discussed, after which we discuss the regulation of DDR mechanisms in and by chromatin, with a particular focus on the role of ATP-dependent chromatin remodeling complexes.

a) Mismatch repair (MMR)

MMR plays a significant role in genomic stability and is a highly conserved mechanism (Iyer et al, 2006). The average spontaneous mutation frequency in humans is only 1 per 109-1010 base pairs per replication round. DNA mismatch repair is an important contributor to this high-fidelity copying process as it recognizes and repairs erroneous insertions, deletions and misincorporation of bases that emerge during DNA replication, recombination or the repair of DNA damage. Genetic inactivation of the mismatch repair system elevates spontaneous mutability 50-1000-fold.

DNA mismatch repair in eukaryotes is initiated by mismatch recognition during replication (Li, 2008; Modrich, 2006). First, the mismatch is bound either by a heterodimer of MutSα homologues (MSH2/MSH6), in case of base–base mismatches or one or two base loops, or by a heterodimer of MutSβ homologues (MSH2/MSH3), in case of larger loops of 2–14 bp. Then an ATP-dependent conformational change takes place that results in the recruitment of a MutLα (MLH1/PMS2) or MutLβ (MLH1/PMS1) heterodimer that is important to discriminate between the old and newly synthesized DNA strand. MutL heterodimers trans-

locate in either direction to search for strand discontinuity and nick the 3' or 5' side of the mismatched base on the discontinuous strand identified as newly synthesized. The stretch of DNA containing the mismatch is degraded by the EXO1 exonuclease, in cooperation with the single-stranded DNA-binding protein RPA. RPA also protects the ssDNA following excision and together with the clamp loader RFC and DNA clamp PCNA, facilitates DNA resynthesis by DNA polymerase δ. Finally, the resynthesized fragment is ligated by DNA ligase I.

b) Base-excision repair (BER)

BER mainly deals with non-bulky small nucleobase lesions, by excising and replacing in correct or damaged bases (Dianov & Hubscher, 2013; Kim & Wilson, 2012). One of the major causes of such lesions are reactive oxygen species (ROS) produced by normal cellular metabolism. They are also induced by oxidation, deamination and alkylation of nucleotides, resulting in the formation of damaged nucleotide derivatives, such as 8-oxo-7,8-dihydroguanine (8–oxoG), 3-methyladenine and hypoxanthine. Especially 8-oxoG lesions are harmful as these can lead to GC to TA transversions due to mispairing during replication, which are therefore quite common somatic mutations found in human cancers. Besides dealing with nucleobase lesions, the downstream steps of BER are also utilized to repair single-strand breaks.

DNA damage recognition in BER is performed by a group of different DNA glycosylases, each recognizing a specific type of (or group of structurally related) damaged bases. DNA-glycosylase enzymes recognize and remove damaged bases by 'base flipping' and subsequent cleavage of the N-glycosidic bond between the substrate base and the 2'-deoxyribose. Base flipping is a process in which DNA glycosylases gently pinch DNA while scanning, bending DNA at the site of a damaged base which causes the damaged base to flip out of the double helix and to enter the binding site of the enzyme. Subsequently, AP-endonucleases cleave the resulting apurinic/apyrimidinic (AP) site of the sugar-phosphate backbone. In mammals, the major AP endonuclease is APE1 and it performs more than 95% of the total cellular AP site incision activity (Demple & Sung, 2005). The resulting single-strand break can then be processed by either short-patch BER (SP-BER) or long-patch BER (LP-BER). In SP-BER, the single nucleotide gap is filled by DNA polymerase β, and the XRCC1-DNA ligase IIIα complex seals the DNA ends (Dianov & Hubscher, 2013). In LP-BER, the APE1-induced nick 5' to the AP site leads to the recruitment of PCNA and DNA polymerase δ, which displace the strand while polymerizing 2 to 8 nucleotides of new DNA. Since strand displacement by the polymerase produces a DNA flap that is refractory to ligation, FEN1, which is a flap endonuclease, is needed to degrade the displaced DNA fragment. Finally, the DNA ligase I seals the newly incorporated nucleotides (Robertson et al, 2009).

c) Nucleotide Excision Repair (NER)

NER removes many diverse helix-distorting DNA lesions, including the major UV-light induced CPD and 6-4PP photoproducts, and bulky monoadducts and intrastrand crosslinks induced by various chemicals such as some nitrosamines and cancer therapeutics. NER consists of two subpathways that differ only in the initiating damage recognition step: Global genome repair (GG-NER), which detects damage throughout the whole genome, and transcription-coupled repair (TC-NER), which repairs damage specifically in the transcribed strand of active genes (Scharer, 2013). The biological relevance of NER is manifested in patients suffering from rare UV-hypersensitive cancer prone and/or progeroid syndromes

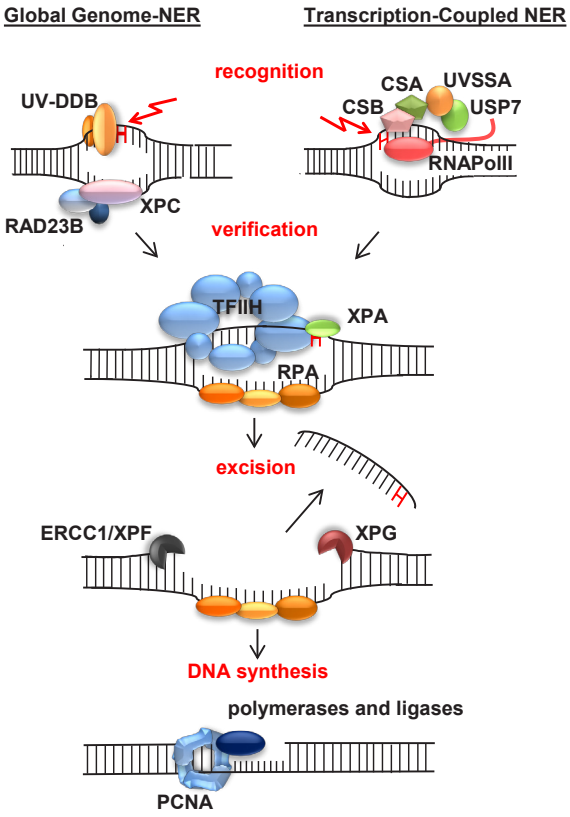

Figure 2. Mammalian nucleotide excision repair (NER).
NER consists of two different damage-detection mechanisms. The majority of lesions are removed by GG-NER, which removes lesions located anywhere in the genome and is initiated by the UV-DDB ubiquitin ligase complex and the XPC/RAD23/CETN2 complex. TC-NER is initiated by stalling of RNAPolII on a lesion present in the transcribed strand of active genes and depends on recruitment of the CSA, CSB and UVSSA proteins. After damage recognition, subsequent steps are similar in both pathways. Damage recognition leads to the recruitment of TFIIH to the damage which opens the DNA around the damage in an ATP-dependent manner using its XPB and XPD helicase subunits and verifies the presence of DNA damage. Then, XPA and RPA are recruited and stabilize the repair complex and properly orient the structure specific endonucleases XPF/ERCC1 and XPG to excise the damaged strand. The incision of the DNA on the 5' side is performed by XPF/ERCC1, while on the 3' side it is performed by XPG. The resulting 25-30 nt single strand DNA gap is filled by the replication machinery which includes RPA, PCNA, RF-C, DNA Pol (δ, ε and κ) and ligation is performed by DNA-LIGI or LIGIIIα-XRCC3. Adapted from (Lans et al, 2012).

caused by NER deficiency. Xeroderma pigmentosum (XP) is specifically caused by defects in genes involved in GG-NER. XP is characterized by pigmentation abnormalities induced by solar UV light, a 2000 times increased risk for skin cancer compared to the general population and increased risk of internal tumors (DiGiovanna & Kraemer, 2012). Cockayne syndrome (CS) and UV-sensitive syndrome (UVSS) are both caused by genetic defects in TC-NER, but display different clinical symptoms (Schwertman et al, 2013). CS is manifested as severe developmental, neurological and premature aging features. UVSS patients, on the other hand, have much milder symptoms than CS patients and only display UV sensitivity. Specific mutations leading to a reduced amount of the TFIIH complex, which is crucial for both NER and transcription, cause trichothiodystrophy (TTD). TTD is also a severe premature-ageing condition of which affected patients display brittle hair and nails, ichthyosis, and progressive mental and physical retardation but no skin cancer predisposition (DiGiovanna & Kraemer, 2012). It is currently not completely understood why defects in the same pathway, i.e. NER, or even in the same sub-pathway, e.g. TC-NER, lead to clinically distinct diseases.

Functional analysis of the proteins that make up the core machinery of NER has led to a detailed molecular model of the multi-step NER mechanism (Scharer, 2013). GG-NER is initiated through cooperative damage detection by the UV-DDB and XPC/RAD23/CETN2 protein complexes (Fig. 2). The XPC protein continuously probes DNA for the presence of lesions (Hoogstraten et al, 2008), having affinity for DNA but a preference for damaged DNA (Sugasawa et al, 1998). XPC is able to recognize the destabilized DNA duplex that is

common to many bulky DNA lesions and binds to the undamaged DNA strand opposite of lesions (Min & Pavletich, 2007). Upon detection of DNA damage, XPC is polyubiquitinated which increases its affinity for DNA (Sugasawa et al, 2005). The UV-DDB complex, which consists of DDB1 and DDB2/XPE bound to a larger CUL4-ROC1 ubiquitin-ligase complex (Groisman et al, 2003), also has high affinity for damaged DNA and is proposed to induce a kink in DNA which increases the recruitment of XPC/RAD23 (Scrima et al, 2008; Tang & Chu, 2002). Its activity is especially important for facilitating the recognition of CPD photolesions by the XPC complex. In contrast to (6-4)PP lesions, which are easily recognized by the XPC complex, CPD lesions only moderately distort the DNA and therefore depend on the activity of the UV-DDB complex to be efficiently detected (Fitch et al, 2003; Moser et al, 2005).

TC-NER is initiated upon stalling of RNA polymerase II (RNApolII) at a damaged site, which serves as a signal to attract the essential TC-NER factors CSA, CSB and UVSSA (Vermeulen & Fousteri, 2013) (Fig. 2). CSA and CSB are key regulators for the further assembly of the TC-NER machinery, which includes core NER and some TC-NER specific factors (Fousteri et al, 2006). Although CSB recruitment to damage is independent of UVSSA, its stability is dependent on the deubiquitinating activity of USP7, which is recruited by UVSSA (Nakazawa et al, 2012; Schwertman et al, 2012; Zhang et al, 2012).

Following DNA damage recognition, TC-NER and GG-NER use the same machinery for the subsequent steps. Damage recognition leads to the recruitment of the transcription factor IIH (TFIIH) (Volker et al, 2001; Yokoi et al, 2000) to open the DNA helix around the damage in an ATP-dependent manner using its XPB and XPD helicase subunits and to verify the presence of DNA damage (Sugasawa et al, 2009). Then, XPA and RPA are recruited and stabilize the repair complex and properly orient the structure specific endonucleases XPF/ERCC1 and XPG to excise the damaged strand. The incision of the DNA on the 5' side is performed by XPF/ERCC1, while on the 3' side it is performed by XPG. The resulting 25-30 nt single strand DNA gap is filled by the replication machinery which includes RPA, PCNA, RFC and DNA polymerases (δ, ε and κ). Finally, ligation is performed by DNA Ligase I or Ligase IIIα-XRCC3 (Moser et al, 2007; Ogi et al, 2010).

d) Double-Strand Break (DSB) Repair

DSBs are induced by IR, specific chemicals and by-products of the cell's metabolism such as reactive oxygen compounds, or because of replication stalling on different types of lesions of which processing may eventually lead to DSBs. Also, they are created as part of natural processes such as meiotic and V(D)J recombination Since both DNA strands are damaged, DSBs are very hazardous to the cell and can lead to chromosome instability and genome rearrangements if not repaired properly. There are two main mechanisms that repair DSBs: Homologous recombination (HR) (San Filippo et al, 2008) and Non-homologous end joining (NHEJ) (Lieber, 2010).

HR is an error-free pathway that uses the sister chromatid as template for repair of the break and therefore only takes place in S and G2 phase when this sister chromatid is present (Fig. 3). HR is initiated when the MRE11/Rad50/NBS1 (MRN) complex binds to broken DNA ends, which is important to hold the broken pieces together, and activates the ATM protein kinase (de Jager et al, 2001; Lavin, 2008; San Filippo et al, 2008). MRN acts together with the nucleases CtIP and EXO1 to resect the DNA ends at the break site, leaving a 3' ssDNA overhang to which RPA binds. Subsequently, BRCA2 promotes the exchange of RPA for

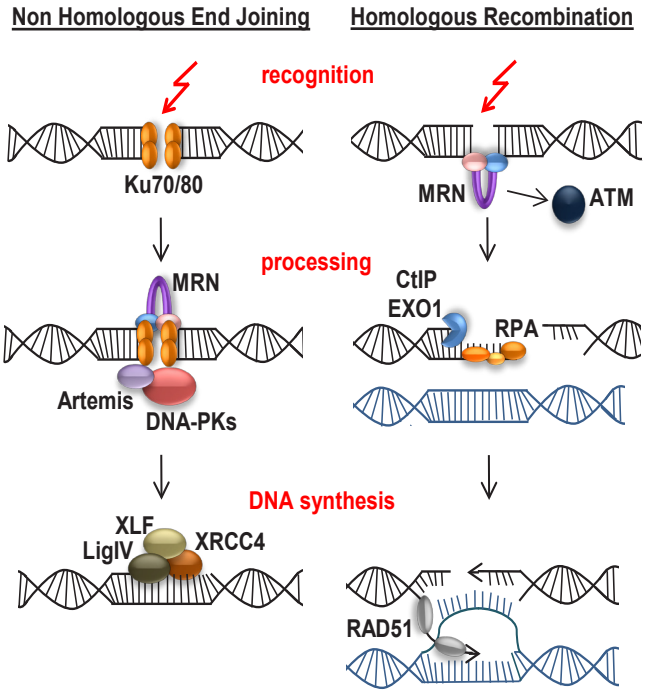

Figure 3. Model for DSB repair by non-homologous end joining (NHEJ) and homologous recombination (HR).
DSBs are predominantly repaired by either NHEJ or HR. In NHEJ, first the broken DNA ends are detected and bound by the Ku70/80 heterodimer. The Ku70/80/DNA complex recruits the Artemis-DNA-PKs complex and the MRN complex, which consists of MRE11, RAD50 and NBS1, to process DNA ends. The XLF-XRCC4-LigaseIV complex finally ligates the processed ends together. HR is initiated when the MRN complex binds to broken DNA and activates ATM protein kinase. MRN together with CtIP and EXO1 resects the DNA ends at the break site, which leads to RPA binding to the ssDNA created. Subsequently, RPA is exchanged with RAD51 which mediates strand invasion into the homologous sister, followed by DNA branch migration. The newly synthesized DNA strand either dissociates or holiday junctions are resolved by specific endonucleases. Finally ligation takes place to complete the repair. The figure is adapted from Figure 3 from (Lans et al, 2012).

RAD51, which forms nucleoprotein filaments on the ssDNA and mediates strand invasion into the homologous sister template, followed by DNA branch migration and the formation of a joint molecule called a D-loop. The invading DNA end is extended by novel DNA synthesis. Next, the second DNA end invades the homologous template and the resultant joint structures called holiday junctions are resolved by specific endonucleases. Finally ligation takes place to complete repair. Alternatively, the first invading DNA strand dissociates and re-anneals with its original template in a process called synthesis-dependent strand annealing.

In contrast to HR, NHEJ can take place in any cell cycle phase (Lieber, 2008). In NHEJ, the broken DNA ends are initially detected and bound by the Ku70/80 heterodimer (Fig. 3), which serves as a node at which subsequent repair factors can dock. Among these factors are a complex formed by the nuclease Artemis and the PI3 kinase DNA-PK and the MRN complex which facilitates DNA end-processing, and finally the XLF-XRCC4-Ligase IV complex, which ligates the processed ends together. Importantly, to allow the repair of a diverse array of DSB end configurations, the loading of these repair factors can occur in any order and the two DNA ends can be independently processed. Furthermore, the XLF-XRCC4-Ligase IV complex can ligate one strand independently of the other strand. In addition, NHEJ is often error-prone because of the DNA end-processing that needs to take place before DNA ends are compatible to be rejoined, resulting in loss of nucleotides prior to ligation.

e) Interstrand Cross-Link (ICL) Repair

DNA interstrand cross-links (ICLs) can be formed by endogenous or exogenous reactive compounds or radiation, and covalently connect the two strands of DNA. Because of this, they are thought to form absolute blocks for DNA unwinding and are thus considered to be barriers to the transcription and replication machineries. As a consequence, ICLs are extremely cytotoxic, par-

ticularly to dividing cells. Therefore, ICL-inducing agents are also widely used in chemotherapy. Cells from patients with Fanconi anemia (FA), a disease characterized by leukemia, bone marrow failure and congenital developmental defects, display genomic instability and hypersensitivity to DNA crosslinking compounds (Kee & D'Andrea, 2010). So far, 16 causative FA proteins have been identified (Clauson et al, 2013), which function cooperatively, with the aid of some proteins involved in DSB repair, translesion synthesis (TLS) and NER, in the repair of ICLs to maintain chromosome stability.

The FA pathway is started by binding of FANCM to a stalled replication fork, whose remodeling activity leads to RPA binding to unwound DNA (Kee & D'Andrea, 2012; Kim & D'Andrea, 2012). RPA then induces an ATR-mediated signal at the stalled replication fork which activates the 12 subunit FA core complex of which the FANCL subunit monoubiquitinates the FANCD2/FANCI (D2/I) heterodimer. Subsequently, the D2/I complex is recruited to damage sites, forming visible discrete nuclear foci, to coordinate the action of downstream repair factors. Monoubiquitinated D2/I functions as a docking site for the recruitment of multiple structure-specific nucleases, including ERCC1-XPF, MUS81–EME1, SLX1 and FAN1, via an interaction with the scaffold protein FANCP/SLX4. The two covalently linked nucleotides are incised on either side leaving an unhooked crosslink still tethered to the complementary strand. SLX4 interacts with each of the above mentioned nucleases, but it is currently unknown how the different nucleases are coordinated in this unhooking reaction. TLS polymerases such as REV1 and Pol ζ (see below) are recruited to bypass the unhooked crosslink. Since a gap is formed on the incised strand, downstream FA proteins induce RAD51-dependent strand invasion and the gap is repaired by homologous recombination. Following homologous recombination, the recombinant intermediates are resolved and NER removes remaining unhooked adducts and fills the resulting gap (see below). Finally, the USP1/UAF1 complex deubiquitinates D2/I to complete the pathway.

When replication forks are stalled by DNA damage, the damage is normally either repaired prior to the resumption of DNA synthesis (McGlynn & Lloyd, 2002), or bypassed (Friedberg et al, 2005; Sale, 2012). Although ICLs are considered to be absolute barriers to the replication apparatus and therefore need to be repaired, interestingly recent evidence suggests that ICLs can also be traversed during replication (Huang et al, 2013). This occurs without lesion repair and depends on the FANCM protein, which indicates that translocase-based mechanisms enable DNA synthesis to continue past ICLs and that these lesions do not necessarily have to be absolute blocks to replication. However, the mechanism behind this observation is still unclear.

f) DNA Damage Signaling and Cell Cycle Arrest

An important mechanism within the DDR is the signaling of the presence of DNA damage, to activate e.g. cell cycle checkpoints and the apoptosis machinery. Cell cycle checkpoints are control mechanisms that verify whether all processes during each cell cycle phase are properly completed before proceeding to the next one. When DNA damage is encountered, signaling is activated and the cell cycle progression is temporarily halted by one of the activated checkpoints, i.e. between G1 and S, in S or between G2 and M phase, until DNA repair is completed (Zhou & Elledge, 2000). In addition, if the damage load is too high for efficient removal, apoptosis is activated to ensure elimination of these damaged cells. Both these DDR branches are important to avoid mutagenesis and genome instability and form a crucial barrier against DNA damage-induced oncogenic trans-

formation. Single and double strand DNA breaks initiate DNA damage signaling by activating phosphoinositide 3-kinase-like kinases, including the well-known DNA-PK, ATM and ATR. ATM is normally catalytically inactive, but is activated when the MRN complex binds DSBs. Activation of ATM requires its auto-phosphorylation on amino acid S1981 and also acetylation by the acetyltransferase Tip60 (Sun et al, 2010). ATR is in a complex with ATRIP and is activated in response to DNA damage induced by different factors such as IR, UV, alkylating agents and replication stress. Processing of these types of DNA damage generates ssDNA which is swiftly coated by RPA. The ATRIP/ATR complex binds to RPA coated ssDNA, which activates ATR-mediated signaling (Zou & Elledge, 2003).

ATM and ATR play key roles in DNA damage signaling by inducing a cascade of phosphorylation events to downstream effector molecules, implicated in activating cell cycle checkpoints, apoptosis or DNA-repair. Among their substrates are histone H2AX and signaling mediator 53BP1 and the checkpoint kinases Chk1 and Chk2.

g) Translesion Synthesis (TLS)

Since replicative DNA polymerases cannot use damaged DNA as template, DNA synthesis is blocked when a lesion is encountered during replication. Therefore, any DNA lesion should either be repaired or bypassed, because it can otherwise lead to the collapse of the replication fork and genetic instability and might finally cause cell death (Batista et al, 2009). TLS allows the replication machinery to tolerate DNA damage without repairing it by replacing replicative DNA polymerases with specialized TLS polymerases with lower fidelity, which are able to synthesize DNA over a lesion that blocks the replication machinery. This replacement is mediated by the ubiquitination of PCNA, in eukaryotes (Waters et al, 2009). The main class of TLS polymerases is the Y-family, which includes Pol ι (iota), Pol κ (kappa), Pol η (eta) and REV1 (Sale et al, 2012), although so-called X and A classes of polymerases also weakly exhibit TLS activity. Although TLS can lead to mutation formation, resuming DNA replication can sometimes be more crucial for cell survival than protecting the accuracy of the genomic information. Therefore TLS is very important for the survival of the cell in the presence of DNA damage. In eukaryotes, TLS is thought to occur in two ways, either by 'polymerase-switching' during replication or 'gap-filling' after replication (Waters et al, 2009).

The polymerase-switching model proposes that TLS comes into play during active DNA replication. When the replication fork is blocked at a lesion, the E2/E3 ubiquitin ligase complex Rad18/Rad6 is thought to bind to the ssDNA generated and to monoubiquitinate PCNA, which serves as a signal for TLS by strengthening the interaction between PCNA and TLS polymerases. Next, an exchange of the replicative polymerase with a TLS polymerase takes place and a nucleotide is inserted across and past the lesion. Finally, a second polymerase switch takes place to relocalize the replicative DNA polymerase. When the lesion is bypassed and the replication fork restarts, repair machineries most probably will remove the lesion.

In the gap-filling model TLS does not need a blocked replication fork but acts on DNA damage which resides in single-stranded gaps that have been left by the replication machinery when it restart directly downstream of a lesion. In this model, the aim of TLS is not to resume progression of the replication fork, but rather to seal these gaps. Although there is no blocked replication fork which signals for the recruitment of TLS polymerases, signaling may involve some of the same factors also used in the polymerase switching model. Once the gap is bypassed and filled, the remaining lesion will most probably be removed by DNA repair pathways.

3) Chromatin and its Remodeling

1

a) Chromatin

Although the main mechanisms of the different repair systems have been elucidated, how the efficiency and crosstalk within the DDR is controlled remains elusive. It is becoming increasingly clear that chromatin poses a barrier and important regulatory platform for repair and as such plays a critical role in controlling DDR efficiency. The experimental work described in this thesis focuses on the role of chromatin remodeling in DDR, specifically those related to NER. To provide the necessary background on this emerging field, the next part of this introduction summarizes the current state-of-art of chromatin modifications associated with the DDR.

The eukaryotic genome is organized in nucleosomes. One nucleosome consist of 147 bp DNA wrapped around a histone octamer consisting of two copies of histones H2A, H2B, H3 and H4. Nucleosomes are joined by a stretch of naked linker DNA of which the length varies, depending on the organism and cell type (Clapier & Cairns, 2009). Histone H1 is not part of the nucleosome, but interacts with the linker DNA and DNA entry site of the nucleosome and compacts chromatin into a more condensed configuration. This way of DNA packaging into chromatin provides the structural basis of wrapping the extremely long DNA molecules into the restricted nuclear volume. However, these structures also form a barrier for many DNA transacting processes such as transcription, replication and DNA repair. Therefore, chromatin needs to be modified to regulate access of proteins to DNA during these processes and also to be restored afterwards. At the same time, chromatin serves as an optimal regulation platform for DNA transacting processes by mediating signaling events, providing docking sites for proteins and controlling their activity. Histone modifiers and ATP-dependent chromatin remodelers are two main classes of enzymes that remodel chromatin.

b) Histone modifying enzymes

Histone modifying enzymes regulate chromatin condensation and DNA accessibility, changing the structure and function of histones, by catalyzing the covalent attachment or removal of functional groups or small proteins to residues in the protruding histone tails (Kouzarides, 2007). The addition and/or removal of these posttranslational modifications (PTMs) to histones, catalyzed by a large group of histone-modifying enzymes (so-called ‘writers’) is highly regulated and is crucial for regulating DNA transacting processes. Since these histone modifying enzymes have such important roles in gene regulation, DNA repair and development, it is no surprise that alterations in their activity are correlated with various human diseases such as Parkinson’s and Huntington’s diseases, diabetes type 1 and rheumatoid arthritis (Portela & Esteller, 2010). The presence of specific histone modifications is recognized by a group of proteins (so-called ‘readers) that carry specific domains with affinity for certain histone PTMs. For example several proteins harbor a bromodomain that specifically recognizes and binds to acetyl groups on histone tails. Recognition and binding of histone modifications by these ‘readers’ can have a broad range of effects, depending on the type of modification, ranging from DNA repair signaling as in the case of H2AX phosphorylation, to the activation/repression of proteins such as transcription factors in regulating gene expression. In other words, histone modifications serve as docking and signaling sites for many chromatin related proteins.

The different types of modifications found on histone tails include acetylation, methylation, phosphorylation, ubiquitination, SUMOylation and poly(ADP-ribosyl)ation (PARylation), which play important roles in DNA repair and DNA damage signaling (Luijsterburg & van Attikum, 2011). For example, phosphorylation of histone variant H2AX at serine 139 (γH2AX) is one the major hallmarks of DNA damage signaling, being induced upon DSB induction, UV damage and replication stress (Marteijn et al, 2009; Rogakou et al, 1998; Ward & Chen, 2001). Induction of γH2AX is a prerequisite for the binding of DNA damage signaling mediator MDC1 and the subsequent recruitment of many DDR proteins at the site of damage (Jungmichel & Stucki, 2010; Scully & Xie, 2013).

Histone acetylation is one of the marks indicative of the transcriptional status of chromatin, since acetylation modulates higher order chromatin structure. Because acetylation promotes the accessibility of chromatin for proteins, proper regulation of the enzymes that attach or remove an acetyl group, i.e. the histone acetyl transferases (HATs) and deacetylases (HDACs), is crucial for proper functioning of DNA. Acetylation also regulates DDR processes. The best known example is acetylation by Tip60, which is a HAT subunit of the INO80 complex TRRAP/Tip60. Together with its cofactor Trrap, Tip60 acetylates ATM (Sun et al, 2005) and the histones H2A/H2AX (Ikura et al, 2007) and H4 (Bird et al, 2002) upon DNA damage, likely to mediate chromatin relaxation needed for efficient DSB repair. Histone methylation recruits proteins which contain chromo and tudor domains. The best known example of regulation through this PTM in DDR is the DNA repair factor 53BP1, which has a high affinity for dimethylated H4K20 (Botuyan et al, 2006), a histone mark that is induced by the histone methyltransferase MMSET upon DNA damage (Pei et al, 2011).

In addition to the methylation, acetylation and phosphorylation of H2A/H2AX and H4, histones H2A and H2AX in damaged chromatin are modified by the attachment of chains of the 8 kDa ubiquitin protein, i.e. ubiquitylation, upon DNA damage induction. Ubiquitylation has been associated with proteolysis for a long time, but different types of ubiquitylation chains are now known to lead to different fates of the target protein, which can be degradation but also regulation of activity and/or differential interaction with other proteins (Panier & Durocher, 2009). The E3 ubiquitin ligases RNF8 and RNF168 ubiquitylate H2A and H2AX histones on K13-15 residues in response to DSB-induced phosphorylation of H2AX, forming K63-linked ubiquitin chains, which orchestrates the recruitment of DDR proteins such as BRCA1 and 53BP1 (Doil et al, 2009; Huen et al, 2007; Kolas et al, 2007; Mailand et al, 2007; Mattiroli et al, 2012; Pinato et al, 2009; Stewart et al, 2009). Similar RNF8-controlled signaling via H2A/H2AX ubiquitylation also takes place following NER-mediated incision of UV-induced DNA damage (Bergink et al, 2007; Marteijn et al, 2009). There is some crosstalk during DSB response between ubiquitylation and the addition of SUMO, a similar small protein that can be covalently attached to proteins. BRCA1 is SUMOylated by the PIAS1 and PIAS4 SUMO E3 ligases in response to genotoxic stress, which is required for its ubiquitin ligase activity *in vitro* and in cells (Morris et al, 2009). However, although SUMOylation by these E3 ligases is essential for regulating ubiquitylation cascades mediated by RNF8, RNF168 and BRCA1 (Galanty et al, 2009; Morris et al, 2009), there is currently no evidence for involvement of histone SUMOylation in the DDR.

Finally, PARylation of histones is likely involved in DDR. For example, the PAR polymerase PARP1 is among the first proteins to recognize DNA breaks (Mortusewicz et al, 2007). Following its interaction with DNA damage, PARP1 catalyzes the addition of ADP-ribose

moieties to a variety of proteins including histone tails and itself creating long and branched negatively charged PAR polymers (D'Amours et al, 1999; Kim et al, 2005). PARylated PARP1 and histones regulate the subsequent recruitment of diverse DDR factors to DNA lesion sites, such as the repair protein XRCC1 to single strand breaks (El-Khamisy et al, 2003). ATM and PAR interact via PAR-binding domains which have been suggested to modulate DSB signaling and repair (Haince et al, 2007). Consistently, the disruption of the ATM–PAR interaction prevents the proper localization of ATM to DNA breaks and significantly reduces the phosphorylation of several ATM targets, such as p53, SMC1 and H2AX (Aguilar-Quesada et al, 2007; Haince et al, 2007). PARylation of chromatin is also thought to specifically regulate the recruitment of ATP-dependent chromatin remodeling proteins and complexes to DNA damage sites, such as ALC1 (Ahel et al, 2009; Gottschalk et al, 2009), SMARCA5 (Smeenk et al, 2013) and the NuRD complex (Chou et al, 2010; Polo et al, 2010). Also during NER, PARP1 associates with the UV-DDB2 complex and has been implicated in PARylation of chromatin to regulate recruitment of the ALC1 chromatin remodeler to facilitate repair (Luijsterburg et al, 2012; Pines et al, 2012).

c) ATP-dependent chromatin remodeling

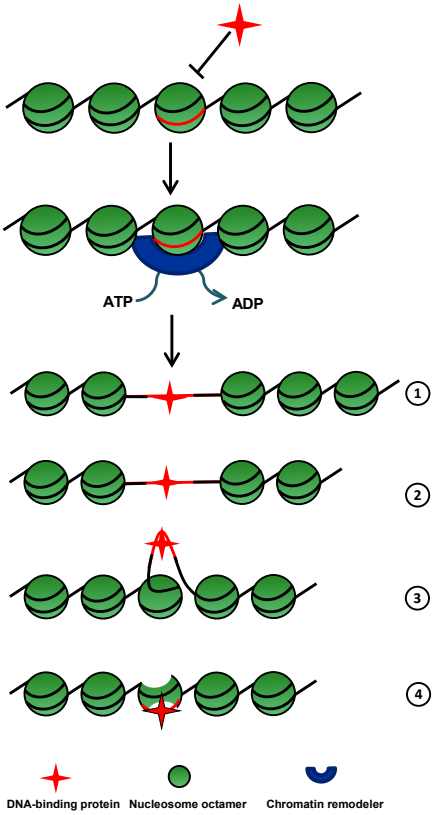

Figure 4. Mechanisms of ATP-dependent chromatin remodeling.
Schematic presentation of the possible distinct modes that ATP-dependent chromatin remodeling complexes use to rearrange chromatin by ATP-hydrolysis, which alters the DNA-nucleosome contacts to facilitate or restrict the access of DNA binding proteins to DNA. Nucleosomes are either repositioned (1) or evicted (2) or unwrapped (3) or their composition is altered by histone replacement or ejection (4). The figure is adapted from (Kasten et al, 2011).

ATP-dependent chromatin remodeling complexes modify chromatin by catalyzing the disruption of DNA-histone contacts and slide or evict nucleosomes or alter their composition by using the energy from ATP hydrolysis (Clapier & Cairns, 2009) (Fig. 4). Four structurally related families of conserved ATP-dependent complexes are identified: SWI/SNF, INO80, CHD and ISWI. Each family is characterized by one or more typical SWI2/SNF2-family subunits. These subunits harbor an ATPase domain belonging to the superfamily of DEAD/H-helicases, consisting of two subdomains, DExx and HELICc, which is responsible for the catalytic mobilization of nucleosomes (Bork & Koonin, 1993; Eisen et al, 1995). The remodeling families are functionally different due to the additional unique domains residing within or adjacent to the ATPase domains, which regulate their activity (Fig. 5). In addition, functional differences between these ATP-dependent chromatin remodelers are defined by their associated subunits (Table 1). The composition of ATP-dependent chromatin remodeling complexes is however dynamic and may vary between different activities and cell types.

In recent years, it has become clear that ATP-dependent chromatin re-

Table 1. Mammalian ATP-dependent chromatin remodeling families. Adapted from (Clapier & Cairns, 2009).

SWI/SNF	**Complex**	**BAF**	**PBAF**					
	ATPase Subunit	BRM or BRG1	BRG1					
	Noncatalytic Subunits	BAF250a, b	BAF180					
			BAF200					
		BAF155, BAF170	BAF155, BAF170					
		BAF60a, b, c	BAF60a, b, c					
		SNF5/BAF47/INI1	SNF5/BAF47/INI1					
		BAF57	BAF57					
		BAF53a, b	BAF53a, b					
		β-actin	β-actin					
ISWI	**Complex**	**CHRAC**	**ACF**	**RSF**	**WICH**	**NORC**	**NURF**	**CERF**
	ATPase Subunit	SMARCA5	SMARCA5	SMARCA5	SMARCA5	SMARCA5	SMARCA1	SMARCA1
	Noncatalytic Subunits	ACF1	ACF1	RSF1	WSTF	TIP5	BPTF	CECR2
		CHRAC15					RbAp46 or 48	
		CHRAC17						
CHD	**Subfamily**	**Subfamily I**	**Subfamily II**	**Subfamily III**				
	Complex		**NuRD**					
	ATPase Subunit	CHD1 or CHD2	Mi-2α/CHD3 or Mi-2β/CHD4	CHD5, CHD6, CHD7, CHD8, CHD9				
	Noncatalytic Subunits		MBD3					
			MTA1, 2, 3					
			HDAC1, 2					
			RbAp46 or 48					
			p66α,β					
INO80	**Complex**	**INO80**	**SRCAP**	**TRRAP/Tip60**				
	ATPase Subunit	hINO80	SRCAP	p400				
	Noncatalytic Subunits	RUVBL1,2/Tip49a, b	RUVBL1,2/Tip49a,b	RUVBL1,2/Tip49a,b				
		BAF53a	BAF53a	BAF53a				
		Arp 5,8	Arp 6	Actin				
		hIes2,6	GAS41	GAS41				
			DMAP1	DMAP1				
			YL-1	YL-1				
			H2AZ,H2B	Brd8/TRC/p120				
			ZnF-HIT1	TRRAP				
				Tip60				
				MRG15				
				FLJ11730				
				MRGBP				
				EPC1				
				ING3				

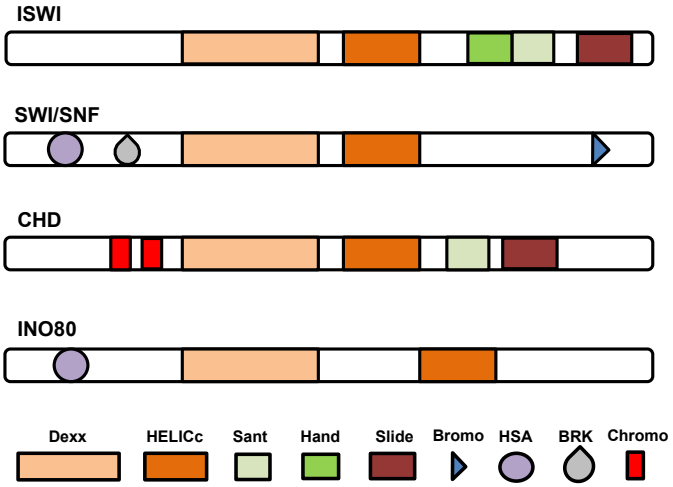

Figure 5. Schematic overview of the mammalian SWI2/SNF2 superfamily of ATP-dependent chromatin remodelers.
All four remodeler families contain a SWI2/SNF2-family ATPase subunit characterized by an ATPase domain that is split in two parts: DExx and HELICc. The typical differences between each family member are the unique domains residing within, or adjacent to, the ATPase domain, which determines the specificity of each protein such as chromodomains for CHD, a bromodomain for SWI/SNF and a HAND domain for ISWI family.

modeling complexes play important roles in the DDR, regulating access to DNA, efficient recruitment of repair factors and DNA damage signaling (Dinant et al, 2008; Lans et al, 2012). In the remainder of this chapter, the current knowledge on the role of ATP-dependent chromatin remodeling complexes in DDR will be summarized. Furthermore, in this thesis, chapters II, III and IV will specifically focus on the role of ISWI and chapter V on the role of SWI/SNF complexes in mammalian NER.

i) ISWI Family

The ISWI (imitation switch) family remodeler complexes were first discovered in Drosophila melanogaster (Ito et al, 1997; Tsukiyama et al, 1995; Tsukiyama & Wu, 1995; Varga-Weisz et al, 1997) and later shown to be conserved in many other organisms (Corona & Tamkun, 2004). In humans, seven separate ISWI complexes have been identified: ACF1, CHRAC, RSF, NoRC, WICH, CERF and NURF (Toto et al, 2014) (Table 1;Fig. 6). Each of the complexes contain one of two conserved ATPase subunits: SMARCA5 (SWI-SNF-Related Matrix-Associated Actin-Dependent Regulator Of Chromatin A5, also known as SNF2H) or SMARCA1 (SWI-SNF-Related Matrix-Associated Actin-Dependent Regulator Of Chromatin A1, also known as SNF2L). In addition to their ATPase domain located in the N-terminal half of the proteins, SMARCA5 and SMARCA1 contain regulatory HAND, SANT and SLIDE domains in minus (Grune et al, 2003) (Fig. 5). Although the exact function of these regulatory domains is still ambiguous, they appear to interact with DNA flanking nucleosomes (Dang & Bartholomew, 2007; Yamada et al, 2011) and regulate the activity of the ATPase domain (Mueller-Planitz et al, 2013). Furthermore, in chapter III, we provide evidence that the SLIDE and ATPase domain are essential for recruitment of SMARCA5 to UV-damaged chromatin while the HAND domain is involved in re-localization at sites UV-induced DNA damage. Based on the very mobile nature of ISWI remodeling complexes, as has been their C terobserved in living cell nuclei with only a small fraction transiently interacting with chromatin, it was hypothesized that these complexes continuously sample nucleosomes through transient binding (Erdel & Rippe, 2011a). It was hypothesized that during this sampling the ISWI complexes are targeted to remodeling substrates by specific cues which could be DNA sequences, histone variants, histone PTMs or other chromatin associated proteins (Erdel & Rippe, 2011b). Our results described in chapter III support such a target localization mechanism for SMARCA5 localization to sites of stalled transcription, involving stalled

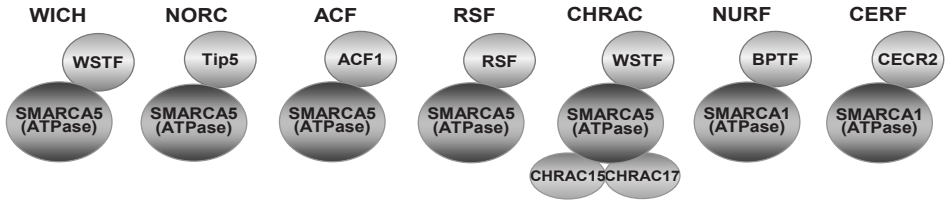

Figure 6. Subunit composition of mammalian ISWI family complexes.
The human ISWI family consists of seven separate complexes: ACF1, CHRAC, RSF, NoRC, WICH, CERF and NURF. Each of the complexes contains one of two conserved ATPase subunits: SMARCA5 (SNF2H) or SMARCA1 (SNF2L). The composition of each ISWI complex is shown.

RNAPolII, histone PTMs and DNA binding as targeting cues. Many ISWI family complexes such as ACF and CHRAC optimize nucleosome spacing to promote chromatin assembly and in many cases are associated with repression of transcription (Varga-Weisz et al, 1997; Yang et al, 2006). However, certain complexes, such as NURF can randomize spacing (Tsukiyama & Wu, 1995), which may assist RNAPolII activation, showing the diversity that can be imparted by attendant subunits.

There is ample evidence that implicates ISWI remodeling in DNA repair and signaling in response to DSBs. SMARCA5 and its associated subunit ACF1 are recruited to DSBs and are necessary for survival and efficient HR and NHEJ after DNA damage induction (Lan et al, 2010; Nakamura et al, 2011; Sanchez-Molina et al, 2011; Smeenk et al, 2013). Furthermore, ISWI subunits are essential for UV survival and regulate recruitment of the TC-NER initiating protein CSB to the site of UV damage (Aydin et al, 2014; Lans et al, 2010)(Chapter III). The role of ISWI in the DNA damage response is discussed in chapter II in detail.

ii) SWI/SNF Family

The SWI/SNF (switching defective/sucrose nonfermenting) family is involved in many cellular processes like transcription (Mohrmann & Verrijzer, 2005), DNA replication (Flanagan & Peterson, 1999), V(D)J recombination (Kwon et al, 2000), viral integration (Yung et al, 2001), cell differentiation, development and tumor suppression (Amankwah et al, 2013; Robinson et al, 2012). It was demonstrated that subunits of SWI/SNF are mutated in approximately 20% of human cancers (Shain & Pollack, 2013), illustrating its significance and suggesting that SWI/SNF may be implicated in genome maintenance and/or regulating transcriptional networks that control cell cycle progression.

The SWI/SNF family chromatin remodelers form large complexes composed of more than 8 subunits (Table 1) (Reisman et al, 2009). Two functionally different SWI/SNF complexes in mammals were identified: PBAF and BAF, depending on the inclusion of either

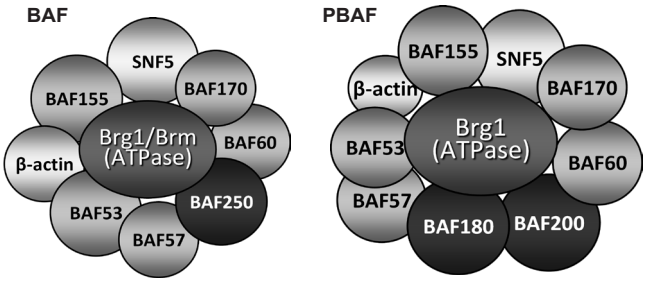

Figure 7. Subunit composition of human SWI/SNF family complexes.
The human SWI/SNF family consists of two main separate complexes: BAF and PBAF, depending on the inclusion of noncatalytic subunits BAF180 and BAF200 (PBAF) or BAF250A or BAF250B (BAF). Furthermore, PBAF contains BRG1 and BAF contains BRG1 or BRM as the catalytic ATPase. The composition of each ISWI complex is shown.

BAF180 and BAF200 (PBAF) or BAF250a or BAF250b (BAF) as noncatalytic subunits (Fig. 7). Furthermore, PBAF contains BRG1 (also called SMARCA4) and BAF contains either BRG1 or BRM (also called SMARCA2) as the catalytic ATPase (Table 1). Besides the conserved DEAD/H-helicase ATPase domain, these ATPases also contain a helicase-SANT (HSA) domain, which is thought to mediate DNA binding, a C-terminal bromodomain, which binds acetylated histones and thus regulates chromatin association of SWI/SNF and a BRK domain whose function is unknown (Fig. 5). SWI/SNF complexes are specifically thought to increase chromatin accessibility by continuous repositioning of nucleosomes along DNA (Schnitzler et al, 1998; Shundrovsky et al, 2006).

SWI/SNF mediated chromatin remodeling has been implicated in mammalian DDR efficiency by increasing chromatin accessibility. Loss of either BRG1 or BRM renders cells sensitive to DSB-inducing agents and impairs damage-induced phosphorylation of H2AX and DSB repair (Park et al, 2009; Park et al, 2006). Furthermore, both BRG1 and BRM ATPases are recruited to damaged chromatin where BRG1 interacts with γH2AX and acetylated H3, which is induced by the HAT GCN5 (Lee et al, 2010; Ogiwara et al, 2011). Histone acetylation by the HATs CBP and p300 was also shown to stimulate chromatin remodeling by SWI/SNF at DSB sites to facilitate the subsequent recruitment of NHEJ factors. Moreover, after damage the interaction between SWI/SNF and the early DDR protein BRIT1which is required for the expression of both BRCA1 and the checkpoint kinase Chk1 and phosphorylation of NBS1 (Lin et al, 2005), is enhanced through phosphorylation of the SWI/SNF core protein BAF170 by ATM/ATR, which likely regulates the recruitment and maintenance of SWI/SNF at sites of damage (Peng et al, 2009). This may be important as loss of BRIT1 leads to chromatin condensation and impaired recruitment of DSB signaling proteins such as MDC1, 53BP1 and ATM (Rai et al, 2006; Wood et al, 2007).

Our laboratory identified different subunits of SWI/SNF complexes, including orthologs of mammalian BRG1 and BRM, SNF5, PBRM1 and BAF155/SMARCC1, which are essential for optimal UV survival of somatic cells in *Caenorhabditis elegans* (Lans et al, 2010) (Chapter V). BRG1/BRM is also important to the UV response of germ cells, but SNF5, PBRM1 and BAF155 are not. These observations are particularly interesting since in *C. elegans* germ cells and early embryos, GG-NER is the major subpathway that is necessary for normal development and survival after exposure to UV-light, whereas in somatic cells TC-NER is sufficient. Therefore, these findings suggest that the involvement of individual SWI/SNF subunits in DDR may differ between cell types. In mammals, SWI/SNF also seems to promote NER in chromatin by stimulating CPD repair but its precise activity is still controversial. It has been proposed that BRG1 stimulates recruitment of XPC to sites of DNA damage (Zhang et al, 2009) but also the reciprocal has been suggested, i.e. that XPC stimulates BRG1 recruitment, which in turn stimulates recruitment of later NER factors (Zhao et al, 2009). Also, BRG1 may transcriptionally regulate the UV-induced G1/S checkpoint, as loss of BRG1 leads to increased UV-induced apoptosis (Gong et al, 2008). Furthermore, the SWI/SNF subunit SNF5 was also suggested to interact with XPC and to promote CPD repair (Ray et al, 2009), but this is also debated (McKenna et al, 2008). In Chapter V, we provide evidence that both BRG1 and BRM interact with DDB2, but not with XPC, and that BRG1 regulates the association of DDB2 to damaged chromatin.

iii) INO80 Family

The INO80 (inositol requiring 80) proteins are characterized by an HSA domain, which reg-

ulates the association with specific Arp subunits of INO80 complexes, and a unique long insertion between the Dexx and HELICc subdomains of the conserved DEAD/H-helicase ATPase domain, which also mediates specific interactions that regulate unique activities of the complexes (Watanabe & Peterson, 2010) (Fig. 4). The mammalian INO80 family consists of three complexes, INO80, SRCAP and TRRAP/Tip60, harboring respectively the INO80, SRCAP and p400 ATPases (Table 1). This family is implicated in transcription (Cai et al, 2007), DNA replication and chromosome segregation during cell division (Hur et al, 2010).

Different roles have also been proposed for INO80 in DSB repair, such as the promotion of H2AX phosphorylation by the INO80 ATPase itself together with the subunit Arp5 (Kitayama et al, 2009). The mammalian INO80 complex is also involved in HR, being recruited to DSBs independently of γH2AX (Kashiwaba et al, 2010; Wu et al, 2007). Also the TRRAP/Tip60 complex regulates DSB repair as its p400 ATPase and associated HAT Tip60 are both recruited to DSBs and histone and ATM acetylation by Tip60 are needed for efficient DSB repair (Bird et al, 2002; Ikura et al, 2007; Murr et al, 2006; Sun et al, 2005). Furthermore, recruitment of p400 to DSBs was shown to decrease nucleosome stability and promote RNF8-mediated chromatin ubiquitylation to regulate 53BP1 and BRCA1 recruitment at sites of damage (Xu et al, 2010). Besides DSB repair, the INO80 complex was also suggested to be involved in NER. INO80-deficient cells are sensitive to UV irradiation (Wu et al, 2007). Moreover, the mammalian INO80 complex was proposed to function during early NER steps by facilitating the recruitment of XPC and XPA to sites of DNA damage and to stimulate efficient 64PP and CPD removal, being localized to damaged DNA independently of XPC (Jiang et al, 2010).

iv) CHD Family

The CHD (chromodomain, helicase, DNA binding) family's characteristic is that the catalytic subunits, next to their conserved DEAD/H-helicase ATPase domain, also harbor two tandemly arranged chromodomains in the N terminus (Fig. 5). Chromodomains, which are unique for the CHD family and not found in any of the other SWI2/SNF2 family members, are most probably responsible for locating these remodelers to methylated histones as chromodomains bind specifically to methylated lysines such as di- or trimethylated H3K4 which are associated with active chromatin (Flanagan et al, 2005; Sims et al, 2005).

In mammals there are three subfamilies of CHD complexes described (Marfella & Imbalzano, 2007) (Table 1). One subfamily contains the ATPases CHD1 and CHD2 and another subfamily harbors the ATPases CHD5 to CHD9, but of both these subfamilies not much is known. The best characterized subfamily is that which contains the ATPases Mi-2α/CHD3 or Mi-2β/CHD4 which are mutually exclusive catalytic components of the NuRD complex (Table 1). The NuRD complex, among whose other subunits are the histone deacetylases HDAC1 and HDAC2 and the methyl CpG-binding domain protein MBD3, is unique in combining ATP-driven chromatin remodeling with post-translational histone modifications, i.e. deacetylation and demethylation (Lai & Wade, 2011). The NURD complex was shown to have a repressive role in transcription (Denslow & Wade, 2007). The different functions of the CHD family may rely, in part, on chromodomain diversity as well as differential subunit association.

CHD complexes have been shown to have different roles in the DDR. Loss of subunits of the NuRD complex leads to increased IR sensitivity and to defective DSB repair in mammalian cells (Larsen et al, 2010; Polo et al, 2010; Smeenk & van Attikum, 2011; Smeenk et al,

2010). In particular, NuRD subunits CHD4 and MTA1 are recruited to DSBs in a PARP-dependent but γH2AX-independent manner (Larsen et al, 2010; Polo et al, 2010; Smeenk et al, 2010). Both CHD4 and the regulatory subunit MTA2 facilitate the accrual of RNF168 and BRCA1 at the damage site by stimulating RNF8/RNF168-dependent formation of ubiquitin conjugates. Furthermore, there are indications that CHD4 is a regulator of the cell cycle checkpoint activation upon DSB by controlling p53 deacetylation. In contrast to CHD4, CHD3 seems to have a more restricted role in the DDR as it was suggested to specifically function in heterochromatin. CHD3 was shown to dissociate from DSBs in heterochromatin upon KAP-1 phosphorylation by ATM, to induce chromatin relaxation and promote DSB repair in heterochromatin (Goodarzi et al, 2011). Currently, no strong evidence is yet provided for a possible role of CHD complexes in the UV-induced DDR. However, CHD2 deficiency was shown to lead to both UV and IR hypersensitivity (Nagarajan et al, 2009), but the role of this ATPase in the DDR has not yet been elucidated in detail.

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Chapter II

ISWI chromatin remodeling complexes in the DNA damage response

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Cell Cycle, 2014

Abstract

Regulation of chromatin structure is an essential component of the DNA damage response (DDR), which effectively preserves the integrity of DNA by a network of multiple DNA repair and associated signaling pathways. Within the DDR, chromatin is modified and remodeled to facilitate efficient DNA access, to control the activity of repair proteins and to mediate signaling. The mammalian ISWI family has recently emerged as one of the major ATP-dependent chromatin remodeling complex families that function in the DDR, as it is implicated in at least three major DNA repair pathways: homologous recombination, non-homologous end-joining and nucleotide excision repair. In this review, we discuss the various manners through which different ISWI complexes regulate DNA repair and how they are targeted to chromatin containing damaged DNA.

Introduction

DNA damage has a major impact on health and is believed to be the underlying cause of both cancer and aging (Marteijn et al, 2014). To protect against the adverse effects of DNA damage, organisms are equipped with diverse mechanisms of DNA repair and associated DNA damage signaling pathways, collectively called the DNA damage response (DDR) (Giglia-Mari et al, 2011). Although the repair mechanisms of most DDR pathways are known in detail, how they function within chromatin and how chromatin configuration and reconfiguration facilitates the DDR has only recently obtained wider attention. In this review, we discuss novel insights that demonstrate an essential function for the ISWI family of ATP-dependent chromatin remodelers in mammalian DDR regulation. As changes and defects in both chromatin remodeling and in the DDR are linked to human malignancies and aging (Garraway & Lander, 2013; Hoeijmakers, 2009; O'Sullivan & Karlseder, 2012), it is crucial to understand the connection between both processes.

Chromatin remodeling

The basal packaging units of the eukaryotic genome are nucleosomes, which each consist of 147 bp DNA wrapped around a histone octamer containing two copies of each histone (H2A, H2B, H3 and H4). This way of packaging of DNA into chromatin not only provides space for this large molecule but also serves as an efficient and important mechanism to actively regulate DNA transacting processes such as transcription, replication and DNA repair (Soria et al, 2012). Modification of chromatin regulates access of proteins to DNA and also provides cues to attract proteins or initiate signaling cascades.

Chromatin is modified by the specific activity of histone modifiers, histone chaperones and ATP-dependent chromatin remodelers. Histone modifying enzymes predominantly covalently modify the N-terminal tails of histone proteins. This in turn may lead to the modulation of nucleosome dynamics by altering histone-DNA contacts or to the chromatin association of proteins that act on DNA or mediate signaling responses (Zentner & Henikoff, 2013). Many different types of histone modifications play essential roles in orchestrating DNA repair and DNA damage signaling, including acetylation, methylation, phosphorylation, ubiquitylation and ADP-ribosylation (Luijsterburg & van Attikum, 2011).

ATP-dependent chromatin remodeling complexes catalyze the disruption of DNA-histone contacts and can slide or evict nucleosomes by using the energy from ATP hydrolysis

(Clapier & Cairns, 2009). In addition, they control nucleosome assembly and composition, in cooperation with histone chaperones, by exchanging histones and histone variants. Four structurally related, evolutionary conserved families have been described among various chromatin remodeling complexes: SWI/SNF, INO80, CHD and ISWI. Central to these complexes is a catalytic subunit harboring a SWI2/SNF2-family ATPase domain (Eisen et al, 1995). The remodeling families are functionally different because of the unique domains that reside within or adjacent to this domain. In addition, tissue context and specific subunits that are associated with most ATPases determine the particular function of each complex. Some ATP-dependent chromatin remodeling complexes, such as SWI/SNF, form large protein assemblies composed of up to 14 subunits, while others consist of only one or a few additional subunits, such as ISWI and some CHD complexes. In spite of their differences, all four families are involved in transcription, DNA replication (Clapier & Cairns, 2009) and DDR (Lans et al, 2012) by regulating access to DNA, efficient recruitment of repair factors and DNA damage signaling. In this review, we specifically focus on recent findings that implicate ISWI chromatin remodeling complexes in the DDR.

ISWI family of ATP-dependent chromatin remodelers

ISWI (Imitation SWItch) family remodeling complexes were first discovered in *Drosophila melanogaster* (Ito et al, 1997; Tsukiyama et al, 1995; Tsukiyama & Wu, 1995; Varga-Weisz et al, 1997) and later shown to be conserved in many other organisms. So far, seven different mammalian ISWI complexes have been described: WICH, NoRC, RSF, ACF, CHRAC, NURF and CERF (Fig. 1A) (Erdel & Rippe, 2011b; Toto et al, 2014). Each of the complexes contains one of two conserved ATPase subunits: SMARCA5 (SWI-SNF-related Matrix-associated Actin-dependent Regulator of Chromatin A5, also known as SNF2H) or SMARCA1 (also known as SNF2L) besides one or more accessory subunits. SMARCA5 is found in all ISWI complexes except CERF (Banting et al, 2005) and NURF (Barak et al, 2003), which contain SMARCA1 (Fig. 1A), although this ATPase selectivity may in part be cell type specific (Thompson et al, 2012). The ATPase domain of both ATPases, which is comprised of a DExx and HELICc region, belongs to the superfamily of DEAD/H-helicases (Bork & Koonin, 1993; Eisen et al, 1995) and is located in the N-terminal half of the proteins (Fig. 1B). In addition, both proteins contain a HAND, SANT and SLIDE domain in the C terminus (Grune et al, 2003), which regulate the activity and specificity of the ATPase domain (Fig. 1B) (Mueller-Planitz et al, 2013). In line with this, the remodeling activity of Drosophila and yeast ISWI complexes is reduced when either the SANT domain or the SLIDE domain is deleted.(Grune et al, 2003; Hota et al, 2013) Especially the SLIDE domain, which interacts with extranucleosomal DNA, stimulates ATPase activity and is required to move DNA along the nucleosome (Dang & Bartholomew, 2007; Hota et al, 2013).

ISWI complexes exhibit various activities, e.g. ACF and CHRAC and yeast Isw regularly space nucleosomes, whereas others, such as NURF, show the opposite activity (Gkikopoulos et al, 2011; Tsukiyama & Wu, 1995; Varga-Weisz et al, 1997; Yang et al, 2006). By controlling nucleosome spacing, these complexes regulate transcription, either by repression or activation, stimulate replication through heterochromatin and generate and maintain higher order chromatin and chromosome organization (Collins et al, 2002; Corona & Tamkun, 2004; Poot et al, 2004). The accessory subunits in each complex most probably modulate the ATPase's function and activity and are important for target recognition and specificity (Banting et al, 2005; Langst & Becker, 2001; Narlikar et al, 2002).

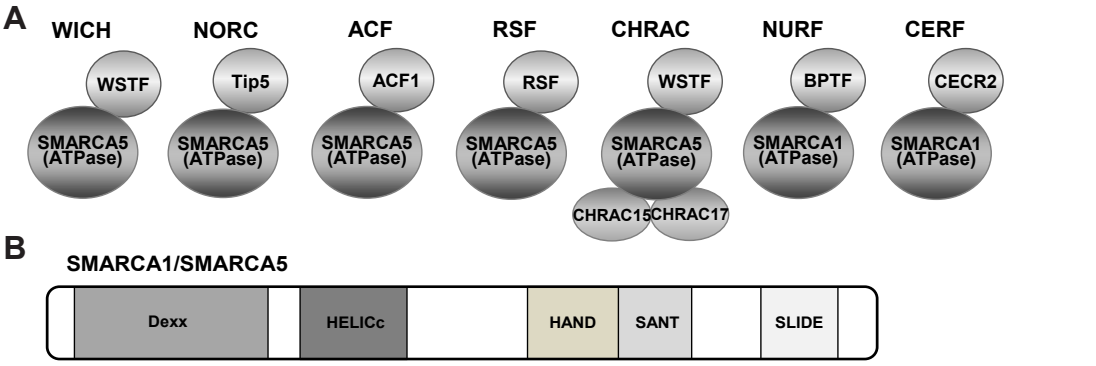

Figure 1. Mammalian ISWI family chromatin remodeling complexes.
(A) Depicted are the seven currently known mammalian ISWI family ATP-dependent chromatin remodeling complexes. WICH, NoRC, RSF, ACF1 and CHRAC all share the SMARCA5 ATPase subunit. NURF and CERF share the SMARCA1 ATPase subunit. (B) Schematic representation of the domains within SMARCA1/SMARCA5. The catalytic activity of both SMARCA1 and SMARCA5 is defined by its ATPase domain that is split in two parts: DExx and HELICc. This domain, which is located in the N-terminal half, is part of the superfamily of DEAD/H-helicases and is shared by SWI2/SNF2-like ATP dependent chromatin remodelers. In addition, both proteins contain HAND, SANT and SLIDE domains that are characteristic for the ISWI family in their C-terminus. These domains regulate the activity of the ATPase domain and are involved in DNA binding.

ISWI function in base excision repair

The continuous induction of a wide diversity of DNA lesions necessitates the action of distinct DNA repair mechanisms, which remove DNA damage depending on the type of lesion, location in the genome and cell cycle phase in which lesions are encountered (Jackson & Bartek, 2009). The major DNA repair mechanisms that have thus far been associated with ISWI chromatin remodeling are Base Excision Repair (BER) and Nucleotide Excision Repair (NER), which both repair lesions that only affect one DNA strand, and Double Strand Break (DSB) repair (Fig. 2).

BER mainly deals with non-bulky small nucleobase lesions, such as oxidative and alkylated bases, by excising and replacing incorrect or damaged bases (Dianov & Hubscher, 2013; Kim & Wilson, 2012). Lesion-specific DNA-glycosylase enzymes recognize specific types of DNA damage, flip the damaged base out of the double helix and cleave the N-glycosidic bond between the substrate base and the 2'-deoxyribose. Next, AP-endonuclease cleaves the abasic site in the sugar-phosphate backbone. The resulting single strand break is then filled in by BER-specific DNA polymerase β and ligated by the XRCC1/ligase III complex (short patch BER). Alternatively, PCNA-dependent DNA polymerase δ polymerizes several nucleotides while displacing the nicked DNA strand, which is removed by the flap-structure endonuclease FEN1, after which DNA ligase I seals the ends (long-patch BER).

How BER functions within chromatin and the role of chromatin remodeling in BER is only poorly understood. *In vitro*, BER efficiency is inhibited by the presence of nucleosomes and compact chromatin, which can be overcome by ATP-dependent chromatin remodeling (Beard et al, 2003; Menoni et al, 2007; Menoni et al, 2012; Nilsen et al, 2002). Evidence for a potential involvement of ISWI in BER comes from an *in vitro* study showing that gap-filling by DNA Polymerase β in oligonucleosome arrays is stimulated by yeast ISWI complexes ISW1 and ISW2 (Nakanishi et al, 2007). This may indicate that ATP-dependent nucleosome displacement facilitates access of DNA polymerase β to DNA. Although it is not understood whether ISWI complexes are similarly required *in vivo*, SMARCA5, SMARCA1

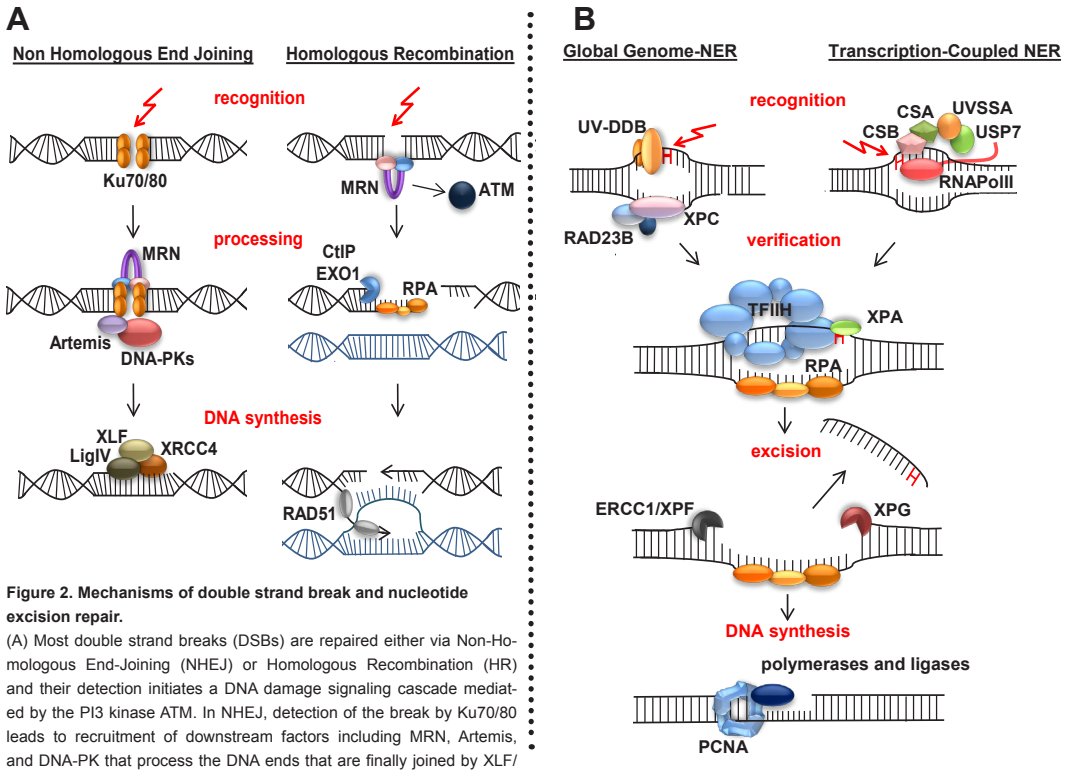

Figure 2. Mechanisms of double strand break and nucleotide excision repair.

(A) Most double strand breaks (DSBs) are repaired either via Non-Homologous End-Joining (NHEJ) or Homologous Recombination (HR) and their detection initiates a DNA damage signaling cascade mediated by the PI3 kinase ATM. In NHEJ, detection of the break by Ku70/80 leads to recruitment of downstream factors including MRN, Artemis, and DNA-PK that process the DNA ends that are finally joined by XLF/XRCC4/LigIV. In HR, detection of the break by MRN leads to 5'-3' end resection, assisted by CtIP and EXO1, which creates ssDNA coated by RPA. RPA is replaced by RAD51, which mediates strand invasion and pairing of a homologous DNA strand that serves as template for error-free repair. The invading DNA end is extended by novel DNA synthesis and either dissociates and re-anneals with its original template, or a second DNA end invades the homologous template after which the resultant joint structures are resolved by specific endonucleases. Finally ligation takes place to complete repair. (B) UV-induced photolesions, monoadducts and other bulky lesions that distort the double helix are repaired by Nucleotide Excision Repair (NER). Lesions are either detected by the global genome NER machinery, via the concerted action of the UV-DDB and XPC/RAD23B complexes, or by the transcription-coupled NER machinery, involving CSA, CSB and UVSSA/USP7 that are recruited to DNA-damage-stalled RNA Polymerase II (RNApolII). Lesion detection leads to recruitment of the transcription factor H (TFIIH) complex, which unwinds DNA and verifies the presence of DNA damage. XPA binds to the lesion in the unwound DNA and RPA covers the non-damaged strand. The endonucleases ERCC1/XPF and XPG bind at both sites of the lesion and excise the damaged strand. The resulting gap is filled in by DNA synthesis and sealed by ligases.

and ACF1 are shown to be recruited to 405 nm laser induced DNA damage in human cells (Erdel & Rippe, 2011a). This type of irradiation mainly induces oxidative damage and single strand breaks (Kielbassa et al, 1997; Lan et al, 2005), which are lesions that are commonly repaired by BER and a variant of BER called single-strand break repair. These findings suggest that human ISWI complexes may assist *in vivo* BER.

Double strand break repair

DSBs can be induced by specific chemicals or reactive oxygen compounds produced by e.g. ionizing radiation or the cell's metabolism. In addition, processing of stalled replication on different types of lesions may eventually lead to the formation of DSBs. As both DNA strands are damaged, DBSs are very hazardous to the cell and can cause chromosome instability and genome rearrangements if not repaired properly. Two main mechanisms are employed to repair DSBs: Homologous Recombination (HR)(San Filippo et al, 2008) and Non-Homologous End Joining (NHEJ) (Lieber, 2010).

HR only takes place in S and G2 phase of the cell cycle when the sister chromatid is present, which is used as template to repair DSBs (Fig. 2A), thereby providing a principally error-free repair of these hazardous lesions. HR is initiated when the MRE11/Rad50/NBS1 (MRN) complex binds to a DSB and together with CtIP and EXO1 resects the broken DNA ends. The created 3'overhangs are bound by the single strand binding protein RPA, which is subsequently exchanged for RAD51 by BRCA2. The resulting RAD51 nucleoprotein filament mediates strand invasion into the homologous sister leading to strand exchange such that the homologous sister chromatid serves as template for novel DNA synthesis. Hereafter, the invading DNA strand dissociates or the other DNA end migrates as well and the resultant joined structures are resolved. Finally, ligation takes place to complete repair (San Filippo et al, 2008). In the NHEJ pathway, which can take place in any cell cycle, broken DNA ends are detected by the Ku70/80 heterodimer, which recruits subsequent repair factors (Fig. 2A)(Lieber, 2008). Following detection, the free DNA ends are processed by Artemis, DNA-PK and the MRN complex and finally joined together by the XLF/XRCC4/LigaseIV complex. NHEJ can be more error-prone than HR, because during end-processing nucleotide loss may occur prior to ligation.

DSBs further lead to an extensive signaling cascade in which chromatin serves as an important signal amplifier. This cascade is initiated by the binding of the MRN complex to DSBs which recruits and activates the PI3-kinase ATM. Activated ATM phosphorylates a range of downstream target proteins, including histone variant H2AX at residue S139 and the E3 ubiquitin ligase BRCA1 (Lukas et al, 2011). H2AX phosphorylation is a key signaling event that spreads into adjacent chromatin, thereby amplifying the signal, and is readily visible under the microscope when stained with specific antibodies - as discernable sub-nuclear foci referred to as ionizing radiation induced foci (Rogakou et al, 1999). The signaling protein MDC1 binds to phosphorylated H2AX and recruits the E3 ubiquitin ligases RNF8 and RNF168 that ubiquitylate H2A/H2AX, after which a range of signaling proteins are recruited, among which are 53BP1 and BRCA1. BRCA1 interacts with several DNA repair proteins including CtIP and, together with 53BP1, regulates whether DSBs are repaired by either NHEJ or HR (Wilm & Mann, 1996; Yun & Hiom, 2009). BRCA1 furthermore regulates cell cycle arrest in concert with cell cycle checkpoint proteins that are also phosphorylated by ATM in response to DNA damage (Yarden et al, 2002). DSB repair and signaling also involve poly(ADP-ribose) (PAR) polymerases PARP1, PARP2 and PARP3, which are thought to PARylate both histone and non-histone proteins to regulate the recruitment of specific PAR-binding repair and chromatin remodeling proteins to stimulate HR or NHEJ (Pines et al, 2013).

ISWI chromatin remodelers in double strand break repair

Most evidence for a role of ATP-dependent chromatin remodeling complexes in DDR comes from studies of DSB repair (Lans et al, 2012; Luijsterburg & van Attikum, 2011). Research on DSB repair is attractive because of its significance for cancer biology. Moreover, cellular DSB repair studies are particularly boosted by the developed technology to induce a single break in a cell which allows the analysis of DSB repair factor binding in intact cells (Lisby et al, 2003; Soutoglou et al, 2007). Several studies have shown that different chromatin remodelers, including SMARCA5 and its binding partners, function in DSB repair. Intriguingly, however, many different activities have been ascribed to these complexes, pointing to multiple parallel functions (Fig. 3) (Lan et al, 2010; Nakamura et al, 2011; Sanchez-Molina et al, 2011; Smeenk et al, 2013). Their interplay and the exact

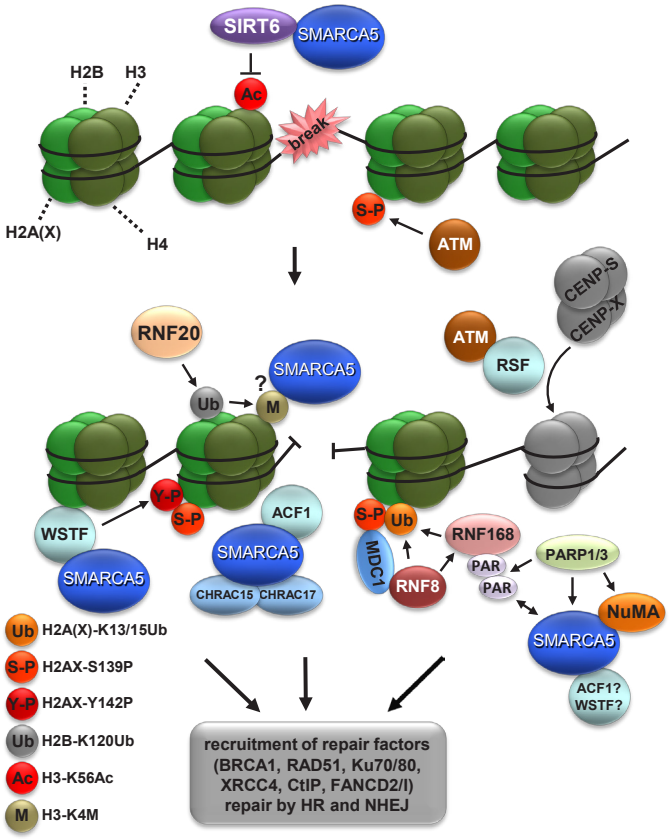

Figure 3. ISWI chromatin remodeling complexes in double strand break repair.
Depicted is a model that includes the various functions of different ISWI complexes at sites of double strand break (DSB) repair. DSBs activate the PI3 kinase ATM, which phosphorylates histone variant H2AX at S139 (indicated with S-P), leading to the recruitment of MDC1, RNF8 and RNF168, which mediate a ubiquitylation signaling cascade. RNF168 is PARylated by PARP1 and interacts with SMARCA5. SMARCA5, whose recruitment is also regulated by NuMA and PARP3, stimulates RNF168-mediated histone ubiquitylation (indicated with Ub). At DSBs, SIRT6 deacetylates H3 at K56 (indicated with Ac), after which RNF20 is recruited to ubiquitylate H2B at K120 (indicated with Ub), coinciding with the methylation of H3 at K4 (indicated with M) and recruitment of SMARCA5, which interacts with SIRT6. SMARCA5 is furthermore recruited together with ACF1, CHRAC15 and CHRAC17 as part of the CHRAC complex and with WSTF as part of the WICH complex. WSTF interacts with and phosphorylates H2AX at Y142 (indicated with Y-P) to maintain S139 phosphorylation (indicated with S-P). Finally, RSF promotes the loading of histone-fold proteins CENP-S and CENP-X at or near DSB sites independently of SMARCA5. The recruitment of SMARCA5, ACF1, WSTF and RSF likely leads to the remodeling of chromatin that is necessary for efficient recruitment of repair factors, including BRCA1, RAD51, Ku70/80 and XRCC4, to facilitate repair by homologous recombination and non-homologous end-joining.

molecular mechanism in which they actually remodel chromatin is currently not well understood.

Both human ISWI ATPases SMARCA5 and SMARCA1 are rapidly recruited to DSBs and their knockdown renders cells hypersensitive to DNA damage (Lan et al, 2010; Nakamura et al, 2011; Sanchez-Molina et al, 2011; Smeenk et al, 2013; Toiber et al, 2013; Vidi et al, 2014). SMARCA5 is recruited to DSBs together with RNF168, in a PARP1-dependent manner, to regulate HR and NHEJ (Smeenk et al, 2013). SMARCA5 binds to PARylated RNF168 and stimulates RNF168-mediated histone ubiquitylation that leads to the recruitment of BRCA1. Another study showed that SMARCA5 recruitment is regulated by PARP3 and the structural nuclear protein NuMA, which interacts with SMARCA5 and is needed for efficient HR (Vidi et al, 2014). Single and dual silencing of NuMA and SMARCA5 led to similar defects in the DSB-induced appearance of ubiquitin foci and CtIP, BRCA1 and RAD51 recruitment, suggesting that both proteins act in a common pathway. Furthermore, SMARCA5 was found to be recruited to DSBs depending on the E3 ubiquitin ligase RNF20 (Nakamura et al, 2011). RNF20 is recruited to DNA damage and ubiquitylates residue K120 of H2B at DSBs to promote the methylation of H3K4. As yeast Isw1 is recruited to chromatin by methylated H3K4 (Santos-Rosa et al, 2003), this suggests that methylated H3K4 also facilitates the binding of mammalian SMARCA5 at sites of DNA damage. Depletion of RNF20 and SMARCA5 or expression of a ubiquitylation-defective H2B mutant leads to impaired DNA end resection and RAD51 and BRCA1 foci formation

and thus defective HR repair. This RNF20-associated defective HR repair may relate to the frequently observed mutations in RNF20 which are seen in colorectal cancers (Barber et al, 2008). Finally, SMARCA5 recruitment to DSBs was shown to depend on an interaction with the deacetylase SIRT6, which localizes early to DSBs to deacetylate H3K56 and is together with SMARCA5 required for efficient DSB signaling and repair (Toiber et al, 2013). As RNF20 recruitment and H2B K120 ubiquitylation were impaired in SIRT6 knockout cells, it was suggested that SIRT6 acts upstream of RNF20 in DSB repair. Intriguingly, SIRT6-deficient mice showed reduced chromatin enrichment of SMARCA5, though only in brain and pancreas but not in liver and heart tissue. These data point to tissue-specific differences in genomic organization involving SMARCA5, which may not only influence tissue-specific transcription, but also DDR.

The importance of ISWI remodeling complexes in genome maintenance is further confirmed by the implication of additional ISWI subunits in chromatin reorganization during DSB repair. ACF1 (ATP-utilizing Chromatin assembly and remodeling Factor 1, also known as BAZ1A) is a noncatalytic subunit of the human ISWI complexes ACF and CHRAC (Fig. 1A). It is thought to enhance the efficiency of nucleosome sliding and to regulate the template specificity of SMARCA5, depending on the DNA flanking the nucleosomes (Eberharter et al, 2001; He et al, 2008; Ito et al, 1999). The ACF complex assists DNA replication through heterochromatin (Collins et al, 2002) and regulates transcription in concert with other histone modifying enzymes and transcriptional regulators (Yasui et al, 2002). The CHRAC complex additionally contains the histone-fold proteins CHRAC15 and CHRAC17 (Fig. 1A), which facilitate ATP-dependent nucleosome sliding by SMARCA5 and ACF1 (Kukimoto et al, 2004). ACF1 is implicated in G2/M checkpoint control in response to DSBs and replication stress (Sanchez-Molina et al, 2011) and functions together with CHRAC15, CHRAC17 and SMARCA5 in both HR and NHEJ (Lan et al, 2010). ACF1 is also rapidly recruited to DSBs, where it interacts with and stimulates the binding of NHEJ-joining KU70/80 proteins to DSBs. However, the interaction of ACF1 with SMARCA5 is only partially required for ACF1 recruitment (Sanchez-Molina et al, 2011) and SMARCA5 is dispensable for KU70/80 recruitment (Lan et al, 2010) This may indicate that SMARCA5 recruitment follows that of ACF1. Since SMARCA5 recruitment is regulated by SIRT6, RNF20, PARP and NuMA, it would be interesting to test whether ACF1 binding to DSBs also depends on these proteins and to determine whether the involvement of ACF1 and SMARCA5 in DSB repair is part of the same process or whether both proteins can act (partially) independent.

Another ISWI complex, WICH, consists of SMARCA5 and the noncatalytic subunit WSTF (Williams syndrome transcription factor, also known as BAZ1B), which is a paralog of ACF1 (Fig. 1A) (Bozhenok et al, 2002) WSTF interacts with the sliding clamp PCNA during replication to promote an open chromatin structure together with SMARCA5 (Poot et al, 2004). WSTF is also recruited to DSBs (Sanchez-Molina et al, 2011) and implicated in DDR signaling by regulating phosphorylation of H2AX (Xiao et al, 2009). In unchallenged conditions, WSTF interacts with H2AX and harbors an unexpected intrinsic kinase activity that constitutively phosphorylates Tyr142 of H2AX, which upon DNA damage induction is needed for the maintenance and amplification of the canonical Ser139 phosphorylation and the sustained recruitment of ATM and MDC1 at damaged sites (Xiao et al, 2009). Remarkably, however, in response to DNA damage, Tyr142 of H2AX is dephosphorylated by the tyrosine phosphatases EYA1 and EYA3 which is a prerequisite for the initial efficient MDC1 binding to phosphorylated Ser139 of H2AX (Cook et al, 2009; Krishnan et al, 2009). Because the pro-apoptotic kinase JNK1 associates with H2AX when it is phosphorylated

on both Ser139 and Tyr142, it was therefore suggested that Tyr142 phosphorylation acts as a molecular switch for a cell to decide between repair and apoptosis (Stucki, 2009). Thus, besides their molecular activity in providing access for repair proteins, on a larger scale ISWI complexes may function in concert with other proteins, such as signaling kinases and phosphatases, to determine cell fate in response to DNA damage.

RSF is another ISWI complex implicated in regulating cell fate. It consists of SMARCA5 and RSF1 (Remodeling and spacing factor 1, also known as HBXAP), a protein that is frequently found to be overexpressed in many types of cancer. Also RSF seems to be involved in genome maintenance, because RSF1 overexpression induces DNA strand breaks via an unknown mechanism (Sheu et al, 2010). If p53 is defective, as is the case for most ovarian serous carcinomas in which RSF1 is overexpressed, DNA damage checkpoint signaling is absent and tumor cells proliferate in the presence of DNA strand breaks, resulting in chromosomal aberrations. Thus, this suggests that the expression of chromatin remodelers must be tightly regulated to prevent genomic instability and inadequate repair. RSF1 was also shown to actively participate in DDR itself, although, surprisingly, independently of SMARCA5. Upon DSB induction, RSF interacts with and is phosphorylated by ATM (Pessina & Lowndes, 2014). Furthermore, RSF1 accumulates at DSB sites without SMARCA5 and promotes NHEJ and HR by facilitating the loading of centromeric histone proteins CENP-S and CENP-X at damaged chromatin, which assist the recruitment of the NHEJ factor XRCC4/LigIV (Helfricht et al, 2013) and the interstrand crosslink repair proteins FANCD2 and FANCI with which RSF also interacts (Pessina & Lowndes, 2014). Furthermore, RSF1 was found to promote HR through a distinct, but unresolved mechanism not involving the loading of CENP-S or CENP-X (Helfricht et al, 2013).

The current evidence suggests that distinct ISWI complexes have multiple functions in DSB repair (Fig. 3). The significance of these complexes for the DDR is emphasized by the notion that HR and NHEJ are impaired when SMARCA5 or associated subunits are depleted. It remains however to be determined whether the association of ISWI complexes with different components of the repair machinery, such as with H2AX, RNF20, RNF168 and Ku70/80, are all part of the same chromatin remodeling event or whether these represent distinct or subsequent steps of the repair reaction.

ISWI chromatin remodelers in nucleotide excision repair

NER removes a large variety of single-stranded helix-distorting DNA lesions, including UV induced DNA damage (Marteijn et al, 2014; Scharer, 2013). It consists of two damage recognition subpathways (Fig. 2B): Global genome repair (GG-NER), which detects damage throughout the whole genome, and transcription-coupled repair (TC-NER), which detects and repairs damage specifically in the transcribed strand of active genes. GG-NER is initiated by the damage detecting protein complex XPC/HR23B, which is - for certain less bulky lesions - assisted by the UV-DDB complex. The UV-DDB complex is particularly important for regulating chromatin organization during GG-NER initiation, as it recruits several ATP-dependent chromatin remodeling proteins (Jiang et al, 2010; Pines et al, 2012; Zhang et al, 2009) and induces chromatin decondensation (Luijsterburg et al, 2012). TC-NER is initiated upon stalling of RNA Polymerase II (RNApolII) at a DNA lesion, which serves as a signal to attract the TC-NER factors CSA, CSB and UVSSA/USP7. Subsequent steps are similar for TC-NER and GG-NER. First, the transcription factor IIH

unwinds the DNA helix and verifies the presence of DNA damage, after which XPA and RPA stabilize the repair complex and properly orient the structure specific endonucleases XPF/ERCC1 and XPG to excise the damaged strand (Fig. 2B). The resulting ~30 nt single stranded DNA gap is filled by DNA synthesis and ligated.

Several studies suggest a role for ATP dependent chromatin remodelers in the initiation of mammalian NER, including SWI/SNF, INO80 and the TC-NER protein CSB itself, which harbors a functional SWI2/SNF2 domain necessary for its function (Citterio et al, 1998; Jiang et al, 2010; Lans et al, 2012; Selzer et al, 2002; Zhang et al, 2009). Because NER intermediates induce a similar DNA damage signaling response as is observed during DSB repair, involving H2AX phosphorylation leading to MDC1 recruitment and an RNF8/RNF168 and ubiquitin-mediated signaling cascade (Hanasoge & Ljungman, 2007; Marini et al, 2006; Marteijn et al, 2009), it is to be expected that ISWI complexes function similarly in this response when induced by NER. Strangely, however, although SMARCA5 and RNF168 interact after ionizing radiation, they do not interact after UV (Smeenk et al, 2013). Intriguingly, recent evidence from our lab shows that ISWI complexes have yet another additional function in regulating UV damage repair, which differs from their identified roles in DSB repair. Within a genetic screen, isw-1, the *C. elegans* ortholog of SMARCA1/SMARCA5, was identified as a novel chromatin-associated protein involved in the UV-induced DDR (Lans et al, 2010) Follow up experiments in mammals showed that knockdown of SMARCA5, but also of ACF1 and WSTF, renders cells sensitive to UV irradiation and defective in the TC-NER dependent re-initiation of transcription after UV (Aydin et al, 2014). SMARCA5, ACF1 and WSTF are recruited to photolesions induced by 266 nm UV-C laser irradiation and like CSB, SMARCA5 recruitment is transcription-dependent. Moreover, SMARCA5, ACF1 and WSTF are needed for efficient recruitment of CSB to UV damage, suggesting that chromatin remodeling by at least two distinct ISWI complexes, ACF1/CHRAC and WICH, facilitates TC-NER (Fig. 4).

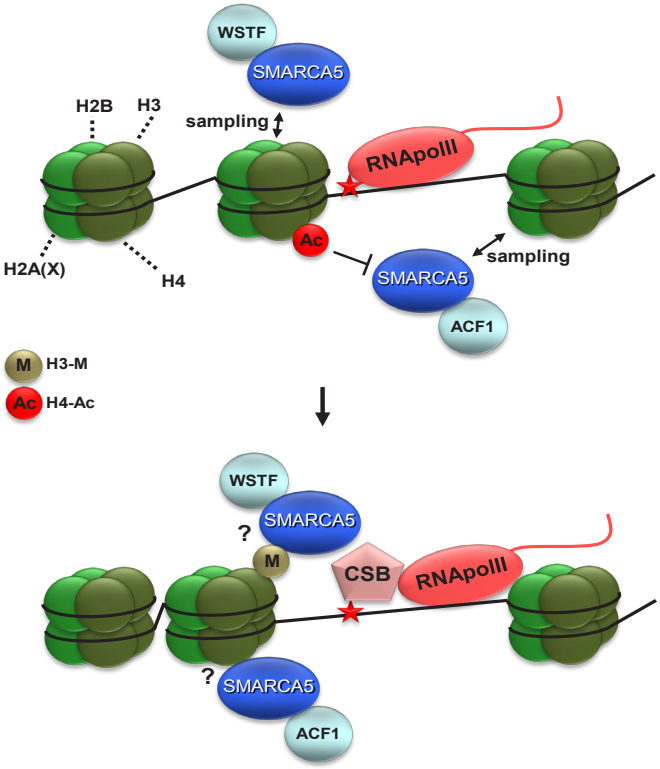

Figure 4. ISWI chromatin remodeling during NER.
Depicted is a hypothetical model of how ISWI complexes function in transcription-coupled nucleotide excision repair. ISWI complexes, including ACF (SMARCA5 and ACF1) and WICH (SMARCA5 and WSTF), continuously sample DNA and only associate with chromatin when encountering specific signals. Such signals could be dependent on RNA polymerase II (RNApolII) arrest at DNA damage and could involve histone deacetylation (acetylation is indicated with Ac) and methylation (indicated with M). DNA damage arrested RNApolII binds with more affinity to the essential repair protein CSB and is itself reverse translocated, likely to make the lesion accessible for repair. SMARCA5 is necessary for efficient loading of CSB at sites of UV-induced transcriptional arrest, suggesting that chromatin remodeling facilitates access to DNA. Reverse translocation of RNApolII probably also requires chromatin remodeling.

Targeting ISWI complexes to DNA damage

Despite the clear evidence that ISWI complexes function in DDR, several questions remain, such as: 1) How do ISWI complexes find sites of DNA damage and identify their target nucleosomes?; 2) What are the exact functional differences between the different ISWI complexes when it comes to actual chromatin remodeling in specific cases?; 3) What determines the choice of chromatin remodeling complex to be used?; 4) What are the interactions of the different complexes *in vivo*? It appears that ISWI complexes localize in a different manner to DSBs, repaired by HR and NHEJ, than to UV-C induced photolesions, repaired by NER. The rapid recruitment to DSBs involves an interaction with RNF168 and depends on PARP activity but not on transcription (Nakamura et al, 2011; Smeenk et al, 2013). In contrast, SMARCA5 does not interact with RNF168 after UV and its recruitment to 266 nm induced UV-C damage is independent of PARP but dependent on active transcription (Aydin et al, 2014; Smeenk et al, 2013). Furthermore, the SLIDE but not the ATPase domain of SMARCA5 is sufficient for DSB recruitment (Lan et al, 2010), whereas in contrast both domains of SMARCA5 are required for recruitment to UV-C damage (Aydin et al, 2014). Finally, however, recruitment to DSBs and UV-C damage may both depends on H3 methylation (Aydin et al, 2014; Lan et al, 2010).

ISWI chromatin remodelers were proposed to continuously sample nucleosomes by transient binding to chromatin until they encounter a signal that permits them to bind longer and with higher affinity to allow remodeling (Erdel et al, 2010). Particularly, specific chromatin modifications or targeting molecules may represent such a cue to target and activate ISWI complexes. Indeed, SMARCA5 activity is stimulated by unacetylated histone H4 tails and specifically inhibited by H4K16 acetylation (Alenghat et al, 2006; Clapier et al, 2001; Corona et al, 2002; Shogren-Knaak et al, 2006). Furthermore, H3K4 methylation was shown in yeast to recruit Isw1 during transcription (Santos-Rosa et al, 2003). In addition, binding and orientation of yeast SMARCA5 orthologs depends on the presence of a specific length of extranucleosomal DNA, involving an interaction with the SLIDE domain (Fig. 1B) (Dang & Bartholomew, 2007; Dang et al, 2006; Kagalwala et al, 2004; Stockdale et al, 2006; Zofall et al, 2004). Thus, multiple cues involving different histone modifications and DNA configurations may act synergistically in determining binding site selectivity and initiation of remodeling activity.

The relatively high concentration of SMARCA5 and its dynamic sampling of chromatin, as observed in live cell imaging experiments, was suggested to ensure efficient and fast loading of SMARCA5 onto its targets, including DNA damaged chromatin (Erdel & Rippe, 2011a; Erdel et al, 2010). This sampling model also provides a kinetic framework for the swift localization of ISWI complexes to UV-C damaged DNA, which represent sites where RNApolII is stalled (Fig. 4). SMARCA5 recruitment to UV-C damage is attenuated when specific cues cannot be detected, i.e. following treatment with chemical inhibitors of histone deacetylation or methylation and when the SLIDE domain that interacts with extranucleosomal DNA is deleted (Aydin et al, 2014). Because recruitment to UV-C damage is also dependent on transcription, these specific histone mark cues that recruit SMARCA5 may be induced as a consequence of lesion-stalled RNApolII. A similar selection mechanism involving histone modifications could also be envisioned for DSB repair, as H3K4 methylation and H3K56 deacetylation coincide with SMARCA5 enrichment at DSBs (Nakamura et al, 2011; Toiber et al, 2013). Additionally, these recruitment cues could be formed by specific protein interactions, such as with SIRT6, PARylated RNF168 and NuMA.

It is also likely that accessory subunits such as ACF1 participate in site selection, as it was shown that a small ACF1 interaction motif in SMARCA5 is necessary for its localization to DSBs (Lan et al, 2010).

The recruitment of SMARCA5 to UV-C damage, but not to DSBs, depends on ATP hydrolysis (Aydin et al, 2014; Lan et al, 2010). SMARCA5 was proposed to use the energy of ATP to associate to substrate nucleosomes, as part of a kinetic proofreading mechanism (Blossey & Schiessel, 2011; Narlikar, 2010). In this model, ISWI complexes act as dimers, containing two ATPases (Racki et al, 2009) in which the first ATP-hydrolysis is used to become committed to a nucleosomeal target, after sampling for cues, which is followed by a second ATP-hydrolysis event that is used for DNA-nucleosome translocation (remodeling). This model is supported by the observation that ATP hydrolysis promotes a more stable and extensive binding of yeast ISW1a and ISW2 to template nucleosomes, before actual translocation takes place (Gangaraju et al, 2009). Thus, ATPase dependent recruitment to UV-C damage may imply that SMARCA5 employs both a sampling as well as a proofreading mechanism to identify and subsequently associate with substrate nucleosomes near or containing UV-damaged DNA.

Chromatin remodeling activity of ISWI complexes at sites of DNA damage

Following target identification, several observations suggest that SMARCA5, as expected, indeed remodels nucleosomes to promote an open chromatin environment to stimulate repair. The requirement of RNF20, SIRT6 and SMARCA5 in HR can be bypassed by forced chemical chromatin relaxation and DNA near a DSB is less accessible to nuclease digestion if SIRT6 is knocked down (Nakamura et al, 2011; Toiber et al, 2013). Furthermore, ATPase activity of SMARCA5 is necessary for both BRCA1 accumulation (Smeenk et al, 2013) and its stimulation of NHEJ (Lan et al, 2010). Both at DSBs and at UV-C lesions, SMARCA5 has been observed to re-localize to the periphery of a DNA damaged area upon initial recruitment which may represent the remodeling of chromatin. In case of DSBs, this re-localization depends on PARP1 activity and involves chromatin expansion and spreading of SMARCA5 and downstream DSB factors throughout damaged chromatin (Smeenk et al, 2013). Although PARP1 also stimulates chromatin remodeling and the re-localization of histones during GG-NER initiation (Pines et al, 2012; Luijsterburg et al., 2012), a similar chromatin expansion is not observed at sites of laser-induced UV-C damage (Dinant et al, 2013) and SMARCA5 spreading to the periphery of these sites also does not depend on PARP activity (Aydin et al, 2014). This suggests that chromatin remodeling activities of ISWI complexes at sites of DSBs and UV lesions are different, but it is not known which substrates define these differences. Dedicated research is thus required to disclose the molecular mechanisms of nucleosome and/or histone displacement by ISWI complexes at different DNA lesions. Furthermore, understanding how ISWI complexes localize to DNA damage may prove to be very helpful to uncover how exactly chromatin remodeling complexes identify their target nucleosomes *in vivo*.

Concluding remarks

In summary, it appears that distinct ISWI complexes carry out a variety of different functions in the mammalian DDR and have multiple different ways of accommodating chromatin structure to facilitate efficient DNA repair and signaling. Importantly, chromatin organization involving SMARCA5 complexes may be an important determinant for cancer development

and treatment. SMARCA5 expression is regulated by the miR-99 family of microRNAs, consisting of miR-99a and miR-100, which is induced upon DNA damage induction (Sun et al, 2011) but shows reduced expression in more advanced cancers (Mueller et al, 2013). Differences in radiation sensitivity between breast tumor cell lines were attributed to the ability of cells to express the miR-99 family in response to DNA damage (Mueller et al, 2013). Upregulated miR-99a and miR-100 expression leads to SMARCA5 downregulation which in turn reduces DNA repair, making cells more sensitive to DNA damage and especially to multiple rounds of DNA damage induction such as used in cancer therapy. Therefore, decreased expression of this miRNA family in cancers may be a mechanism for tumor cells to upregulate DNA repair and acquire resistance to DNA damaging agents. Thus, SMARCA5 may be a component in cancer resistance to DNA damaging agents and therefore a potential therapeutic target. Interestingly, inhibition of the other ISWI ATPase, SMARCA1, selectively activates the DDR and leads to growth inhibition and apoptosis of highly malignant tumor cells (Ye et al, 2009), implying that ISWI complexes in general may be promising targets for cancer treatment.

Acknowledgements

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Chapter III

Human ISWI complexes are targeted by SMARCA5 ATPase and SLIDE domains to help resolve lesion-stalled transcription

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Abstract

Chromatin compaction of DNA presents a major challenge to the detection and removal of DNA damage. Helix-distorting DNA lesions that block transcription are specifically repaired by transcription-coupled nucleotide excision repair, which is initiated by binding of the CSB protein to lesion-stalled RNA polymerase II. Using live cell imaging, we identify a novel function for two distinct mammalian ISWI ATP-dependent chromatin remodeling complexes in resolving lesion-stalled transcription. Human ISWI isoform SMARCA5/SNF2H and its binding partners ACF1 and WSTF are rapidly recruited to UV-C induced DNA damage to specifically facilitate CSB binding and to promote transcription recovery. SMARCA5 targeting to UV-C damage depends on transcription and histone modifications and requires functional SWI2/SNF2-ATPase and SLIDE domains. After initial recruitment to UV damage, SMARCA5 re-localizes away from the center of DNA damage, requiring its HAND domain. Our studies support a model in which SMARCA5 targeting to DNA damage-stalled transcription sites is controlled by an ATP-hydrolysis dependent scanning and proofreading mechanism, highlighting how SWI2/SNF2 chromatin remodelers identify and bind nucleosomes containing damaged DNA.

Introduction

DNA is continuously damaged by environmental agents and endogenous factors. DNA damage interferes with transcription and replication, causing cell death, chromosomal aberrations or mutations, eventually leading to aging and tumorigenesis (Hoeijmakers, 2009). To protect against the adverse effects of DNA damage, organisms are equipped with diverse DNA repair and associated DNA damage signaling pathways, collectively called the DNA damage response (DDR) (Jackson & Bartek, 2009).

Nucleotide Excision Repair (NER) removes different types of helix-distorting DNA lesions, including UV-induced cyclobutane pyrimidine dimers (CPD) and pyrimidine (6-4) pyrimidone photoproducts (6-4PP). Its biological relevance is illustrated by the severe cancer prone and/or progeroid features presented by patients suffering from rare hereditary NER-deficient syndromes (Hoeijmakers, 2009). NER consists of two damage recognition pathways: Global genome repair (GG-NER) and transcription-coupled repair (TC-NER). GG-NER detects lesions located anywhere in the genome and is initiated through cooperative damage detection by the UV-DDB and XPC/HR23B protein complexes (Sugasawa, 2010). TC-NER repairs transcription blocking damage and is initiated by RNA Polymerase II (RNApolII) stalling at lesions, which attracts the essential TC-NER factors CSA and CSB and the UVSSA/USP7 complex (Vermeulen & Fousteri, 2013). Damage recognition leads to the recruitment of the transcription factor IIH to verify the damage and open the surrounding DNA helix. Next, XPA and RPA bind to stabilize the repair complex and properly orient the structure specific endonucleases XPF/ERCC1 and XPG, which excise the damaged strand. The resulting 30 nucleotide single strand DNA gap is filled and sealed by DNA synthesis and ligation (Huang et al, 2013).

Chromatin interferes with DNA binding of proteins implicated in DNA-transacting processes such as transcription, replication and DNA repair. For efficient execution of these processes chromatin is commonly modified to regulate access of proteins to DNA. ATP-dependent chromatin remodeling complexes modify chromatin by catalyzing the disruption of DNA-histone contacts and can slide or evict nucleosomes or alter their composition (Clapier &

Cairns, 2009). Four structurally related conserved families of ATP-dependent chromatin remodeling complexes have been described: SWI/SNF, INO80, CHD and ISWI. Next to their established roles in transcription and replication it has recently become clear that these remodeling complexes are also implicated in the DDR, including NER (Lans et al, 2012; McGlynn & Lloyd, 2002). The mammalian and yeast SWI/SNF ATPase BRG1 and several regulatory subunits interact with GG-NER initiation factors XPC or DDB2 and stimulate efficient repair of CPDs (Gong et al, 2006; Ray et al, 2009; Zhang et al, 2009; Zhao et al, 2009). INO80 was also found to play a role in GG-NER, in yeast to restore repair-induced nucleosome loss (Sarkar et al, 2010) and in mammals to regulate XPC recruitment and efficiency of repair (Jiang et al, 2010). It was speculated that chromatin compaction may not be a major hurdle for TC-NER as chromatin is already opened because of transcription (Hanawalt & Spivak, 2008). However, several chromatin modifying factors have been linked to this process as well. The histone acetyl-transferase p300 and HMGN1 were found to associate with TC-NER complexes in a CSB-dependent manner (Fousteri et al, 2006). In addition, efficient restart of transcription after TC-NER was found to depend on histone methyltransferase DOT1L (Oksenych et al, 2013) and on accelerated histone H2A exchange and new histone H3.3 deposition, mediated by the FACT and HIRA histone chaperones, respectively (Adam et al, 2013; Dinant et al, 2013). Furthermore, the TC-NER key factor CSB exhibits ATP-dependent chromatin remodeling activity *in vitro* (Citterio et al, 2000; Lake et al, 2010), which is stimulated by the histone chaperones NAP1L1 and NAP1L4 (Kee & D'Andrea, 2010). Although in *C. elegans* (Lans et al, 2010) as well as in yeast (Gari & Constantinou, 2009) ATP-dependent chromatin remodeling was suggested to promote TC-NER, it is still unknown whether and how ATP-dependent chromatin remodeling plays a role in mammalian TC-NER.

Using genetic screening in the nematode *C. elegans* to find novel chromatin-associated proteins involved in the UV-induced DDR, we have previously identified isw-1 (Lans et al, 2010). isw-1 is orthologous to mammalian SNF2H/SMARCA5 (Andersen et al, 2006), the major catalytic ATPase subunit of several ISWI-type chromatin remodeling complexes (Aihara et al, 1998), suggesting that these complexes play an important role in the cellular response to UV-induced DNA damage. Here, we used a live cell imaging approach to identify a new function for SMARCA5 and its binding partners WSTF and ACF1 in mammalian TC-NER, which is mechanistically distinct from its role in response to double strand DNA breaks (DSBs) (Friedberg et al, 2005; Lan et al, 2010; Nakamura et al, 2011; Sanchez-Molina et al, 2011; Smeenk et al, 2013; Xiao et al, 2009). Our findings indicate that ISWI chromatin remodeling complexes utilize ATP-hydrolysis and the SMARCA5 SLIDE domain to associate with UV-damaged chromatin to specifically promote CSB recruitment and to resolve damage-stalled transcription.

Material and methods

Cell culture

U2OS, HeLa, MRC5, TA24 (UVSSA deficient), XP4PA (XPC deficient) and CS1AN (CSB deficient) cell lines were cultured in a 1:1 mixture of Ham's F10 (Lonza) and Dulbecco's modified Eagle's medium (DMEM) (Lonza) supplemented with antibiotics and 10% fetal calf serum (FCS) at 37°C, 20% O2, and 5% CO2. Primary wild-type human C5RO fibroblasts were cultured in Ham's F10 (Lonza) supplemented with antibiotics and 15% fetal calf serum. U2OS and MRC5 cells expressing GFP-fusion proteins were generated by transfection

and isolation of stable colonies and FACS sorting. To inhibit transcription, cells were treated with α-amanitin (25 μg/ml) for 12 hrs or with DRB (75 μM) for 1 hr. To inhibit methylation, cells were treated with AdOX (Adenosine Dialdehyde) (20 μM or 100 μM) for 12 hrs. For PARP inhibition experiments, cells were treated with olaparib (AZD2281, 10 μM) or PJ34 (10 μM) for 1 hr (Pines et al, 2012; Smeenk et al, 2013). Efficient PARP inhibition by both inhibitors (Supplementary Fig. S4B) was demonstrated by immunofluorescence (IF) using monoclonal PAR antibody 10H (Alexis Biochemicals) following 5 min 50 mM H2O2 treatment, which induces granular nuclear PAR staining (Dinant et al, 2013; Rogakou et al, 1998). To inhibit deacetylation, cells were treated with HDAC inhibitors, TSA (Trichostatin A, 45 nM) for 20 hrs or Na-Bu (Sodium Butyrate, 10 mM) for 2 hrs.

Plasmids and siRNAs

Cloning details for SMARCA5-GFP, ACF1-GFP, GFP-WSTF and CPD-Photolyase-mCherry are available upon request. TA24 cells expressing UVSSA-GFP (Schwertman et al, 2012) and CS1AN cells expressing GFP-HA-CSB (van den Boom et al, 2004) were described before. Site-directed mutagenesis was used to generate the ATPase inactivating SMARCA5 mutant, by changing Lys211 in the nucleotide-binding motif to Arg (Brestovitsky et al, 2011), and to generate SMARCA5 deletion mutants of the HAND (aa 743-843), SANT (aa 741-890) and SLIDE (aa 898-1012) domains. To stably knock down protein expression, cells were transduced with MISSION shRNA (Sigma-Aldrich; Clone ID SHC002 for control; TRCN0000016776 for CSB; TRCN0000083194 for XPA; TRCN0000013217 for SMARCA5; TRCN0000013342 for WSTF), by lentiviral transduction (Dull et al, 1998) and selection with puromycin. Transient siRNA-mediated knockdown was achieved using Lipofectamine RNAiMAX (Invitrogen) transfection according to the manufacturer's instruction. siRNAs used were from Dharmacon: control (D-001210-05), SMARCA5 (L-011478-00), ACF1 (L-006941-00 and J-006941-05), CSB (L-004888-00), WSTF (custom, AAGCCCGCUUGGAAAGGUACA), XPC (custom, CUGGAGUUUGAGACAUAUCUU).

Colony survival

To determine colony survival, approximately 300 cells were plated in 6-well plates in triplicate. After 12-16 hrs, cells were irradiated with a single dose of UV irradiation (0-8 J/m^2; 254 nm; Philips TUV lamp). After 7 days, colonies were fixed and stained with 0.1% Brilliant Blue R (Sigma) and counted. The survival was plotted as the mean percentage of colonies obtained after treatment compared to the mean number of colonies from the non-irradiated samples.

UV-induced UDS and RRS

UDS was measured following UV-C irradiation (16 J/m^2) of C5RO primary fibroblasts grown on 24 mm cover slips and transfected with siRNA. Irradiated cells were incubated for 2 hrs in the presence of 0.1 mM 5-ethynyl-29-deoxyuridine (EdU; Invitrogen) after UV irradiation. Recovery of RNA Synthesis (RRS) was performed in siRNA-transfected HeLa or U20S cells 16 hrs after UV-C irradiation. Unirradiated and irradiated cells were incubated for 2 hrs in the presence of 0.1 mM 5-ethynyl-uridine (EU). EdU and EU incorporation was visualized using Click-iT Alexa Fluor 594 according to the manufacturer's protocol (Invitrogen). UDS and RRS levels were quantified by measuring and averaging fluorescence intensities for >100 cells with ImageJ software of images obtained with a Zeiss LSM700 confocal microscope.

Immunofluorescence and western blotting

For IF, cells were grown on 24 mm coverslips for 3 days prior to the experiments and fixed using 2% paraformaldehyde in the presence of 0.1% Triton X-100. Cells were immunostained as described previously (Rademakers et al, 2003) and embedded in Vectashield mounting medium with DAPI (Vector Laboratories, Burlingame, CA). Images (Supplementary Fig. S2C and S4B) were obtained using a LSM510 META confocal microscope (Carl Zeiss, Inc.). Antibodies used for IF and western blotting were: anti-SMARCA5 (SNF2H; Abcam), anti-CSB (E18, SantaCruz), anti-ACF1 (Novus) and anti-WSTF (affinity purified as described in (Poot et al, 2000)), anti-CPD (TDM-2; MBL International).

Live cell confocal laser-scanning microscopy

All live cell confocal laser-scanning images were obtained at 37°C using a Leica TCS SP5 microscope (with Leica Application Suite), except Supplementary Figs. S3C and S4D, which were obtained using a Zeiss LSM 510 (with LSM image browser), both equipped with a 100× quartz objective. Local UV-C damage was induced by laser irradiation at 266 nm (Rapp OptoElectronic, Hamburg GmbH), which specifically creates CPD and 6-4PP DNA lesions that are repaired by NER but no strand breaks, as described previously (Dinant et al, 2007). To quantify the recruitment of SMARCA5 in the center and periphery of a damaged area, we used three different regions of interest (ROIs), one in the middle, one in the periphery and one in an area of the nucleus not exposed to DNA damage (‘outside’) to check for monitor bleaching (Supplementary Fig. S3B). All three ROIs were quantified with ImageJ software and curves were normalized to the first data points prior to damage induction. For every curve, at least 10 cells were measured and all results were confirmed by independent duplicate experiments. Statistical difference between curves was determined by one-way ANOVA comparison of areas under each curve.

Immunoprecipitation

CSB (Fig. 5C), ACF1 and WSTF (Supplementary Fig. S5A and B) were immunoprecipitated using chromatin-enriched nuclear extracts from 10 14-cm culture dishes of GFP-CSB expressing CS1AN cells or 5 14-cm culture dishes of U2OS cells expressing ACF1-GFP or GFP-WSTF. Cells were collected 20 (CSB) or 5 min (ACF1/WSTF) after irradiation (20 J/m^2) by scraping in 3 ml of PBS containing protease inhibitor cocktail (Roche), centrifuged for 5 min at 1500 rpm and washed again with PBS. Cells were swollen in 5x pellet volume of Hepes buffer (10 mM HEPES pH 7.6, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, protease inhibitor cocktail) for 10 min. Nuclei were isolated by douncing cells with a type A pestle and centrifugation at 3000 rpm for 10 min at 4°C. Nuclei were washed and resuspended in 1.5 x pellet volumes of Hepes buffer (100 mM HEPES pH 7.6, 1.5 mM $MgCl_2$ 150 mM NaCl, 25% glycerol, protease inhibitor, 0.5 mM DTT) and subsequently dounced using a pestle type B. Next, chromatin was digested with 25 U Microccocal nuclease (MNase; Sigma) for 1 hr at 4°C. These conditions were chosen such that DNA was digested to mononucleosome size. The resulting chromatin-enriched nucleoplasmic fraction was cleared from insoluble nuclear material by centrifugation at 15000 rpm for 15 min. For immunoprecipitation of SMARCA5 mutants (Fig. 7C), extracts were prepared by scraping cells from a 14-cm dish in RIPA buffer (PBS containing 1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS; Roche protease inhibitor cocktail) followed by sonication (to obtain DNA fragments <800 bp) and 16000g

centrifugation at 4°C for 10 min to remove insoluble material. Extracts were incubated with GFP-trap beads (Chromotek) for 2 hrs at 4 °C. Subsequently, beads were washed four times in Hepes Buffer and boiled in Laemmli sample buffer for analysis by western blotting.

Results

SMARCA5 functions in the transcription-coupled response to UV

Following the identification of isw-1 in the UV response of *C. elegans* (Lans et al, 2010), we tested whether its mammalian ortholog SMARCA5 is also involved in the UV-DDR. Stable knockdown of SMARCA5 renders HeLa cells hypersensitive to UV, similar as CSB knockdown (Fig. 1A and B). These data suggest that SMARCA5 has an evolutionary conserved function in the UV-DDR.

Because chromatin remodeling is thought to be required to facilitate access for NER damage detection proteins (Lans et al, 2012), we subsequently investigated whether SMARCA5 specifically regulates GG-NER, TC-NER or both. First, we determined UV-induced unscheduled DNA synthesis (UDS) as a measure of GG-NER (Nakazawa et al, 2010). Cells treated with siRNA against SMARCA5 (Fig. 1A) exhibited a similar UDS level as control treated cells, whereas knockdown of XPC caused a strong UDS reduction (Fig. 1C). These data suggest that SMARCA5 is not involved in GG-NER. Next, we measured

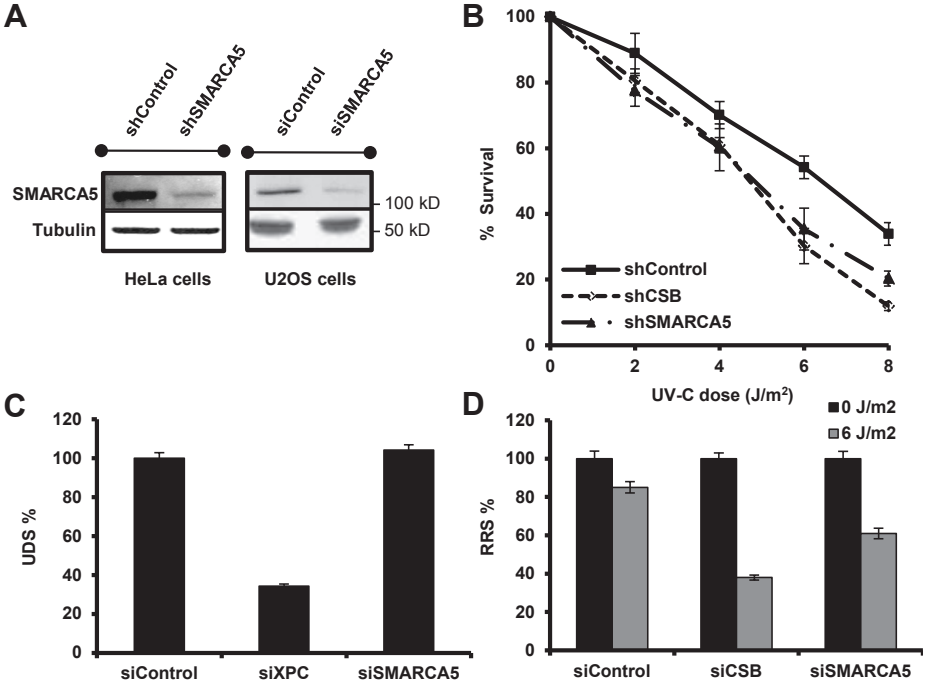

Figure 1. SMARCA5 functions in transcription-coupled repair.

(A) Immunoblots show reduced SMARCA5 expression levels in HeLa cells stably expressing shRNA and U2OS cells treated with siRNA against SMARCA5. Tubulin was used as loading control. (B) SMARCA5 depletion sensitizes cells to UV. Colony survival of HeLa cells stably expressing shRNA against SMARCA5 or CSB following UV irradiation. The percentage of surviving cells is plotted against the applied UV-C dose (J/m^2). (C) DNA repair synthesis (UDS) after UV-irradiation (16 J/m^2), determined by EdU incorporation, as a measure for GG-NER, in wild-type primary fibroblasts (C5RO) (>100 cells for each sample) treated with siRNA. Plotted are, respectively, control (set at 100% UDS), XPC and SMARCA5 siRNAs. (D) Recovery of RNA synthesis (RRS), as a measure for TC-NER, determined by EU incorporation 16 hrs after UV-irradiation (0 and 6 J/m^2) in HeLa cells (>100 cells) treated with respectively control (set at 100% at 0 J/m^2), CSB and SMARCA5 siRNAs. Error bars denote standard error of the mean. Results of all experiments were confirmed at least twice.

recovery of RNA synthesis (RRS) after UV-induced inhibition, as a measure of TC-NER (Nakazawa et al, 2010). Cells depleted for both SMARCA5 and CSB showed reduced RRS levels after UV (Fig. 1D). Similar results were obtained with an shRNA targeting a different part of the SMARCA5 mRNA, ruling out off-target effects (Supplementary Fig. S1A). Importantly, transcription in non-damaged cells was not affected, suggesting that the decrease in RRS is not caused by a general transcription reduction induced by SMARCA5 knockdown (Supplementary Fig. S1B). These results indicate that SMARCA5 is specifically involved in TC-NER and/or regulates transcription restart after TC-NER.

SMARCA5 accumulates at local UV-C damage

TC-NER factors such as CSB localize to DNA damage induced by a 266 nm UV-C laser, which specifically induces CPD and 6-4PP photolesions (Fig. 2A) (Dinant et al, 2007; Schwertman et al, 2012). Stably expressed GFP-tagged SMARCA5 also rapidly accumulated at local UV-C damage, in both U2OS and MRC5 cells (Fig. 2A, Supplementary Fig. S2A), in a dose dependent manner (Supplementary Fig. S2B). This was confirmed by local UV damage induction using a microporous filter (Supplementary Fig. S2C)(Volker et al, 2001). The association of TC-NER factors with TC-NER complexes depends on stalling of RNApolII complexes and thus on active transcription (van den Boom et al, 2004; Vermeulen & Fousteri, 2013). Inhibition of RNApolII activity using α-amanitin (Brueckner & Cramer, 2008) indeed decreased the accumulation of both CSB and SMARCA5 at local damage (Fig. 2B). SMARCA5 recruitment was also attenuated by the transcription elongation inhibitor DRB (Supplementary Fig. S3A, in which both recruitment to the center and periphery of the damaged area is quantified as explained below) (Zhu et al, 1997). These results confirm a function of SMARCA5 in TC-NER and suggest that this protein may localize to UV damage depending on RNApolII stalling.

TC-NER and suggest that this protein may localize to UV damage depending on RNApolII stalling. Intriguingly, however, the accumulation characteristics of SMARCA5-GFP were different from those of GFP-CSB. Whereas CSB remained localized in the center of the damage, SMARCA5 swiftly spread from the center to the periphery of the UV damaged area (Fig. 2A and Supplementary Fig. S3B). Binding kinetics at the periphery were slower but eventually reached a higher level than at the damage center. Fluorescence intensity measurements across the damage confirmed that SMARCA5, but not CSB, re-localized around the damage center (Fig. 2C). Furthermore, co-expression of SMARCA5-GFP with mCherry-fused Potorous tridactylus CPD-photolyase, which specifically binds to CPDs (Chigancas et al, 2004), also showed that SMARCA5 moves away from the area with the highest damage concentration and accumulates at the periphery (Supplementary Fig. S3C). This peculiar re-localization was never observed for any of the tested NER proteins (Vermeulen, 2011).

DSB induction by laser micro-irradiation also triggers SMARCA5 recruitment and spreading into adjacent chromatin, which is dependent on poly(ADP-ribose) polymerase (PARP) activity (Smeenk et al, 2013). However, PARP inhibition by olaparib (Fig. 2D) or PJ-34 (Supplementary Fig. S4A and B) did not affect SMARCA5 recruitment and spreading, indicating that its recruitment and function at UV-induced DNA damage involve a different mechanism than at DSBs. As GFP-CSB recruitment was also unaffected by PARP inhibition (Fig. 2D), these results suggests that poly(ADP) ribosylation does not play a role in the assembly of TC-NER factors.

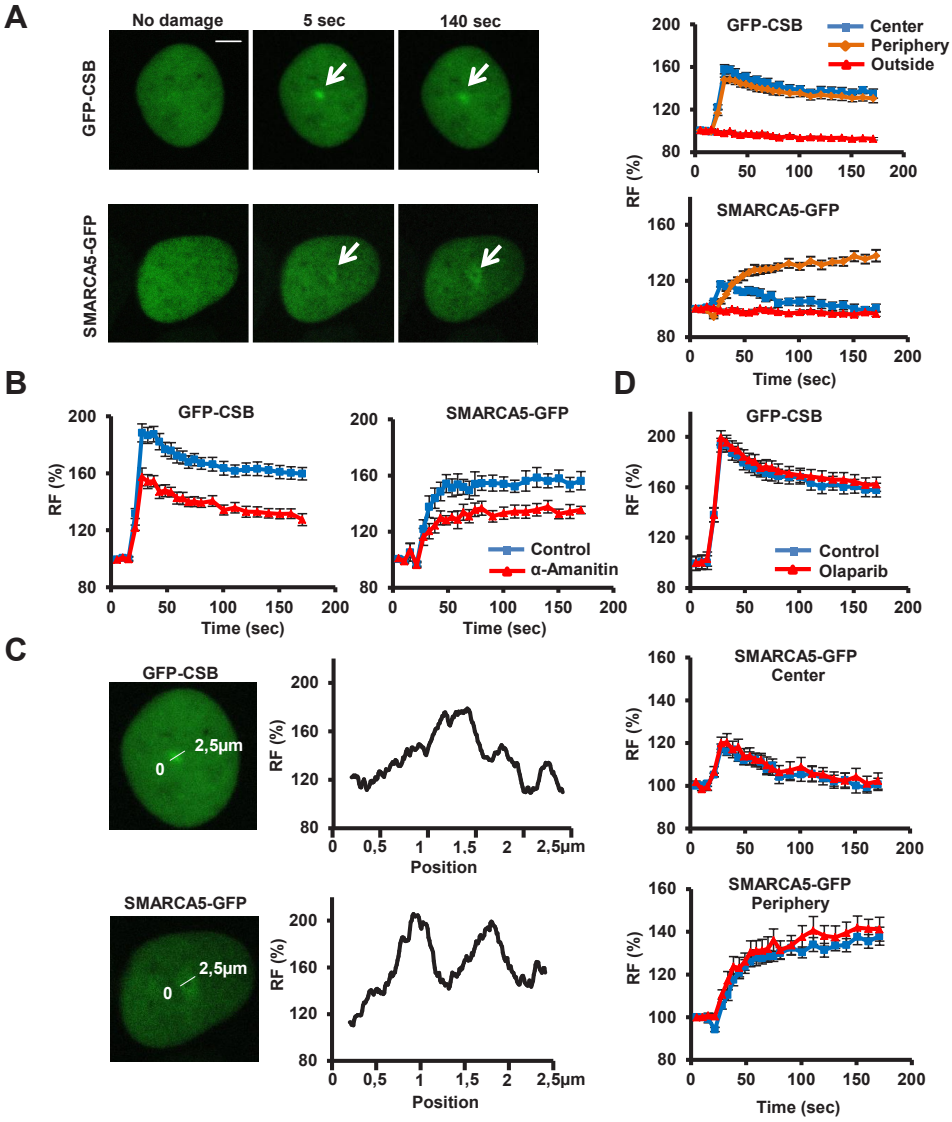

Figure 2. Transcription-dependent SMARCA5 (re)localization to UV-C damage.
(A) Live cell images (left) before, 5 and 140 seconds after UV-C (266 nm) laser-induced local damage (arrows) of GFP-CSB (expressed in CSB deficient CS1AN fibroblasts) and SMARCA5-GFP (expressed in U2OS cells). Scale bar is 5 µm. Graphs (right) show the normalized fluorescence intensities (n>10 cells) that indicate recruitment to the damage center (blue), the damage periphery (orange) and outside the damaged area (red; mean ± standard error of the mean) of GFP-CSB (top) and SMARCA5-GFP. (B) Treatment with α-amanitin impairs the binding to DNA damage sites of GFP-CSB ($p<0.01$ compared to control) and SMARCA5-GFP (peripheral recruitment, $p=0.018$ compared to control)). (C) Line scans of GFP-CSB and SMARCA5-GFP intensity along the indicated line in the image (n=5 cells). (D) GFP-CSB ($p=0.363$ compared to control) and SMARCA5-GFP recruitment (center $p=0.682$, periphery $p=0.36$ compared to control) to DNA damage is unaffected upon PARP inhibition using olaparib (n>10 cells, mean ± standard error of the mean). RF denotes Relative Fluorescence. All results were confirmed using independent, duplicate experiments.

SMARCA5 recruitment depends on histone modifications but not on NER

DNA damage-induced binding of CSB to stalled RNApolII complexes is essential for the subsequent assembly of most other repair factors (Fousteri et al, 2006). Surprisingly, however, depletion of CSB by siRNA did not affect the recruitment of SMARCA5 (Fig. 3A). Local damage accumulation of SMARCA-GFP in CSB-deficient CS1AN fibroblasts

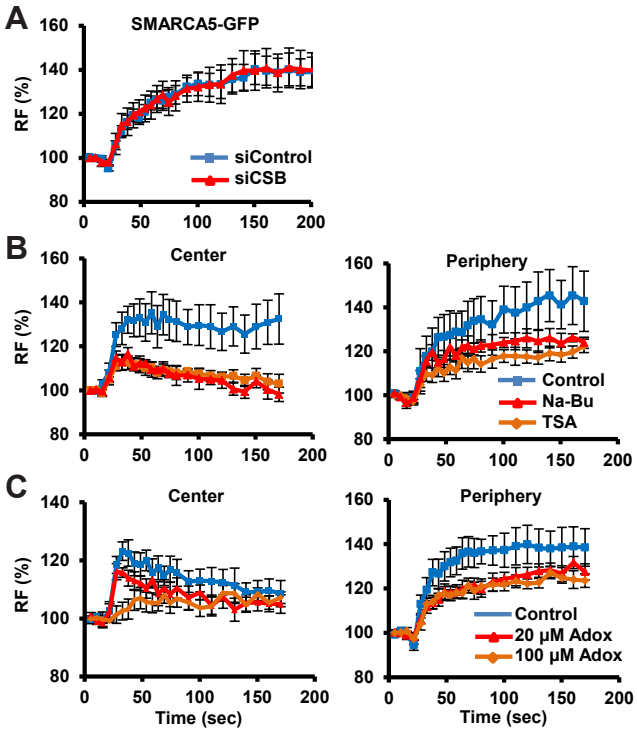

Figure 3. DNA damage association of SMARCA5 requires histone modifications but not NER.

(A) SMARCA5-GFP recruitment is not affected by siRNA-mediated knock-down of CSB in U2OS cells (p=0.97 compared to control). (B, C) SMARCA5-GFP recruitment to DNA damage, both at the center (left) and at the periphery (right), is impaired after HDAC inhibition by TSA (center p=0.006, periphery p=0.040 compared to control) and Na-Bu (center p=0.006, periphery p=0.187 compared to control) treatment (B) and by methyltransferase inhibition using Adox (C; 100 μM center p=0.064, periphery p=0.041; 20 μM center p=0.197, periphery p=0.076 compared to control). For each experiment, the mean of n>10 cells ± standard error of the mean are shown. Graphs depict the normalized fluorescence intensity indicating DNA damage recruitment at the damage center or periphery. Results were confirmed using independent, duplicate experiments. RF denotes Relative Fluorescence.

further confirmed that CSB is not required to bring SMARCA5 to UV damaged chromatin (Supplementary Fig. S4C). SMARCA5 also accumulated normally to UV damage in TC-NER defective UVSSA fibroblasts and GG-NER defective XPC fibroblasts (Supplementary Fig. S4C). This suggests that SMARCA5 recruitment does not depend on TC-NER or GG-NER initiation and that SMARCA5 functions upstream or in parallel to TC-NER.

The activity of many ATP-dependent chromatin remodelers is regulated by post-translational modifications of histones. For instance, lysine acetylation of histone H4 N-terminal tails was found to interfere with *Drosophila* and mammalian ISWI/SMARCA5 binding and function (Alenghat et al, 2006; Corona et al, 2002; Shogren-Knaak et al, 2006). Increasing histone acetylation using the histone deacetylase (HDAC) inhibitors TSA and NaBu indeed reduced SMARCA5 recruitment to UV damage, both at the center as well as at the periphery (Fig. 3B). Methylation of histone H3 is another post-translational modification that was shown to be necessary for SMARCA5 association with chromatin during transcription and DSB repair (Nakamura et al, 2011; Santos-Rosa et al, 2003). We found that inhibition of histone methylation using Adox also reduced recruitment of SMARCA5 to UV damage (Fig. 3C). Together, these results indicate that chromatin is modified by both histone (de)acetylation and methylation to regulate SMARCA5 localization to UV damage.

ATPase, SLIDE and HAND domains regulate UV damage-induced accumulation and re-localization of SMARCA5

Next, we determined which SMARCA5 domains are responsible for its damage accumulation and subsequent re-localization. SMARCA5 harbors an ATPase domain at the N-terminus, for ATP hydrolysis, and HAND, SANT and SLIDE domains at the C-terminus (Fig. 4A), which were suggested to associate with linker DNA to control nucleosome sliding (Grune et al, 2003; Hota et al, 2013). To test involvement of the ATPase domain, we introduced

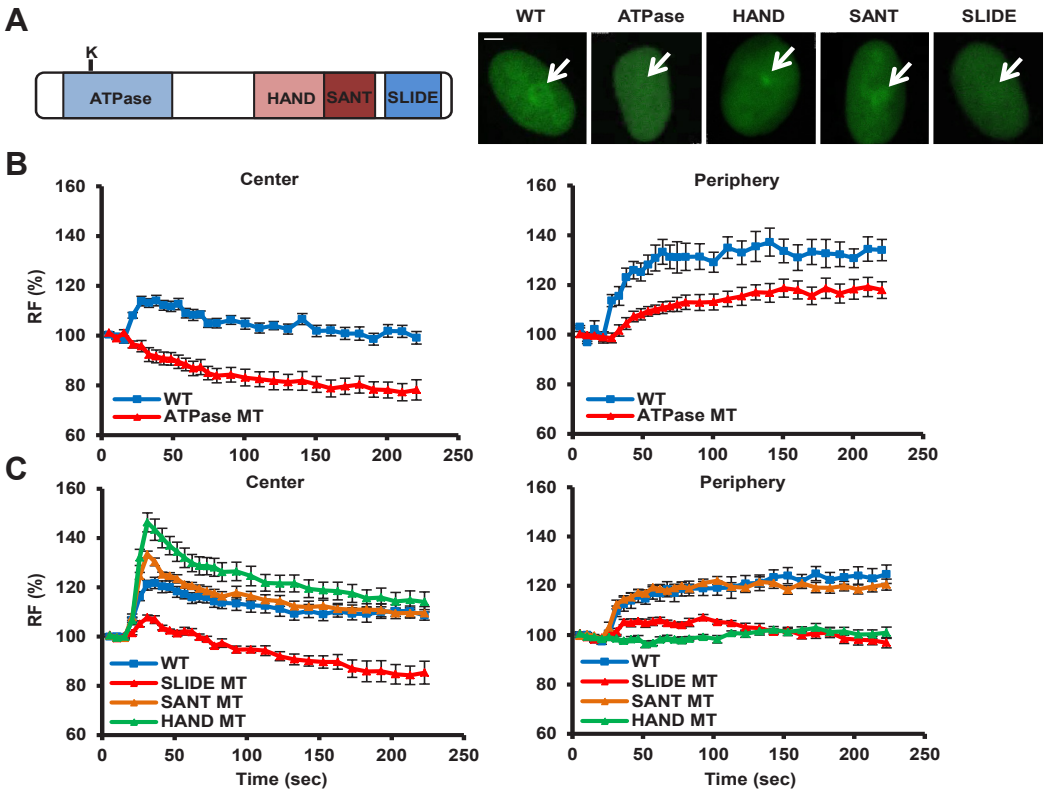

Figure 4. The ATPase, SLIDE and HAND domains of SMARCA5 regulate its damage recruitment.
(A) Schematic representation of SMARCA5 domains (left). The invariant Lysine 211 in the ATPase domain is indicated by a 'K'. Representative images (right) show live cell accumulation pattern of wild type (WT), ATPase-dead, HAND, SANT and SLIDE deletions mutants at local UV-C damage in U2OS cells. Scale bar is 5 μm. (B) The SMARCA5 ATPase mutant shows impaired recruitment to DNA damage. Graph of the normalized fluorescence intensity of wild type and ATPase mutant (MT) at the damage center (left) and periphery (right; mean ± standard error of the mean; n>10 cells). (C) Graphs of the normalized fluorescence intensity of wild type (WT), HAND, SANT and SLIDE deletion mutants (MT) at the damage center (left) and periphery (right; mean ± standard error of the mean; n>10 cells). Recruitment of the SLIDE domain mutant is impaired, whereas the HAND domain mutant is only recruited to the center of damage. All results were confirmed using independent, duplicate experiments. RF denotes Relative Fluorescence.

an inactivating mutation by replacing Lys211 in the nucleotide-binding motif with Arg (Brestovitsky et al, 2011). Intriguingly, ATPase dead SMARCA5-GFP did not localize to the center of UV damage and was even depleted from this area (Fig. 4A and B). Furthermore, a reduced and delayed recruitment to the periphery of the damage was observed. This suggests that ATP hydrolysis directs SMARCA5 targeting to UV-C induced DNA damage.

Next, we deleted each of the C-terminal HAND, SANT or SLIDE domains to analyze their involvement. Deletion of the SANT domain did not affect damage binding (Fig. 4A and C). Accumulation of the HAND deletion mutant to the center of damage, however, was strikingly higher than wild type and it showed no re-localization to the periphery (Fig. 4A and C). In contrast, the SLIDE deletion mutant did not localize to the center of the damage at all, nor to the periphery (Fig. 4A and C). Because the SLIDE domain alone is necessary and sufficient for SMARCA5 recruitment to DSBs induced by laser micro-irradiation (Lan et al, 2010), we also tested UV-C damage recruitment of this domain only. GFP tagged SLIDE, however, showed a very weak and transient recruitment to UV-C damage, with no re-localization (Supplementary Fig. S4D), contrasting the recruitment of this domain to DSBs and that of the whole SMARCA5 protein to UV-C damage. Our results therefore

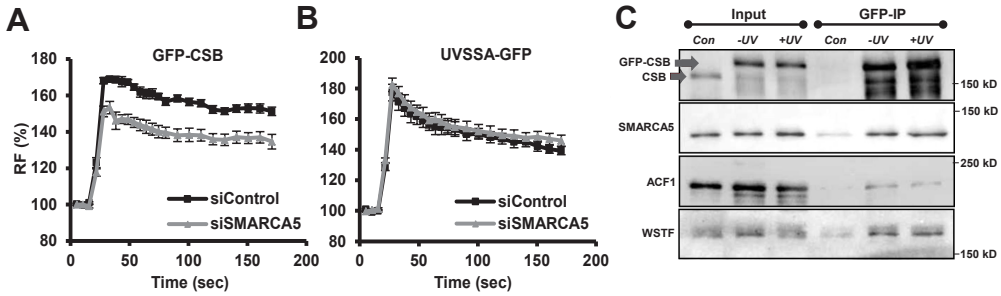

Figure 5. SMARCA5 interacts with CSB and regulates its recruitment.
(A, B) Graphs of the normalized fluorescence intensity indicating local UV-C laser induced DNA damage recruitment of (A) GFP-CSB ($p<0.01$ compared to control) and (B) UVSSA-GFP ($p=0.513$ compared to control) in cells siRNA depleted for SMARCA5. n>10 cells, error bars denote standard error of the mean. RF denotes Relative Fluorescence. (C) GFP immunoprecipitation of GFP-CSB in MNase treated nuclear extracts shows that SMARCA5, ACF1 and WSTF co-purify with CSB , both in unchallenged conditions (-UV) and 20 min after UV irradiation (+UV). Ctrl is control. All results were confirmed using independent, duplicate experiments.

indicate that both the ATPase and the SLIDE domains are involved in recognition and binding of SMARCA5 to its nucleosomal target, whereas the HAND domain seems to be involved in SMARCA5 re-localization after initial binding.

SMARCA5 facilitates CSB binding to UV induced damage

The UV damage recruitment of SMARCA5 suggests that its chromatin remodeling activity may facilitate access and function of subsequent repair factors. Indeed, as shown in Fig. 5A, siRNA-mediated knock-down of SMARCA5 attenuated the recruitment of CSB to local UV damage. Importantly, this reduction was not due to transcription inhibition, because overall transcription levels were similar in cells with and without SMARCA5 (Supplementary Fig. S1B). UVSSA recruitment, however, was not dependent on SMARCA5 (Fig. 5B), indicating that the effect of SMARCA5 depletion on CSB is not an indirect consequence of changes in chromatin compaction or transcription. Rather, these data point to an important, specific regulatory function of SMARCA5 in TC-NER to facilitate recruitment of CSB, possibly by chromatin remodeling.

Because SMARCA5 regulates CSB, we tested whether both proteins interact by performing native co-immunoprecipitation using nuclear extracts of GFP-CSB expressing cells, which were enriched for chromatin proteins by MNase treatment, and GFP as bait. We found that CSB co-purifies with SMARCA5, in untreated as well as UV irradiated cells (Fig. 5C). This CSB and SMARCA5 interaction, which may be either direct or indirect, confirms a role for SMARCA5 in TC-NER and suggests that both proteins simultaneously promote TC-NER.

ACF1 and WSTF function to regulate TC-NER

SMARCA5 is the catalytic subunit of several ISWI family ATP dependent chromatin remodeling complexes including ACF (Ito et al, 1997) and WICH (Bozhenok et al, 2002). Both complexes were previously shown to be involved in DSB repair (Lan et al, 2010; Xiao et al, 2009), while Drosophila ACF complex was also shown to facilitate NER of DNA damage in linker DNA *in vitro* (Ura et al, 2001). The human ACF complex consists of SMARCA5 and ACF1 (LeRoy et al, 2000), whereas the WICH complex consists of SMARCA5 and WSTF (Bozhenok et al, 2002) (Supplementary Fig. S5A and B). As shown in Fig. 6A and B, knockdown of ACF1 and WSTF (Supplementary Fig. S5C) rendered cells hypersensitive

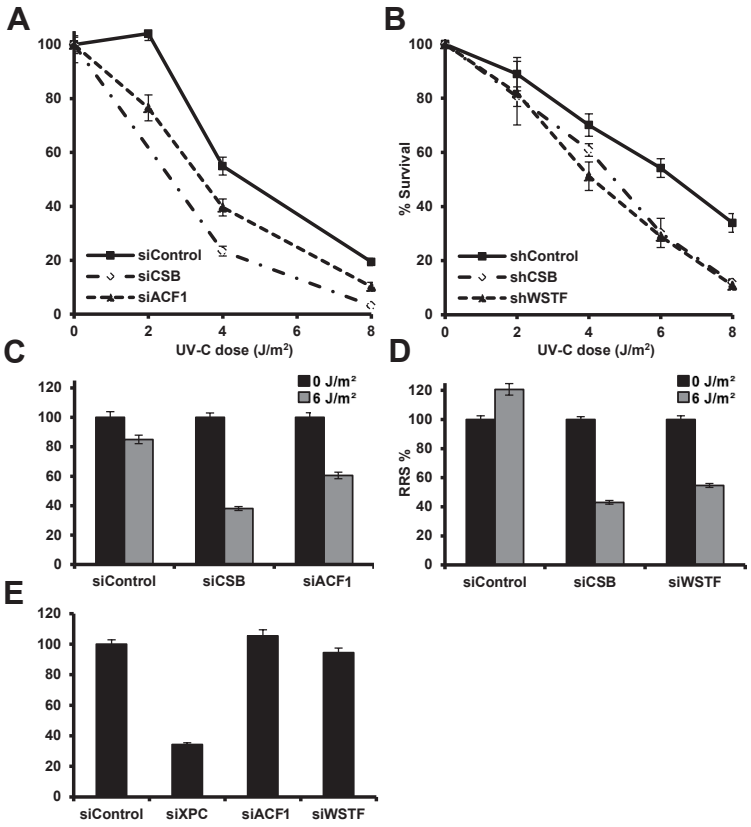

Figure 6. ACF1 and WSTF function in the transcription-coupled response to UV.
Depletion of ACF1 and WSTF renders cells hypersensitive to UV and impairs RRS. Colony survival of U2OS cells treated with siRNAs against ACF1 and CSB (A) and HeLa cells stably expressing shRNAs against WSTF and CSB (B). The percentage of surviving cells is plotted against the applied UV-C dose (J/m^2). (C) and (D) depict impaired RRS, 16 hrs after 6 J/m^2 UV-C irradiation, in U2OS cells treated with siRNA against ACF1 or WSTF as measured by EU incorporation. (E) siRNA treatment against ACF1 or WSTF in primary C5RO fibroblasts does not affect UDS, as measured by EdU incorporation after 16 J/m^2 UV-C irradiation. Error bars denote standard error of the mean. All results were confirmed using independent, duplicate experiments.

to UV. In addition, loss of ACF1 and WSTF clearly reduced RRS (Fig. 6C and D) but not UDS (Fig. 6E). This was achieved using different siRNAs, ruling out off-target effects (Supplementary Fig. S5C). These results indicate that ACF1 and WSTF are both involved in TC-NER but not GG-NER, consistent with a function in complex with SMARCA5. Based on these results, we hypothesize that both the ACF and the WICH complex may remodel chromatin during initiation of TC-NER.

GFP-tagged ACF1 and WSTF, stably expressed in U2OS cells, were both recruited to local UV damage (Fig. 7A and B). However, their recruitment exhibited a strikingly different accumulation pattern. ACF1-GFP quickly located to the center of the damage spot, after which it spread to the periphery of the damage, similarly as SMARCA5 (Fig. 7A). Contrary, GFP-WSTF was even depleted from the center of the damage immediately after damage induction, while it showed a very strong subsequent recruitment to the periphery (Fig. 7B). These results implicate both ACF1 and WSTF at sites of UV damage, although with distinct (re)distribution kinetics. To test whether this difference reflects a dynamic interaction with SMARCA5, which may first bind to ACF1 in the center and be handed over to WSTF in the periphery, we performed co-immunoprecipitation after UV. We did not observe any detectable change in each of the different complexes shortly after UV irradiation (Supplementary Fig. S5A and B), suggesting that there is no change in the interaction of SMARCA5 with ACF1 or WSTF upon DNA damage induction. Furthermore, we tested whether both subunits could still associate with the SMARCA5 ATPase, HAND and SLIDE domain mutants that show different recruitment patterns (Fig. 4). Interestingly, both WSTF and ACF1 co-immunoprecipitated with the ATPase and HAND domain mutants, whereas

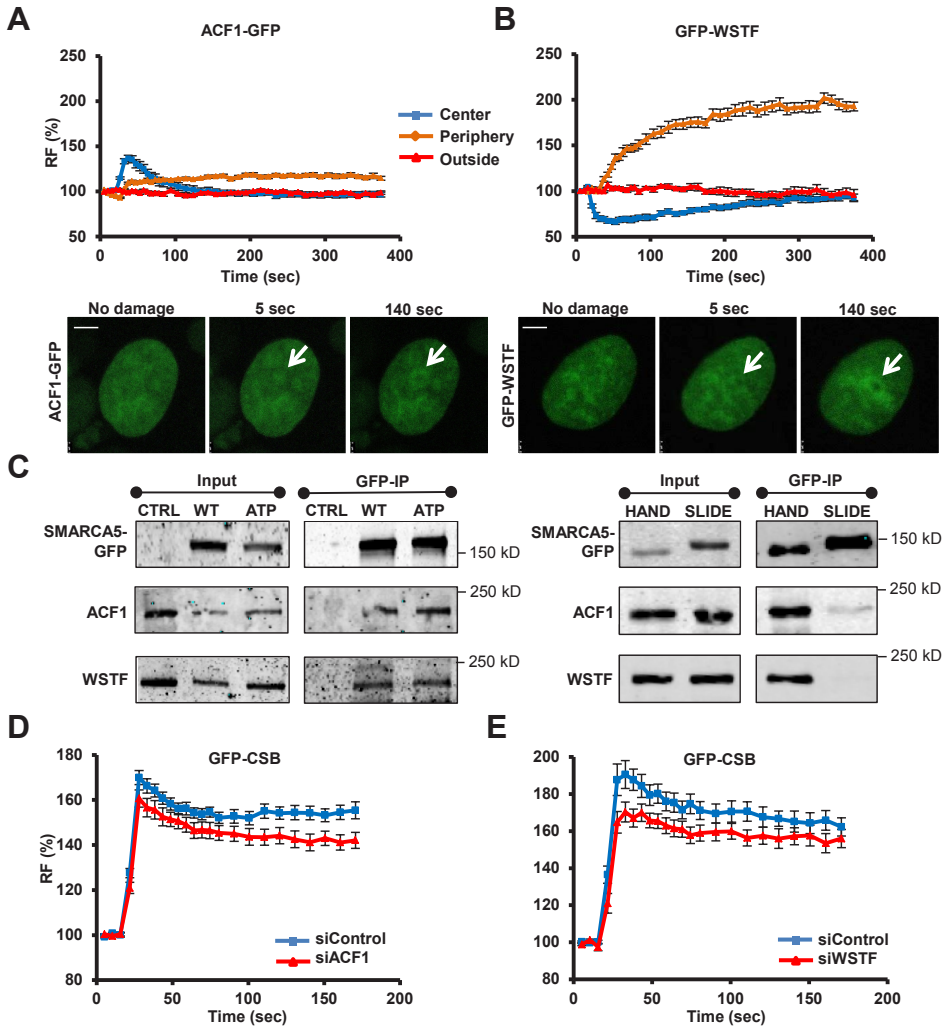

Figure 7. WSTF and ACF1 are recruited to UV damage to regulate CSB recruitment.
ACF1-GFP (A) and GFP-WSTF (B) are recruited to DNA damage induced by UV-C (266 nm) laser. Graphs depict normalized fluorescence intensities indicating DNA damage recruitment in the damage center (blue), the damage periphery (orange) and outside the damaged area (red) (mean ± standard error of the mean; n>10 cells). Representative images of the accumulation of ACF1-GFP and GFP-WSTF at sites of UV damage are shown below the graphs (scale bar is 5 µm). (C) GFP immunoprecipitation of GFP-tagged SMARCA5 (WT) and ATPase (ATP), HAND and SLIDE domain deletion mutants. CTRL is control. Only deletion of the SLIDE domain impairs the interaction of SMARCA5 with ACF1 and WSTF. (D) and (E) depict graphs of the normalized fluorescence intensity indicating DNA damage re**cruitm**ent of GFP-CSB in cells in which ACF1 (C; p=0.033 compared to control) or WSTF (D; p=0.117 compared to control) are depleted by siRNA (mean ± standard error of the mean; n>10 cells). Results were confirmed using independent, duplicate experiments.

specifically loss of the SLIDE domain disrupted the interaction of SMARCA5 with ACF1 and WSTF (Fig. 7C). These data suggest that the impaired recruitment of the SLIDE domain mutant (Fig. 4C) may be related to the inability of SMARCA5 to form complexes with ACF1 and/or WSTF.

Immunoprecipitation of GFP-CSB on MNase treated nuclear extracts showed that CSB also co-purifies with ACF1 and WSTF (Fig. 5C). Furthermore, the recruitment of CSB was attenuated when ACF1 or WSTF were depleted by siRNA (Fig. 7D and E), albeit to a lesser extent than for SMARCA5 knockdown (Fig. 5A). These findings confirm our results with SMARCA5 and indicate that at least two different ISWI chromatin remodeling complexes,

ACF and WICH, function together at sites of DNA damage-stalled transcription to stimulate efficient TC-NER.

Discussion

Chromatin remodeling during DNA damage repair is thought to be particularly important in regulating the efficiency of lesion recognition (Lans et al, 2012). Here, we show, in living human cells, that the ATP-dependent chromatin remodeler SMARCA5 facilitates binding of CSB to active TC-NER complexes and controls the repair efficiency of transcription-stalling UV lesions. Furthermore, we show that SMARCA5 binding partners from two distinct ISWI complexes, ACF1 and WSTF, regulate TC-NER initiation in two different discernable kinetic steps. The TC-NER organizing factor CSB also possesses ATP-dependent chromatin remodeling activity (Citterio et al, 2000), and several additional chromatin remodeling proteins were recently implicated in TC-NER and transcription resumption after UV (Adam et al, 2013; Cho et al, 2013; Dinant et al, 2013; Fousteri et al, 2006; Oksenych et al, 2013). Together, these observations suggest that extensive chromatin remodeling needs to take place when RNApolII encounters a lesion.

SMARCA5, ACF1 and WSTF function in DSB repair as well (Lan et al, 2010; Nakamura et al, 2011; Smeenk et al, 2013; Xiao et al, 2009), indicating that ISWI is generally important to maintain genome stability. Nevertheless, we identify a novel role for ISWI in TC-NER which is mechanistically distinct from its role in DSB repair, where it stimulates NHEJ and HR (Lan et al, 2010) and promotes the RNF168-mediated ubiquitin signaling response (Smeenk et al, 2013). Recruitment and chromatin spreading of SMARCA5 in response to DSBs depends on PARP activity, whereas we did not observe any PARP dependency of SMARCA5 loading and re-localization at sites of UV-C induced DNA damage. Furthermore, we find that the SMARCA5 ATPase mutant does not localize to UV-C induced DNA damage, while it does localize to DSBs (Lan et al, 2010).

Although the exact molecular activity of chromatin remodeling complexes in response to lesion-stalled transcription, including that of ISWI, remains elusive, we do propose a speculative model for SMARCA5 function in this process (Fig. 8). ISWI is thought to mediate regular positioning of nucleosomes, especially behind the transcriptional start site of genes (Gkikopoulos et al, 2011; Langst & Becker, 2001; Sala et al, 2011). It is thus conceivable that in TC-NER, ISWI may function to regulate transcriptional activity upon UV damage. Furthermore, both ACF1 and WSTF have been suggested to maintain an open chromatin structure during mammalian replication (Collins et al, 2002; Poot et al, 2004). In line with these observations and the reduced CSB recruitment following ISWI knockdown, we propose that the chromatin remodeling capacity of ISWI complexes facilitates an open chromatin structure for efficient CSB association to lesion stalled transcription complexes. As CSB is necessary for the recruitment of most subsequent TC-NER factors (Fousteri et al, 2006), we suggest that chromatin remodeling by ISWI stimulates efficient TC-NER.

The different recruitment kinetics of ACF1 and WSTF to sites of TC-NER (Fig. 7A) suggests a functional difference between both subunits in regulating this process. Indeed, different functions for ACF1 and WSTF have been described in literature. In the DSB response, ACF1 was found to interact with and regulate recruitment of Ku70 (Lan et al, 2010), whereas WSTF was found to interact with and phosphorylate histone H2AX on Tyr142 and to regulate maintenance of Ser139 phosphorylation (Xiao et al, 2009). Moreover,

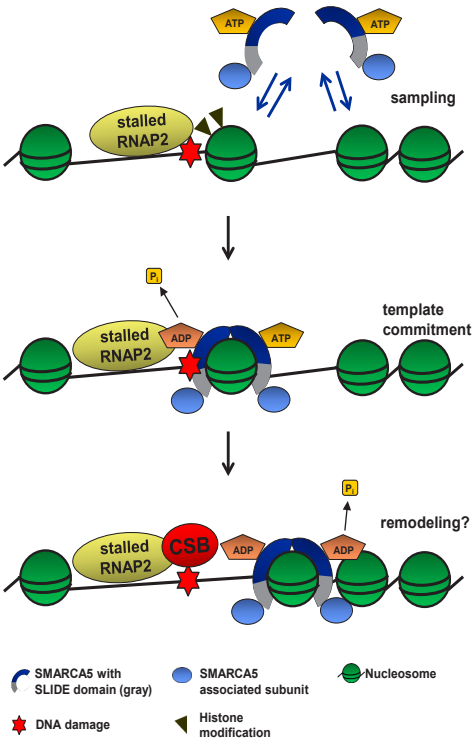

Figure 8. Model for ISWI recruitment and function in TC-NER. SMARCA5 utilizes ATP-hydrolysis and its SLIDE domain, which is necessary for the association with ACF1 and WSTF subunits, to scan for and bind to target nucleosomes in the vicinity of lesion-stalled RNApolII. Its recruitment depends on both active transcription and histone modifications. SMARCA5 may remodel chromatin to facilitate efficient CSB association with stalled transcription sites. See discussion for details.

non-catalytic subunits such as ACF1 and WSTF are supposed to regulate the activity and template specificity of the catalytic SMARCA5 subunit, depending on the DNA flanking the nucleosomes (He et al, 2008). Thus, ACF1- and WSTF-containing complexes may have temporally and spatially separated functions during TC-NER as well, to facilitate CSB binding by remodeling chromatin and/or lesion-stalled RNApolII.

Intriguingly, we find that SMARCA5 is first rapidly recruited to the central area of a locally induced DNA damage spot, after which it re-localizes to the damage periphery. This is in striking contrast to CSB and other NER proteins, which accumulate in a concentrated spot. The peripheral re-localization of SMARCA5 may be indicative of actual chromatin remodeling. Surprisingly, deletion of the HAND domain led to a strong initial accumulation of SMARCA5 at local UV damage, but prevented its re-localization to the periphery. Although the function of the SMARCA5 HAND domain is not known, it was postulated to control the directionality of nucleosome sliding due to its contact with the DNA entry/exit site of the nucleosome (Dang & Bartholomew, 2007). Thus, it may be that in the absence of the HAND domain, SMARCA5 still associates with nucleosomal targets in damaged chromatin, but its subsequent activity, i.e. chromatin remodeling, is impaired. ACF1 followed a similar initial binding pattern as SMARCA5, but its re-localization to the periphery was less prominent. In contrast, WSTF did not even recruit to the center of damage, but immediately accumulated at the periphery. The initial central localization of SMARCA5 therefore may reflect its association with ACF1, whereas its subsequent peripheral re-localization may reflect its association with WSTF. We tested for a possible handover of SMARCA5 between the different complexes by immunoprecipitation (Supplementary Fig. S5A and B), but did not observe a quantifiable change in subunit composition following UV. Although these observations argue against this handover model, it should be noted that the applied procedure, i.e. precipitating the bulk of WSTF- and ACF1-containing complexes, may not be sufficiently sensitive to reveal temporarily changes in composition of only a small fraction of the resident complexes being actively engaged in TC-NER.

Strikingly, SMARCA5 localization to UV-induced DNA damage is independent of NER. It is, however, dependent on transcription, suggesting a direct association of SMARCA5 with lesion-stalled transcription, similar as CSB (van den Boom et al, 2004; van Gool et al, 1997) and UVSSA (Schwertman et al, 2012). Alternatively, SMARCA5 may continuously

scan nucleosomes and bind only to nucleosomal substrates in damaged chromatin, much like XPC scans for DNA damage in GG-NER (Hoogstraten et al, 2008). Previous FRAP and FCS analyses have shown that SMARCA5 complexes are highly mobile and that only a low percentage is transiently bound to chromatin at any given time (Erdel et al, 2010). These findings support a model in which the majority of SMARCA5 molecules continuously sample nucleosomes and only a minor fraction binds to and translocates those nucleosomes that contain a specific cue such as a posttranslational modification. Furthermore, ISWI complexes were suggested to use an ATP-hydrolysis driven kinetic proofreading mechanism to recognize substrate nucleosomes (Blossey & Schiessel, 2011; Narlikar, 2010). Both electron microscopy (Racki et al, 2009) and single molecule FRET studies (Blosser et al, 2009) suggest that ISWI complexes contain dimers of SMARCA5 that first utilize ATP-hydrolysis to associate with nucleosomeal targets and then utilize a second ATP-hydrolysis event to translocate DNA. Yeast ISW1a and ISW2 chromatin remodeling complexes also bind more stably to nucleosomes depending on ATP-hydrolysis (Gangaraju et al, 2009). Thus, the impaired recruitment of the ATPase-inactive SMARCA5 mutant likely implies that SMARCA5 employs a probing and proofreading mechanism to associate with substrate nucleosomes near damaged DNA in an ATP-hydrolysis dependent manner (Fig. 8).

Besides the ATPase domain, deletion of the SLIDE domain interferes with binding to DNA damage sites. This domain in yeast SMARCA5 orthologs was suggested to help anchor SMARCA5 to the nucleosome through its interaction with extranucleosomal DNA, which facilitates DNA movement into the nucleosome (Dang & Bartholomew, 2007; Hota et al, 2013; Kagalwala et al, 2004; Sale, 2012). Our results support this idea and indicate that similarly human SMARCA5 utilizes its SLIDE domain, besides ATP-hydrolysis, to recognize and bind to nucleosomal targets in the context of damaged DNA. This property of the SLIDE domain may involve ACF1 and WSTF as we show that the SLIDE domain is necessary for the interaction of SMARCA5 with these subunits. The SLIDE domain dependent interaction with ACF1 is in agreement with the identification of a small motif at the end of the Drosophila ISWI SLIDE domain as the ACF1-interacting domain (Ward & Chen, 2001). This same motif is deleted in our human SLIDE mutant and could be necessary for the interaction with WSTF besides ACF1 as well. Furthermore, the above mentioned ISWI dimerization could explain that in spite of the depletion at the center, ATPase and SLIDE domain mutants still showed slight recruitment to the periphery, as these mutants may still dimerize and travel with functional, endogenous SMARCA5. Conversely, it could be that the ATPase and/or SLIDE mutants are not able to dimerize anymore and therefore show defective DNA damage localization.

The transcription-dependent SMARCA5 translocation to DNA damage suggests that this chromatin remodeler, while continuously probing chromatin, is recruited to a cue that is both DNA damage and transcription dependent. Chromatin targeting and activity of ISWI complexes was shown to depend on histone modifications, such as di- and trimethylation of H3 lysine 4 (Santos-Rosa et al, 2003) and hypo-acetylation of the H4 tail (Alenghat et al, 2006; Clapier et al, 2001; Corona et al, 2002; Gangaraju et al, 2009; Shogren-Knaak et al, 2006). Importantly, H4 acetylation levels decrease after UV damage (Oksenych et al, 2013). In accordance with these findings, we show that inhibition of histone methyltransferase and deacetylase activity interferes with SMARCA5 binding to DNA damage sites. Therefore, we propose a model in which ISWI chromatin remodeling complexes accumulate at sites of UV damage early during TC-NER, in an ATP-hydrolysis and transcription-dependent manner,

which is further stimulated by posttranslational histone modifications (Fig. 8). Most likely this recruitment results in chromatin remodeling to facilitate efficient CSB recruitment.

Several other ATP-dependent chromatin remodeling factors, i.e. SWI/SNF (Zhang et al, 2009; Zhao et al, 2009), INO80 (Jiang et al, 2010) and ALC1 (Pines et al, 2012), were recently implicated in mammalian GG-NER. The specificity of the ISWI ATP-dependent chromatin remodeling complexes for TC-NER suggests that specific chromatin configurations characteristic for either GG- or TC-NER require alternative types of chromatin remodeling events.

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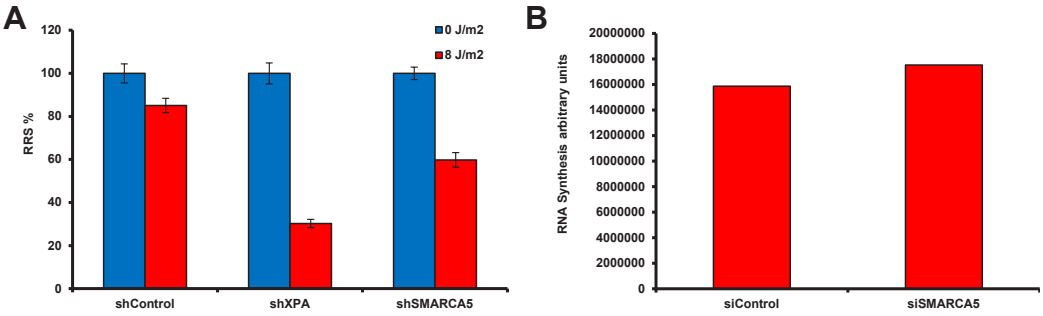

Figure S1. SMARCA5 functions in the transcription-coupled UV response.
(A) RRS levels determined by EU incorporation 16 hours after 8 J/m² UV in HeLa cells stably expressing shRNA against SMARCA5 show that loss of SMARCA5 leads to reduced recovery of RNA synthesis after UV irradiation. (B) Fluorescence intensity of incorporated EU in SMARCA5 siRNA treated cells is comparable to that of control siRNA treated cells, showing that global transcription levels in non-challenged cells are not affected by SMARCA5 knockdown.

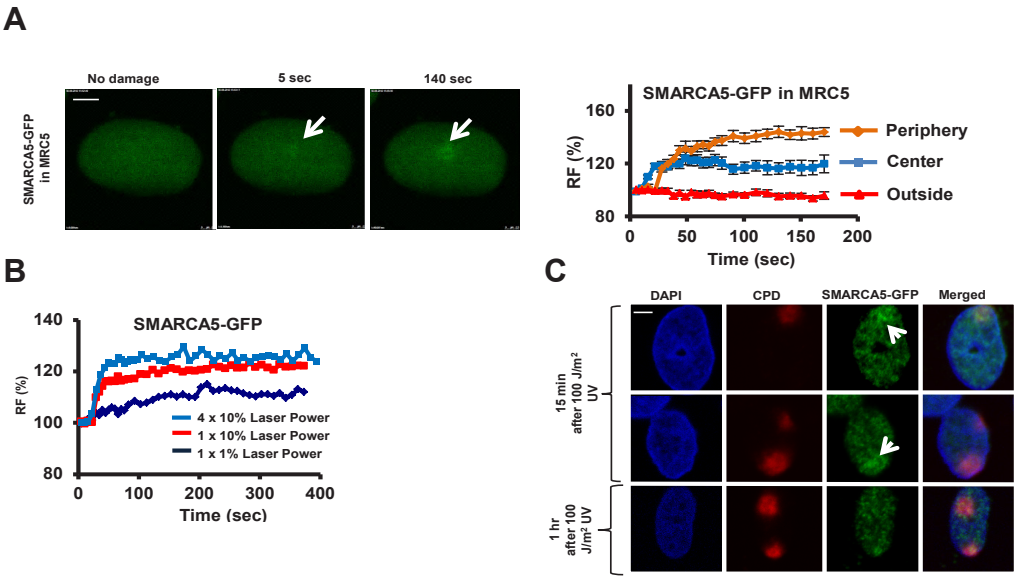

Figure S2. SMARCA5 recruitment to UV-C induced DNA damage.
(A) Images (left) show live cell recruitment of SMARCA5-GFP in MRC5 cells to UV-C (266 nm) laser-induced DNA damage (arrows). Graph (right) depicts the normalized fluorescence intensities (n>10 cells) that indicate recruitment at the damage center (blue), the damage periphery (orange) and outside the damaged area (red; mean ± standard error of the mean). (B) Recruitment of SMARCA5 to UV-C induced DNA damage is dose dependent. Normalized SMARCA5-GFP fluorescence intensities at the damage periphery in cells exposed to different UV-C (266 nm) laser intensities: 1% or 10% laser power for 5 seconds (1x) and 10% laser power for 20 seconds (4x; mean ± standard error of the mean, n > 10 cells). RF is Relative Fluorescence. (C) Recruitment of SMARCA5-GFP to local damage induced by irradiation (100 J/m²) through a microporous filter with a 254 nm UV-C lamp. U2OS cells were fixed 15 minutes and 1 hour after UV. Immunofluorescence staining was performed using antibodies against CPD (red) and SMARCA5 (green). SMARCA5-GFP clearly accumulated at local DNA damage 15 minutes after the damage but 60 minutes after UV irradiation, it was hardly detectable.

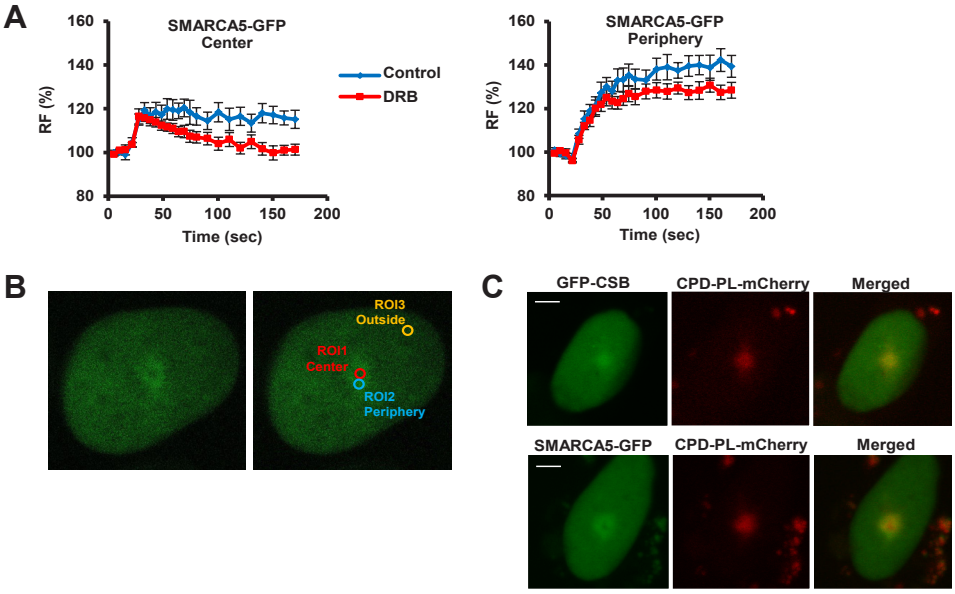

Figure S3. Efficient SMARCA5 recruitment depends on transcription and shows a dynamic re-localization to the periphery.
(A) Graph of the normalized fluorescence intensity indicating recruitment of SMARCA5-GFP to the center (p=0.029 compared to control) and periphery (p=0.114 compared to control) of a UV-C laser-induced DNA damage area in U2OS cells in which transcription is inhibited with DRB (n > 10 cells, mean ± standard error of the mean). RF is Relative Fluorescence. (B) An example cell with SMARCA5-GFP accumulation at local 266 nm UV-induced DNA damage. In the right image, the 'center', 'periphery' and 'outside' regions of interest (ROIs) are indicated that are used to quantify the recruitment of SMARCA5. (C) Co-expression and co-localization of GFP-CSB or SMARCA5-GFP with CPD-Photolyase-mCherry in U2OS cells after local UV-C laser irradiation. SMARCA5 is localized to the periphery of the DNA damage spot indicated by the presence of CPD-photolyase. Scale bar, 5 µm..

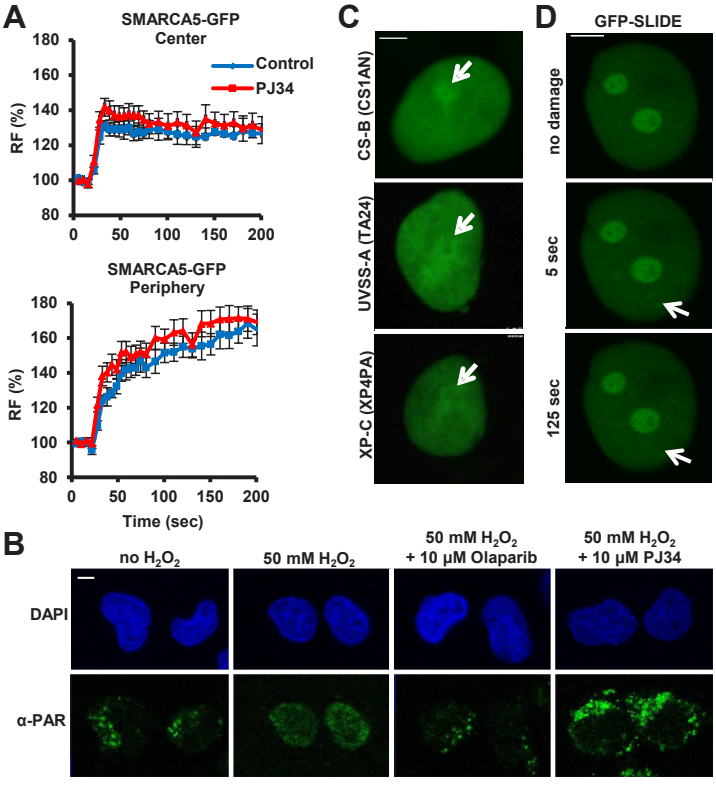

Figure S4. SMARCA5 recruitment does not depend on PARP or NER activity.
(A) Graph of the normalized fluorescence intensity indicating recruitment of SMARCA5-GFP to the center (p=0.480 compared to control) and periphery (p=0.314 compared to control) of a UV-C laser-induced DNA damage area in U2OS cells treated with PARP inhibitor PJ34 (n > 10 cells, mean ± standard error of the mean). RF is Relative Fluorescence. (B) Immunofluorescence of PAR shows that 5 minutes treatment with 50 mM H_2O_2 induces PARylation in the nucleus, which is inhibited by treatment with PARP inhibitors Olaparib and PJ34, demonstrating the effectiveness of both inhibitors. Scale bar is 5 µm. (C) SMARCA5-GFP recruitment to UV-C induced DNA damage is normal in CSB-deficient CS1AN fibroblasts, UVSSA-deficient TA24 and XPC-deficient XP4PA fibroblasts. (D) The SMARCA5 SLIDE domain tagged to GFP shows a very weak accumulation signal at UV-C induced DNA damage which disappears within 2 minutes. Scale bar is 5 µm.

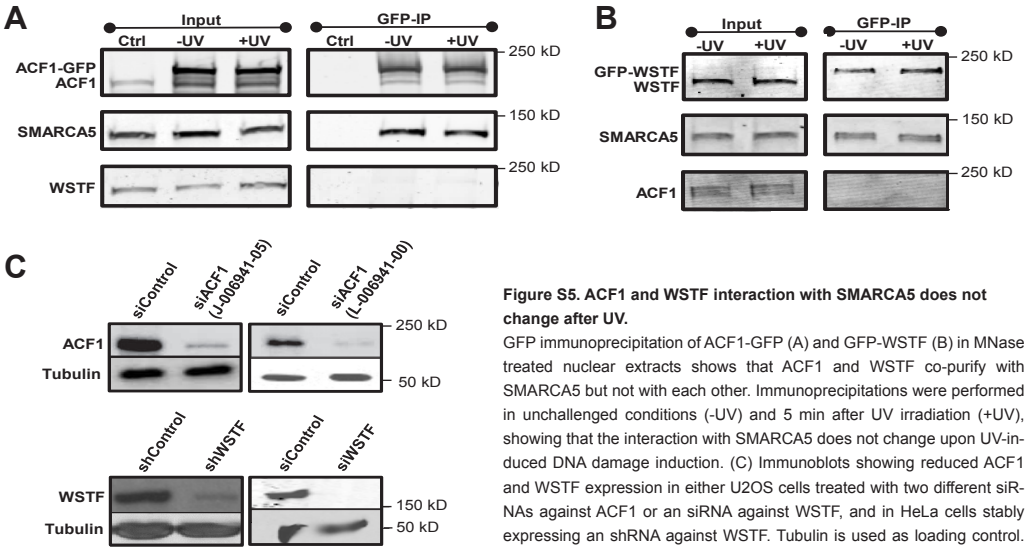

Figure S5. ACF1 and WSTF interaction with SMARCA5 does not change after UV.

GFP immunoprecipitation of ACF1-GFP (A) and GFP-WSTF (B) in MNase treated nuclear extracts shows that ACF1 and WSTF co-purify with SMARCA5 but not with each other. Immunoprecipitations were performed in unchallenged conditions (-UV) and 5 min after UV irradiation (+UV), showing that the interaction with SMARCA5 does not change upon UV-induced DNA damage induction. (C) Immunoblots showing reduced ACF1 and WSTF expression in either U2OS cells treated with two different siRNAs against ACF1 or an siRNA against WSTF, and in HeLa cells stably expressing an shRNA against WSTF. Tubulin is used as loading control.

3

Chapter IV

Quantitative proteomics analysis of SMARCA5 suggests a role in mRNA metabolism after DNA damage

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Chapter V

SWI/SNF facilitates Nucleotide Excision Repair

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Manuscript in preparation

Chapter VI

General Discussion & Perspectives

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Chapter VII

*Summary

*Nederlandse samenvatting

*List of Abbreviations

*Curriculum Vitae

*List of Publications

*PhD Portfolio

*Acknowledgements

Summary

Endogenous cellular processes as well as environmental chemicals and radiation continuously damage DNA and thereby compromise the function of the genome. The integrity of DNA is protected by the DNA damage response (DDR), which consists of a network of DNA repair and associated signaling pathways. Remodeling and modification of chromatin is an integral part of the DDR, as it regulates access of repair proteins to DNA and mediates damage signaling by providing docking sites for signaling proteins to control their activity. Despite detailed knowledge on the molecular mechanisms of most DDR processes, relatively little is known on the interplay between DDR and chromatin remodeling. The work that is described in this thesis is focused on the role of ATP-dependent chromatin remodeling in the DDR, specifically in the Nucleotide Excision Repair (NER) pathway. NER removes a wide variety of DNA lesions, including those induced by UV irradiation. Within NER two damage recognition sub-pathways are operational: global genome repair (GG-NER), which detects lesions located anywhere in the genome; and transcription-coupled repair (TC-NER), which removes transcription blocking DNA lesions from the transcribed strand of active genes.

In Chapter I first general background information on the DDR is provided and the current state-of-art of chromatin modifications and the involvement of ATP-dependent chromatin remodeling complexes in the DDR are reviewed. In Chapter II, a more detailed description on the versatile roles of the mammalian ISWI family of ATP-dependent chromatin remodeling complexes in the DDR is presented. Various essential and novel functions of ISWI complexes in regulating homologous recombination, non-homologous end-joining, NER and DNA damage signaling are discussed. In addition, recent insight on how ISWI complexes are targeted to sites of DNA repair is described, which improves understanding of how chromatin remodeling complexes identify and associate with substrate nucleosomes *in vivo*. In the subsequent Chapters III to V the experimental work performed on chromatin-remodelers in NER are being described.

In Chapter III, we provide evidence for a novel function of two distinct mammalian ISWI ATP-dependent chromatin remodeling complexes in resolving lesion-stalled transcription. We show that human SMARCA5 and its binding partners ACF1 and WSTF are rapidly recruited to UV-C induced DNA damage to specifically facilitate the binding of TC-NER factor CSB and to promote transcription recovery. Moreover, SMARCA5 recruitment characteristics to UV-C damage suggest that SMARCA5 continuously samples chromatin and only associates with nucleosomes near DNA damage if there is a transcription-dependent histone modification present, which requires the activity of the SMARCA5 ATPase and the presence of the SLIDE domain. Intriguingly, after initial recruitment to UV damage, SMARCA5 relocalizes away from the center of DNA damage, which depends on its HAND domain and may be indicative of actual chromatin remodeling.

In Chapter IV, we aim to identify the proteins with which SMARCA5 interacts in mammalian cells to obtain a deeper understanding of the mechanisms underlying SMARCA5 function in the UV-DDR. To this end, two different proteomics experiments are described, both before and after DNA damage induction by UV irradiation. The identified interacting proteins are analyzed with bioinformatical tools and subsequent database analyses are used to depict the protein-protein interactions among the SMARCA5 interacting proteins and to identify the biological pathways in which they function. These analyses reveal increased asso-

ciation of SMARCA5 with the transcription machinery in response to UV-light. Surprisingly however, also the pathways of RNA processing and export and translation appeared to be enriched after UV-induced DNA damage. These data suggest that SMARCA5 functions as part of a complex network of proteins and is possibly involved in modulating transcription and translation in response to UV, besides chromatin remodeling.

In Chapter V, we focus on the involvement of SWI/SNF ATP-dependent chromatin remodeling complexes in the UV-DDR. Several subunits of these complexes were identified in a *C. elegans* screen for genes that protect against UV irradiation. Our findings confirm that the SWI/SNF BRM and BRG1 ATPases and several additional subunits are also essential for UV survival in mammals. Both ATPases are also recruited to local UV-C damage. BRG1 associates with GG-NER-initiation factor DDB2 and regulates its mobility after UV irradiation, whereas BRM associates with DDB2 specifically in a UV dependent manner. These data suggest that BRM and BRG1 function to regulate the initial steps of GG-NER by interacting with DDB2. Furthermore, we showed that both catalytic subunits play a role in the transcription recovery after UV, suggesting that they a function in TC-NER as well.
Finally, Chapter VI concludes and evaluates all the obtained results and highlights how the results described in this thesis contribute towards a further understanding of the importance of chromatin remodeling in the UV-DDR. Furthermore, possible future directions for research of chromatin remodeling involvement in the UV-DDR are discussed.

Nederlandse Samenvatting

Endogene cellulaire processen en omgevingsfactoren als straling en chemicaliën beschadigen voortdurend DNA, waardoor de functie van het genoom verstoord raakt. De integriteit van DNA wordt bewaakt en bewaard door de DNA schade respons (Engels: DNA Damage Respons, DDR). Dit is een netwerk van verschillende DDR en bijbehorende DNA schade signaleringsmechanismen. Een integraal onderdeel van de DDR is de herstructurering (Engels: remodeling) en chemische verandering (modificatie) van chromatine. Chromatine remodeling en modificatie vinden plaats zodat DDR eiwitten beter toegang tot beschadigd DNA kunnen krijgen en om DNA schade signalen efficiënt te kunnen doorgegeven, door middel van het creëren van bindingsplaatsen voor signaal-eiwitten en het reguleren van hun activiteit. Ondanks dat er veel bekend is over de moleculaire mechanismen van DDR processen, is er relatief gezien maar weinig bekend over het samenspel tussen chromatine remodeling en deze processen. In dit proefschrift wordt onderzoek naar de rol van ATP-afhankelijke chromatine remodeling eiwitcomplexen in de DDR beschreven, in het bijzonder hun rol in het Nucleotide Excision Repair (NER) DNA schade herstel proces. NER verwijdert veel verschillende typen DNA schade, inclusief die door UV-straling wordt geïnduceerd. NER bestaat uit twee verschillende DNA schade herkenningsmechanismen: 'Global Genome NER' (GG-NER), dat DNA beschadigingen opspoort die verspreid liggen in het complete genoom, en 'Transciption-Coupled NER' (TC-NER), dat specifiek DNA schade in de getranscribeerde streng van actieve genen detecteert.

In hoofdstuk I wordt de achtergrond, de functie en het belang van de DDR in het algemeen besproken en worden de belangrijkste DDR mechanismen kort samengevat. Daarnaast wordt een actueel overzicht van de betrokkenheid van chromatine modificaties en ATP-afhankelijke chromatine remodeling eiwitcomplexen in de DDR gegeven.

Hoofdstuk II gaat specifiek in op de functies van de ISWI familie van ATP-afhankelijke chromatine remodeling complexen in de DDR van zoogdieren. Nog maar recent is ontdekt dat dit type chromatine remodeling complexen verschillende belangrijke functies vervult voor het efficiënt laten verlopen van uiteenlopende DDR processen, zoals 'homologous recombination', 'non-homologous end-joining', NER en DNA schade signalering. In dit hoofdstuk wordt ook recent onderzoek besproken dat laat zien hoe ISWI eiwitcomplexen mogelijk plekken van DNA schade in het genoom kunnen vinden. Kennis hierover geeft meer inzicht in hoe chromatine remodeling eiwitcomplexen in het algemeen hun substraateiwitten - de nucleosomen – identificeren en binden. In de daaropvolgende hoofdstukken III tot en met V wordt het experimentele werk aan chromatine remodelers in relatie tot NER gepresenteerd en bediscussieerd.

In hoofdstuk III wordt aangetoond dat twee afzonderlijke ISWI ATP-afhankelijke chromatine eiwitcomplexen betrokken zijn bij het herstel van transcriptie als deze door DNA schade is geblokkeerd. Dit is een nieuwe, nog niet eerder beschreven functie voor beide complexen. We laten zien dat in humane cellen zowel het ISWI katalytische ATPase eiwit SMARCA5 als twee bindingspartners van dit eiwit, ACF1 en WSTF, zich snel naar UV-C geïnduceerde DNA schade begeven om het binden van het essentiële TC-NER eiwit CSB en het herstel van transcriptie te stimuleren. De manier waarop SMARCA5 bij DNA schade ophoopt, suggereert dat dit ATPase eiwit continu het chromatine aftast en sterker aan nucleosomen dichtbij DNA schade bindt als er een transcriptie afhankelijke chromatine modificatie aanwezig is. Hiervoor zijn zowel ATPase activiteit als het SLIDE eiwitdomein van SMARCA5

belangrijk. Na de snelle beweging naar DNA schade toe, beweegt SMARCA5 zich weer van de DNA schade af. Deze beweging is afhankelijk van het 'HAND' domein en geeft bovendien sterke aanwijzingen dat er daadwerkelijk chromatine remodeling plaatsvindt op de plaats van DNA schade.
In hoofdstuk IV worden de eiwitten waarmee SMARCA5 in humane cellen een interactie aangaat geïdentificeerd, om een beter begrip te krijgen van de mogelijke functies van SMARCA5 in de DDR. In dit hoofdstuk beschrijven we twee proteomica onderzoeken, één onder normale condities en één na de inductie van DNA schade door middel van UV-straling, die we vervolgens analyseren met behulp van bioinformatica hulpmiddelen en eiwitdatabanken die de mogelijke functie van eiwitten beschrijven. Op deze manier kunnen de eiwit-eiwit interacties van de SMARCA5-bindende eiwitten en de biologische processen waarin deze functioneren, worden onderzocht. Tot onze verrassing laat deze analyse niet alleen zien dat SMARCA5 na de inductie van DNA schade betrokken lijkt te zijn bij transcriptie, maar ook bij RNA maturatie en export en translatie processen. Deze analyse suggereert daarom dat SMARCA5 als onderdeel van een complex netwerk van eiwitten functioneert en als gevolg van DNA schade niet alleen chromatine maar ook mogelijk transcriptie en translatie reguleert.

In hoofdstuk V gaan we dieper in op de rol van SWI/SNF ATP-afhankelijke chromatine remodeling complexen in de DDR. In een onderzoek in het modelorganisme *C. elegans* is gevonden dat een aantal eiwitten van deze SWI/SNF complexen bescherming bieden tegen UV-straling. Onze bevindingen bevestigen dat de SWI/SNF katalytische ATPase eiwitten BRM en BRG1, en ook enkele andere eiwitten uit deze complexen, belangrijk zijn voor de overleving van humane cellen na UV-straling. Beide ATPase eiwitten bewegen zich ook naar UV-straling geïnduceerde lokale (dit is in een klein gedeelte van de celkern) DNA schade in de cel toe. BRG1 bindt en reguleert de mobiliteit van het GG-NER initiatie eiwit DDB2 na UV-straling. BRM bindt ook aan DDB2, op een UV-straling afhankelijke manier. Deze bevindingen suggereren dat BRM en BRG1 de eerste stappen van GG-NER beïnvloeden door een interactie aan te gaan met DDB2. Beide ATPasen zijn verder ook nodig voor het opnieuw opstarten van transcriptie nadat deze door UV-geïnduceerde DNA schade is geblokkeerd. Dit wijst er mogelijk op dat SWI.SNF complexen ook betrokken zijn bij TC-NER.

In hoofdstuk VI worden tenslotte de belangrijkste bevindingen gepresenteerd en bediscussieerd en enkele algemene conclusies getrokken aan de hand van de resultaten beschreven in dit proefschrift. Dit laat zien hoe ons onderzoek bijdraagt om de belangrijke functie van chromatine remodeling tijdens de UV-straling geïnduceerde DDR beter te begrijpen. Als laatste worden de verschillende mogelijkheden besproken voor toekomstig onderzoek naar de rol van chromatine remodeling in de UV-straling geïnduceerde DDR.

List of Abbreviations

6-4PP	pyrimidine-(6-4)-pyrimidone product
ACF1	ATP-utilizing chromatin assembly and remodeling factor 1
ARID1A	AT-rich interactive domain-containing protein 1A
ARID1B	AT-rich interactive domain-containing protein 1B
ATM	ataxia telangiectasia-mutated
ATR	ataxia telangiectasia and Rad3-related kinase
BAZ1A	Bromodomain adjacent to zinc finger domain protein 1A
BAZ1B	Bromodomain adjacent to zinc finger domain protein 1B
BRG1	brahma-related gene 1
BRM	brahma
BER	base excision repair
CPD	cyclobutane pyrimidine dimer
CHRAC	chromatin accessibility complex
DSB	double strand break
DDR	DNA damage response
GG-NER	Global genome nucleotide excision repair
γH2AX	phosphorylated histone H2AX
HDAC	histone deacetylase
HR	homologous recombination
ICL	interstrand crosslink
IR	ionizing radiation
IRIF	ionizing radiation induced foci
ISWI	imitation SWI

MMR	mismatch repair
NER	nucleotide excision repair
NHEJ	non-homologous end joining
NuRD	nucleosome remodeling and histone deacetylation
PAR	poly(ADP-ribose)
PARG	poly(ADP-ribose) glycohydrolase
PARP	poly(ADP-ribose) polymerase
PIKK	phosphoinositide-3-kinase-related protein kinase
PTM	post translational translation
RNAi	RNA interference
RSF1	Remodeling and Spacing Factor 1
SMARCA1	SWI/SNF related matrix associated actin dependent regu lator of chromatin subfamily A member 1
SMARCA2	SWI/SNF related matrix associated actin dependent regu lator of chromatin subfamily A member 2
SMARCA5	SWI/SNF related matrix associated actin dependent regu lator of chromatin subfamily A member 5
SNF2H	sucrose non-fermenting protein 2 homolog
SWI/SNF	SWItch/Sucrose NonFermentable
TC-NER	Transcription-coupled nucleotide excision repair
TLS	Translesion synthesis
UV	Ultraviolet
WSTF	Williams syndrome transcription factor

Curriculum Vitae

Name : Özge Zelal Aydın
Date of birth : 22-01-1982
Place of birth : Ankara – Turkey

Education	**Year**
Doctor of Philosophy in Genetics Prof.dr.Jan Hoeijmakers, Prof.dr.Wim Vermeulen Erasmus Medical Centre, the Netherlands	2009-2014
Erasmus Exchange program in Molecular Cell Biology Dr.Marcel Schaaf Leiden University, the Netherlands	2008
Master of Science in Biology Prof.dr.Feride Severcan Middle East Technical University, Turkey	2006-2009
Bachelor of Science in Biology Middle East Technical University, Turkey	1999-2004
Secondary and High School Ankara Anadolu Lisesi	1992-1999

Work experience	**Year**
PhD candidate (Under supervision of Dr.Hannes Lans and Dr. Wim Vermeulen) Erasmus MC, Rotterdam, the Netherlands	2009-2014
Application Specialist Engin Healthcare, Ankara, Turkey	2006-2008
Application Specialist Makro Healthcare, Ankara, Turkey	2005-2006
Contract Research Associate Optimum CRO, Ankara, Turkey	2004-2005

List of Publications

- Aydin ÖZ, Marteijn JA, Ribeiro-Silva C, Rodríguez López A, Wijgers N, Smeenk G, van Attikum H, Poot RA, Vermeulen W, Lans H. Human ISWI complexes are targeted by SMARCA5 ATPase and SLIDE domains to help resolve lesion-stalled transcription (2014) *Nucleic Acids Research* 42(13):8473-85

- Aydin ÖZ, Vermeulen W, Lans H. ISWI chromatin remodeling complexes in the DNA damage response (2014) *Cell Cycle* doi 10.4161/15384101.2014.956551

- Aydin ÖZ, Cano D, van de Veer M, Ribeiro-Silva C, Wijgers N, Vermeulen W, Lans H. SWI/SNF facilitates Nucleotide Excision Repair. *Manuscript in preparation*

Portfolio

Name	: Özge Zelal Aydın
PhD period	: June 2009 – April 2014
Promotor	: Prof.dr.Jan Hoeijmakers
Copromotor	: Prof.dr.Wim Vermeulen, Dr.Hannes Lans

1. PhD training — Year

General courses

Safely Working in the Laboratory	2010
CDB course	2010
Genetics Course	2010
Biochemistry and biophysics course	2010
Literature course	2010

Specific courses

Epigenetic regulation in health and disease	2010
Genome maintenance and cancer	2010
In vivo imaging from molecule to organism	2010
The Microscopic Image Analysis: From Theory to Practice	2011
Biomedical English Writing and Communication	2012

Seminars and workshops

3rd Annual CGC scientific meeting	2010
17th MGC workshop	2010
20th MGC Symposium	2010
CGC/CBG meeting “Molecular Mechanisms in Cancer”	2010
Photoshop and Illustrator CS4	2010
21th MGC symposium	2011
19th MGC PhD workshop	2012
22th MGC symposium	2012

Presentations

18th MGC workshop	2011
MGC DNA Repair Meeting	2012

(Inter)national conferences

Chemistry related to Biological & Medical sciences Veldhoven, the Netherlands. Poster presentation	2009

Responses to DNA damage:from molecular mechanism to human disease Egmond een Zee, the Netherlands. Poster presentation	2011
EMBO Workshop on Chromosome Damage, Structure and Repair Cape Sounio, Greece.	2011
9th Dutch Chromatin Meeting Groningen, the Netherlands. Poster presentation	2011
the Life Sciences Momentum Rotterdam, the Netherlands	2011
10th Dutch Chromatin Meeting Amsterdam, the Netherlands. Poster presentation	2012
GRC Mammalian DNA Repair Ventura, the USA. Poster presentation	2013
Joint Dutch Chromatin Meeting & NVBMB Fall Symposium Rotterdam, the Netherlands. Poster presentation	2013

2. Supervising practicals and excursions & Tutoring

Bachelor student	September 2012 t/m April 2013
Master student	February t/m August 2011
Junior science programme	1 t/m 5 November 2010
Werkcollege Genetics	01-10-2012

Acknowledgments

Now, it is time to be open and honest about everything happened in my professional life between 2009 and 2014. It was a whole new life and a very big change for me to leave everything behind and come to the Netherlands for PhD. It was difficult as well, especially first half. I was thinking and thinking that I made a mistake, coming to the NL for many reasons, particularly socializing was very difficult here. Also technically, I was having difficulties in my project, no matter what I do, no matter what I try, nothing was working. But then things changed, and it was more like down the hill this time! Maybe I adapted, maybe I was luckier, maybe I changed my mentality… New people (mostly international) came to the lab and also I started to have some interesting data from my experiments. In the end, this entire journey taught me a lot about life, people and myself, but above all about chromatin remodelers.

I want to acknowledge here some valuable people who shared nice moments with me, but please be aware that I cannot acknowledge you guys enough here, especially knowing myself and Turkish friends that we never feel satisfied enough from the things written for us. I will try my best….

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nobody else was caring about these difficulties. Maybe you forgot, but I didn't and I feel indebted to you for that. When you walk in the corridor, be careful, if someone hugs you out of blue, this might be me. Maikel, my favorite Dutch :) , of course we will be in contact with each other, but thanks for being that sweet and warm. I will always remember that you and me were playing (and pipetting/writing in the meanwhile) Franz Ferdinand, Placebo, Massive Attack out loud every day in the lab as soon as everybody left. Bert-Jaap, you are a great person, very helpful, very sweet, and very very FRAP. I've never seen anyone who doesn't like/trust you and thanks for always being there! Petra and Loes, thanks for all the nice moments we shared and technical helps you gave me, wish you the best! My last roommates Barbara, Alice, Mariangela, Cristina, Mark and other room tenants Karen, Yasemin, Imke, Franzi and Serena, you changed the atmosphere in the lab a lot, and made my last year full of fun in the lab. I like all of you A LOT!!! Mariangela, volareee oooo, cantaree oooo ♪♫ I wish you started earlier and we knew each other before. You are a great person! We'll be in contact. Nils, thanks for helping me in computer and for nice chats. Maria Tresini, I am grateful for the helpful hints you gave me… Pim Visser, it's a pity that you got retired that early. Thanks for being patient when I was trying to speak Dutch, I will miss a lot our chats on the corridor and your friendship. Cintia and Ricardo, I've already missed you guys and it was very nice to get to know you; one day I will visit you in São Paulo. Hervé, beside everyday lunch rituals we had, and our gossips in the back row during workdiscussions, it was also unforgettable for me to have a trip to California! When we went to Universal Studios, we were like 2 kids and it was one of the biggest fun I've ever had. Good luck in France… Marinke, wish you the best in your future life; you were very good student and made a very good job… David, the hot Colombian, I must tell you that it is always great to dance with you and Dusseldorf would be dull if you were not there; good luck in Groningen. Mireille, my dear, you are one of the bravest persons I've ever known, I hope everything will work out as you wish. Aida Rodriguez, Weike, Alice and Mark, I had so much fun with you guys, sorry that I was teasing you that much, I will miss you all…Karen Sap, your positivity and compatibility always impressed me; thanks for always being very friendly and I will miss our 'next to centrifuge chats'!!! Aida Farshadi, you were always very nice to me and I understood better with you the similarities between our cultures; take care my ex-house ;). Luca, our transfer from 10th floor for lunch, we have met years ago in MGC symposium and you've always been very nice, friendly to me, and I could talk to you for hours. I will miss you, best wishes! Aristea, I will always remember you with your unique ways. You are a genuine 'delikanlı' (ask Nesrin what it means)… Indriati, thanks for all organizations you made, and crazy Blender nights. Fanny, bebişim, I don't understand how you could endure us all these years (peygamber sabrı), I mean, not many people could stand our high volume Turkish chats, Turkish-French translations and dances and songs… Merci pour tout, nous serons en contact. Ali Imam, you are very warm and interesting person and I wish I got to know you earlier. I seriously think if one day you are sick of biology, you can earn your life as a historian or social anthropologist. Best wishes. Romana, Tommy, Michelle, Sander, Roel, Pim, Berina, Peter, Annelieke, Ralph, Xiao, Andrea Conidi, Eric Soler, Wouter Koole, Guillome, Maria Mikropoulou, Maria Vouyioukalaki, Magda, Klaas, Julie, Alireza, Ali Refah, Sophia, Olaf, Haleh, Ülkü, KC, Marcel, Kostas, Yanto, Charlie, Inger, Gosia, Humberto, Kishan, Akos, Mijke Visser, guys thanks you all for your friendship. Adriaan, Gert van Cappellen, Erik-Jan, Alex Nigg, Gert-Jan, Jasperina, Marike, Job Lassche; thanks for all your help and nice tips. 'Theater Erasmus' guys, Thomas, Chris Vink, Cheryl, Polynikis, Johan, Maaike, Ruben, it was fun to train with you. Dear Marcel Schaaf and Antonia, I think you have a big impact on my decision of going on with PhD, it was very nice to work with you for 5 months and thank you for everything. Sevgili Erdal Abi, yardımların için çok teşekkürler.

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