A role of SUMOylation in proteostasis, centromere integrity and the DNA damage response

Liebelt, F.

CHAPTER 1

General Introduction

Frauke Liebelt[1]

[1]Department of Cell and Chemical biology, Leiden University Medical Center, 2300 RC Leiden, The Netherlands.

POST-TRANSLATIONAL MODIFICATIONS

The average adult human body consists of approximately 37.2 trillion cells[1]. All that we are and everything that we do is orchestrated within and between them. Each cell carries an elaborate manual that is essential for everything. The manual, our genetic material or deoxyribonucleic acid (DNA), describes how to build other macromolecules that form the actual workforce of the cell. An important group of macromolecules, which are encoded by our DNA are polypeptides, also called proteins. To build a protein, the DNA of a particular gene, which is the entity of the DNA that carries the instructions for the synthesis of one particular protein, must first be transcribed into messenger ribonucleic acid (mRNA), which is subsequently translated into a protein[2-4]. Due to processes like alternative promotors, alternative splicing or RNA editing, the transcription of around 20,000 genes of the human genome result in about 100,000 different mRNAs[5-10]. After or during the time when a protein is translated it can be altered by the covalent attachment of small chemical entities or other proteins. These processes are called co-translational and post-translational protein modification (PTM), respectively, but are often collectively referred to as PTMs[11,12]. PTMs further increase the complexity and functionality of the proteome[13,14]. As PTMs are reversible they are ideal for fast and transient signal transductions for example when the cell has to rapidly adapt to changing environments. PTMs can be divided into two major classes. The first class of PTMs comprises the covalent attachment of small chemical groups to a substrate protein. The best studied and most abundant chemical PTM is phosphorylation, by which a small phosphoryl group is added to a serine, threonine or tyrosine residue within the substrate by enzymes called kinases[15,16]. Protein phosphatases are responsible for the reversibility of this PTM as they can remove the phosphoryl group from their substrates[17]. Thus far it is estimated that 230,000 phosphorylation sites exist on around 8-10,000 substrate proteins within the human proteome, including substrate proteins involved in most of the cellular signalling pathways[18]. Other abundant chemical modifications include acetylation, methylation and glycosylation, which are all conjugated and deconjugated via specific sets of enzymes[19-21].

The second class of PTMs consist of the covalent attachment of small proteins to a substrate protein[22]. Here, the best studied and most abundant modification is the attachment of ubiquitin to lysine residues, a process referred to as ubiquitination[23,24]. After the discovery of ubiquitin, a whole class of structurally related small proteins were identified, which share not only the structural component of a globular β- grasp fold, called the ubiquitin fold, but are also used for covalent attachment to lysine residues in substrate proteins utilizing their C-terminal Glycine-Glycine motif (di-Gly). This class of ubiquitin-like proteins (UbLs) include the Small Ubiquitin-like Modifiers (SUMOs), Nedd8, Isg15, Fat10, Fub1, Ufm1, Urm1, Atg12 and Atg8[25-35]. Like other PTMs the addition of ubiquitin and UbLs to target proteins is mediated by specialized enzymes. Three distinct groups of enzymes act sequentially to form the enzymatic cascade resulting in the conjugation of ubiquitin and UbLs to a substrate protein. The E1 activating enzymes, the E2 conjugating enzymes and the E3 ligases[36-39]. Modification specific proteases are used to remove the small protein entities from the substrate once the aim of the modification is fulfilled. Despite the structural similarity to each other and the resemblance of the mechanisms used to attach and remove them from substrates, ubiquitin and UbLs all have different primary sequences, different surface charge distributions, different sets of E1, E2 and E3 enzymes and diverse biological significances and consequences[40].

SUMOYLATION

SUMO paralogues

The small ubiquitin-like modifiers, SUMOs, structurally differ from other Ubls due to their flexible N-terminus, which also contains the major site for SUMO chain formation. All eukaryotes express at least one SUMO paralogue, like yeast, C. elegans and Drosophila. Mammals express four SUMO paralogues, SUMO1-4. SUMO2 and SUMO3 share 97% of sequence identity and are thus far not distinguishable by antibodies and often referred to as SUMO2/3. SUMO1 has 47% sequence identity with SUMO2. It is still questionable if the SUMO4 precursor can be processed to expose the C-terminal diGly motif needed for the conjugation to substrate proteins, as none of the known SUMO proteases seem to be able to do so[41]. The mature version of SUMO4 was however identified in lysates of serum starved cells, suggesting possible unknown SUMO proteases that are specifically expressed under these circumstances[42].

All SUMO paralogues are structurally very similar, but differ in expression levels. SUMO2 is the most abundant SUMO family member and knockout of SUMO2 is embryonic lethal in mice, while SUMO1 and SUMO3 knockout mice show only mild phenotypes, possibly due to the ability of SUMO2 to compensate for the loss of either SUMO1 or SUMO3[43,44]. Also, the different family members have different susceptibilities to SUMO specific isopeptidases for both, processing of the precursors and deconjugation[45]. Another difference between SUMO1 and SUMO2/3 is their ability to form SUMO chains. SUMO2 and SUMO3 harbour a lysine residue at position 11 (K11) within their flexible N-terminus that is located in a sequence motif that is preferentially targeted for SUMOylation and referred to as SUMO consensus motif[46]. This enables SUMO2/3 to polymerize[47]. Although the main site of SUMO chain formation seems to be K11, site-specific mass spectrometry approaches have identified several other SUMO acceptor lysines within SUMO2, SUMO3 and even SUMO1[48]. SUMO1 possesses an N-terminal lysine at position 7 that is located in an inverted SUMO consensus motif and low efficiency SUMO1 chain formation was demonstrated in vitro and by site-specific mass spectrometry in vivo, although the biological relevance and stoichiometry of SUMO1 chains remain to be established [48-51]. Rather than forming a chain itself, SUMO1 was suggested to function as a capping factor, terminating SUMO2/3 chain formation[47,52,53].

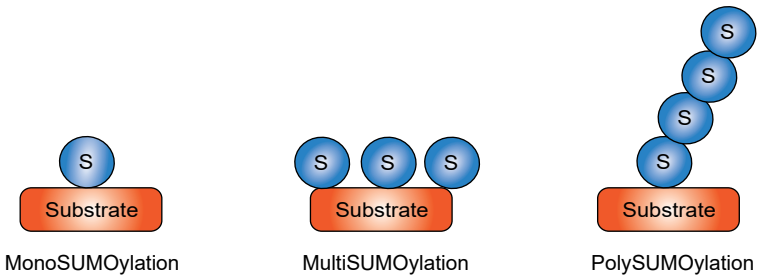

Figure 1. Possibilities of SUMO modification. SUMO substrates can be modified by a single SUMO moiety on a single lysine (mono-SUMOylation). Or single SUMO moieties can be conjugated to multiple lysines within the same substrate (multi-SUMOylation). A substrate can also be modified by a SUMO chain (poly-SUMOylation).

The SUMO conjugation cycle

SUMO can be conjugated to a single lysine within a substrate protein as single moiety (mono-SUMOylation), target multiple lysines within the substrate (multi-SUMOylation) or form a SUMO chain on a lysine residue (poly-SUMOylation) (Figure 1). The SUMO paralogues are translated into premature proteins (pre-SUMO), which have to be matured by the activity of a group of SUMO specific isoeptidases (SENPs). The SENPs remove a couple of C-terminal amino acids to expose the C-terminal di-Gly motif, which is needed for the conjugation to target lysine residues of the substrate. Conjugation and deconjugation of SUMO to a substrate is achieved via the activity of an enzymatic cascade involving SUMO specific E1, E2 and E3 enzymes and isopeptidases (Figure 2). The heterodimeric SUMO E1 enzyme consists of the SUMO-activating enzyme subunit 1 (SAE1) and subunit 2 (SAE2, also called UbA2). In an ATP-dependent two-step process, the mature C-terminus of SUMO is first adenylated, followed by the formation of a SAE2~SUMO thioester bond[36,54]. The single known SUMO E2 conjugating enzyme, Ubc9 renamed as UbE2I, is able to bind the SAE2s ubiquitin fold and this interaction stimulates the transfer of SUMO to Ubc9[55]. Unlike E2 enzymes involved in ubiquitin conjugation, Ubc9 has the unique ability to directly contribute to SUMO substrate selection[56]. Ubc9 has a low affinity to a so-called SUMO consensus motif, which is present in many substrate proteins and defined by a hydrophobic residues (ψ) upstream of the target lysine followed by any amino acid and an acidic residue downstream of the modification site (ψKx(D/E))[57,58]. In vitro a high concentration of Ubc9 together with the E1 enzyme is often sufficient to SUMOylate a substrate, given the presence of a SUMO consensus motif within the target protein[49,57,59]. In vivo, however, most substrates seem to require the presence of a SUMO E3 ligase for efficient SUMOylation.

Bona fide SUMO E3 ligases facilitate the transfer of SUMO to its target lysine by enhancing the interaction between the SUMO-charged Ubc9 and the substrate and by positioning the SUMO-Ubc9 in an orientation ideal for the nucleophilic attack by the target lysine[60]. Compared to the ubiquitin system in which hundreds of E3 ligases have been identified, only a few SUMO E3 ligases have been discovered thus far. The majority of SUMO E3 ligases belong to a family of proteins that carry a SP-RING (Siz/Pias -Really Interesting New Gene) domain that binds to Ubc9[61]. In humans this group consists of six members, the protein inhibitor of activated STAT (PIAS) 1, PIASxα, PIASxβ, PIAS3, PIAS4 (also known as PIASγ) and Nse2[55,62]. In contrast to the ubiquitin E3 ligases, which confer substrate specificity to the ubiquination system, the SUMO E3 ligases seem to exhibit a significant redundancy exemplified by knockout studies in mice[63,64]. The nuclear pore complex (NPC) component RanBP2, another unrelated, well studied SUMO E3 ligase, does not harbour a SP-RING domain but instead carries different motifs that bind SUMO and Ubc9. RanBP2 is involved in nuclear export/import as well as having a critical role during mitosis[49,65]. Additional to the SP-RING family and RanBP2, a third group of E3 ligases was recently discovered. The vertebrate- and SUMO2/3 paralogue- specific ZNF451 family comprised of ZNF451-1 and ZNF451-2, which are very similar, the more distinct isoform ZNF451-3 and the primate-specific KIAA1586, which shares a nearly identical N-terminus, including catalytic tandem-SUMO interaction motifs (SIMs), with the other family members[66,67]. Thus far the ZNF451 family has been implicated in SUMO chain formation and could be of particular importance during stress-induced SUMO conjugation after proteasome inhibition or DNA damage[66].

Additional proteins have been suggested to be E3 ligases as they are able to enhance SUMOylation of one or more substrates. These include the human polycomb protein Pc2/CBX4[68], the topoisomerase I-binding RING finger protein Topors[69], the transcription factor

Krox20[70], the tumour suppressor p14/Arf[71], the histone deacetylase HDAC4[72] and the Ras homologue enriched in striatum (Rhes)[73]. Whether they are bona fide SUMO E3 ligases must further be evaluated.

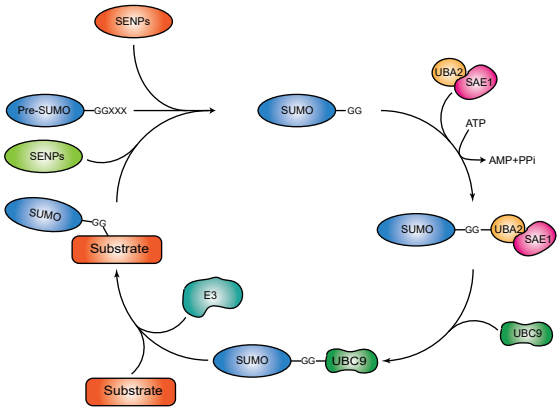

Figure 2. The SUMO conjugation cycle. SUMO is attached to lysine residues within a substrate by an enzymatic cascade comprised of the heterodimeric SUMO E1 activating enzyme, SAE1 and UbA2, the single SUMO E2 conjugation enzyme, Ubc9, and a handful of SUMO E3 ligases. SUMO specific proteases, SENPs, are responsible for the maturation of pre-SUMO and the deconjugation of SUMO from the substrate. SUMO and substrate can re-enter the cycle.

SUMO chains

The discovery of the SUMO chain-stimulating E3 ligase ZNF451 supports the notion of a specific physiological role of poly-SUMOylation, although knowledge about SUMO chain signalling remains limited compared to ubiquitin chain signalling. Ubiquitin has seven internal lysine residues that can be used for ubiquitin conjugation, resulting in different chain linkages with distinct biological consequences[74].

It is considered that the predominant SUMO chain linkage in vivo is via the lysine at position[11], although site specific mass spectrometry has identified multiple additional SUMO acceptor lysines, which could result in different linkages or branched SUMO chains[48]. However, so far there has been no indication that different SUMO chain linkages fulfil distinct roles within the cell. The main consequence of SUMO conjugation is the alteration of binding surfaces of the substrate, which can either hinder or promote intra- or intermolecular interaction. The same holds true for poly-SUMOylation. SUMO is able to promote molecular interaction due to its affinity to SUMO-interacting motifs, which are short peptide sequences mostly located in unstructured regions of the modified protein itself or interacting proteins. The sequences of SIMs consist of a stretch of conserved large hydrophobic amino acids that are either preceded or followed by an acidic patch or phosphorylation sites[75]. Proteins possessing multiple adjacent SIMs can bind to poly-SUMOylated proteins with enhanced affinity.

For example, the mammalian kinetochore protein CENP-E harbours multiple SIMs and its critical localization at the kinetochore during mitosis depends on the SUMOylation of unknown substrates at the kinetochore. SENP2 overexpression, as well as Ubc9 depletion resulted in incorrect CENP-E localization and consequently cell cycle arrest. Although CENP-E preferably binds SUMO2/3 chains in vitro, the necessity for poly-SUMOylation rather than multi-SUMOylation in vivo has not been confirmed with certainty[76]. A more direct need for poly-SUMOylation has however been demonstrated for the accurate localization of the yeast protein Zip1. Zip1 is a subunit of the synaptonemal complex (SC), which bridges homologues chromosomes during meiosis. Depleting the ability of the yeast SUMO paralogue Smt3 to form SUMO chains resulted in a lower sporulation efficiency, which was due to the mislocalization of Zip1 and deformation of the SC. Interestingly, as in the case of CENP-E,

the actual responsible poly-SUMOylated protein remains unidentified[77]. Other proteins that bind SUMO chains are SUMO-targeted ubiquitin ligases and they will be discussed in more detail below. Interestingly, cellular stressors such as DNA damage or heat shock induce global SUMO2/3 modification and most likely the formation of SUMO chains[78-80], hinting towards a global function of poly-SUMOylation during the response to such stressors[81-84].

SUMO-targeted ubiquitin ligases

SUMO chains on target substrates can be a signal for the recruitment of SUMO-targeted ubiquitin ligases (STUbLs). The STUbLs thus far identified all contain a RING domain and SIMS, which are responsible for the interaction with ubiquitin E2 conjugating enzymes and promote the preference of STUbLs for SUMOylated proteins, respectively. Due to the presence of multiple adjacent SIMs, STUbls prefer poly-SUMOylated substrates[85,86]. The most extensively studied human STUbL is the RING-finger protein 4 (RNF4). RNF4 possesses at least three closely spaced SIMs and shows a clear preference for substrates that are modified by a SUMO chain of at least three SUMO moieties[86]. RNF4 thus binds poly-SUMOylated substrates and mediates their ubiquitination by K48- or K63-linked ubiquitin chains, which signal for proteasomal degradation or recruitment of ubiquitin-binding motif-containing proteins, respectively. This mechanism has thus far been implicated in a variety of cellular processes including promyelocytic leukemia (PML) nuclear body (NB) integrity, mitosis and the DNA damage response[86-89]. The second known human STUbL RNF111 (also called Arkadia) was discovered by a bioinformatics approach, screening ubiquitin ligases for potential SIMs[90]. Its ubiquitin ligase activity was previously described in the context of TGF-β signalling but whether RNF111 functions as a STUbL in this signalling pathway is not certain. Like RNF4, RNF111 is preferably recruited to poly-SUMOylated proteins and also targets the nuclear body component PML, suggesting an overlapping pool of SUMO substrates targeted by RNF4 and RNF111[90,91]. How only some poly-SUMOylated proteins are targeted by STUbLs, whether RNF4 and RNF111 have target preferences and if more as yet unidentified STUbLs exist are most certainly interesting topics for future studies.

SUMO Group modification

A major conundrum in the SUMOylation field is the observation that hundreds of proteins have been identified to be SUMOylated but only a relatively small number of SUMO pathway enzymes have been identified. This led to the question of how specificity is achieved within the SUMO system compared to the ubiquitin system, which contains hundreds of different E3 ligases and deubiquitinating enzymes that confer specificity to the system. Another observation is that SUMOylation-deficiency of a single substrate often lacks an observable phenotype, although SUMOylation in general is essential for cell survival[92,93]. The concept of SUMO group modification might explain both of the above mentioned observations[94-96]. It is the idea that multiple proteins within a protein complex or functionally connected pathway are modified by SUMOylation and other proteins carrying SIMs interact with enhanced affinity to the SUMOylated proteins. Since this would result in multiple SUMO-SIM interactions, disrupting one single SUMO-SIM interaction can be easily compensated for by others. Also, the SUMO modification would not need to be extremely specific and therefore locally targeting one of the few SUMO ligases or SUMO proteases to the physically or functionally associated protein group would be sufficient, explaining the limited number of these enzymes. The concept was first proposed by Psakhye and Jentsch in 2012 who showed that in yeast DNA double-strand break (DSB) induction leads to the SUMOylation

of multiple proteins involved in homologues recombination (HR) by the E3 ligase Siz2. Site-directed mutation of single SUMOylation sites did not result in any observable phenotype. Only the mutation of several SUMO acceptor lysines in multiple physically connected HR proteins resulted in reduced repair efficiency[96].
Despite the novelty of the idea that SUMO targeting protein groups is a general mechanism of SUMOylation, observations of multiple SUMO substrates within a protein complex were observed earlier. For example, in yeast three of the five septins, components of a ring structured protein complex located at the bud neck of mitotic yeast cells, are SUMOylated during mitosis by the E3 ligase Siz1, which localizes to the septin ring prior to mitosis[95,97,98]. Additional examples promoting the SUMO group-modification hypothesis can be found in this thesis as in chapter 5 UV irradiation stimulates SUMOylation of multiple subunits of the transcription initiation TFIID complex or in chapter 4, where we demonstrate that SENP6 targets multiple members of the constitutive centromere-associated network (CCAN) for deSUMOylation.

SUMO proteases
SUMOylation of target substrates can be reversed by the activity of SUMO specific proteases. But their role is not limited to deconjugation. Certain SUMO proteases have a dual function within the SUMO cycle and are additionally responsible for the maturation of the SUMO precursor. All up to now identified SUMO proteases are cysteine isopeptidases but they all differ in their preference for SUMO paralogues and for maturation or deconjugation (Figure 3). The known mammalian SUMO proteases can be divided into three main groups. The first group and the largest group is the family of Sentrin (SUMO)-specific proteases (SENPs). The second group that comprises only one member is the recently discovered Ubiquitin-specific protease-like 1 (USPL1). And the third group contains the two related deSUMOylation isopeptidases (DeSIs).

The Ulp/SENP family
The first identified SUMO protease was the yeast protein UbL-specific protease 1 (Ulp1). Based on sequence similarities to Ulp1 the second yeast SUMO protease Ulp2 and the six mammalian SUMO proteases SENP1, 2, 3, 5, 6 and 7 were identified. The Ulp/SENP family shares a conserved catalytic domain located at the C-terminus of the proteins, with their active-site cysteine embedded in the catalytic triad His- Asp- Cys. The mammalian SENP1, 2, 3 and 5 are closer related to yeast Ulp1, whereas SENP6 and SENP7 more closely resemble Ulp2. Furthermore, SENP1 is closest related to SENP2, SENP3 is closest related to SENP5 and SENP6 and SENP7 share the highest sequence identity with each other and their catalytic domains diverge from the remaining SENP family members. The catalytic domains of SENP6 and SENP7 are interrupted by four conserved loop insertions, of which Loop1 protrudes out to make contact with residues in SUMO2 and SUMO3 that are absent in SUMO1, explaining the enzymes SUMO paralogue specificity.
Target specificity of the Ulp/SENP family is thought to be mainly determined by cellular localization that is mediated by their N-termini. Although a small fraction of some SENPs can be found in the cytoplasm, the majority of the enzymes are located within the nucleus, which is also the main site of SUMO conjugation and deconjungation. The yeast SUMO protease Ulp1 and the mammalian SUMO proteases SENP1 and SENP2 are concentrated at the nuclear pore during interphase, while they relocate to the kinetochore during mitosis[99-101]. The major localization site of SENP3 and SENP5 is the nucleolus[102,103]. SENP6

and SENP7 are diffusely localized throughout the nucleoplasm[104,105]. SENP1, SENP2 and catalytically inactive SENP6 are also reported to accumulate in nuclear foci, partly but not exclusively co-occurring with PML bodies[99,106].
SENP1, 2 and 5 have demonstrated SUMO precursor processing activity and show a considerable paralogue specificity based on how well the amino acids C-terminal of the di-Gly motif of the premature SUMO can be placed into the binding pockets of the SUMO proteases. SENP1 prefers SUMO1 for precursor processing as well as for deconjugation in vivo, while in vitro SENP1 deconjugates SUMO1,2 and 3 with equal efficiency[107-110]. SENP2 deconjugates and processes SUMO2 more efficiently than SUMO1 or SUMO3[111-113]. The precursor activity of SENP3 remains to be evaluated. The precursor processing activity of SENP5 is higher for SUMO3 than for the other paralogues and SENP6 and SENP7 do not possess the ability to process precursor SUMOs[114,115]. SENP6 and SENP7 also show a very limited activity towards deconjugating a single SUMO moiety from a target protein, whereas they are very efficient in disassembling SUMO chains[115-117] (Figure 3).

Biological functions of SENP1, SENP2, SENP3 and SENP5
SENP1 has been demonstrated to regulate multiple important transcription factors including ELK1, STAT5, IRF8, Bcl11b and Hif1α[118-122]. For example, the deSUMOylation of Hif1α by SENP1 is crucial for erythropoiesis in early murine embryonic development as it stabilizes the transcription factor and therefore promotes the expression of Hif1α target genes during hypoxic conditions[122]. Interestingly, SENP1 seems to be regulated by a positive feedback loop as its transcription increases upon hypoxia depended on Hif1α activity[123]. Transcription factors are however not the only reported targets of SENP1, as for example it was demonstrated that SENP1 activity at the kinetochore during mitosis is important for faithful sister chromatid separation[99].
SENP2 plays a role during early cardiac development in mice. Here SENP2 deSUMOylates the polycomb transcriptional repression complex subunit PRC1, which reverses the repressive effect of PRC1 on GATA4 and GATA6 expression, important genes for cardiac development[124]. SENP2 activity has also been implicated in the response to DNA damage upon which SENP2 deSUMOylates the Nuclear Factor κ B (NFκB) essential modulator NEMO, which leads to the restriction of NFκB regulated pro-survival genes transcription[125].
SENP3 and SENP5 have been implicated in the regulation if ribosome biogenesis by multiple studies, which fits with their nucleolar localization[102,126,127]. Additionally, SENP3 was shown to colocalize with and deSUMOylate Borealin, a component of the chromosomal passenger complex, in the nucleolus during interphase. SUMOylated Borealin is found at the centromeres during mitosis indicating a role for SENP3 in cell cycle regulation[128]. SENP5 seems to additionally be involved in the regulation of mitochondrial fission and fusion during mitosis[129,130].

SUMO chain regulation by SENP6
As SUMO can modify itself, a multitude of SUMO substrates are targets for poly-SUMOylation. The SUMO specific proteases SENP6 and SENP7 have a preference for deconjugating SUMO chains and are therefore tightly connected to pathways that are regulated by poly-SUMOylation. Thus far poly-SUMOylation is mainly reported to indirectly signal for protein degradation due to the recruitment of STUbLs.
SUMO chain deconjugation by SENP6 to prevent the recruitment of the mammalian STUbL RNF4 has been reported in multiple studies (Figure 4A). For example, the kinetochore

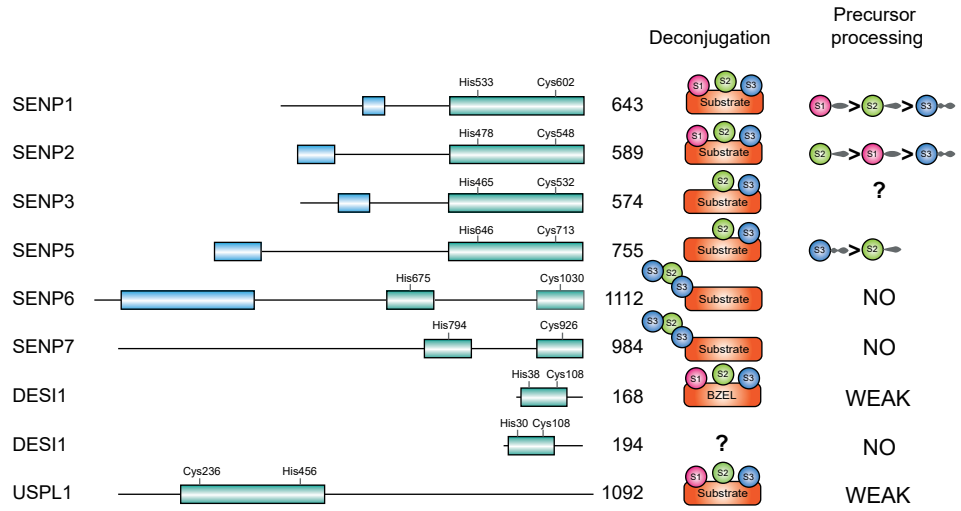

Figure 3. Mammalian SUMO specific proteases. Mammals express six SUMO proteases that belong to the family of Sentrin-specific proteases (SENPs). SENP1, 2 and SENP5 have the additional ability to process pre-SUMO. SENP6 and SENP7 are unique as their catalytic domain has conserved insertions and they have a preference for the deconjugation of SUMO chains. Recently discovered DeSI proteases and USPL1 show no pre-SUMO processing activity but have no SUMO paralogue preference for deconjugation. Blue rectangles indicate position of regulatory N-terminal domains. Green rectangles represent catalytic domains containing indicated catalytic residues. BZEL (ZBTB46) is the only identified target of DeSI1.

proteins CENP-I and Mis18BP1 are deSUMOylated by SENP6, and it has been suggested that accumulation of SUMO chains due to SENP6 depletion stimulates RNF4-mediated ubiquitination and degradation of CENP-I and Mis18BP1 with the consequence of incoherent chromosome congression during metaphase and the delocalization of the epigenetic centromere marker and histone variant CENP-A, respectively[88,131]. Another example of the counterbalancing relationship between SENP6 and RNF4 is their regulation of the Fanconi anemia ID complex (FANCI and FANCD2). Here, SENP6 and RNF4 seem to balance the level of DNA loaded ID complex and the subsequent nuclease activity upon the induction of replication fork-stalling lesions[132]. And finally, it was shown that SENP6 depletion in osteochondroprogenitor cells (OCPs) of mice, led to accumulation of SUMOylated TRIM28 and reduction of TRIM28 total levels, which together with an earlier report that identified TRIM28 as a RNF4 target, suggest SUMO chain induced- and RNF4-mediated degradation of TRIM28[133,134]. As co-repressor TRIM28 negatively controls P53 signalling and SENP6 depletion consequently leads to hyperactive P53 signalling, enhanced senescence and apoptosis of OCPs, which causes the observed premature aging phenotype in adult mice upon SENP6 depletion[134]. Interestingly, SENP6 also deSUMOylates proteins that are known RNF4 targets but SENP6 deficiency does not appear to induce RNF4- dependent degradation, like in the case of the promyolocytic leukemia protein (PML)[86,87,106]. This observation might suggest that not all SUMO chains that are targeted by SENP6 also recruit RNF4 and that chains with different properties might be present on the same protein. Whether these different properties are encoded by different architecture of SUMO chains remains to be investigated. However, STUbLs are not the only effector proteins that can be recruited to SENP6-regulated SUMO chains. For example, the replication protein RPA70 is usually deSUMOylated by

SENP6 during S-phase but upon replication stress-induced DNA damage, the interaction of SENP6 with RPA70 is disrupted. As a consequence, SUMO chains accumulate on RPA70 that subsequently recruit factors that are necessary for DNA damage repair[135] (Figure 4B).
Yet another mechanism of SENP6 regulation is exemplified by the role of SENP6 during Toll-like receptor (TLR) signalling. Here, the SUMOylation of NEMO, a negative regulator of NFκB, interferes with the binding and activity of the deUbiquitinase CYLD, which is needed to activate NEMO and inhibit NFκB signalling. By deSUMOylating NEMO, SENP6 allows CYLD binding and subsequent NEMO activation and attenuation of NFκB signalling[136] (Figure 4C).

The biological functions of SENP7
The closest relative to SENP6 is SENP7 and they share the preference for deconjugating poly-SUMOylated substrates. SENP7 plays an important role in the regulation of chromatin. For example, SENP7 stimulates homologous recombination after DNA damage by deSUMOylating the chromatin associated protein KAP1. SUMOylated KAP1 is able to recruit proteins that induce chromatin condensation. In contrast, deSUMOylation of KAP1 leads to relaxation of chromatin and improved accessibility for DNA damage repair factors[137]. Furthermore, SENP7 is involved in regulating the integrity of pericentric DNA regions, although this might be independent of its protease activity[138,139]. SENP7 was also recently implicated in innate immunity due to SENP7s regulation of the cytoplasmic DNA-sensor cGAS. Here, SUMOylation of cGAS inhibited its activation by DNA located in the cytoplasm. SENP7 reverses this inhibition and therefore stimulates cGAS activity, which ultimately leads to the transcriptional activation of interferon genes[140].

USPL1
USPL1 distantly resembles deubiquitylating enzymes (DUbs) but has SUMO-specific deconjugation activity[141]. USPL1 is the only SUMO protease that localizes to Cajal bodies within the nucleus. Its broad deSUMOylation activity in-vitro suggests that its specificity is conferred by its localization as its prime in-vivo function seems to be in Cajal body integrity[142]. Interestingly, knockdown of USPL1 results in impaired cell growth but as this phenotype can be rescued by exogenous USPL1 without protease activity it is likely that USPL1 has SUMO protease-independent functions as well[141].

DeSI
The SUMO proteases DeSI1 and DeSI2 were discovered in 2012. They belong to the permuted papain fold peptidases of double-stranded RNA viruses and eukaryotes (PPPDE) class of proteases[143]. The DeSI proteases seem to function as homodimers and their active site is formed by the catalytic dyad of Cys-His[144]. DeSI1 and DeSI2 are diffusely located throughout the cytoplasm and the nucleus and specifically deSUMOylate the transcriptional repressor BZEL (BTB-ZF protein expressed in lymphocytes)[143,144]. No other targets have been identified thus far and DeSIs therefore are the only highly specific SUMO proteases.

SUMO and DNA damage
SUMOylation is involved in most nuclear processes. It plays an important role in the regulation of transcription, mitosis, RNA biogenesis and also the cellular response to DNA damage[145-149]. The integrity of our DNA is constantly challenged by endogenous and exogenous DNA insults. Cells are equipped with complex machineries to repair damaged DNA and thereby prevent either cell death or DNA mutations that could lead to diseases

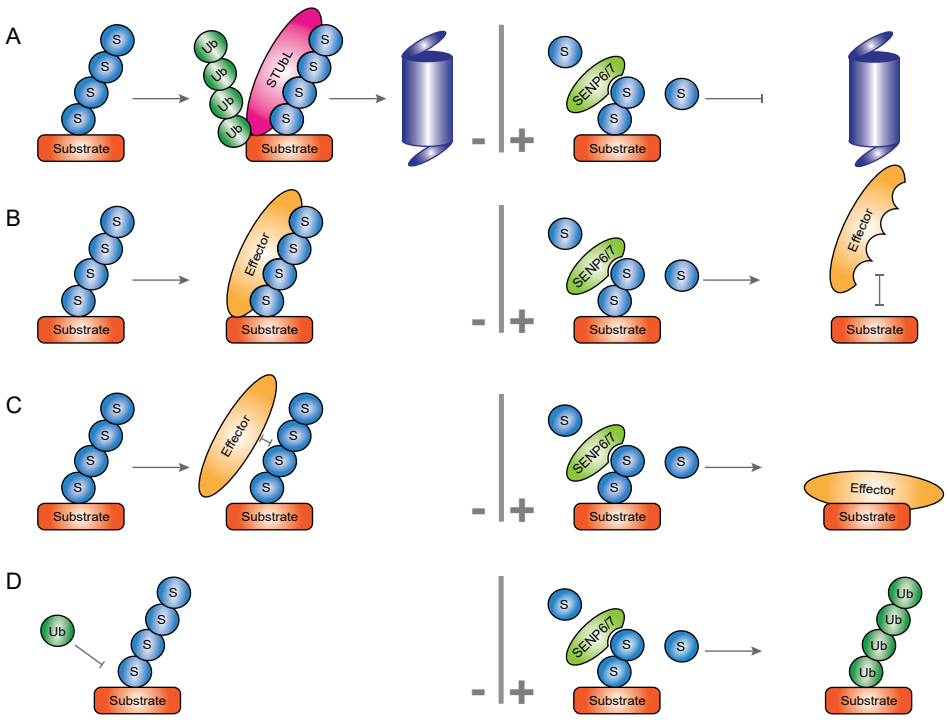

Figure 4. Mechanisms of SUMO chain regulation without (-) and with (+) SENP6. (**A**) SUMO chains can recruit STUbLs, which ubiquitinate and target the substrate to the 26S proteasome for degradation. SENP6 can stabilize the substrate by deconjugation. (**B**) SUMO chains can recruit other effector proteins. SENP6 can inhibit effector binding to the substrate by deconjugation. (**C**) SUMO chains can prevent binding of effector proteins. SENP6 can facilitate binding of the effector protein to the substrate by deconjugation. (**D**) SUMO chains can compete with ubiquitin for the same target lysine, thereby preventing ubiquitination of the substrate. SENP6 can stimulate substrate ubiquitination or any other lysine-targeting PTM by deconjugation.

such as cancer[150]. Different DNA lesions activate different DNA repair pathways. Oxidized, alkylated or deaminated bases are removed by Base-excision repair (BER). Highly cytotoxic double-strand breaks (DSB) can be repaired via several mechanism including homologous recombination (HR), non-homologous end-joining (NHEJ) or alternative NHEJ pathways. UV irradiation-induced cyclobutane pyrimidine dimers (CPDs) or 6-4 photoproducts (6-4 PPs) and other bulky helix-distorting DNA lesions are repaired by nucleotide excision repair (NER)[151]. PTMs, including SUMOylation, play an important role in regulating these pathways.

Base Excision Repair

The first link between SUMO and DNA damage repair was the discovery that the Base-excision repair (BER) protein Thymine-DNA glycosylase (TDG) is SUMOylated. TDG recognises G:T and G:U mismatches and subsequently removes the incorrect base. The resulting abasic site is then further processed by AP endonucleases, DNA polymerases and ligases. The SUMOylation of TDG stimulates an intramolecular interaction between the covalently attached SUMO1 and a SIM. This interaction leads to a conformational change that results in the dissociation of TDG from the established abasic site. This dissociation is needed in order to allow the endonuclease activity of APE1, which is the subsequent step of BER[152,153].

Double-Strand Break break repair

Further, SUMOylation has been implicated to play an important role during the repair of DNA double-strand breaks (DSB). DSBs are the most toxic DNA lesion and functional repair mechanisms are essential to ensure survival of cells. In mammalian cells, several components of the SUMO machinery are recruited to DSB sites, including SUMO1, SUMO2/3, Ubc9, PIAS1 and PIAS4. The SUMO E3 ligases PIAS1 and PIAS4 were shown to be important for the accumulation of crucial DNA repair factors at the DNA damage site, including the pivotal ubiquitin E3 ligase RNF168[154,155]. Upon DNA damage, RNF168 is recruited by ubiquitination events that are mediated by RNF8, another ubiquitin E3 ligase[156]. Together RNF8 and RNF168 create a ubiquitination wave along the sites of DSBs, which is necessary for the accumulation of multiple highly important repair proteins, such as 53BP1 and BRCA1[157]. Consequently, knockdown of PIAS1 and PIAS4 led to a reduced DSB repair efficiency and sensitized cells to various DSB-inducing agents[154]. The STUbL RNF4 is also recruited to DSBs, most likely by the SUMOylation of substrates by PIAS1 and PIAS4. RNF4 recruitment to DSBs results in the K48-linked ubiquitination of SUMOylated proteins and their subsequent proteasomal degradation[158]. In conclusion, SUMOylation regulates both recruitment and degradation of important DNA damage response factors during the repair of DSBs.

Nucleotide Excision Repair

UV-induced DNA lesions and other bulky DNA adducts are repaired by NER. The NER pathway can be divided into two sub-pathways. Global genome NER (GG-NER) removes UV-induced lesions throughout the entire genome while transcription-coupled NER (TC-NER) selectively repairs lesions located within actively transcribed genes. SUMOylation as well as ubiquitination modify and regulate important proteins of both sub-pathways[151,159,160].

GG-NER is activated by the binding of the protein Xeroderma pigmentosum group C (XPC) to the bulky DNA lesion 161. In contrast to 6-4PPs, CPDs, which are the most common UV-induced photolesions, only mildly disturb the DNA helix and are therefore not efficiently recognized by XPC alone[162-166]. UV-radiation DNA damage-binding protein 2 (DDB2) supports XPC in the recognition of CPDs and 6-4PPs[167,168]. DDB2 also forms the substrate recognition subunit of a ubiquitin E3 ligase complex, together with the adaptor DDB1, the scaffold Cullin4A and the RING protein Roc1 (CRL4 complex)[169]. This E3 ligase complex is also called the UV-DDB complex. After lesion recognition by DDB2, UV-DDB targets XPC, the core histones H2A, H3 and H4 as well as DDB2 itself for ubiquitination[170-175]. While ubiquitinated DDB2 is targeted for degradation, the ubiquitination of XPC enhances its DNA binding[171,176]. XPC is highly regulated by PTMs including SUMOylation upon UV damage. This SUMOylation triggers the STUbL RNF111 to add K63-linked ubiquitin chains to XPC, which seems essential for the release of DDB2 from the lesion site and the subsequent hand-over to downstream GG-NER factors[177-180].

The binding of XPC to the lesion recruits the transcription initiation factor IIH (TFIIH) complex. TFIIH contains 10 subunits, including the ATPase XPB and the DNA helicase XPD, which separate the complementary DNA strands around the lesion to create an open repair 'bubble'[181,182]. Subsequently the repair bubble is stabilized by XPA and RPA before the short stretch of DNA containing the lesion is excised by the endonucleases XPF-ERCC1 and XPG and the gap is closed by the joined activity of DNA polymerases and DNA ligases[181,183-185] (Figure5).

TC-NER relies on a different strategy to recognize UV-induced lesion. In actively transcribed

genes, UV-induced lesions will block the elongating RNA polymerases, which is the initial trigger for the recruitment of the proteins Cockayne-Syndrome B (CSB) and CSA. Lesion-bound CSB and CSA are necessary to recruit TFIIH for subsequent repair steps, which are common to both GG-NER and TC-NER 186 (Figure 5). Interestingly, analogous to DDB2, CSA is a substrate adaptor of a ubiquitin E3 ligase, containing DDB1, Cullin4A and Roc1 (CRL)[187]. Targets of the CSA-CRL complex, however, remain scarce and debated. CSB has been suggested to be targeted for ubiquitination by CSA-CRL complex and subsequently be degraded by the proteasome[187]. Recently, a new player in TC-NER has been identified, the UV stimulated scaffold protein A (UVSSA)[188-191]. It was suggested that UVSSA is responsible for the recruitment of the deubiquitinase USP7 to the site of the UV-induced lesion. Subsequently, USP7 deubiquitinates and stabilizes CSB and therefore counteracts the proposed activity of CSA[188,190].

Interestingly, analogous to the GG-NER damage recogniser XPC, CSB has also been identified to be SUMOylated upon UV irradiation. The SUMOylation of CSB is important for TC-NER efficiency, but detailed insight into the function of this SUMOylation event is missing[159] (this thesis chapter 5).

Additional to the SUMOylation and ubiquitination of CSB, the ubiquitination of RNA polymerase II (RNAPII) plays a pivotal role during TC-NER. The lesion stalled RNAPII covers approximately 35 nucleotides and therefore blocks access of downstream repair factors to the lesion[192]. Multiple mechanisms were suggested explaining how the stalled RNAPII is regulated to allow DNA repair. These mechanisms include dissociation of RNAPII from the damaged DNA, which may or may not be followed by proteasomal degradation, or RNAPII backtracking, a known process common in transcriptional proofreading and transcriptional pausing[193,194].

Polyubiquitination and proteasomal degradation of RPB1, the major catalytic subunit of RNAPII, is considered to be a last resort only when TC-NER fails to repair the lesion[193,195]. The ubiquitination of RPB1 involves a two-step mechanism with multiple layers of regulation. Initially, the ubiquitin E3 ligase NEDD4 mediates the monoubiquitination of RNAPII[196,197]. This monoubiquitination event was suggested to subsequently facilitate the polyubiquitination and proteasomal degradation of RPB1. Multiple ubiquitin E3 ligases were proposed to be responsible for the polyubiquitination of RPB1. The Elongin A/B/C complex together with Cullin 5 (CUL5) and RING-box protein 2 (RBX2) form a ubiquitin E3 ligase that selectively modifies monoubiquitinated RPB1 with K48-linked ubiquitin chains[197]. Additionally, the pVHL-ElonginB/C-Cul2-Rbx1 ubiquitin E3 ligase was able to bind and ubiquitinate RPB1[198]. Besides the Elongin containing complexes, the DNA damage associated ubiquitin E3 ligase BRCA1, and the CSA contain E3 ligase complex were suggested to be involved in polyubiquitination of RPB1[199-202]. However, reduced ubiquitination of RPB1, specifically observed during later time points after UV irradiation, in cells depleted of CSA could alternatively be explained by a drastic decrease of transcription upon UV damage in those cells and the consequent absence of new RNAPII molecules encountering the damage lesions[196]. Although the possibility that CSA directly targets RPB1 for ubiquitination at a later time point after damage induction is not convincingly excluded and remains a subject of active discussion.

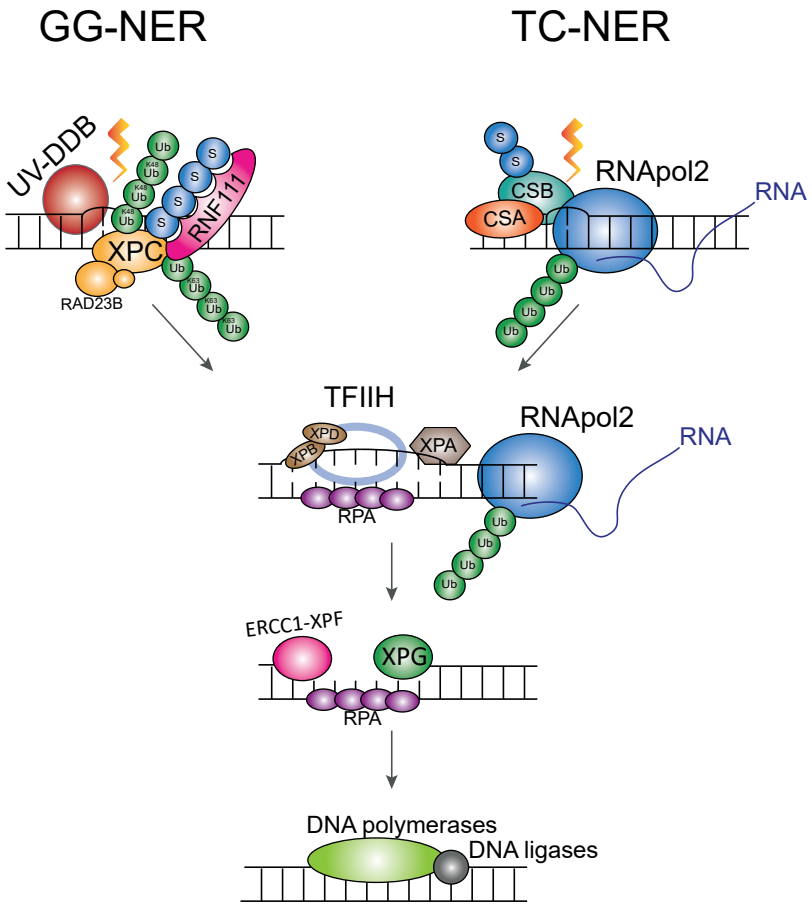

Figure 5. Nucleotide excision repair is regulated by PTMs. Nucleotide excision repair (NER) can be subdivided into two pathways. Global genome (GG) - NER relies on the activity of XPC and UV-DDB to recognise the UV-induced lesion. UV-DDB adds K48-linked ubiquitin chains to XPC. XPC s also SUMOylated, which recruits the STUbL RNF111, which adds K63-linked ubiquitin chains to XPC. In TC-NER the UV-lesion is recognised by the stalling of the processing RNA polymerase 2 (RNApol2) at the lesion. CSA and CSB are recruited to the stalled RNApol2 and are responsible to facilitate the subsequent steps of the pathway. CSB is SUMOylated upon UV damage and RNApol2 is ubiquitinated. Subsequent repair steps include the unwinding of the DNA by the TFIIH subunits XPB and XPD, the stabilization of the unwound DNA by XPA and RPA, the excision of the damaged DNA by the endonucleases ERCC1-XPF and XPG and the closing of the DNA gap by DNA polymerases and DNA ligases.

STUDYING SUMOYLATION

To study how SUMO regulates cellular processes, we first have to identify the proteins in the cell that are targeted for SUMOylation. Mass spectrometry offers an ideal tool to gain a system-wide overview of SUMOylation substrates. During the past decade huge advances in the development of SUMO proteomics were made and multiple hurdles were crossed. However, some challenges remain unsolved.

The identification of SUMO substrates in a complex protein sample is hampered by the low stoichiometry of this modification. Several strategies have been developed to enrich for SUMOylated proteins before identifying them by mass spectrometry, each with their own advantages and disadvantages. Especially the identification of endogenous SUMOylation targets continues to be challenging. Antibodies against SUMO do exist and were used to immunoprecipitate SUMOylated proteins from cell extracts, but due to the mild buffer conditions needed to ensure the integrity of the antibody, the background remains high and the yield poor[203]. In another approach researchers purified endogenous poly-SUMOylated proteins by using a RNF4 fragment including its four SIMs, which has affinity to polySUMOylated but not monoSUMOylated proteins. Here, the structural integrity of the RNF4 fragment was also of high importance for the purification and non-denaturing conditions had to be used and resulted in a co-purification of proteins merely interacting with polySUMOylated proteins that bound to the immobilized RNF4 SIM fragment[204].

Mild buffer conditions might not only lead to high background but also do allow SUMO proteases to deconjugate SUMO from its substrates during the lysis. The inclusion of cysteine protease inhibitors like N-Ethylmaleimide or Iodoacetamide inhibits SUMO proteases and helps to retain SUMOylated species. Additionally, methods have been developed that purify SUMOylated proteins under highly denaturing conditions, reducing non-specific background and maximizing yield. The drawback of these purification methods is that they are all based on the exogenous expression of an epitope tagged SUMO, which can lead to non-physiologically relevant SUMOylated proteins and restrict the identification of SUMOylated proteins to model systems that allow such exogenous protein expression. Multiple different epitope tags have been used including FLAG, TAP, Strep, His6 and His10 epitopes. These purification methods followed by mass spectrometry enabled the identification of hundreds of SUMOylated substrates.

The technique used throughout this thesis, is based on the exogenous expression of a His10-tagged SUMO2. This tagged SUMO is conjugated to substrate proteins and low expression levels of the exogenous His10-SUMO2 reduced possible overexpression artefacts. Although background binding is low, including a parental cell line not harbouring the His10-SUMO2 construct and identifying background binders is an important aspect of the identification of putative SUMO substrates, as only proteins that are highly enriched within the purified sample compared to the background binder control qualify.

Two different methods have been employed in connection to SUMO proteomics to gain quantitative information. Stable isotope labelling by amino acids in cell culture (SILAC) was used in multiple studies but allows a maximum of three experimental conditions to be compared. The recent development in robust computational label-free quantification allows an unlimited number of experimental conditions to be compared and consistent relative quantifications to be obtained[48,205].

However, direct mass spectrometry-based proof that a protein is truly SUMOylated can only be achieved by the identification of the SUMO conjugated peptides of substrate proteins. This, however, presents quite a challenge. For shot-gun proteomics, proteins are routinely digested by proteases, resulting in peptides that can be resolved and identified by mass spectrometry. The most common proteases used are lysyl endopeptidase (Lys-C), which cleaves C-terminal of lysines and trypsin, which cleaves C-terminal of arginines and lysines. If digested with trypsin, mammalian SUMO2/3 leaves behind a 32 amino acid long C-terminal peptide conjugated to the remaining peptide of the target substrate. This tryptic remnant cannot efficiently be resolved and identified by the mass spectrometer. Multiple approaches

were established to overcome this problem. Introduction of an additional cleavage site for trypsin by site-specific mutations of residues close to the C-terminus of SUMO (Q87R or T90R) shortened the tryptic remnants but, without purification of the SUMOylated peptides, resulted in the identification of only a few SUMO acceptor sites[206,207].

An increased number of SUMOylation sites were identified by expressing a His tagged SUMO mutant with all lysines mutated to arginines in combination with the Q87R mutation. This protected the His-tagged SUMO from being digested with Lys-C and enabled post-digestion purification of the SUMOylated peptides, thereby decreasing sample complexity and enhancing the identification of acceptor sites. Subsequently, multiple strategies were developed that combined the expression of SUMO mutants, highly efficient enrichment of SUMOylated proteins prior to tailored enzymatic digestion and re-purification of SUMOylated peptides, leading to the identification of thousands of SUMO acceptor lysines[48].

SUMO proteomics is now a widely used tool to study global SUMOylation events in the cell under specific circumstances and is also the starting point of each chapter within this thesis. It enabled us to have a system-wide look at dynamics and regulations and helps us to study how groups of proteins are simultaneously regulated by SUMO.

References

1. Bianconi, E., Piovesan, A., Facchin, F. et al. An estimation of the number of cells in the human body Annals of human biology 40, 463-471, (2013).
2. CRICK, F. H. On protein synthesis. Symp. Soc. Exp. Biol 12, 138-163, (1958).
3. Davidson, E. H. and Britten, R. J. Organization, transcription, and regulation in the animal genome. Q. Rev. Biol 48, 565-613, (1973).
4. Hershey, J. W. Translational control in mammalian cells. Annu. Rev. Biochem 60, 717-755, (1991).
5. Ayoubi, T. A. andVan de Ven, W. J. Regulation of gene expression by alternative promoters. FASEB J 10, 453-460, (1996).
6. Tischer, E., Mitchell, R., Hartman, T. et al. The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. J. Biol. Chem 266, 11947-11954, (1991).
7. Ota, T., Suzuki, Y., Nishikawa, T. et al. Complete sequencing and characterization of 21,243 full-length human cDNAs. Nat. Genet 36, 40-45, (2004).
8. Hiesel, R., Wissinger, B., Schuster, W. and Brennicke, A. RNA editing in plant mitochondria. Science 246, 1632-1634, (1989).
9. Pan, Q., Shai, O., Lee, L. J., Frey, B. J. and Blencowe, B. J. Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. Nat. Genet 40, 1413-1415, (2008).
10. Carninci, P., Kasukawa, T., Katayama, S. et al. The transcriptional landscape of the mammalian genome. Science 309, 1559-1563, (2005).
11. Uy, R. and Wold, F. Posttranslational covalent modification of proteins. Science 198, 890-896, (1977).
12. Wold, F. In vivo chemical modification of proteins (post-translational modification). Annu. Rev. Biochem 50, 783-814, (1981).
13. Jensen, O. N. Modification-specific proteomics: characterization of post-translational modifications by mass spectrometry. Curr. Opin. Chem. Biol 8, 33-41, (2004).
14. Walsh, C. T., Garneau-Tsodikova, S. and Gatto, G. J., Jr. Protein posttranslational modifications: the chemistry of proteome diversifications. Angew. Chem. Int. Ed Engl 44, 7342-7372, (2005).
15. Kruger, R., Kubler, D., Pallisse, R., Burkovski, A. and Lehmann, W. D. Protein and proteome phosphorylation stoichiometry analysis by element mass spectrometry. Analytical chemistry 78, 1987- 1994, (2006).
16. Ubersax, J. A. and Ferrell, J. E., Jr. Mechanisms of specificity in protein phosphorylation. Nature reviews. Molecular cell biology 8, 530-541, (2007).
17. Barford, D., Das, A. K. and Egloff, M. P. The structure and mechanism of protein phosphatases: insights into catalysis and regulation. Annual review of biophysics and biomolecular structure 27, 133-164, (1998).
18. Vlastaridis, P., Kyriakidou, P., Chaliotis, A. et al. Estimating the total number of phosphoproteins and phosphorylation sites in eukaryotic proteomes. GigaScience 6, 1-11, (2017).
19. Drazic, A., Myklebust, L. M., Ree, R. and Arnesen, T. The world of protein acetylation. Biochimica et biophysica acta 1864, 1372-1401, (2016).
20. Murn, J. and Shi, Y. The winding path of protein methylation research: milestones and new frontiers. Nature reviews. Molecular cell biology 18, 517-527, (2017).
21. Ohtsubo, K. and Marth, J. D. Glycosylation in cellular mechanisms of health and disease. Cell 126, 855-867, (2006).
22. Kerscher, O., Felberbaum, R. and Hochstrasser, M. Modification of proteins by ubiquitin and ubiquiin-like proteins. Annu. Rev. Cell Dev. Biol 22, 159-180, (2006).
23. Hochstrasser, M. Ubiquitin-dependent protein degradation. Annu. Rev. Genet 30, 405-439, (1996).
24. Pickart, C. M. Mechanisms underlying ubiquitination. Annu. Rev. Biochem 70, 503-533, (2001).
25. Mahajan, R., Delphin, C., Guan, T., Gerace, L. and Melchior, F. A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. Cell 88, 97-107, (1997).
26. Matunis, M. J., Coutavas, E. and Blobel, G. A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex. J. Cell Biol 135, 1457-1470, (1996).
27. Muller, S., Hoege, C., Pyrowolakis, G. and Jentsch, S. SUMO, ubiquitin's mysterious cousin. Nat. Rev. Mol. Cell Biol 2, 202-210, (2001).

28. Liakopoulos, D., Doenges, G., Matuschewski, K. and Jentsch, S. A novel protein modification pathway related to the ubiquitin system. EMBO J 17, 2208-2214, (1998).
29. D'Cunha, J., Jr, K. E., Haas, A. L., Truitt, R. L. and Borden, E. C. Immunoregulatory properties of ISG15, an interferon-induced cytokine. Proc. Natl. Acad. Sci. U. S. A 93, 211-215, (1996).
30. Liu, Y. C., Pan, J., Zhang, C. et al. A MHC-encoded ubiquitin-like protein (FAT10) binds noncovalently to the spindle assembly checkpoint protein MAD2. Proc. Natl. Acad. Sci. U. S. A 96, 4313- 4318, (1999).
31. Michiels, L., Van der Rauwelaert, E., Van, H. F., Kas, K. and Merregaert, J. fau cDNA encodes a ubiquitin-like-S30 fusion protein and is expressed as an antisense sequence in the Finkel-Biskis-Reilly murine sarcoma virus. Oncogene 8, 2537-2546, (1993).
32. Komatsu, M., Chiba, T., Tatsumi, K. et al. A novel protein-conjugating system for Ufm1, a ubiquitin-fold modifier. EMBO J 23, 1977-1986, (2004).
33. Van der Veen, A. G., Schorpp, K., Schlieker, C. et al. Role of the ubiquitin-like protein Urm1 as a noncanonical lysine-directed protein modifier. Proc. Natl. Acad. Sci. U. S. A 108, 1763-1770, (2011).
34. Hochstrasser, M. Biochemistry. All in the ubiquitin family. Science 289, 563-564, (2000).
35. Vierstra, R. D. The expanding universe of ubiquitin and ubiquitin-like modifiers. Plant Physiol 160, 2-14, (2012).
36. Schulman, B. A. and Harper, J. W. Ubiquitin-like protein activation by E1 enzymes: the apex for downstream signalling pathways. Nat. Rev. Mol. Cell Biol 10, 319-331, (2009).
37. Wenzel, D. M., Stoll, K. E. and Klevit, R. E. E2s: structurally economical and functionally replete. The Biochemical journal 433, 31-42, (2011).
38. van Wijk, S. J. and Timmers, H. T. The family of ubiquitin-conjugating enzymes (E2s): deciding between life and death of proteins. FASEB J 24, 981-993, (2010).
39. Nagy, V. and Dikic, I. Ubiquitin ligase complexes: from substrate selectivity to conjugational specificity. Biol. Chem 391, 163-169, (2010).
40. Van der Veen, A. G. and Ploegh, H. L. Ubiquitin-like proteins. Annu. Rev. Biochem 81, 323-357, (2012).
41. Owerbach, D., McKay, E. M., Yeh, E. T., Gabbay, K. H. and Bohren, K. M. A proline-90 residue unique to SUMO-4 prevents maturation and sumoylation. Biochem. Biophys. Res. Commun 337, 517-520, (2005).
42. Guo, D., Han, J., Adam, B. L. et al. Proteomic analysis of SUMO4 substrates in HEK293 cells under serum starvation-induced stress. Biochem. Biophys. Res. Commun 337, 1308-1318, (2005).
43. Evdokimov, E., Sharma, P., Lockett, S. J., Lualdi, M. and Kuehn, M. R. Loss of SUMO1 in mice affects RanGAP1 localization and formation of PML nuclear bodies, but is not lethal as it can be compensated by SUMO2 or SUMO3. J. Cell Sci 121, 4106-4113, (2008).
44. Wang, L., Wansleeben, C., Zhao, S. et al. SUMO2 is essential while SUMO3 is dispensable for mouse embryonic development. EMBO Rep 15, 878-885, (2014).
45. Hickey, C. M., Wilson, N. R. and Hochstrasser, M. Function and regulation of SUMO proteases. Nat. Rev. Mol. Cell Biol 13, 755-766, (2012).
46. Rodriguez, M. S., Dargemont, C. and Hay, R. T. SUMO-1 conjugation in vivo requires both a consensus modification motif and nuclear targeting. J. Biol. Chem 276, 12654-12659, (2001).
47. Tatham, M. H., Jaffray, E., Vaughan, O. A. et al. Polymeric chains of SUMO-2 and SUMO-3 are conjugated to protein substrates by SAE1/SAE2 and Ubc9. J. Biol. Chem 276, 35368-35374, (2001).
48. Hendriks, I. A. and Vertegaal, A. C. A comprehensive compilation of SUMO proteomics. Nat. Rev. Mol. Cell Biol 17, 581-595, (2016).
49. Pichler, A., Gast, A., Seeler, J. S., Dejean, A. and Melchior, F. The nucleoporin RanBP2 has SUMO1 E3 ligase activity. Cell 108, 109-120, (2002).
50. Yang, M., Hsu, C. T., Ting, C. Y., Liu, L. F. and Hwang, J. Assembly of a polymeric chain of SUMO1 on human topoisomerase I in vitro. J. Biol. Chem 281, 8264-8274, (2006).
51. Pedrioli, P. G., Raught, B., Zhang, X. D. et al. Automated identification of SUMOylation sites using mass spectrometry and SUMmOn pattern recognition software. Nat. Methods 3, 533-539, (2006).
52. Chung, T. L., Hsiao, H. H., Yeh, Y. Y. et al. In vitro modification of human centromere protein CENP-C fragments by small ubiquitin-like modifier (SUMO) protein: definitive identification of the modification sites by tandem mass spectrometry analysis of the isopeptides. J. Biol. Chem 279, 39653-39662, (2004).
53. Vertegaal, A. C., Ogg, S. C., Jaffray, E. et al. A proteomic study of SUMO-2 target proteins. J. Biol. Chem 279, 33791-33798, (2004).

54. Dye, B. T. and Schulman, B. A. Structural mechanisms underlying posttranslational modification by ubiquitin-like proteins. Annu. Rev. Biophys. Biomol. Struct 36, 131-150, (2007).

55. Flotho, A. and Melchior, F. Sumoylation: a regulatory protein modification in health and disease. Annu. Rev. Biochem 82, 357-385, (2013).

56. Pichler, A., Fatouros, C., Lee, H. and Eisenhardt, N. SUMO conjugation - a mechanistic view. Biomol. Concepts 8, 13-36, (2017).

57. Bernier-Villamor, V., Sampson, D. A., Matunis, M. J. and Lima, C. D. Structural basis for E2-mediated SUMO conjugation revealed by a complex between ubiquitin-conjugating enzyme Ubc9 and RanGAP1. Cell 108, 345-356, (2002).

58. Yunus, A. A. and Lima, C. D. Lysine activation and functional analysis of E2-mediated conjugation in the SUMO pathway. Nat. Struct. Mol. Biol 13, 491-499, (2006).

59. Okuma, T., Honda, R., Ichikawa, G., Tsumagari, N. and Yasuda, H. In vitro SUMO-1 modification requires two enzymatic steps, E1 and E2. Biochem. Biophys. Res. Commun 254, 693-698, (1999).

60. Reverter, D. and Lima, C. D. Insights into E3 ligase activity revealed by a SUMO-RanGAP1-Ubc9-Nup358 complex. Nature 435, 687-692, (2005).

61. Yunus, A. A. and Lima, C. D. Structure of the Siz/PIAS SUMO E3 ligase Siz1 and determinants required for SUMO modification of PCNA. Mol. Cell 35, 669-682, (2009).

62. Andrews, E. A., Palecek, J., Sergeant, J. et al. Nse2, a component of the Smc5-6 complex, is a SUMO ligase required for the response to DNA damage. Mol. Cell Biol 25, 185-196, (2005).

63. Roth, W., Sustmann, C., Kieslinger, M. et al. PIASy-deficient mice display modest defects in IFN and Wnt signaling. J. Immunol 173, 6189-6199, (2004).

64. Wong, K. A., Kim, R., Christofk, H. et al. Protein inhibitor of activated STAT Y (PIASy) and a splice variant lacking exon 6 enhance sumoylation but are not essential for embryogenesis and adult life. Mol. Cell Biol 24, 5577-5586, (2004).

65. Dawlaty, M. M., Malureanu, L., Jeganathan, K. B. et al. Resolution of sister centromeres requires RanBP2-mediated SUMOylation of topoisomerase IIalpha. Cell 133, 103-115, (2008).

66. Eisenhardt, N., Chaugule, V. K., Koidl, S. et al. A new vertebrate SUMO enzyme family reveals insights into SUMO-chain assembly. Nat. Struct. Mol. Biol 22, 959-967, (2015).

67. Abascal, F., Tress, M. L. and Valencia, A. Alternative splicing and co-option of transposable elements: the case of TMPO/LAP2alpha and ZNF451 in mammals. Bioinformatics 31, 2257-2261, (2015).

68. Kagey, M. H., Melhuish, T. A. and Wotton, D. The polycomb protein Pc2 is a SUMO E3. Cell 113, 127-137, (2003).

69. Weger, S., Hammer, E. and Heilbronn, R. Topors acts as a SUMO-1 E3 ligase for p53 in vitro and in vivo. FEBS Lett 579, 5007-5012, (2005).

70. Garcia-Gutierrez, P., Juarez-Vicente, F., Gallardo-Chamizo, F., Charnay, P. and Garcia-Dominguez, M. The transcription factor Krox20 is an E3 ligase that sumoylates its Nab coregulators. EMBO Rep 12, 1018-1023, (2011).

71. Tago, K., Chiocca, S. and Sherr, C. J. Sumoylation induced by the Arf tumor suppressor: a p53-independent function. Proc. Natl. Acad. Sci. U. S. A 102, 7689-7694, (2005).

72. Zhao, X., Sternsdorf, T., Bolger, T. A., Evans, R. M. and Yao, T. P. Regulation of MEF2 by histone deacetylase 4- and SIRT1 deacetylase-mediated lysine modifications. Mol. Cell Biol 25, 8456-8464, (2005).

73. Subramaniam, S., Mealer, R. G., Sixt, K. M. et al. Rhes, a physiologic regulator of sumoylation, enhances cross-sumoylation between the basic sumoylation enzymes E1 and Ubc9. J. Biol. Chem 285, 20428-20432, (2010).

74. Komander, D., Clague, M. J. and Urbe, S. Breaking the chains: structure and function of the deubiquitinases. Nat. Rev. Mol. Cell Biol 10, 550-563, (2009).

75. Vogt, B. and Hofmann, K. Bioinformatical detection of recognition factors for ubiquitin and SUMO. Methods Mol. Biol 832, 249-261, (2012).

76. Zhang, X. D., Goeres, J., Zhang, H. et al. SUMO-2/3 modification and binding regulate the association of CENP-E with kinetochores and progression through mitosis. Mol. Cell 29, 729-741, (2008).

77. Cheng, C. H., Lo, Y. H., Liang, S. S. et al. SUMO modifications control assembly of synaptonemal complex and polycomplex in meiosis of Saccharomyces cerevisiae. Genes Dev 20, 2067-2081, (2006).

78. Golebiowski, F., Matic, I., Tatham, M. H. et al. System-wide changes to SUMO modifications in response to heat shock. Sci. Signal 2, ra24, (2009).
79. Xiao, Z., Chang, J. G., Hendriks, I. A. et al. System-wide analysis of SUMOylation dynamics in response to replication stress reveals novel SUMO target proteins and acceptor lysines relevant for genome stability. Mol. Cell Proteomics 14, 1419-1434, (2015).
80. Hendriks, I. A., D'Souza, R. C., Yang, B. et al. Uncovering global SUMOylation signaling networks in a site-specific manner. Nat. Struct. Mol. Biol 21, 927-936, (2014).
81. Srikumar, T., Lewicki, M. C., Costanzo, M. et al. Global analysis of SUMO chain function reveals multiple roles in chromatin regulation. J. Cell Biol 201, 145-163, (2013).
82. Zhou, W., Ryan, J. J. and Zhou, H. Global analyses of sumoylated proteins in Saccharomyces cerevisiae. Induction of protein sumoylation by cellular stresses. J. Biol. Chem 279, 32262-32268, (2004).
83. Skilton, A., Ho, J. C., Mercer, B., Outwin, E. and Watts, F. Z. SUMO chain formation is required for response to replication arrest in S. pombe. PLoS One 4, e6750, (2009).
84. Saitoh, H. and Hinchey, J. Functional heterogeneity of small ubiquitin-related protein modifiers SUMO-1 versus SUMO-2/3. J. Biol. Chem 275, 6252-6258, (2000).
85. Uzunova, K., Gottsche, K., Miteva, M. et al. Ubiquitin-dependent proteolytic control of SUMO conjugates. J. Biol. Chem 282, 34167-34175, (2007).
86. Tatham, M. H., Geoffroy, M. C., Shen, L. et al. RNF4 is a poly-SUMO-specific E3 ubiquitin ligase required for arsenic-induced PML degradation. Nat. Cell Biol 10, 538-546, (2008).
87. Lallemand-Breitenbach, V., Jeanne, M., Benhenda, S. et al. Arsenic degrades PML or PML-RARalpha through a SUMO-triggered RNF4/ubiquitin-mediated pathway. Nat. Cell Biol 10, 547-555, (2008).
88. Mukhopadhyay, D., Arnaoutov, A. and Dasso, M. The SUMO protease SENP6 is essential for inner kinetochore assembly. J. Cell Biol 188, 681-692, (2010).
89. Yin, Y., Seifert, A., Chua, J. S. et al. SUMO-targeted ubiquitin E3 ligase RNF4 is required for the response of human cells to DNA damage. Genes Dev 26, 1196-1208, (2012).
90. Sun, H. and Hunter, T. Poly-small ubiquitin-like modifier (PolySUMO)-binding proteins identified through a string search. J. Biol. Chem 287, 42071-42083, (2012).
91. Erker, Y., Neyret-Kahn, H., Seeler, J. S. et al. Arkadia, a novel SUMO-targeted ubiquitin ligase involved in PML degradation. Mol. Cell Biol 33, 2163-2177, (2013).
92. Hayashi, T., Seki, M., Maeda, D. et al. Ubc9 is essential for viability of higher eukaryotic cells. Exp. Cell Res 280, 212-221, (2002).
93. Nacerddine, K., Lehembre, F., Bhaumik, M. et al. The SUMO pathway is essential for nuclear integrity and chromosome segregation in mice. Dev. Cell 9, 769-779, (2005).
94. Cremona, C. A., Sarangi, P., Yang, Y. et al. Extensive DNA damage-induced sumoylation contributes to replication and repair and acts in addition to the mec1 checkpoint. Mol. Cell 45, 422-432, (2012).
95. Johnson, E. S. and Blobel, G. Cell cycle-regulated attachment of the ubiquitin-related protein SUMO to the yeast septins. J. Cell Biol 147, 981-994, (1999).
96. Psakhye, I. and Jentsch, S. Protein group modification and synergy in the SUMO pathway as exemplified in DNA repair. Cell 151, 807-820, (2012).
97. Johnson, E. S. and Gupta, A. A. An E3-like factor that promotes SUMO conjugation to the yeast septins. Cell 106, 735-744, (2001).
98. Reindle, A., Belichenko, I., Bylebyl, G. R. et al. Multiple domains in Siz SUMO ligases contribute to substrate selectivity. J. Cell Sci 119, 4749-4757, (2006).
99. Cubenas-Potts, C., Goeres, J. D. and Matunis, M. J. SENP1 and SENP2 Affect Spatial and Temporal Control of Sumoylation in Mitosis. Mol. Biol. Cell 24, 3483-3495, (2013).
100. Leisner, C., Kammerer, D., Denoth, A. et al. Regulation of mitotic spindle asymmetry by SUMO and the spindle-assembly checkpoint in yeast. Curr. Biol 18, 1249-1255, (2008).
101. Takahashi, Y., Mizoi, J., Toh, E. and Kikuchi, Y. Yeast Ulp1, an Smt3-specific protease, associates with nucleoporins. J. Biochem 128, 723-725, (2000).
102. Haindl, M., Harasim, T., Eick, D. and Muller, S. The nucleolar SUMO-specific protease SENP3 reverses SUMO modification of nucleophosmin and is required for rRNA processing. EMBO Rep 9, 273-279, (2008).

103. Gong, L. and Yeh, E. T. Characterization of a family of nucleolar SUMO-specific proteases with preference for SUMO-2 or SUMO-3. J. Biol. Chem 281, 15869-15877, (2006).

104. Mukhopadhyay, D., Ayaydin, F., Kolli, N. et al. SUSP1 antagonizes formation of highly SUMO2/3-conjugated species. J. Cell Biol 174, 939-949, (2006).

105. Shen, L. N., Geoffroy, M. C., Jaffray, E. G. and Hay, R. T. Characterization of SENP7, a SUMO-2/3-specific isopeptidase. Biochem. J 421, 223-230, (2009).

106. Hattersley, N., Shen, L., Jaffray, E. G. and Hay, R. T. The SUMO protease SENP6 is a direct regulator of PML nuclear bodies. Mol. Biol. Cell 22, 78-90, (2011).

107. Reverter, D. and Lima, C. D. A basis for SUMO protease specificity provided by analysis of human Senp2 and a Senp2-SUMO complex. Structure 12, 1519-1531, (2004).

108. Shen, L. N., Dong, C., Liu, H., Naismith, J. H. and Hay, R. T. The structure of SENP1-SUMO-2 complex suggests a structural basis for discrimination between SUMO paralogues during processing. Biochem. J 397, 279-288, (2006).

109. Shen, L., Tatham, M. H., Dong, C. et al. SUMO protease SENP1 induces isomerization of the scissile peptide bond. Nat. Struct. Mol. Biol 13, 1069-1077, (2006).

110. Sharma, P., Yamada, S., Lualdi, M., Dasso, M. and Kuehn, M. R. Senp1 is essential for desumoylating Sumo1-modified proteins but dispensable for Sumo2 and Sumo3 deconjugation in the mouse embryo. Cell Rep 3, 1640-1650, (2013).

111. Reverter, D. and Lima, C. D. Structural basis for SENP2 protease interactions with SUMO precursors and conjugated substrates. Nat. Struct. Mol. Biol 13, 1060-1068, (2006).

112. Bekes, M., Prudden, J., Srikumar, T. et al. The dynamics and mechanism of SUMO chain deconjugation by SUMO-specific proteases. J. Biol. Chem 286, 10238-10247, (2011).

113. Mikolajczyk, J., Drag, M., Bekes, M. et al. Small ubiquitin-related modifier (SUMO)-specific proteases: profiling the specificities and activities of human SENPs. J. Biol. Chem 282, 26217-26224, (2007).

114. Di, B. A., Ouyang, J., Lee, H. Y. et al. The SUMO-specific protease SENP5 is required for cell division. Mol. Cell Biol 26, 4489-4498, (2006).

115. Lima, C. D. and Reverter, D. Structure of the human SENP7 catalytic domain and poly-SUMO deconjugation activities for SENP6 and SENP7. J. Biol. Chem 283, 32045-32055, (2008).

116. Kolli, N., Mikolajczyk, J., Drag, M. et al. Distribution and paralogue specificity of mammalian deSUMOylating enzymes. Biochem. J 430, 335-344, (2010).

117. Drag, M., Mikolajczyk, J., Krishnakumar, I. M., Huang, Z. and Salvesen, G. S. Activity profiling of human deSUMOylating enzymes (SENPs) with synthetic substrates suggests an unexpected specificity of two newly characterized members of the family. Biochem. J 409, 461-469, (2008).

118. Witty, J., Aguilar-Martinez, E. and Sharrocks, A. D. SENP1 participates in the dynamic regulation of Elk-1 SUMOylation. Biochem. J 428, 247-254, (2010).

119. Van, N. T., Angkasekwinai, P., Dou, H. et al. SUMO-specific protease 1 is critical for early lymphoid development through regulation of STAT5 activation. Mol. Cell 45, 210-221, (2012).

120. Chang, T. H., Xu, S., Tailor, P., Kanno, T. and Ozato, K. The small ubiquitin-like modifier-deconjugating enzyme sentrin-specific peptidase 1 switches IFN regulatory factor 8 from a repressor to an activator during macrophage activation. J. Immunol 189, 3548-3556, (2012).

121. Zhang, L. J., Vogel, W. K., Liu, X. et al. Coordinated regulation of transcription factor Bcl11b activity in thymocytes by the mitogen-activated protein kinase (MAPK) pathways and protein sumoylation. J. Biol. Chem 287, 26971-26988, (2012).

122. Cheng, J., Kang, X., Zhang, S. and Yeh, E. T. SUMO-specific protease 1 is essential for stabilization of HIF1alpha during hypoxia. Cell 131, 584-595, (2007).

123. Xu, Y., Zuo, Y., Zhang, H. et al. Induction of SENP1 in endothelial cells contributes to hypoxia-driven VEGF expression and angiogenesis. J. Biol. Chem 285, 36682-36688, (2010).

124. Kang, X., Qi, Y., Zuo, Y. et al. SUMO-specific protease 2 is essential for suppression of polycomb group protein-mediated gene silencing during embryonic development. Mol. Cell 38, 191-201, (2010).

125. Lee, M. H., Mabb, A. M., Gill, G. B., Yeh, E. T. and Miyamoto, S. NF-kappaB induction of the SUMO protease SENP2: A negative feedback loop to attenuate cell survival response to genotoxic stress. Mol. Cell 43, 180-191, (2011).

126. Yun, C., Wang, Y., Mukhopadhyay, D. et al. Nucleolar protein B23/nucleophosmin regulates the vertebrate SUMO pathway through SENP3 and SENP5 proteases. J. Cell Biol 183, 589-595, (2008).
127. Finkbeiner, E., Haindl, M. and Muller, S. The SUMO system controls nucleolar partitioning of a novel mammalian ribosome biogenesis complex. EMBO J 30, 1067-1078, (2011).
128. Klein, U. R., Haindl, M., Nigg, E. A. and Muller, S. RanBP2 and SENP3 function in a mitotic SUMO2/3 conjugation-deconjugation cycle on Borealin. Mol. Biol. Cell 20, 410-418, (2009).
129. Zunino, R., Schauss, A., Rippstein, P., Andrade-Navarro, M. and McBride, H. M. The SUMO protease SENP5 is required to maintain mitochondrial morphology and function. J. Cell Sci 120, 1178-1188, (2007).
130. Zunino, R., Braschi, E., Xu, L. and McBride, H. M. Translocation of SenP5 from the nucleoli to the mitochondria modulates DRP1-dependent fission during mitosis. J. Biol. Chem 284, 17783-17795, (2009).
131. Fu, H., Liu, N., Dong, Q. et al. SENP6-mediated M18BP1 deSUMOylation regulates CENP-A centromeric localization. Cell Res 29, 254-257, (2019).
132. Gibbs-Seymour, I., Oka, Y., Rajendra, E. et al. Ubiquitin-SUMO Circuitry Controls Activated Fanconi Anemia ID Complex Dosage in Response to DNA Damage. Mol. Cell 57, 150-164, (2015).
133. Kuo, C. Y., Li, X., Kong, X. Q. et al. An arginine-rich motif of ring finger protein 4 (RNF4) oversees the recruitment and degradation of the phosphorylated and SUMOylated Kruppel-associated box domain-associated protein 1 (KAP1)/TRIM28 protein during genotoxic stress. J. Biol. Chem 289, 20757-20772, (2014).
134. Li, J., Lu, D., Dou, H. et al. Desumoylase SENP6 maintains osteochondroprogenitor homeostasis by suppressing the p53 pathway. Nat Commun 9, 143, (2018).
135. Dou, H., Huang, C., Singh, M., Carpenter, P. B. and Yeh, E. T. Regulation of DNA repair through deSUMOylation and SUMOylation of replication protein A complex. Mol. Cell 39, 333-345, (2010).
136. Liu, X., Chen, W., Wang, Q., Li, L. and Wang, C. Negative regulation of TLR inflammatory signaling by the SUMO-deconjugating enzyme SENP6. PLoS Pathog 9, e1003480, (2013).
137. Garvin, A. J., Densham, R. M., Blair-Reid, S. A. et al. The deSUMOylase SENP7 promotes chromatin relaxation for homologous recombination DNA repair. EMBO Rep 14, 975-983, (2013).
138. Romeo, K., Louault, Y., Cantaloube, S. et al. The SENP7 SUMO-Protease Presents a Module of Two HP1 Interaction Motifs that Locks HP1 Protein at Pericentric Heterochromatin. Cell Rep 10, 771-782, (2015).
139. Maison, C., Romeo, K., Bailly, D. et al. The SUMO protease SENP7 is a critical component to ensure HP1 enrichment at pericentric heterochromatin. Nat. Struct. Mol. Biol 19, 458-460, (2012).
140. Cui, Y., Yu, H., Zheng, X. et al. SENP7 Potentiates cGAS Activation by Relieving SUMO-Mediated Inhibition of Cytosolic DNA Sensing. PLoS Pathog 13, e1006156, (2017).
141. Schulz, S., Chachami, G., Kozaczkiewicz, L. et al. Ubiquitin-specific protease-like 1 (USPL1) is a SUMO isopeptidase with essential, non-catalytic functions. EMBO Rep 13, 930-938, (2012).
142. Hutten, S., Chachami, G., Winter, U., Melchior, F. and Lamond, A. I. A role for the Cajal-body-associated SUMO isopeptidase USPL1 in snRNA transcription mediated by RNA polymerase II. J. Cell Sci 127, 1065-1078, (2014).
143. Shin, E. J., Shin, H. M., Nam, E. et al. DeSUMOylating isopeptidase: a second class of SUMO protease. EMBO Rep 13, 339-346, (2012).
144. Suh, H. Y., Kim, J. H., Woo, J. S. et al. Crystal structure of DeSI-1, a novel deSUMOylase belonging to a putative isopeptidase superfamily. Proteins 80, 2099-2104, (2012).
145. Verger, A., Perdomo, J. and Crossley, M. Modification with SUMO. A role in transcriptional regulation. EMBO Rep 4, 137-142, (2003).
146. Dasso, M. Emerging roles of the SUMO pathway in mitosis. Cell Div 3, 5, (2008).
147. Cuijpers, S. A. G. and Vertegaal, A. C. O. Guiding Mitotic Progression by Crosstalk between Post-translational Modifications. Trends in biochemical sciences 43, 251-268, (2018).
148. Finkbeiner, E., Haindl, M., Raman, N. and Muller, S. SUMO routes ribosome maturation. Nucleus 2, 527-532, (2011).
149. Jackson, S. P. and Durocher, D. Regulation of DNA damage responses by ubiquitin and SUMO. Mol. Cell 49, 795-807, (2013).
150. Jackson, S. P. and Bartek, J. The DNA-damage response in human biology and disease. Nature 461, 1071-1078, (2009).
151. Marteijn, J. A., Lans, H., Vermeulen, W. and Hoeijmakers, J. H. Understanding nucleotide excision repair and its roles in cancer and ageing. Nat Rev Mol Cell Biol 15, 465-481, (2014).

152. Baba, D., Maita, N., Jee, J. G. et al. Crystal structure of thymine DNA glycosylase conjugated to SUMO-1. Nature 435, 979-982, (2005).

153. Steinacher, R. and Schar, P. Functionality of human thymine DNA glycosylase requires SUMO-regulated changes in protein conformation. Curr. Biol 15, 616-623, (2005).

154. Galanty, Y., Belotserkovskaya, R., Coates, J. et al. Mammalian SUMO E3-ligases PIAS1 and PIAS4 promote responses to DNA double-strand breaks. Nature 462, 935-939, (2009).

155. Morris, J. R., Boutell, C., Keppler, M. et al. The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress. Nature 462, 886-890, (2009).

156. Bekker-Jensen, S. and Mailand, N. Assembly and function of DNA double-strand break repair foci in mammalian cells. DNA repair 9, 1219-1228, (2010).

157. Lukas, J., Lukas, C. and Bartek, J. More than just a focus: The chromatin response to DNA damage and its role in genome integrity maintenance. Nature cell biology 13, 1161-1169, (2011).

158. Galanty, Y., Belotserkovskaya, R., Coates, J. and Jackson, S. P. RNF4, a SUMO-targeted ubiquitin E3 ligase, promotes DNA double-strand break repair. Genes Dev 26, 1179-1195, (2012).

159. Sin, Y., Tanaka, K. and Saijo, M. The C-terminal Region and SUMOylation of Cockayne Syndrome Group B Protein Play Critical Roles in Transcription-coupled Nucleotide Excision Repair. J. Biol. Chem 291, 1387-1397, (2016).

160. Puumalainen, M. R., Ruthemann, P., Min, J. H. and Naegeli, H. Xeroderma pigmentosum group C sensor: unprecedented recognition strategy and tight spatiotemporal regulation. Cellular and molecular life sciences : CMLS 73, 547-566, (2016).

161. Sugasawa, K., Ng, J. M., Masutani, C. et al. Xeroderma pigmentosum group C protein complex is the initiator of global genome nucleotide excision repair. Molecular cell 2, 223-232, (1998).

162. Kim, J. K., Soni, S. D., Arakali, A. V., Wallace, J. C. and Alderfer, J. L. Solution structure of a nucleic acid photoproduct of deoxyfluorouridylyl-(3'-5')-thymidine monophosphate (d-FpT) determined by NMR and restrained molecular dynamics: structural comparison of two sequence isomer photoadducts (d-U5p5T and d-T5p5U). Nucleic acids research 23, 1810-1815, (1995).

163. Jing, Y., Kao, J. F. and Taylor, J. S. Thermodynamic and base-pairing studies of matched and mismatched DNA dodecamer duplexes containing cis-syn, (6-4) and Dewar photoproducts of TT. Nucleic acids research 26, 3845-3853, (1998).

164. McAteer, K., Jing, Y., Kao, J., Taylor, J. S. and Kennedy, M. A. Solution-state structure of a DNA dodecamer duplex containing a Cis-syn thymine cyclobutane dimer, the major UV photoproduct of DNA. Journal of molecular biology 282, 1013-1032, (1998).

165. Sugasawa, K., Okamoto, T., Shimizu, Y. et al. A multistep damage recognition mechanism for global genomic nucleotide excision repair. Genes & development 15, 507-521, (2001).

166. Reardon, J. T. and Sancar, A. Recognition and repair of the cyclobutane thymine dimer, a major cause of skin cancers, by the human excision nuclease. Genes & development 17, 2539-2551, (2003).

167. Tang, J. Y., Hwang, B. J., Ford, J. M., Hanawalt, P. C. and Chu, G. Xeroderma pigmentosum p48 gene enhances global genomic repair and suppresses UV-induced mutagenesis. Molecular cell 5, 737-744, (2000).

168. Wakasugi, M., Shimizu, M., Morioka, H. et al. Damaged DNA-binding protein DDB stimulates the excision of cyclobutane pyrimidine dimers in vitro in concert with XPA and replication protein A. The Journal of biological chemistry 276, 15434-15440, (2001).

169. Groisman, R., Polanowska, J., Kuraoka, I. et al. The ubiquitin ligase activity in the DDB2 and CSA complexes is differentially regulated by the COP9 signalosome in response to DNA damage. Cell 113, 357-367, (2003).

170. Scrima, A., Fischer, E. S., Lingaraju, G. M. et al. Detecting UV-lesions in the genome: The modular CRL4 ubiquitin ligase does it best! FEBS Lett 585, 2818-2825, (2011).

171. Sugasawa, K., Okuda, Y., Saijo, M. et al. UV-induced ubiquitylation of XPC protein mediated by UV-DDB-ubiquitin ligase complex. Cell 121, 387-400, (2005).

172. Kapetanaki, M. G., Guerrero-Santoro, J., Bisi, D. C. et al. The DDB1-CUL4ADDB2 ubiquitin ligase is deficient in xeroderma pigmentosum group E and targets histone H2A at UV-damaged DNA sites. Proceedings of the National Academy of Sciences of the United States of America 103, 2588-2593, (2006).

173. Wang, H., Zhai, L., Xu, J. et al. Histone H3 and H4 ubiquitylation by the CUL4-DDB-ROC1 ubiquitin ligase facilitates cellular response to DNA damage. Molecular cell 22, 383-394, (2006).

174. Guerrero-Santoro, J., Kapetanaki, M. G., Hsieh, C. L. et al. The cullin 4B-based UV-damaged DNA-binding protein ligase binds to UV-damaged chromatin and ubiquitinates histone H2A. Cancer research 68, 5014-5022, (2008).
175. Nag, A., Bondar, T., Shiv, S. and Raychaudhuri, P. The xeroderma pigmentosum group E gene product DDB2 is a specific target of cullin 4A in mammalian cells. Molecular and cellular biology 21, 6738-6747, (2001).
176. Matsumoto, S., Fischer, E. S., Yasuda, T. et al. Functional regulation of the DNA damage-recognition factor DDB2 by ubiquitination and interaction with xeroderma pigmentosum group C protein. Nucleic acids research 43, 1700-1713, (2015).
177. Wang, Q. E., Zhu, Q., Wani, G. et al. DNA repair factor XPC is modified by SUMO-1 and ubiquitin following UV irradiation. Nucleic Acids Res 33, 4023-4034, (2005).
178. Poulsen, S. L., Hansen, R. K., Wagner, S. A. et al. RNF111/Arkadia is a SUMO-targeted ubiquitin ligase that facilitates the DNA damage response. J. Cell Biol 201, 797-807, (2013).
179. Akita, M., Tak, Y. S., Shimura, T. et al. SUMOylation of xeroderma pigmentosum group C protein regulates DNA damage recognition during nucleotide excision repair. Scientific reports 5, 10984, (2015).
180. van Cuijk, L., van Belle, G. J., Turkyilmaz, Y. et al. SUMO and ubiquitin-dependent XPC exchange drives nucleotide excision repair. Nature communications 6, 7499, (2015).
181. Evans, E., Moggs, J. G., Hwang, J. R., Egly, J. M. and Wood, R. D. Mechanism of open complex and dual incision formation by human nucleotide excision repair factors. The EMBO journal 16, 6559-6573, (1997).
182. Wakasugi, M. and Sancar, A. Assembly, subunit composition, and footprint of human DNA repair excision nuclease. Proceedings of the National Academy of Sciences of the United States of America 95, 6669-6674, (1998).
183. Missura, M., Buterin, T., Hindges, R. et al. Double-check probing of DNA bending and unwinding by XPA-RPA: an architectural function in DNA repair. The EMBO journal 20, 3554-3564, (2001).
184. Ogi, T., Limsirichaikul, S., Overmeer, R. M. et al. Three DNA polymerases, recruited by different mechanisms, carry out NER repair synthesis in human cells. Mol. Cell 37, 714-727, (2010).
185. Moser, J., Kool, H., Giakzidis, I. et al. Sealing of chromosomal DNA nicks during nucleotide excision repair requires XRCC1 and DNA ligase III alpha in a cell-cycle-specific manner. Molecular cell 27, 311-323, (2007).
186. Vermeulen, W. and Fousteri, M. Mammalian transcription-coupled excision repair. Cold Spring Harb. Perspect. Biol 5, a012625, (2013).
187. Groisman, R., Kuraoka, I., Chevallier, O. et al. CSA-dependent degradation of CSB by the ubiquitin-proteasome pathway establishes a link between complementation factors of the Cockayne syndrome. Genes Dev 20, 1429-1434, (2006).
188. Schwertman, P., Lagarou, A., Dekkers, D. H. et al. UV-sensitive syndrome protein UVSSA recruits USP7 to regulate transcription-coupled repair. Nat. Genet 44, 598-602, (2012).
189. Nakazawa, Y., Sasaki, K., Mitsutake, N. et al. Mutations in UVSSA cause UV-sensitive syndrome and impair RNA polymerase IIo processing in transcription-coupled nucleotide-excision repair. Nat. Genet 44, 586-592, (2012).
190. Zhang, X., Horibata, K., Saijo, M. et al. Mutations in UVSSA cause UV-sensitive syndrome and destabilize ERCC6 in transcription-coupled DNA repair. Nat. Genet 44, 593-597, (2012).
191. Fei, J. and Chen, J. KIAA1530 protein is recruited by Cockayne syndrome complementation group protein A (CSA) to participate in transcription-coupled repair (TCR). J. Biol. Chem 287, 35118-35126, (2012).
192. Tornaletti, S. and Hanawalt, P. C. Effect of DNA lesions on transcription elongation. Biochimie 81, 139-146, (1999).
193. Wilson, M. D., Harreman, M. and Svejstrup, J. Q. Ubiquitylation and degradation of elongating RNA polymerase II: the last resort. Biochim Biophys Acta 1829, 151-157, (2013).
194. Sigurdsson, S., Dirac-Svejstrup, A. B. and Svejstrup, J. Q. Evidence that transcript cleavage is essential for RNA polymerase II transcription and cell viability. Mol Cell 38, 202-210, (2010).
195. Woudstra, E. C., Gilbert, C., Fellows, J. et al. A Rad26-Def1 complex coordinates repair and RNA pol II proteolysis in response to DNA damage. Nature 415, 929-933, (2002).
196. Anindya, R., Aygun, O. and Svejstrup, J. Q. Damage-induced ubiquitylation of human RNA polymerase II by the ubiquitin ligase Nedd4, but not Cockayne syndrome proteins or BRCA1. Mol. Cell 28, 386-397, (2007).

197. Harreman, M., Taschner, M., Sigurdsson, S. et al. Distinct ubiquitin ligases act sequentially for RNA polymerase II polyubiquitylation. Proceedings of the National Academy of Sciences of the United States of America 106, 20705-20710, (2009).

198. Kuznetsova, A. V., Meller, J., Schnell, P. O. et al. von Hippel-Lindau protein binds hyperphosphorylated large subunit of RNA polymerase II through a proline hydroxylation motif and targets it for ubiquitination. Proceedings of the National Academy of Sciences of the United States of America 100, 2706-2711, (2003).

199. Bregman, D. B., Halaban, R., van Gool, A. J. et al. UV-induced ubiquitination of RNA polymerase II: a novel modification deficient in Cockayne syndrome cells. Proc. Natl. Acad. Sci. U. S. A 93, 11586-11590, (1996).

200. Ratner, N., Bloom, G. S. and Brady, S. T. A role for cyclin-dependent kinase(s) in the modulation of fast anterograde axonal transport: effects defined by olomoucine and the APC tumor suppressor protein. J. Neurosci 18, 7717-7726, (1998).

201. Starita, L. M., Horwitz, A. A., Keogh, M. C. et al. BRCA1/BARD1 ubiquitinate phosphorylated RNA polymerase II. J Biol Chem 280, 24498-24505, (2005).

202. Kleiman, F. E., Wu-Baer, F., Fonseca, D. et al. BRCA1/BARD1 inhibition of mRNA 3' processing involves targeted degradation of RNA polymerase II. Genes Dev 19, 1227-1237, (2005).

203. Becker, J., Barysch, S. V., Karaca, S. et al. Detecting endogenous SUMO targets in mammalian cells and tissues. Nat. Struct. Mol. Biol 20, 525-531, (2013).

204. Bruderer, R., Tatham, M. H., Plechanovova, A. et al. Purification and identification of endogenous polySUMO conjugates. EMBO Rep 12, 142-148, (2011).

205. Hendriks, I. A. and Vertegaal, A. C. Label-Free Identification and Quantification of SUMO Target Proteins. Methods Mol. Biol 1475, 171-193, (2016).

206. Knuesel, M., Cheung, H. T., Hamady, M., Barthel, K. K. and Liu, X. A method of mapping protein sumoylation sites by mass spectrometry using a modified small ubiquitin-like modifier 1 (SUMO-1) and a computational program. Mol. Cell Proteomics 4, 1626-1636, (2005).

207. Wohlschlegel, J. A., Johnson, E. S., Reed, S. I. and Yates, J. R., III. Improved identification of SUMO attachment sites using C-terminal SUMO mutants and tailored protease digestion strategies. J. Proteome. Res 5, 761-770, (2006).