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ORIGINAL PAPER

Role of SUMO/Ubc9 in DNA damage repair and tumorigenesis

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Abstract DNA damage repair is an important cell function for genome integrity and its deregulation can lead to genomic instability and development of malignancies. Sumoylation is an increasingly important ubiquitin-like modification of proteins affecting protein stability, enzymatic activity, nucleocytoplasmic trafficking, and protein-protein interactions. In particular, several important DNA repair enzymes are subject to sumoylation, which appears to play a role in copping with DNA damage insults. Recent reports indicate that Ubc9, the single SUMO E2 enzyme catalyzing the conjugation of SUMO to target proteins, is overexpressed in certain tumors, such as lung adenocarcinoma, ovarian carcinoma and melanoma, suggestive of its clinic significance. This review summarizes the most important DNA damage repair pathways which are potentially affected by Ubc9/SUMO and their role in regulating the function of several proteins involved in the DNA damage repair machinery.

Keywords Ubc9 · SUMO · Sumoylation · DNA repair

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Introduction

DNA integrity is under constant threat by both exogenous and endogenous sources, which may lead to genomic instability and disruption of normal cellular homeostasis. When occuring spontaneously, genomic instability is the driving force of evolution in living organisms across generations. If DNA damage is excessive within a single organism, genomic instability results in cumulative mutations of genes that regulate growth, differentiation and apoptosis, a hallmark of cancer. To ensure DNA fidelity and survival, organisms have developed DNA damage sensor mechanisms which activate diverse cellular responses, such as apoptosis induction and cell cycle arrest. Highly complex and diverse DNA repair systems have evolved to cope with different types of DNA damage. Over the past years an increasing number of proteins either directly involved in the DNA damage repair or associated with cellular responses to DNA damage have been described to undergo SUMO modification or sumoylation (Wood et al. 2005). The present review will summarize the role of sumoylation and more specifically the role of Ubc9, an E2 SUMO conjugating enzyme, in DNA damage repair pathways and genome integrity.

The sumoylation cycle

Posttranslational modification of proteins is an important mechanism regulating the activity, localization, intracellular levels, and interaction with other molecules. Apart from small molecule modifications, such as phosphorylation, acetylation, or methylation, larger

molecules may be attached to target proteins and therefore, provide larger and more chemically diverse surfaces for interaction. Ubiquitin is the most extensively studied large molecule modifier which via a 3-step process is covalently attached to a lysine residue of the target protein and usually marks proteins for proteasomal degradation. Recently, an increasing number of ubiquitin-like modifiers (SUMO) have been described, which are not directly involved in protein degradation (reviewed in Schwartz and Hochstrasser 2003). The SUMO protein exists in four isoforms-paralogues (SUMO-1 through -4) and is covalently attached to the ε-amino group of a lysine residue in the target protein.

Sumoylation involves a 3-step pathway analogous to the ubiquitination pathway: a precursor SUMO protein undergoes 'maturation' by cysteine proteases, termed isopeptidases, which cleave the C-terminus of the precursor form. The mature SUMO protein is then 'activated' by the SUMO E1 enzyme, a heterodimer of the AOS1 and UBA2 proteins, and is conjugated to the target proteins by the SUMO E2 enzyme, termed Ubc9. Although SUMO conjugation can be performed by Ubc9 without E3 ligases in some cases, the reaction efficiency and specificity, both target- and subcellular compartment-specific, can be enhanced by a large number of SUMO E3 ligases. The resulting SUMO conjugation of target proteins is usually short-lived and sumoylated proteins can be rapidly deconjugated, secondary to the action of similar proteases involved in SUMO maturation (Hay et al. 2005). Therefore, the biological consequences of sumoylation are not proportional to the overall levels of sumoylated proteins, but rather to the modification 'history' of a particular protein; a deconjugated but previously sumoylated protein may have a different long term fate from a protein which has never been modified. Proteomic analyses in both lower eukaryotic (Hannich et al. 2005) and mammalian cells (Zhou et al. 2005) have revealed a significant number of sumoylated proteins involved in important nuclear functions, such as chromosome aggregation, DNA replication, transcription, and damage repair.

General function of Ubc9

In contrast with the ubiquitination pathway which utilizes several E2 conjugating enzymes, Ubc9 is the only SUMO E2 enzyme and therefore a key regulator of the sumoylation pathway. Ubc9 was originally described as an important protein for normal mitosis and cell cycle progression in lower eukaryotes because its absence was associated with cell cycle arrest and abortive mitosis (Seufert et al. 1995). A proteomic analysis revealed that after overexpression of the *p*53 *U*pregulated *M*odulator of *A*potosis (PUMA), a BH3-only pro-apoptotic protein that induces BAX-dependent apoptosis, Ubc9 was one of the proteins that were downregulated, suggesting that it may have significant role in the suppression of apoptosis (Gu et al. 2004). In Drosophila, Ubc9 loss-of-function studies similarly showed mitotic defects in hemopoietic tissues (Chiu et al. 2005). Ubc9 yeast mutants are more sensitive to DNA damaging agents (Mao et al. 2000; Jacquiau et al. 2005). In mammalian cells, Ubc9 protein downregulation did not appear to have significant effects in cell cycle but was associated with defects in cytokinesis and furthermore increased the number of apoptotic cells (Hayashi et al. 2002). Moreover, targeted gene inactivation of the mouse Ubc9 is embryonically lethal right after the blastocyst stage secondary to chromosome defects, such as polyploidy and abnormal metaphase plates and anaphase bridges (Nacerddine et al. 2005). In summary, Ubc9 is important for genome integrity especially during mitosis and overall cell survival.

DNA repair pathways

At least four principal, partially overlapping DNA damage repair pathways deal with different types of DNA damage in mammals. They are highly conserved across prokaryotic and lower eukaryotic organisms. For single strand breaks, nucleotide excision repair (NER) pathway repairs bulky DNA adducts; base excision repair (BER) pathway repairs damage inflicted by cellular metabolism and by spontaneous depurination; and mismatch repair (MMR) pathway repairs mispaired nucleotides, insertions or deletions secondary to slippage of DNA polymerase during the normal synthesis of repetitive sequences in replication or recombination. On the other hand, homologous recombination (HR) and non-homologous end joining (NHEJ) are the major pathways for double strand breaks (DSB) (reviewed in Sancar et al. 2004).

Thus, NER, MMR, and BER mechanisms overcome only single rather than double strand DNA lesions during transcription and normal replication. Exogenous (e.g., ultraviolet radiation and polycyclic aromatic hydrocarbons) and endogenous sources predominantly cause lesions utilizing NER and MMR pathways, respectively, whereas both endogenous (e.g., oxygen radicals and spontaneous hydrolysis of DNA bonds) and exogenous sources (e.g., ionizing radiation and

alkylating agents) result in BER lesions. DSBs, however, are particularly dangerous because both strands are affected and therefore no intact complementary strands can be used for templates. Thus, cells have to identify which ends belong together; otherwise, essential genes may be inactivated and lead to cell death. HR usually occurs in S and G2 phase of the cell cycle, when DNA is replicated such that a sister chromatid may be used as a template and, thus, is more accurate. In contrast, the simpler, error-prone NHEJ, which rejoins the two broken ends directly and leads to small DNA sequence deletions, is the dominant double strand DNA repair mechanism during the G1 phase.

Despite the differences in terms of particular proteins involved in each repair pathway the overall concept is similar. 'Architectural' factors constantly survey the DNA structure for abnormal backbone conformations ('indirect readout') or particular DNA sequences ('direct readout') and facilitate access of DNA damage repair factors by remodeling the highly condensed chromatin structure via phosphorylation, ubiquitination, acetylation, and methylation of different histone molecules. DNA repair signal originates *in situ*, at the sites of DNA damage, and activates downstream p53-dependent and p53-independent (p63 and p73) effector pathways, which either induce DNA damage repair or induce cell cycle arrest or apoptosis.

Table 1 summarizes an ever growing list of proteins involved in each type of DNA damage repair. *NER* is the most versatile repair system for lesion recognition and substrate specificity and may either survey the whole genome (global genome NER or GG-NER) or may be coupled with transcription (transcription coupled NER or TC-NER). For *NER* the 'indirect readout' sensing predominates, followed by dual incisions to bracket the lesion, release of the excised oligomer, repair synthesis to fill in the resulting gap and ligation. For *BER* a broad range of DNA glycosylases recognize altered bases as a result of redox reactions, alkylation (methylation), deamination (uracil or xantine) or simple base mismatches and remove these bases to create an apurinic/apyrimidinic (AP) site. The resultant 3′ sugar residue(s) is (are) removed by an APE1 endonuclease and the gap is then filled by a DNA polymerase. Finally, the nick is sealed by an *X*-ray *r*epair *c*ross *c*omplementing (XRCC)-ligase complex. Depending on the extent of DNA damage, short patch or long-patch (2–10 nucleotides) BER pathways are activated. For *MMR* different DNA damage sensors detect mismatches, single-base loops (hMSH2/6) or insertion-deletion loops (hMSH2/3), which naturally occur during replication, especially of microsatellite sequences that consist of either mono- or di-nucleotide repeats, or recombination. These proteins interact with components of the replication machinery for repair (polδ/ε, RPA, PCNA, RFC, exonuclease 1, and endonuclease FEN1). Unrepaired single strand lesions by the above mechanisms may lead to stalling of replication forks, nicks and subsequent development of DSBs.

The existence of two different DSB DNA repair pathways is probably related to two independent DSB DNA damage sensors. For *HR* the Mre11-Rad50-Nbs1 (MRN) complex is comprised of proteins with: (a) DNA structure recognition (Rad50), (b) 3′-to-5′ exonuclease and endonuclease activity to create 3′ single strand overhangs (Mre11) and therefore, the ability to attract Rad51/Rad52, and (c) ability to recruit members of the phosphoinositide-3-kinase-related protein kinase (PIKK) family of proteins (Nbs1), such as the ataxia telangiectasia mutated (ATM) protein. The single strand DNA-binding protein replication protein A (RPA) binds to the ssDNA overhangs, and Rad51/Rad52 are then recruited to DSBs. Both RPA and Rad52 help load Rad51 onto ssDNA to form nucleoprotein filaments which search for the homologous duplex DNA in the undamaged sister chromatid to facilitate strand invasion. The resected 3′ end invades a homologous DNA duplex and is extended by DNA polymerase ('Holiday junctions'). The newly synthesized fragments ends are joined by DNA ligases, and the Holiday junctions are resolved by resolvases. Upon recruitment to DSBs, ATM is activated and phosphorylates-activates p53, phosphorylates-inactivates MDM2, and also phosphorylates a variety of other proteins involved in repair (Rad9, Rad17, p95Nbs1) and checkpoint control (chk1, chk2). Recent evidence suggests that members of the Fanconi anemia (FANC-A, -C, -E, -F) and breast cancer (BRCA-1, -2) complex associate with the M/R/N complex and participate in the DSB HR. For *NHEJ* the Ku heterodimer (Ku70/80) binds to the DSB ends and recruits the DNA-PKcs and the ligase4-XRCC4 heterodimer which ligates the two duplex termini regardless of whether the two ends come from the same chromosome.

DNA repair enzymes subject to sumoylation

Base excision repair (BER)-DNA glycosylases

Thymidine glycosylase (TDG) is one of the several DNA glycosylases involved in BER. It releases thymine or uracil from G·T and G·U mismatches arising from spontaneous hydrolytic deamination. Unrepaired lesions may result in a C-to-T transition after DNA

Table 1 Enzymes involved in different DNA repair pathways

	Strand affected	Cell cycle specificity	DNA damaging sources	Proteins involved	Human syndromes
Base excision repair	Single	Nonspecific	Ionizing radiation, alkylating agents, oxygen free radicals, spontaneous hydrolysis	DNA glycosylases, APE1 endonuclease, polβ, polδ/ε, XRCC1, ligase 1/3, PCNA, FEN1	None reported
Short patch					
Long patch					
Nucleotide excision repair	Single	Nonspecific	Ultraviolet radiation, polycyclic aromatic hydrocarbons, cisplatin	XPA, RPA, XPC, $p48^{DDB2}$, TFIIH, XPG, XPF-ERCC1	Cockayne syndrome
Transcription-coupled				CSA, CSB	Xeroderma pigmentosum Trichothiodystrophy
Global genome	Various forms	Nonspecific	Platinum compounds, ultraviolet irradiation	DDB-2	Xeroderma pigmentosum group E
Mismatch repair	Single	Replication	Mispaired nucleotides by DNA polymerases	hNLH1, hMSH2/3/6, hPMS1/2, RPA, PCNA, RFC, exonuclease 1, and endonuclease	Hereditary nonpolyposis colorectal cancer
		Recombination		FEN1	
Homologous recombination (HR)	Double	S or G2 phase	Reactive oxygen species, ionizing radiation, chemicals	Mre11-Rad50-Nbs1	Ataxia-telangiectasia, Nijmegen breakage syndrome
				Rad51, Rad52, RPA, resolvases	Werner syndrome
				Bloom's helicase, Werner's helicase, RecQ4	Bloom syndrome
					Rothmund Thompson syndrome
					Sporadic cancer
					ATR-Seckel
Non-homologous end joining	Double	G1 phase	Same as HR	Ku70/80 DNA-PKcs XRCC4	LIG4 syndrome
					Radiosensitive severe combined immunodeficiency
Minor	Single	Nonspecific	Methylating carcinogens or alkylating therapeutic agents	0^6-methylguanine methyltransferase	None reported

replication. After base excision TDG remains stably bound to the resultant AP site, protecting this harmful repair intermediate until it is transferred to the AP endonuclease1 for the next step of the repair pathway. TDG associates with SUMO noncovalently (Takahashi et al. 2005) and covalently (Hardeland et al. 2002; Baba et al. 2005). The noncovalent interaction promotes covalent attachment of SUMO to TDG, which favorably interacts with the promyelocytic leukemia (PML) protein and is directed to nuclear bodies. Moreover, PML promotes SUMO conjugation to TDG which reduces its affinity for the AP site. Therefore, covalent modification of TDG with SUMO facilitates its 'recycling' for subsequent rounds of BER (Fig 1). The XRCC1, a molecular scaffold protein that coordinates the assembly of BER complexes at the damaged sites, is also a SUMO substrate but the effect of sumoylation on its function is unknown (Gocke et al. 2005).

Poly (ADP-ribose) polymerase (PARP) enzymes

Poly (ADP-ribose) polymerases are multifunctional proteins which directly bind to damaged DNA and catalyze the synthesis of poly (ADP-ribose) on target proteins, such as histones, transcription factors and polymerases. Although the consequences of poly (ADP-ribosyl)ation are unknown, this posttranslational modification of proteins suppresses their DNA-binding affinity and activity. Through their BRCA-1 carboxy terminus motif, PARP interacts with several DNA repair and cell cycle checkpoint proteins (Bouchard et al. 2003). In response to single strand DNA damage, PARP-1, for example, rapidly associates with chromatin and recruits the mitotic chromosome organizer condesin I to sites of ssDNA damage where it interacts with BER factors, such as XRCC1 (Heale et al. 2006). Among these proteins, Ubc9 was found to interact with PARP in a yeast-two-hybrid system. The importance of this interaction is unknown, but it has been speculated that deceleration of DNA synthesis for DNA repair prior to replication and a possible role in apoptosis are involved (Masson et al. 1997).

Nucleotide excision repair (NER)

Xeroderma pigmentosum protein C (XPC) forms a complex with hHR23B and is an important damage recognition factor of the GG-NER pathway by recognizing architectural abnormalities into dsDNA for subsequent binding of other NER factors. XPC also undergoes SUMO conjugation which prevents its degradation during UV radiation (Wang et al. 2005). It is speculated that XPC sumoylation not only stabilizes the protein but also facilitates its recycling for additional rounds of NER by decreasing its affinity for the

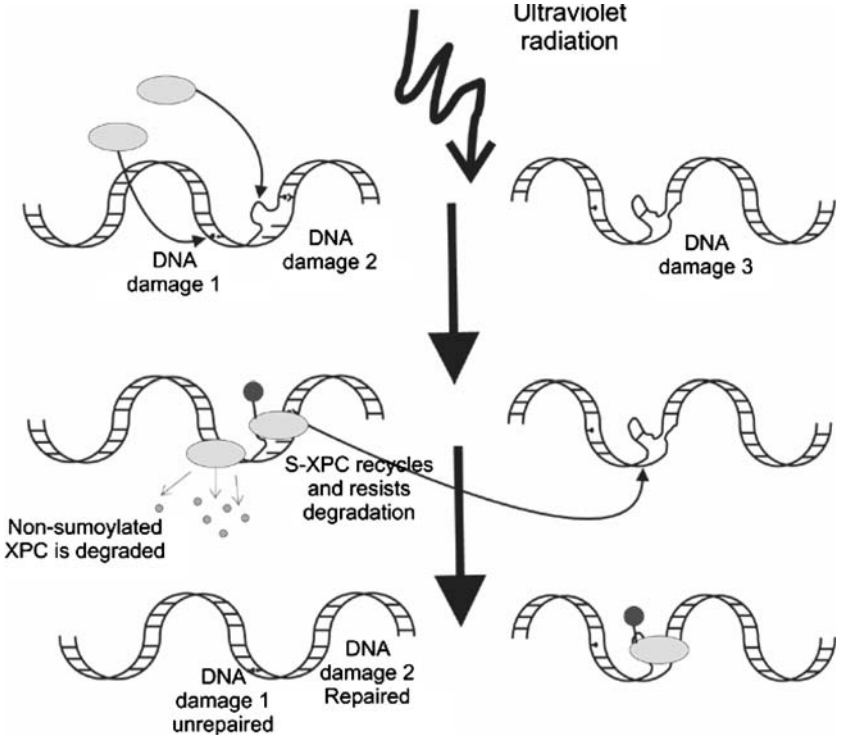

Fig. 1 Role of sumoylation in base excision repair (BER). Only one of the several DNA glycosylases, the thymidine glycosylase (TDG), has shown to be affected by sumoylation. Thus, SUMO modification of TDG reduces affinity of the enzyme with the DNA and therefore enables it to begin another round of BER at another damaged site. The function of scaffold protein XRCC1 is unknown

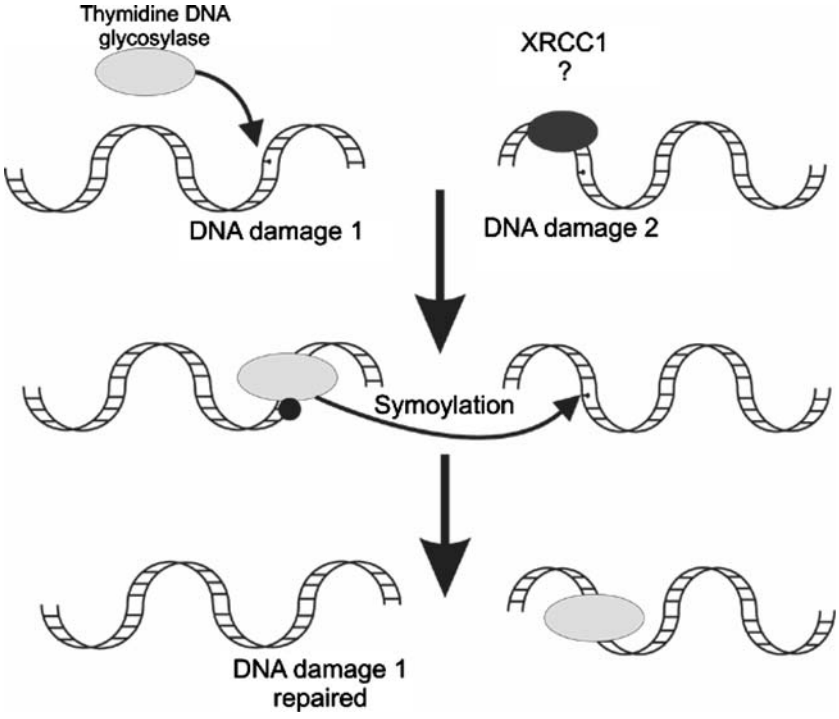

Fig. 2 Role of sumoylation in nucleotide excision repair (NER). Similar with BER, NER proteins, such as XPC, are SUMO substrates which not only become available for subsequent round of DNA repair but are also protected from degradation from DNA damaging agents, such as ultraviolet radiation. S-XPC, sumoylated XPC

NER complex once it has been formed (Wang et al. 2005) (Fig 2).

Homologous recombination (HR)

Early HR-Rad51 and Rad52

The sumoylation system has been shown to play an important role in the repair of DSBs via HR as well as chromosome recombination during normal meiosis. Both Rad51 and Rad52 are key components of the eukaryotic HR machinery, which are involved in the early stages of HR and directly interact with Ubc9 and SUMO-1 (Kovalenko et al. 1996; Shen et al. 1996a; Shen et al. 1996b). Moreover, SUMO-1 may noncovalently interact with Rad51 and decrease radioresistance by down-regulating DSB-induced HR (Li et al. 2000). Nuclear depletion of Ubc9 causes significant disruption of Rad51 intracellular trafficking, such that induction of Rad51 nuclear foci by DNA damaging agents is markedly inhibited (Saitoh et al. 2002). hMMS21 functions together with Rad51 in the repair of DNA DSBs via HR and is both a SUMO substrate and a SUMO ligase for hSMC6 and the DNA repair protein TRAX (Fig 3). Depletion of hMMS21 sensitizes mammalian cells to DNA damage-induced apoptosis secondary to increased foci of DNA damage and hyperactivation of ATM/ATR signaling pathways, suggesting inefficient DNA repair mechanisms (Potts and Yu 2005).

RecQ DNA helicases

The Bloom (BLM) protein belongs to the RecQ family and functions in DNA recombination and maintenance of genome integrity. It was recently shown that the BLM protein also undergoes SUMO modification which affects its nuclear localization (Suzuki et al. 2001; Eladad et al. 2005). More specifically, it has been hypothesized that unmodified BLM normally resides in the PML nuclear bodies from which it constantly moves out to the nucleoplasm to survey intermediate DNA structures of HR. In the absence of such DNA conformations it is sumoylated and thereby re-directed to the PML-NBs. Upon arrival to PML-NBs it is rapidly de-sumoylated to begin another round of DNA substrate surveillance. However, if it encounters any high-affinity DNA structure BLM undergoes conformational changes that prevent its sumoylation. Under this concept, the ability of BLM to undergo sumoylation allows for constant DNA surveillance without inappropriate activation of DNA damage repair pathways (Eladad et al. 2005). Werner's helicase (WRN) is another member of the RecQ family of DNA helicases that is predominantly localized in the nucleolus. Recent studies have shown that WRN is also a SUMO substrate (Woods et al. 2004; Kawabe et al. 2000). It has, therefore, been hypothesized that genotoxic stress induces sumoylation of WRN which may facilitate its migration from the nucleoli to discrete nuclear foci. In

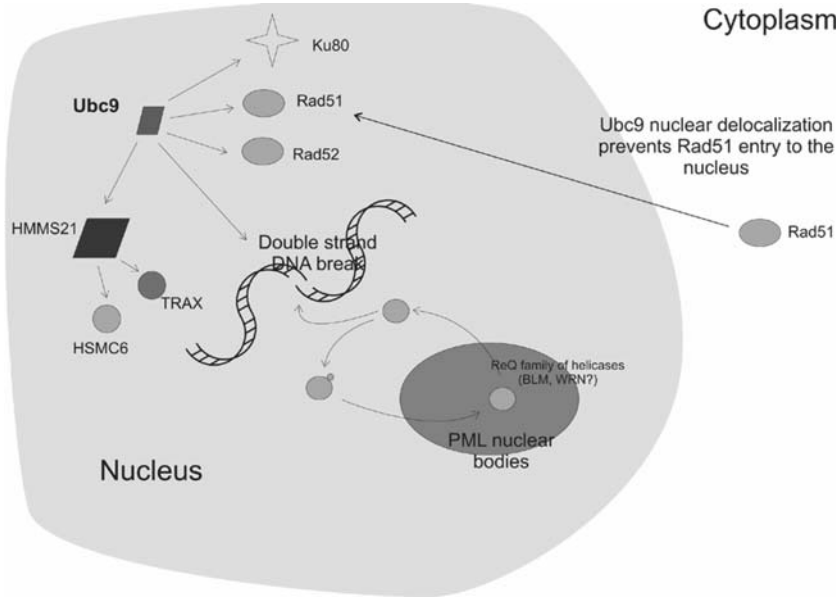

Fig. 3 Role of sumoylation in double strand DNA damage repair (dsDNA DR). Ubc9 is predominantly a nuclear protein that affects intranuclear and nucleocytoplasmic trafficking. For example, if Ubc9 is depleted from the nucleus important dsDNA DR factors are not transported from the cytoplasm to the nucleus. Moreover, sumoylation is fundamental for trafficking of important dsDNA damage recognition proteins, such as members of the RecQ family of proteins. These proteins constantly cycle between subnuclear structure-storage protein depots (PML nuclear bodies) and chromatin screening for those which are involved in the early steps of homologous recombination (Rad51 and Rad52) and the non-homologous end joining (Ku80)

these nuclear foci WRN partially co-localizes with DNA repair proteins, such as Rad51 and Rad52, and may be involved in several nuclear functions, such as HR, restoration of stalled replication forks, repair of DNA strand breaks, and telomere maintenance.

A number of other DNA damage repair proteins are also SUMO substrates with unknown effect on final outcome, such as: (a) the translin-associated factor X (TRAX), a ssDNA binding protein that recognizes consensus sequences found at breakpoint junctions of various chromosomal translocations (Aoki et al. 1997), (b) the Ku80, part of the Ku heterodimer involved in the NHEJ DNA repair pathway (Gocke et al. 2005), (c) the XRCC4, an important protein for NHEJ, which only in response to sumoylation can be translocated from the cytoplasm to the nucleus to complete V(D)J recombination events (Yurchenko et al. 2006).

Postreplicative DNA repair- the Rad6 pathway in lower eukaryotes

Postreplicative DNA repair is an important mechanism for maintaining genome integrity. It is different from other previously described repair mechanisms in that it does not physically remove DNA lesions which normally arise during DNA replication, but rather circumvents them, thereby preventing the severe consequences of stalled replication forks that could otherwise lead to cell-cycle arrest and ultimately death. This repair is mediated by low-fidelity, damage-tolerant polymerases which either synthesize across the lesion (translesion synthesis, error-prone) or rely on the genetic information provided by the newly synthesized sister chromatid. The recruitment of most damage-tolerant polymerases to the replication forks is mediated by the proliferating cell nuclear antigen (PCNA), an essential processivity factor which encircles the DNA as a sliding clamp and serves as an interaction platform for the coordination of replication, repair, chromatin assembly, and cell cycle regulation. PCNA is able to integrate such diverse processes largely due to its ability to undergo several posttranslational modifications, such as mono- and poly-ubiquitination and sumoylation. The conjugation enzymes responsible for these modifications are members of the RAD6 pathway of genes and have been most extensively studied in lower eukaryotic organisms, such as *S. cerevisiae* (reviewed in Ulrich 2005). PCNA monoubiquitination is triggered by DNA damage via a concerted action of RAD6, RAD18, and UBA1 proteins and activates translesion synthesis by a transient exchange of the replicative polymerase for the damage-tolerant polymerases (pol) ζ and η. Persistent DNA damage induces nuclear import of UBC13 and MMS2 proteins

which are recruited to the chromatin by RAD5 and promote PCNA polyubiquitination. Polyubiquitinated PCNA is thus engaged to the error-free repair and involves transient template switch to the undamaged sister chromatid (Hoege et al. 2002).

PCNA also directly interacts with Ubc9 and is sumoylated at the same residue that is ubiquitinated (Hoege et al. 2002). Although the impact of PCNA sumoylation is not yet fully understood one of the functions of PCNA during S phase in the absence of DNA damaging agents is to stimulate the error-prone pol ζ which extends primer termini opposite a variety of lesions or mismatches. Thus sumoylated PCNA overcomes replication fork blocks not caused by DNA damage, but by other refractory DNA structures (Stelter and Ulrich 2003). Moreover, during the normally occurring lesions of the S phase, the SUMO modified PCNA recruits the *s*uppressor of *R*AD *s*ix screen mutant *2* (srs2) RecQ helicase to the replication forks, thereby, preventing access to the RAD52 recombination machinery by disrupting RAD51 nucleoprotein filaments. By doing so, PCNA sumoylation suppresses unwanted and deleterious recombination events during DNA replication and facilitates ubiquitin-dependent damage avoidance (Papouli et al. 2005; Pfander et al. 2005). Although PCNA sumoylation has been described in metazoans (Leach and Michael 2005) no such modification has been described in mammalian organisms (Kannouche et al. 2004), suggesting that there is a degree of plasticity in terms of PCNA modification across different species.

Chromatin-structure and role in DNA damage repair

Chromatin, the highly condensed structure composed of genomic DNA and histones, undergoes several modifications in the process of transcription, replication and DNA repair in order to overcome the natural barrier of DNA accessibility. Covalent modification of histones, a $(H3\text{-}H4)_2$ tetramer associated with the H2A-H2B dimers, is an important mechanism of chromatin structure modifications facilitating these naturally occurring functions mentioned above. In addition to post-translational changes, such as phosphorylation, lysine ubiquitination, acetylation, and methylation, sumoylation of histones is an important mechanism of chromatin modification. In *S. pombe* fission yeast, Ubc9/Hus5 physically interacts and sumoylates the conserved heterochromatin proteins Swi6 and Chp2, two heterochromatin protein 1 analogues which bind to methylated histone H3, as well as the Clr4, a histone H3 methylating enzyme, resulting in efficient silencing and heterochromatin stability (Shin et al. 2005). In mammalian cells, interaction of Ubc9 with transcription factors, such as c-jun, or p53, may approximate Ubc9 to chromatin and mediate sumoylation of histone H4 (Fig 4). Sumoylated H4 may facilitate recruitment of histone deacetylase 1 (HDAC-1) and heterochromatin protein-1 (HP1-γ) leading to transcriptional repression (Shiio and Eisenman 2003). Also, Ubc9 interacts and sumoylates the transcriptional co-repressor N-CoR which may facilitate SUMO-conjugation of N-CoR associated proteins, such as HDACs, leading to overall transcriptional repression (Tiefenbach et al. 2006). Similarly, sumoylation at the cell cycle regulatory domain 1 (CRD1) of p300 transcriptional coactivator facilitates binding of HDAC6 leading (Girdwood et al. 2003); sumoylation at the ETS domain of the Elk-1 transcriptional factor recruits HDAC-2 (Yang and Sharrocks 2004). Both

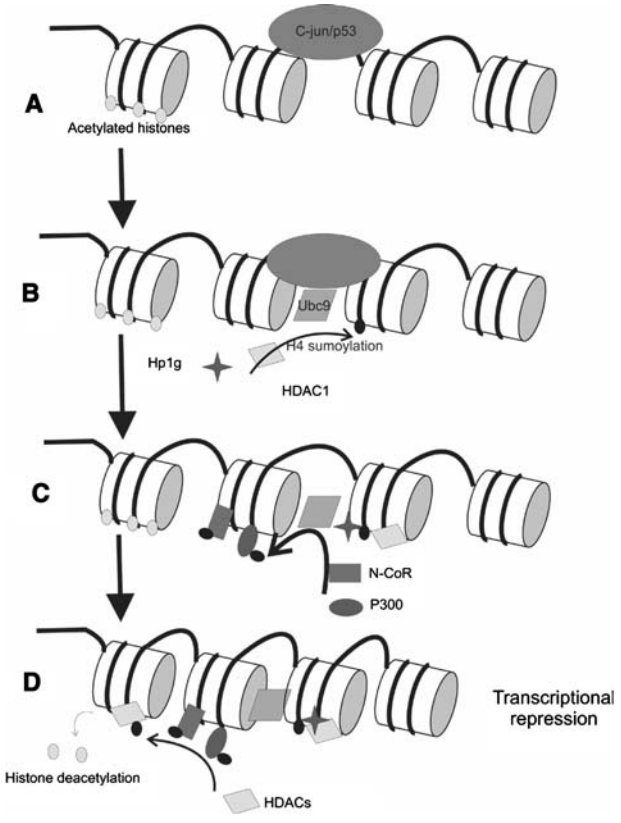

Fig. 4 Role of sumoylation in chromatin function. Transcription factors may facilitate approximation of Ubc9 to chromatin (**B**) and serve as scaffold for subsequent sumoylation of target proteins, such as histone deacetylases (HDACs), and other transcription coactivators/repressors (**C**). Several HDACs appear to be activated by this process and result in transcriptional repression (**D**). Note that sumoylatin of chromatin associated proteins may also result in transcriptional activation

result in histone deacetylation and, therefore, transcriptional repression. Conversely, sumoylation of *de novo* DNA methyltransferase 1 (Dnmt3a), an important enzyme for genomic imprinting and transcriptional silencing, inhibits its interaction with HDAC-1 and HDAC-2 and releases transcriptional repression (Ling et al. 2004). The role of sumoylation of several chromatin modification/remodeling proteins, such as the SPTF-associated factor 65 gamma (STAF65γ), the spliceosome associated protein 130 (SAP130), MOZ2, the histone H3 acetyltransferase GCN5, the chromatin remodeler Mi2, and the histone demethylase BHC110/LSD1/AOF2 is currently unknown (Gocke et al. 2005).

DNA topoisomerase II plays crucial roles in both chromosome condensation and segregation during mitosis. SUMO conjugation of topoisomerase II is important for remodeling of the enzyme on mitotic chromosomes at the metaphase-anaphase transition of Xenopus egg extracts because Ubc9-DN disrupts dissociation of sister chromatids (Azuma et al. 2003) and may facilitate dissociation of the stalled topoisomerase IIβ from DNA (Isik et al. 2003). Sumoylation of topoisomerase I may moderate topoisomerase-induced damage (Mao et al. 2000) probably by inducing nucleolar delocalization and redistribution to other nuclear sites (Mo et al. 2002).

DNA damage response genes

The p53 tumor suppressor gene functions primarily as a sequence-specific transcription factor that modulates the expression of a large number of genes by responding to a variety of signals that impact upon cellular homeostatic mechanisms that monitor DNA replication, DNA damage and cell division (Ljungman et al. 2000). Under normal conditions p53 production matches its degradation via its ubiquitination by the MDM2 protein. Upon genotoxic stress, p53 undergoes a number of possible modifications, such as phosphorylation, acetylation, methylation, ubiquitination or sumoylation, which may increase its half life and its activity. The pattern and extend of p53 modifications varies depending on the type and extend of DNA damage, as well as the tissue type which undergoes the genotoxic stress. The downstream processes following p53 activation may be transactivation-dependent via recognition of highly conserved p53-response elements (reviewed in Harris and Levine 2005) or transactivation-*in*dependent by 'nonspecific sequence' binding to mismatched or bulged DNA structures resulting from DNA damage (reviewed in Sengupta and Harris 2005). The p53-responsive genes may initiate one of three cellular programs leading to cell cycle arrest (at G1 or G2), cellular senescence or apoptosis. Activated p53 may directly enhance the DNA damage repair function acting either as a chromatin-accessibility and repair factor or by promoting the activity of repair enzymes involved in the different repair pathways mentioned above.

Both p53 and its regulatory protein MDM2 directly interact with Ubc9 and are SUMO substrates (Buschmann et al. 2001; Rodriguez et al. 1999). p53 sumoylation is regulated by several SUMO E3 ligases (Schmidt and Muller 2002; Weger et al. 2005) as well as modification of p53 interacting proteins (Chen and Chen 2003). Moreover, p53 sumoylation may be facilitated after interaction with other proteins during genotoxic stress (Kurki et al. 2004). However, the effect of sumoylation on p53 function is controversial (Rodriguez et al. 1999; Schmidt and Muller 2002) and is affected by prior p53 modifications (Lin et al. 2004). It is speculated that p53 sumoylation is transient, applying to the entire but only a small fraction of the p53 pool at a time, and may be restricted at a specific compartment favoring a specific p53 function, such as regulating the half life of specific protein/protein or protein/DNA complexes (Melchior and Hengst 2002).

Potential role of Ubc9 in cancer

The increasing number of DNA damage repair proteins undergoing SUMO conjugation suggests that Ubc9, and by extension sumoylation, are important for maintenance of genome integrity. Genotoxic stress induces sumoylation of a broad number of proteins involved in nuclear functions (Manza et al. 2004) as well as proteins involved in important signaling pathways, such as the nuclear factor kappa B (NF-κB) (Huang et al. 2003). Ubc9 and SUMO-1 were highly expressed in human premalignant conditions in response to low-grade, long-term genotoxic stress, implying that upregulation of sumoylation may be an adaptive process to genotoxic stress (Romanenko et al. 2006). Furthermore, Ubc9 is overexpressed in several malignancies, such as lung adenocarcinoma (McDoniels-Silvers et al. 2002), ovarian carcinoma (Mo et al. 2005), and melanoma (Moschos et al. 2005). Antagonizing Ubc9 function in MCF-7 breast cancer cells transplanted in nude mice inhibited cell growth and increased apoptosis via Bcl-2 dependent mechanisms (Mo et al. 2005). Inactivating mutations of Ubc9's SUMO conjugating activity enhances sensitivity to DNA damaging agents (Jacquiau et al. 2005; Mo et al. 2004). Overall, the data suggests that Ubc9 may be fundamental for tumori-

genesis and tumor progression by preventing activation of apoptotic pathways and by minimizing the acute cellular stress response associated with the accumulating DNA damage of tumor progression. Furthermore, agents antagonizing Ubc9 function may act an important anticancer therapies by reversing chemotherapy resistance observed at some point during cancer therapy (reviewed in Mo and Moschos 2005).

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