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The Ku70/80 ring in Non-Homologous End-Joining: easy to slip on, hard to remove

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1. ABSTRACT

Non-homologous end-joining (NHEJ) is an essential DNA double strand break repair pathway during all cell cycle stages. Deficiency in NHEJ factors can lead to accumulation of unrepaired DNA breaks or faulty DNA repair, which may ultimately result in cell death, senescence or carcinogenesis. The Ku70/80 heterodimer is a key-player in the NHEJ pathway and binds to DNA termini with high affinity, where it helps to protect DNA ends from degradation and to recruit other NHEJ factors required for repair. The mechanism of Ku70/80 detachment from the DNA helix after completion of DNA repair is incompletely understood. Some data suggest that certain DNA repair factors are ubiquitylated and targeted for proteasomal degradation after repair. Recent studies suggest that Ku80 is conjugated to lysine48-linked ubiquitin chains by the Skp1-Cullin-F-box (SCF) complex and/or the RING finger protein 8 (RNF8) ubiquitin-protein ligases, followed by rapid proteasomal degradation. In this review we address the structure and function of the Ku70/80 heterodimer and how ubiquitylation may affect the release of Ku70/80 from chromatin and its subsequent degradation via the ubiquitin-proteasome system.

2. INTRODUCTION

DNA double strand breaks (DSBs) are among the most cytotoxic DNA lesions, resulting in chromosomal breakage and fragmentation. The accumulation of unrepaired DSBs usually triggers initiation of apoptotic or senescence pathways (1). In the absence of such terminal pathways, a cell may survive with deleted, translocated or incorrectly repaired genomic fragments, which increase the potential for carcinogenic events (2). DSBs are caused by either exogenous factors, including exposure to radiation or radiomimetic chemicals, or endogenous factors such as genomic rearrangements, including recombination of gene segments, variable (V), (diversity (D)), joining (J), (V(D)J recombination). The two major DNA repair pathways dedicated to the repair of DSBs are homologous recombination (HR) and non-homologous end-joining (NHEJ). HR is restricted to the late S and G2 phases, where it uses the sister chromatid as a template to guide error-free repair of the DSB. NHEJ, on the other hand, does not require a homologous sequence (3). Due to the lack of a guidance template, this pathway is more prone to introduce errors (4). However, unlike HR, the NHEJ pathway can function throughout the cell cycle (3,5). The NHEJ pathway is essential for a number of cellular processes such as telomere maintenance, V(D)J recombination and DSB repair in postmitotic cells (Figure 1) (6-9). Therefore, defects in NHEJ are generally associated with a spectrum of clinical problems, including severe combined immune deficiency (SCID), radiation-sensitivity, an increased occurrence of cancers, and certain neurodegenerative diseases such as Huntington's disease (10,11).

Proteins involved in DNA repair are tightly regulated by posttranslational modifications (PTMs),

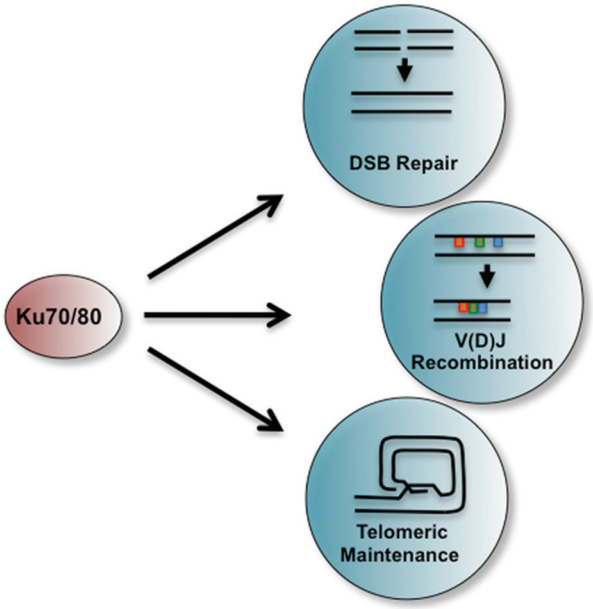

Figure 1. The Ku70/80 heterodimer is vital in several cellular processes. Ku70/80 has a role in DSB repair, V(D)J recombination and telomere maintenance.

including phosphorylation, ubiquitylation, sumoylation, and acetylation (12,13). These PTMs generally serve to regulate the repair factors' subcellular localization, enzymatic activity and recruitment to sites of DNA damage (13). In recent years there has been an increased interest in the regulatory roles of PTMs in DNA repair, e.g. in processes such as detection of DNA damage, recruitment of other repair factors, and post-repair dissociation from the DNA break site (14). Post-repair dissociation of repair enzymes from chromatin is important, since repair factors trapped on re-ligated DNA could interfere with subsequent transcription and replication (15). Some repair proteins bind very efficiently to DNA breaks, but are constrained in their dissociation from the repaired DNA because of structural restrictions, for instance by being wrapped around the DNA such as proliferating cell nuclear antigen (PCNA) and Ku70/80 (16,17). In other words: these proteins are quick to associate with damaged DNA, but are apparently harder to remove after the damage is repaired. Like PCNA, Ku70/80 removal is associated with ubiquitylation catalyzed by at least two different E3 ubiquitin-protein ligases (15,18-20). In this review, we summarize the essential elements of the NHEJ pathway, discuss the pre-repair association of Ku70/80 with DSBs, as well as the post-repair dissociation of Ku70/80 from the DNA, and evaluate the pathways and factors that potentially influence this removal.

3. THE NON-HOMOLOGOUS END-JOINING DNA REPAIR PATHWAY

NHEJ is based on unguided re-ligation of the two DNA termini, which are created as a result of DNA breakage. Ligation takes place either directly or after limited processing of the DNA ends, the latter potentially resulting in deletion or insertion of a few nucleotides at the break site. The central proteins in the process are: Ku70, Ku80, the DNA-Dependent Protein Kinase catalytic subunit (DNA-PKcs), DNA Ligase IV, and the X-Ray Cross-Complementation group 4 protein (XRCC4) (Figure 2). Several NHEJ associated factors facilitate the process at the DSB site such as Artemis, XRCC4-like factor (XLF), polynucleotide kinase/phosphatase (PNKP), aprataxin polynucleotide kinase/phosphatase-like factor (APLF), aprataxin (APTX), paralog of XRCC4 and XLF (PAXX) and the polymerases Pol λ and Pol μ (Figure 2) (21-28). Several factors are essential in cell type specific processes such as Artemis and terminal deoxynucleotidyl transferase (TdT) during the process of V(D)J recombination in lymphocytes (29,30). Most of these NHEJ factors are phylogenetically conserved in higher eukaryotes.

Ku70 (69 kDa) and Ku80 (83 kDa) are two of the central components of the NHEJ pathway. The two proteins form a heterodimer with a ring-shaped structure of which the central canal fits a double-stranded DNA helix. The Ku70/80 heterodimer possesses a high affinity for double stranded DNA ends. After introduction of a DSB, Ku70/80 binds swiftly to DNA termini and subsequently recruits and activates the DNA-PKcs kinase to the damage site. The Ku70/80 – DNA-PKcs complex (also referred to as the DNA-PK complex) protects the DNA ends from degradation (31) and juxtaposes the DNA ends in a synaptic complex (31-33). The DNA-PK complex phosphorylates a large number of substrate proteins on serine and threonine residues after DSB introduction, including the Ku70/80 subunits and itself (34-36). Autophosphorylation of DNA-PKcs at the DSB site has been shown to modify the synaptic complex in such a manner that the DNA termini become accessible for further processing and repair (37-39). Interestingly, although a large number of proteins are phosphorylated by DNA-PKcs, only autophosphorylation of DNA-PKcs has been confirmed to play a functional role in the DSB repair process (36,39,40). DSBs that require limited or no resection can be repaired independently of DNA-PKcs (41,42). Eroded DNA ends require resection by the RecQ helicases; WRN, RECQL1 or RECQL4 (43-51) and the nucleases exonuclease 1 (EXO1) and the MRE11-RAD50-NBS1 (MRN) complex (52-54). Minor DNA processing is catalyzed by DNA Pol λ and Pol μ (23). The final ligation step in the DNA repair process is promoted by APTX, APLF, PNKP, PAXX and is carried out by XLF and Ligase IV/XRCC4 (24-28,55,56). XLF interacts with XRCC4 which stimulates Ligase IV to ligate the nick (57).

3.1. The Ku70/80 heterodimer

The crystal structure of the Ku heterodimer reveals that Ku70 and Ku80 share a similar topology,

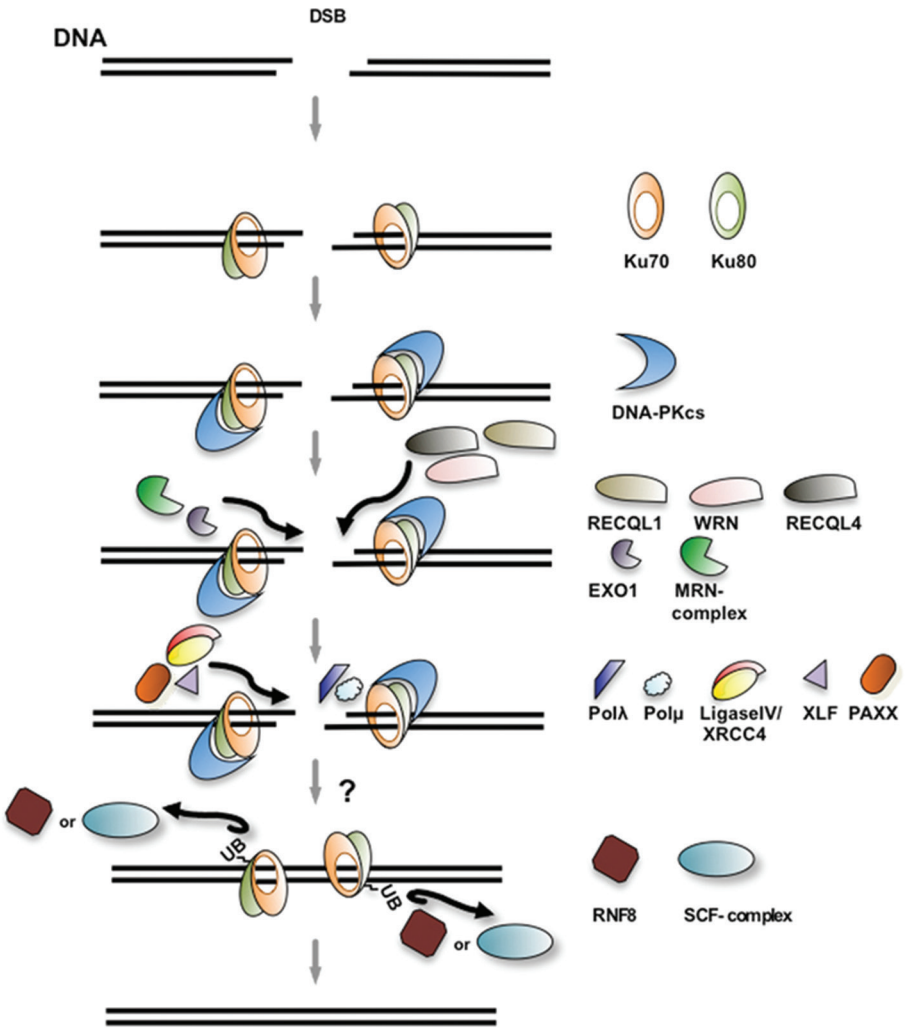

Figure 2. Schematic overview of the NHEJ DSB repair pathway. The Ku70/80 heterodimer recognizes and binds to the DSB, followed by recruitment of DNA-PKcs. Next, the NHEJ associated factors are recruited for processing and repair of the DNA ends. The NHEJ associated factors dissociate after DSB repair (some regulated by PTMs), from the chromatin. The Ku70/80 heterodimer remains and will be removed by ubiquitylation (UB).

forming a toroidal structure. Each subunit can be divided into three domains: an N-terminal α/ß domain which resembles a von Willebrand A (vWA) domain (residues 37-260 in Ku70 and 9-235 in Ku80) (17), a ß-barrel domain (residues 256-438 in Ku70, and 247-424 in Ku80) and a C-terminal extension of each subunit forming a three-helical bundle in Ku70 and a six-helical structure in Ku80 (Figure 3a,b) (17,58). Dimerization of Ku70 and Ku80 is facilitated by the ß-barrel domains; their α-helical extensions and their extended loops which form interconnected ß-strands (antiparallel ß-sheets) in the so-called arms, which are present in both proteins (Figure 3a). Ku70/80 threads onto a DNA end and binds in a DNA-sequence independent-manner (59,60). The heterodimer fits onto the major and minor groves of the DNA helix, which follows a defined path through the protein ring. The Ku70/80 central canal makes contact with only a few phosphates of the DNA backbone, and not with the DNA bases (17). The inner surface of the central canal is positively charged (Figure 3b) which stimulates the heterodimer to slide easily over a double stranded DNA end. Once bound to the DNA termini, the Ku70/80 complex has the ability to move along the DNA molecule in an ATP-independent manner (61-63). Binding of the Ku heterodimer to DNA is mediated by the central regions (including the β barrel domain) of both Ku70 (residues 266-529) and Ku80 (residues 244-543) (Figure 3c) (36). Ku70/80 can bind to ends of duplex DNA, including hairpins, nicked and forked structures (64). Electrophoretic mobility shift assays (EMSA) have revealed that Ku70/80 is not able to bind supercoiled, circular, or linear single-stranded DNA and therefore will not bind on internal positions in chromatin (17,65). Quantitative binding analyses showed that Ku70/80 binds to duplex DNA with dissociation constants in the nM range (66-68). Studies have shown

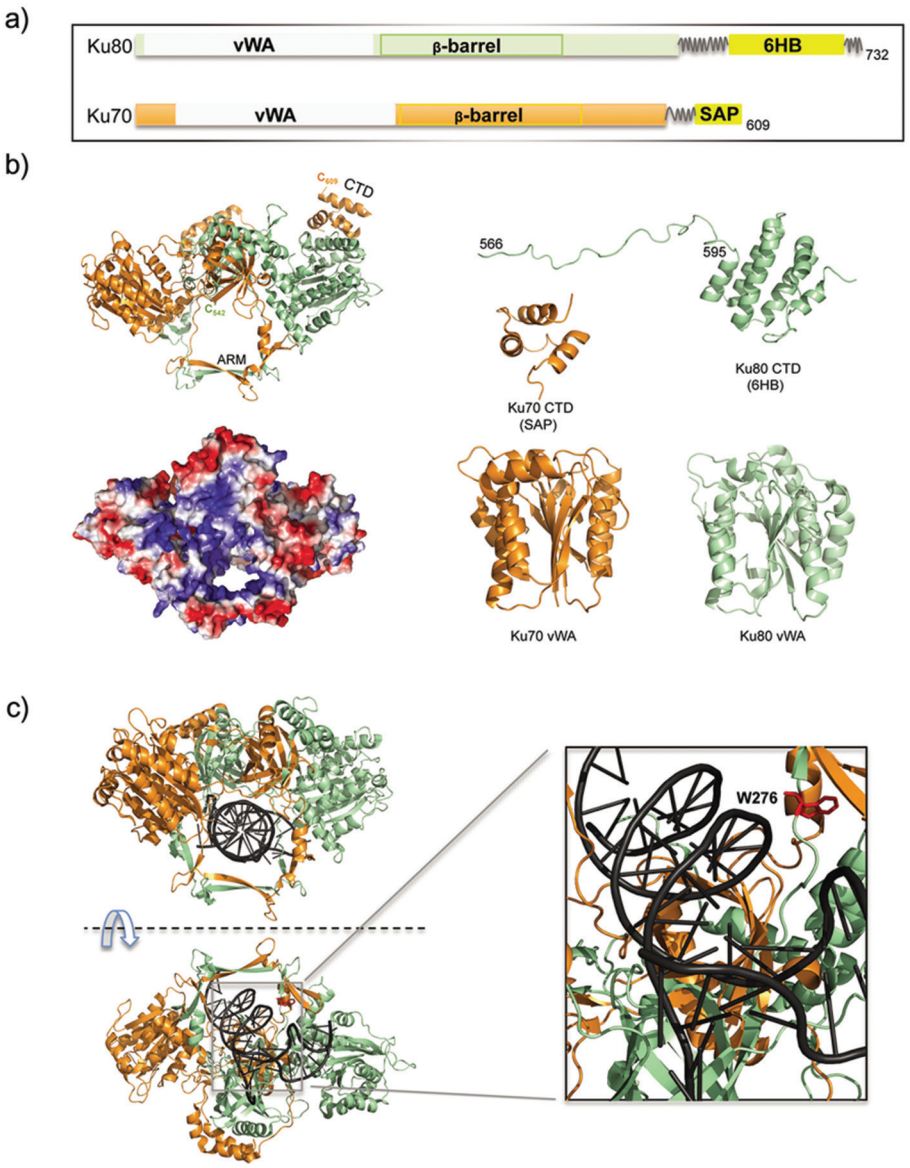

Figure 3. Structures of the Ku70/80 heterodimer alone and with DNA. a) Schematic overview of the two subunits of Ku70/80 indicating the positions and extent of the folded domains (vWAs, β-barrels, SAP and the 6-α-helical bundle (6HB)). Highly flexible linkers are indicated by zig-zag lines. b) Structure of the free Ku70/80 heterodimer (PDB code: 1JEQ) (17), with the vWA domains on the edges, the β-barrels with the arms and the extended helical regions in the core of the heterodimer. Of the C-terminal extensions, only the SAP domain was resolved in this structure. Below, in blue, red and white is shown the electrostatic surface of the heterodimer with blue indicating positive charges and red negative charges. The inner surface of the DNA binding tunnel is positively charged. To the left is shown the NMR solution structures of the isolated CTDs, the SAP domain of Ku70 in orange (PDB code: 1JJR) (71), and the 6HB of Ku80 in green (PDB code: 1RW2) (71). Below them are the two vWA domains with the 5 bladed β-sheet of 4 parallel and 1 antiparallel strand surrounded by helical structures. c) Structure of the DNA-Ku70/80 complex (PDB code: 1JEG) (17), shown from the front (top) and tilted to show the interaction part with W276 and the succeeding loop in green (highlighted in the zoom window with W276 in red sticks). In all illustrations Ku70 is shown in orange, Ku80 in green, and DNA in black.

that when Ku70/80 is bound at a DNA end or break, the Ku70 subunit is located proximally from the DNA end, whereas the Ku80 subunit is more distally located (60). Several atomic force microscopy (AFM) studies have showed that the structure of Ku70/80 enables the protein complex to slide or translocate inward along the length of a DNA molecule, with histones capable of blocking the passage (60,62,63,69).

3.1.1. Regulatory roles of the C-terminal extension of Ku70 and Ku80?

The C-terminal domain (CTD) of Ku70 (residues 530-609) consists of a linker or flexible arm connected to a SAF-A/B, Acinus and PIAS (SAP) domain (residues 560-609) (36,70). The NMR solution structure of the Ku70 SAP domain showed a three α-helix bundle, which in the crystal structure of the DNA-free heterodimer contacts

the vWA domain of Ku80 via a flexible, unresolved linker from residues 536-560 (Figure 3a) (17,71). This domain was not visible in the crystal structure of the DNA-bound heterodimer. Bioinformatics as well as further NMR studies suggested that this SAP domain binds DNA (71-74). Interestingly, one study revealed a structural change in the Ku70 SAP domain upon DNA binding (73). The biological functionality of either Ku80 binding or DNA binding by the Ku70 SAP domain is still not fully resolved.

The CTD of Ku80 (residues 544-732) comprises a folded domain consisting of six α-helices (residues 595-704) flanked by an unstructured linker (residues 544-594) and a flexible C-terminal end (36,72,75). The helices α2 and α4 form a hydrophobic pocket and may represent a protein binding domain (73,75). It has been reported that the last 14 residues of the Ku80 C-terminus are essential in activation of DNA-PKcs kinase activity (76,77). In contrast, biochemical and cellular studies showed that Ku70/80 lacking these Ku80 C-terminal residues was still able to activate DNA-PKcs, albeit to a lower extent (77,78). Additionally, it was reported that the Ku80 C-terminus stimulates Artemis-mediated DNA-end processing (78), and that a Ku70/80 heterodimer lacking the entire Ku80 CTD (truncated at residue 548) binds less efficiently to DNA, which is either caused by minor structural changes in the truncated protein (79) or lack of potential CTD-DNA interaction. Interestingly, it was reported that the Ku80 C-terminal region is involved in dimerization of Ku70/80 and can tether DNA ends in the absence of DNA-PKcs; the latter suggesting an important role in repair of simple DSB lesions which require no processing (61,62). Whether the Ku80 CTD connects with the Ku70 vWA in a similar manner to those interactions formed between Ku70 CTD and the vWA domain of Ku80, or if it binds DNA, has not yet been addressed.

3.1.2. Function of the N-terminal domain of Ku70 and Ku80

Recently, it has been suggested that the vWA domain of the Ku70 subunit is essential in the detection of DNA damage in response to ionizing radiation (IR) (80). Mutagenesis experiments in mouse embryo fibroblasts (MEFs), showed that mutations of S155/D156 in the Ku70 vWA domain unexpectedly increased cell survival after IR and suppressed activation of transcriptional factor 2 (ATF2) (80). The S155A substitution alone was sufficient to confer enhanced survival, whereas alteration to a phosphomimetic residue (S155D) reversed this effect, and induced pronounced hypersensitivity to IR compared to cells lacking Ku70. These findings suggest that S155 may be a critical phosphorylation site, affecting ATF2 as well as downstream apoptotic pathways (80). The Ku80 vWA domain is associated with binding several proteins including APLF (56). Based on a cellular study, mutations in the Ku80 vWA domain (L68R, Y74R and I112R) contribute to a reduced interaction with APLF during repair (56). Additionally, screens in yeast demonstrated that the mutations L240S, Y49H, M16I, and L149R were all defective in telomere silencing, but only minimally perturbed in telomere length (81), suggesting that the Ku80 vWA domain is a site for interaction with proteins important for telomere maintenance.

3.2. Ku70/80 heterodimer in relation to disease

Mice deficient in the *Ku70* or *Ku80* genes display premature aging characterized by osteoporosis, incomplete plate closure, growth failure, atopic skin disease, liver pathology, sepsis, cancer and a shortened life span (82-84). Moreover, $Ku70^{-/-}$ and $Ku80^{-/-}$ mice are defective in V(D)J rearrangement (82,83,85). In contrast to mouse cells, human cells depleted in Ku70 are not viable (86). Human cells depleted in Ku80 display cell death accompanied by massive telomere loss (87,88). Several case studies suggest that down-regulation in mRNA levels of Ku70 or Ku80 can contribute to cancer (89-94). Moreover, several reports indicate that single nucleotide polymorphisms (SNPs) in Ku70 or Ku80 potentially contribute to different types of cancer including breast, lung and gastric track cancers (93-96). This is consistent with data showing that single point mutations in either Ku70 or Ku80 can contribute to enhanced cell survival (allowing for carcinogenesis rather than cell death) or block removal of the Ku70/80 heterodimer DNA post-repair (80,97) (discussed in detail below).

4. THE E3-LIGASES, SCFF-BXL12 AND RNF8 IN RELEATION TO DSB REPAIR

A major function of the ubiquitin system is to target proteins to the 26S proteasome for degradation. However, ubiquitylation also plays important regulatory functions that are independent of protein degradation (98,99). Accordingly, the human genome encodes more than 600 different E3 ubiquitin-protein ligases that are the main factors determining the specificity of the ubiquitylation process. In recent years, several E3-ligases have been associated with DSB repair. The Skp1-Cullin-F-box (SCF) complexes form a large group of really interesting new gene (RING) family E3-ubiquitin-ligases, which primarily functions to ubiquitylate signaling proteins such as those regulating the cell cycle. In the SCF-complexes, various F-box proteins mediate substrate binding and therefore control substrate specificity. The SCF-complex most commonly promotes K48- and K63-linked poly-ubiquitylation (100). In general, K48-linked ubiquitin chains are associated with proteasomal degradation, whereas the K63-linked poly-ubiquitylation plays roles in DNA damage recognition and regulation of DSB repair (101,102). Dysfunction of the SCF-complex is associated with skin and lymphoma cancers, but is also related to neurodegenerative disorders, expanded tri-repeat diseases such as Huntington's disease and Machado-Joseph disease (103). Interestingly, the SCF complex is itself subject to regulation by conjugation

Table 1. Lysine ubiquitylation sites after DNA damage and possible accessible lysines after DNA bind

Protein	Reported ubiquitylation sites in DNA-free dimer	Lysines accessible in the presence of DNA	Remarks	References
Ku70	K117			(113)
Ku70		K317		(73)
Ku80	K195			(113)
Ku80	K265	K265	DNA interactions	(73,113)
Ku80	K481			(113)
Ku80		K534		(73)
Ku80		K543		(73)

The table lists reported lysine (K117, K195, K265 and K481) ubiquitylation sites in Ku70 and Ku80 after pheomycin treatment. Other lysines (K317, K265, K534 and K543) were detected by mass spectrometry to be accessible on Ku70/80 for covalent binding of NHS-biotin after DNA binding

to the ubiquitin-like (UBL) modifier neural precursor cell expressed, developmentally down-regulated 8 (NEDD8). Conjugation of NEDD8 (called neddylation) to the Cullin subunits in the SCF complexes stimulates the SCF ubiquitin-protein ligase activity. Neddylation is an essential process in most organisms and has been associated with DNA damage repair (104-106).

The RNF8 and RNF168 E3 ligases are not SCF-type ligases, but free RING domain family E3s that also regulate DNA damage signaling by ubiquitylation at the DSBs. It is reported that the RNF8 E3 ligase promotes K48- and K63-linked poly-ubiquitylation via differential RING-dependent interactions (107). RNF8 is also involved in telomere regulation; uncapped telomeres accumulate ubiquitylated histone H2A in a manner dependent on the E3 ligase RNF8 and stimulate both accumulation of DNA damage response (DDR) associated proteins tumor suppressor p53-binding protein 1 (53BP1) and phosphorylated ataxia telangiectasia mutated (ATM), and promote NHEJ at the uncapped telomeres (108). Recently, it was suggested that RNF168 promotes K63-linked poly-ubiquitylation of 53BP1 before it localizes to DSBs, and controls its response to DSB independently of the H2A-RNF8 route (109). Depletion of RNF8 or RNF168 reduces telomere-induced genome instability (108). Thus, the RNF8 pathway, which usually suppresses carcinogenesis by promoting DSB repair, possibly also enhances telomere-induced genome instability (108).

4.1. Removal of Ku70/80 from DNA

Post-repair dissociation of Ku70/80 from chromatin has been associated with two different E3-ubiquitin-ligases. A screen for DSB-binding F-box proteins revealed that the F-box protein Fbxl12 was recruited to double stranded DNA in a Ku-sensitive manner (110). Immunodepletion of Fbxl12 prevented Cul1 and Skp1 binding to DSBs and reduced Ku80 ubiquitylation, suggesting that Fbxl12 targets Ku80 for degradation (110). Interestingly, only Ku80 and not Ku70 has been found to be modified with K48-linked ubiquitin chains (97). Moreover, in one study, it was shown that a W276R mutant (numbered W275 in (97)) in the Ku80 vWA domain was functional in NHEJ and could be K48-polyubiquitylated, but was not removed from DNA (97). Therefore, it would be interesting to determine the efficiency of Ku70/80-W276R in NHEJ (97). It is possible that minor structural changes in the Ku80-W276R variant interfere with efficient degradation, but also possible that the Ku80-W276R mutant is unable to interact with chaperones such as p97 that are often required for segregating ubiquitylated proteins from chromatin prior to degradation (111,112). Structurally, W276 is located in the descending limb of the arm with its side chain pointing towards Ku80 and not towards DNA and the succeeding loop from W276 enters the major groove of DNA (Figure 3c). It is possible that insertion of a positive charge leads to rotation of the side chain and that this minor structural change will allow the arginine to interact with the DNA phosphates and hence increase DNA affinity. Such a model certainly needs further testing both in contexts of *in vitro* and *in vivo* settings.

Previously, mass spectrometry data have shown that NEDD8 covalently attaches to DNA-PKcs, but the functional relevance of this modification is unknown (104). Recent data suggest that the ubiquitin-conjugating enzyme E2M (UBE2M) E2 enzyme responsible for NEDD8-conjugation localizes to DNA damage sites, which lends support to the role of the SCF-complex in ubiquitylation of Ku70/80 (110,113). Accordingly, depletion of NEDD8 in cells showed a hypersensitivity to IR (113), and phleomycin treatment followed by mass spectroscopy analysis suggested that K114 of Ku70 and K195, K265 and K481 of Ku80 were increased in ubiquitylation (Table 1) (113). However, when K195, K265 and K481 were mutated to arginine (R), the data showed functional redundancy, suggesting that either yet unidentified lysines of Ku70 and/or Ku80 can be ubiquitylated or that multiple lysines can support the removal of Ku70/80 post-repair. In support, another study on the structural change of Ku70/80 imposed by DNA binding mapped accessible lysines by covalent binding of NHS-biotin and showed that K317 of Ku70 and K534 and K543 of Ku80 were accessible after DNA binding (Table 1) (73). Interestingly, K265 (on Ku80) makes contact with the DNA (17), while the NHS-biotin was conjugated in the absence of DNA, but not in the presence of DNA. Therefore, the reported ubiquitylation of K265 is probably not relevant for DNA-bound Ku70/80 (73,113).

In case of Ku80, its ubiquitylation has also been reported to be catalyzed by the E3 ubiquitin-protein ligase RNF8 (114). Observations support that RNF8 regulates the abundance of Ku80 at sites of DNA damage, and that RNF8 depletion results in prolonged retention of Ku80 (for up to four hours) at damage sites and in impaired NHEJ repair (114). However, it is not uncommon that DNA repair enzymes linger at the DSBs for more than four hours (114), for example it is reported that several DSB repair proteins related to the NHEJ, can remain more than 16 hours at a break site (115-117). Interestingly, RNF8, but not RNF168, promotes degradation of Ku80 (114). The ubiquitylation status of Ku70 was not tested in this study (114).

5. CONCLUSION AND PERSPECTVES

The Ku70/80 heterodimer has a vital role in cellular processes of DNA repair and maintenance. Removal of Ku70/80 from chromatin post-repair is essential to avoid that trapped Ku-complexes accumulate and interfere with transcription and replication. It is known that some proteins can be ubiquitylated by multiple E3 ligases (118,119). It is therefore possible that more than one E3 ligase promotes K48-linked ubiquitylation and subsequent degradation of Ku70 or Ku80. Future studies to address the role of chaperones such as p97 in dissociating ubiquitylated Ku70/80 from DNA could also reveal some analogies to the mechanisms employed for HR-mediated DNA repair. The RNF8 E3-ligase was shown to be essential in DSB repair and in telomere maintenance and is therefore favored over the SCF-complex in post-repair degradation of Ku80. The SCF-complex is primarily a cell cycle regulator, while Ku70/80 operates independently of the cell cycle and is the key DSB repair pathway in postmitotic cells such as neurons (9). However, interestingly, it has been shown that the SCF-complex is essential in both mitotic and postmitotic cells (103,120). In support of SCF-mediated degradation of Ku70/80, a recent study suggested that neddylation stimulates ubiquitylation and DNA dissociation of Ku70/80 post-repair (113). Until now, one point mutation has been suggested to block post-repair removal of Ku70/80 (97). For future studies, it will be important to generate other mutants in Ku70 and/or Ku80, where ubiquitylation and DNA dissociation are uncoupled. In the studies addressing this so-far, the essential lysine(s) in Ku70/80 that is ubiquitylated was not determined, but it is possible that more ubiquitylation sites in Ku70/80 are essential for its degradation (97,110,113,114). Identifying such residues as well as their interdependence and redundancy will be critical to determine the order of events, i.e. if ubiquitylation, as suspected, is a prerequisite for its DNA dissociation.

6. ACKNOWLEDGMENTS

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Abbreviations: 53BP1: Tumor suppressor p53-binding protein 1; AFM: Atomic Force Microscopy; APLF: Aprataxin polynucleotide kinase/phosphatase-like factor; APTX Aprataxin; ATM: Ataxia telangiectasia mutated; CTD: carboxy-terminal domain; DNA-PKcs: DNA-Dependent Protein Kinase catalytic subunit; DSB: Double Strand Breaks; EMSA: Electrophorectic mobility shift assays; HR: Homologous Recombination; MEF: mouse embryo fibroblasts; NEDD8: Neural precursor cell expressed, developmentally down-regulated 8; NHEJ: Non-Homologous End-Joining; PTMs: Posttranslational modifications; RING: Really Interesting New Gene; RNF8: RING finger protein 8; SAP: SAF-A/B, Acinus and PIAS; SCF: Skp1-Cullin-F-box; SCID: Severe combined immune deficiency; SNPs: Single nucleotide polymorphisms; SUMO: Small Ubiquitylation-like MOdifier; TdT: Terminal deoxynucleotidyl transferase; PAXX: Paralog of XRCC4 and XLF; PNKP: polynucleotide kinase/phosphatase; PCNA: Proliferating cell nuclear antigen; Pol λ: DNA polymerase lambda; Pol μ: DNA polymerase mu; PTMs: Posttranslational modifications; UBE2M: ubiquitin-conjugating enzyme E2M; UBLs: Ubiquitylation-like proteins; V(D)J recombination: variable (V), (diversity (D)) joining (J), gene segments recombination; vWA: von Willebrand A domain; WRN: Werner; XLF: XRCC4-like factor; XRCC4: X-Ray Cross-Complementation group 4

Key Words: Ku70, Ku80, Degradation, Double strand break, NEDD8, Non-homologous end-joining, NHEJ, SCF, RNF8, Proteasome, Ubiquitylation, Review

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