## Review Article

# Ubiquitin at work: The ubiquitous regulation of the damage recognition step of NER

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## ARTICLE INFORMATION

## ABSTRACT

Nucleotide excision repair (NER) removes a wide variety of helix distorting DNA lesions. NER comprises two damage recognition sub-pathways: GG-NER operates genome wide, whereas TC-NER specifically removes transcription-blocking lesions from the transcribed strand of actively transcribed genes. NER is a multistep process, which requires the concerted action of 30 proteins that need to be tightly controlled at the right time and place for efficient repair. Post-translational protein modifications (PTMs) are common regulators of complex protein networks. Several NER factors were shown to be modified by ubiquitin, whereas others are actively involved in the ubiquitin–proteasome system itself. PTMs by ubiquitylation can be swiftly induced in a reversible manner and have the ability to regulate protein function, localization or stability. This makes the regulation by ubiquitin highly suitable for the coordination of the complex NER reaction. Accumulating evidence, including proteome wide quantitative proteomics approaches, showed that especially NER factors involved in the damage recognition are regulated by ubiquitin, emphasizing the high level of regulation during the initiation of the NER reaction. In this review we will therefore focus on the different functions of ubiquitylation during the DNA damage recognition steps of NER.

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## Contents

# Introduction

Nucleotide excision repair (NER) removes a remarkable wide variety of DNA lesions, including UV-induced cyclopyrimidine dimers (CPDs) and 6-4 photoproducts (6-4PPs), chemically induced bulky adducts and endogenously generated oxidative lesions [1]. Within NER there are two different modes of damage recognition, which are dependent on the genomic location of the DNA lesion. Global genome NER (GG-NER) detects damage throughout the genome, whereas transcription coupled NER (TC-NER) specifically targets lesions in the transcribed strand of active genes. Inherited defects in NER are associated with different recessive autosomal disorders, illustrating its biological significance. Mutations in genes encoding proteins involved in GG-NER cause xeroderma pigmentosum (XP), which is characterized by extreme sun (UV) sensitivity and ∼2000 fold increased risk of skin cancer [2]. Defects associated with TC-NER can result in Cockayne syndrome (CS) or UV-sensitive syndrome (UVSS). Whereas the phenotype of UVSS patients is mainly restricted to UV hyper-sensitivity, CS patients present premature aging, developmental and neurological abnormalities [2,3].

## Global genome NER

The main damage recognition complex in GG-NER is the hetero-trimer XPC–RAD23–Centrin2 [4]. XPC is the DNA binding subunit, which constantly probes the DNA for helix distorting lesions [5]. When XPC encounters a damage-induced helix distortion it binds the undamaged strand opposite to the damage, which is a common feature for all types of NER-inducing lesions thereby explaining the broad range of lesions cleared by NER. Although XPC is the major DNA damage sensor of GG-NER it hardly recognizes UV-induced CPDs, as these hardly destabilize the DNA helix [6]. In this case XPC depends on the UV-DDB complex, which consists of DDB1 and DDB2 (XPE) [7]. The latter subunit has high affinity for UV-induced DNA damage and is required for CPD repair, but also facilitates the repair of 6-4PPs. Structural studies have shown that the WD40 domain of DDB2 binds the lesion [8]. After the damage recognition, TFIIH is recruited by a direct interaction with XPC [1]. TFIIH was initially identified as a transcription factor consisting of 10 subunits, including two DNA helicases XPB and XPD [9]. XPC binds multiple DNA structures, but the NER reaction can only be finalized upon damage verification most likely depending on the helicase activity of XPD and the XPA protein [10–12]. XPA binds the damage strand, while RPA binds the undamaged strand and positions the endonucleases ERCC1/XPF and XPG on the 5′ and 3′ side of the damage respectively [13]. After the coordinated incision [14] the lesion containing oligonucleotide is removed and the undamaged strand

is used as a template for DNA repair synthesis performed by PCNA and the DNA polymerase δ, ε or κ [15]. Finally the DNA gap is sealed by ligase I or ligase III/XRCC1, which completes the NER reaction [16].

## Transcription coupled NER

Bulky DNA lesions block transcription elongation and may eventually trigger cell death. This cytotoxic effect of transcription-blocking DNA lesions is counteracted by TC-NER, which specifically removes DNA lesions from the transcribed strand, thereby allowing the restart of the blocked transcription [17]. TC-NER is initiated by stalling of elongating RNA pol II on lesions. The first proteins to interact with lesion stalled RNA pol II are the TC-NER proteins UVSSA and CSB. UVSSA is required for stabilization of CSB, which will be discussed in more detail below [18,19]. CSB is responsible for the recruitment of CSA and core NER factors [20]. Efficient TC-NER requires the subsequent recruitment of HMNG1, XAB2 and TFIIS, which most likely assist the remodeling of the stalled RNA Pol II complex, thereby generating access for NER proteins to repair the damage [20].

# Ubiquitylation

NER involves the action of more than 30 proteins to recognize, verify and repair the damage. The repair factors have to assemble into functional repair complexes and dissociate at the right time and location for proper repair. Intricate molecular processes, such as NER, require a tight regulation, which is commonly achieved by post-translational modifications (PTMs) to ensure proper complex assembly and control the function of different protein activities. Their swift induction, reversibility and ability to regulate activity and binding of other proteins make PTMs a very suitable regulator. Phosphorylation, ribosylation and SUMOylation have been reported to play a role in the DNA damage response, including NER [21,22]. Based on a recent literature ubiquitylation seems to be a key regulator for NER [21]. Ubiquitin is a highly conserved protein of 76 amino acid that modifies protein substrates, thereby regulating its stability and function [21,22]. Ubiquitin is covalently attached *via* its C-terminal glycine residue to the ε-amino group of lysine residues in substrates through the concerted action of three enzymes. Ubiquitin is activated in an ATP-dependent manner by an ubiquitin-activating enzyme (E1). Subsequently ubiquitin is transferred to an ubiquitin-conjugating enzyme (E2), which together with an ubiquitin-ligating enzyme (E3) links ubiquitin to the substrate with a high specificity [23]. There are approximately 2 E1, 40 E2 and >600 E3 proteins encoded in the human genome, which illustrates the complexity

and determines the specificity of ubiquitylation [22]. After linkage to one or more sites in the substrate (mono- or multi-ubiquitylation) ubiquitin can use one of its 7 internal lysine residues (K6, K11, K27, K29, K33, K48 and K63) or its N-terminus to form ubiquitin chains (poly-ubiquitylation) [24]. Ubiquitin chains have distinct structures and properties; consequently they have a different impact on the fate of the target protein. For example K48-linked chains result in proteasomal degradation, whereas K63-linked chains are involved in cellular signaling [24]. In addition, ubiquitylated proteins can be recognized by chain specific ubiquitin-binding proteins ("readers"), thereby for example regulating protein–protein interactions [25]. Like most other PTMs ubiquitylation is transient and can be reversed by approximately 100 different deubiquitylating enzymes (DUBs) [26]. The large number of enzymes involved in ubiquitylation and the different ubiquitin chains that can be formed illustrate the diversity of regulation with ubiquitin. Advanced quantitative proteomics in combination with purification methods for ubiquitylated proteins or peptides revealed many UV-induced differential ubiquitylated proteins, confirming the significant involvement of ubiquitin in DNA repair [18,27]. Even though different factors involved in the UV-induced DNA damage response are known to be ubiquitylated e.g. XPA [28] and histone H2A [29], the largest UV-induced ubiquitylation increase was observed for the damage recognition factors of GG-NER and TC-NER, underlining the high regulation of NER initiation. Therefore, in this review we will focus on the role of ubiquitylation in the damage recognition steps of NER.

## Ubiquitylation in GG-NER

### UV-DDB

The UV-DDB heterodimer (DDB1 and DDB2) is part of a multi-subunit ubiquitin E3 ligase complex that contains the scaffold protein CUL4 and the E2-binding subunit RBX-1/ROC1. DDB1 is the adapter subunit, which can interact with different DCAF proteins (DDB1- and CUL4-associated factors). DCAFs are characterized by their WD40 repeats that serve as substrate receptor [30]. DDB2 is one of the best characterized DCAFs and targets this Cullin-RING containing ubiquitin ligase complex (CRL4$^{DDB2}$) to sites of UV-induced DNA damage. The ubiquitin ligase activity of CRL4$^{DDB2}$ is tightly regulated by the COP9 signalosome (CSN), a multisubunit-protease [31]. Under unperturbed conditions the CSN is associated with the CRL4$^{DDB2}$ complex, thereby inhibiting its activity by removing the ubiquitin-like protein Nedd8 from CUL4. In addition, it was shown in vitro that CSN has also deubiquitylating activity, which might target ubiquitin chains formed on this CRL ligase complex itself [31]. Upon UV damage infliction CSN dissociates from the CRL4$^{DDB2}$ complex, stimulating the neddylation of CUL4, thereby activating the E3 ligase activity of this CRL [31]. Several CRL4$^{DDB2}$ substrates have been identified, including XPC and DDB2 itself [32,33]. In addition, to these damage recognition factors the CRL4$^{DDB2}$ complex ubiquitylates the core histones H2A [34,35], H3 and H4 [36]. The UV-induced ubiquitylation of H3 and H4 followed the kinetics of the 6-4PP repair and weakens the interaction between the histones and DNA, which may aid access of NER proteins to DNA lesions [36]. It was shown in vitro that the UV-induced histone H2A

mono-ubiquitylation at K119/K120 by the CRL4$^{DDB2}$ complex also results in nucleosome destabilization [35].

In response to UV-damage DDB2 is auto-ubiquitylated, leading to its proteasomal degradation [33,37]. In addition, in vitro experiments suggested that DDB2 ubiquitylation might also reduce the binding affinity of the E3 ubiquitin complex for damaged DNA [32]. DDB2 is mainly ubiquitylated at multiple N-terminal lysine residues [32]. Deletion of the DDB2 N-terminus (aa 1–40) as well as mutation of the first 7N-terminal lysine residues into arginine resulted in stabilization of DDB2 after UV [32,38]. Ubiquitylated DDB2 is recognized by the ATP-driven molecular chaperone VCP/p97, which facilitates proteasomal degradation or recycling of ubiquitylated proteins [39]. More information on the role of VCP/p97 in the DNA damage response can be found in the review by Dantuma et al. this issue. The recruitment of p97 to sites of DNA damage depends, in addition to CRL ligase activity, on the substrate specific ubiquitin binding-adaptors NPL4, UFD1, and UBXD7. P97 extracts ubiquitylated DDB2 from the chromatin bound CRL4$^{DDB2}$ complex and channels it into the 26S proteasome for degradation (Fig. 1). Knockdown of p97 resulted in an increased recruitment, prolonged residence time of DDB2 and XPC at lesions and a reduced repair activity, suggesting that ubiquitylated DDB2 needs to be removed from the chromatin for efficient NER [39]. In line with this, siRNA mediated knockdown of CUL4A in human cells resulted in an increased retention time of DDB2 at lesions in combination with reduced CPD removal [40]. In contrast, CUL4A knockout mice exhibit enhanced GG-NER activity and increased UV resistance in combination with increased DDB2 and XPC protein levels [41]. These contradicting observations may be explained by the different species in which these studies were performed that are characterized by different DDB2 expression levels [42]. However, together these observations show that CUL4A-dependent DDB2 ubiquitylation not only stimulates damage recognition, but also induces the subsequent p97-dependent clearance of DDB2 that is required for to promote the downstream repair reaction. Moreover, it appeared that expression levels of DDB2 need to be under tight control to warrant efficient NER [43]. It was recently found that the steady state level of DDB2 is regulated by the DUB USP24, which interacts with DDB2 and might counteract CUL4A under unperturbed conditions [44]. The recently identified PARylation of DDB2 adds another level of regulation and complexity to the DDB2 function in NER. It was shown that PARP1-dependent PARylation of DDB2 resulted in its prolonged binding to chromatin and its delayed degradation [45]. This PARylation dependent stabilization of DDB2 thus counteracts CUL4A induced ubiquitylation and the subsequent degradation of DDB2 (Fig. 1). The crosstalk between DDB2 ubiquitylation and PARylation is most likely based on competition of lysine residues. Since both ubiquitylation and PARylation are targeted to lysine residues at the 40 N-terminal amino acids of DDB2 [45].

### XPC

Next to DDB2, also XPC protein levels are controlled by the ubiquitin–proteasome system. High levels of XPC are found to be cytotoxic, most likely caused by its ability to bind a wide variety of DNA structures, thereby interfering with DNA metabolism [46]. An important XPC regulator is its binding partner RAD23. Mammalian cells express two RAD23 paralogs, RAD23A and RAD23B.

Both proteins encode two ubiquitin-associated (UBA) domains, which recognize ubiquitylated proteins, and an ubiquitin-like (UBL) domain, which can interact with the proteasome, thereby targeting bound ubiquitylated proteins for degradation [21]. Although XPC can bind both proteins, it is predominantly associated to the more abundant RAD23B. Interestingly, deletion of
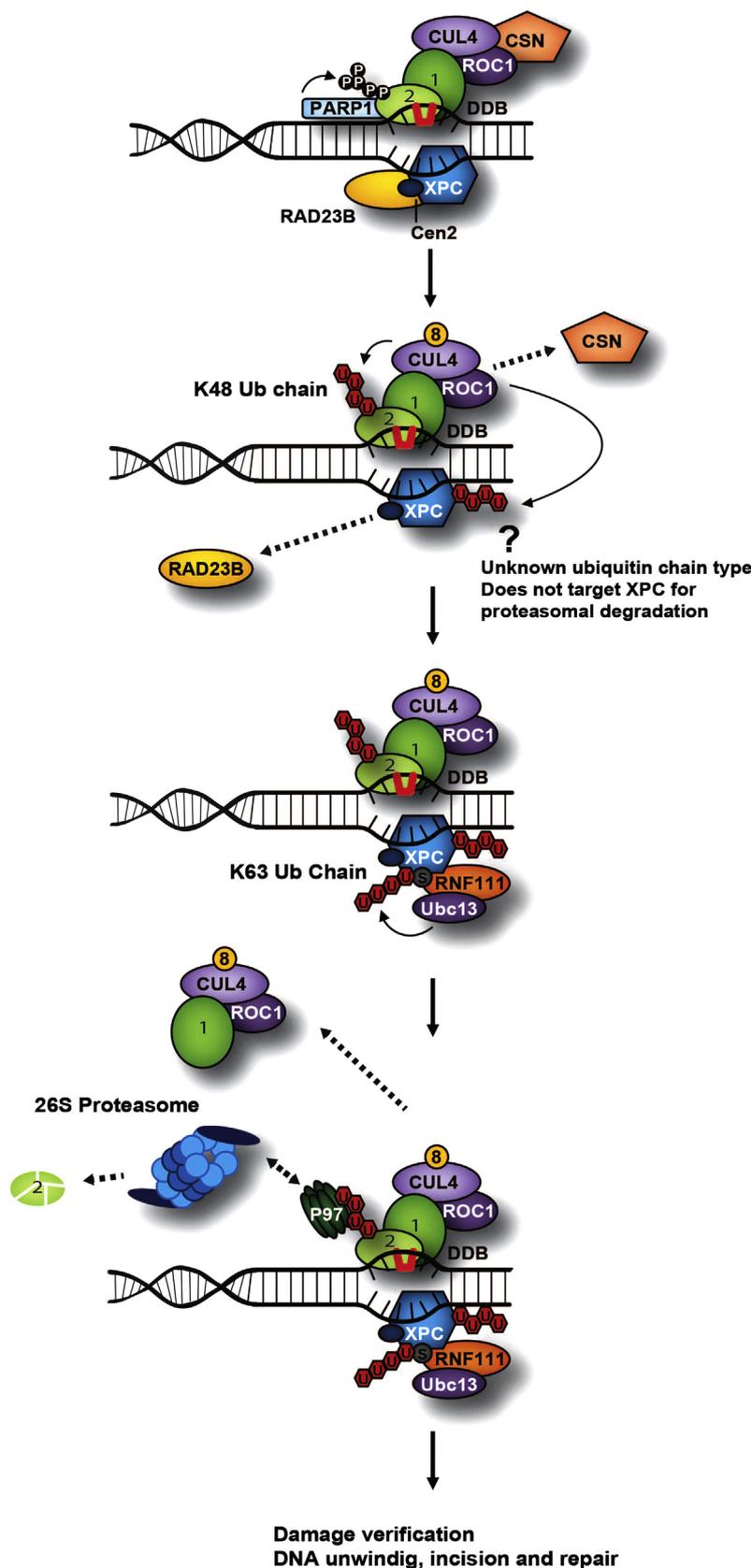
both RAD23 paralogs severely reduced steady-state levels of XPC, causing reduced UV-resistance and DNA repair synthesis comparable with XPC$^{-/-}$ cells [46]. Thus contrary to the expected proteasomal shuttling function of RAD23B, this protein stabilizes XPC rather than targeting XPC for degradation. Overexpression of Rad4, the yeast ortholog of XPC, in rad23 yeast strains only partially complemented the UV sensitivity, suggesting additional roles of RAD23 during NER [47]. Indeed, FRAP analysis of XPC in RAD23 double knock out cells showed that RAD23 is required for XPC binding to UV-induced DNA damage. Although RAD23 is essential for XPC loading, it is not detectable at DNA lesions, since it dissociates from XPC upon damage recognition [48]. This suggests that RAD23 delivers XPC at UV lesions, but is not required to stabilize lesion-bound XPC. XPC is also a well described target of the CRL4$^{DDB2}$ complex upon UV-damage [30,32]. Interestingly, in contrast to DDB2, XPC polyubiquitylation did not result in degradation (Fig. 1). Whether the CRL4$^{DDB2}$ complex forms different poly-ubiquitin chains on DDB2 (K48-linked) and XPC (unknown) is currently not known. However, it has been noted that XPC is modified by K48- as well as K63-linked chains [39,49]. It is suggested that the K48-linked ubiquitylated XPC—like DDB2—is recognized by the ATPase p97 and channeled into the proteasome for degradation [39]. In a yeast-two hybrid screen OTUD4, a deubiquitylating enzyme was identified as an interaction partner of XPC [50]. Knockdown of OTUD4 resulted in an increase of the ubiquitylated form of XPC after UV. Although the exact function of OTUD4 is currently unknown, it may function to trim down CUL4-induced polyubiquitin chains protecting XPC form degradation [50].

XPC is not only ubiquitylated, but also modified by the ubiquitin like modifier SUMO upon UV-damage in a DDB2 dependent manner. The XPC SUMOylation was suggested to result in its stabilization [51]. Recently, another XPC regulating E3 ubiquitin-ligase was identified: RNF111 [49]. It belongs to the class of SUMO-targeted ubiquitin ligases (STUbLs), which provide direct crosstalk between SUMOylation and ubiquitylation. RNF111 specifically binds the UV-induced SUMOylated form of XPC, which results in the RNF111 dependent formation of UBC13-dependent K63-linked chains on XPC (Fig. 1) [49]. Although both the CRL4$^{DDB2}$ complex and RNF111 ubiquitylate XPC in response to UV, they have opposite effects on the damaged DNA binding kinetics of XPC. Whereas knockdown of RNF111 increased the accumulation of XPC-GFP on local UV-induced damage, knockdown of DDB2 resulted in a decrease of XPC-GFP accumulation [49]. Despite this opposing effect on XPC kinetics knockdown of either DDB2 or RNF111 resulted in reduced DNA repair synthesis (a measure for GG-NER), indicating that both E3 ligases are required for efficient GG-NER [49]. Future research is required

to elucidate the interplay between these different E3 ligases, DUBs and ubiquitin binding proteins to provide insight in their exact roles during NER.

## Ubiquitylation in TC-NER

### CSA and CSB

Like DDB2, CSA belongs to the family of DCAF proteins and forms an E3 ubiquitin complex with DDB1–CUL4–ROC1 (CRL4$^{CSA}$) [31]. Whereas the DDB2 containing CRL4 complex is required for proper GG-NER, the CSA containing CRL4 complex is essential for TC-NER and the subsequent transcription restart. The ubiquitin ligase activity of CRL4$^{CSA}$ is also regulated by the CSN [31]. However, CSN remains associated to the CRL4$^{CSA}$ shortly after UV-irradiation and only dissociates at later time points as compared to CRL4$^{DDB2}$ complex [31]. It was shown that CSB is polyubiquitylated and degraded 3 h after UV-irradiation, coinciding with the dissociation of the CSN from CRL4$^{CSA}$ [52]. This CSA-mediated CSB degradation was suggested to occur after repair has been completed and the subsequent CSB-clearance was proposed to allow transcription restart [52]. Next to CRL4$^{CSA}$ also the heterodimer BRCA1–BARD1 E3-ligase complex was suggested to be implicated in TC-NER [53]. Knockdown of BRCA1 resulted in a reduced ubiquitylation and degradation of CSB and inhibited CPD-repair by TC-NER [53]. The existence of two independent pathways resulting in CSB degradation might either indicate redundancy or reflect different roles for CSA and BRCA1 mediated ubiquitylation in TC-NER. The C-terminus of CSB contains an ubiquitin-associated (UBA) domain that can interact with ubiquitin, with the highest affinity for K63-linked chains [54]. Although deletion of the UBA domain does not interfere with TC-NER complex assembly, repair does not occur. Live cell imaging experiments revealed that deletion of the UBA domain completely immobilizes CSB after UV and thereby traps TC-NER complexes at the site of DNA damage, most likely blocking the TC-NER reaction [54]. This suggests that the UBA domain is involved in the release of CSB, indicating that this is a crucial step during repair. However the ubiquitylated interaction partner of the UBA domain, which most likely serves as signal for CSB dissociation, is still unknown.

### UVSSA

Next to exome sequencing of UVSS group A patient cells [55] and correction cloning by chromosome transfer to UVSS-A cells [19], the TC-NER factor UVSSA was recently identified in a quantitative proteomics screen to detect differences in protein ubiquitylation

**Fig. 1** – **The role of ubiquitin in the regulation of global genome NER. GG-NER repairs helix distorting lesions in the entire genome. UV-induced DNA damage can be recognized by DDB2 and XPC. In response to UV, DDB2 is PARylated by PARP1, which stabilizes DDB2 at the site of DNA damage. DDB2 forms an E3 ligase complex with DDB1, CUL4 and ROC1 (CRL4$^{DDB2}$). The E3 ligase activity is tightly regulated by the COP9 signalosome (CSN). Upon damage binding CSN dissociates form the complex, stimulating the neddylation of CUL4, thereby activating the E3 ligase. This results in polyubiquitylation of DDB2 and XPC. Ubiquitylated DDB2 is extracted from the CRL4$^{DDB2}$ complex by p97 and channeled into the 26S proteasome for degradation. XPC, associated with RAD23B and CEN2, binds the undamaged strand and is ubiquitylated by the CRL4$^{DDB2}$ complex, which is a signal to increase its binding affinity for damaged DNA. RAD23 is required for efficient XPC loading and contains two ubiquitin-binding domains and an ubiquitin-like domain. Upon binding of XPC to the damaged DNA, RAD23 is released from the complex. XPC is in response to UV SUMOylated, which results in the recruitment of RNF111 by its SIM domains. This SUMO-targeted E3 ligase subsequently modifies XPC with K63-linked ubiquitin chains, which is crucial for efficient NER.**

upon UV-induced DNA damage [18]. UVSSA was found to be ubiquitylated itself; however this ubiquitylation was independent on DNA damage. Further research suggested that UVSSA co-

purifies with ubiquitylated RNA pol II upon UV-damage and interacts with the TC-NER factors CSA and CSB [18,19,55]. In addition, UVSSA forms a complex with the DUB USP7 in a
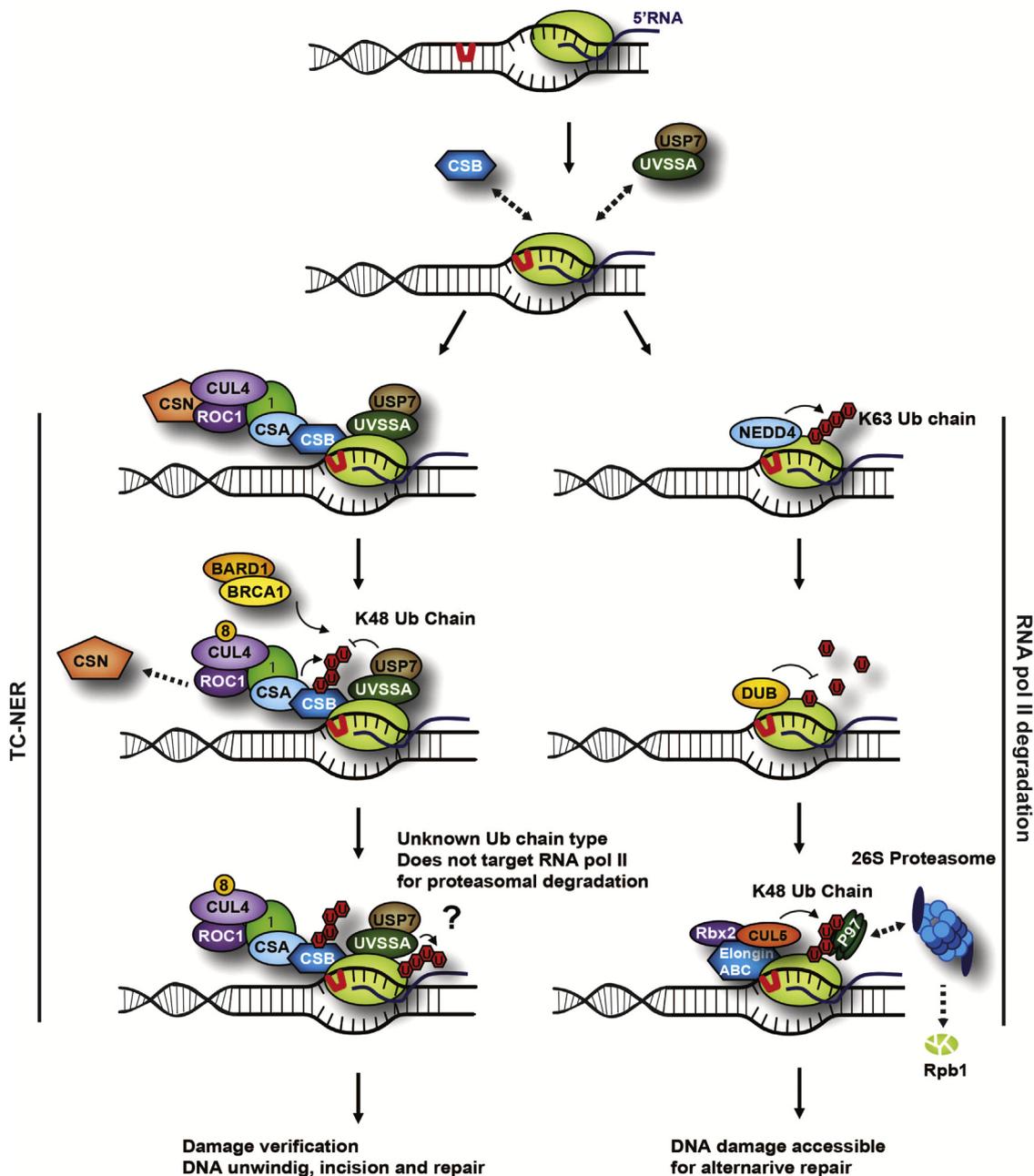


**Fig. 2** – The role of ubiquitin in the regulation of transcription coupled NER. Transcription blocking lesions are repaired by TC-NER. Upon stalling of elongating RNA pol II at the DNA lesion, it is bound by CSB and UVSSA. CSB recruits CSA, which forms an E3 ligase complex together with DDB1, CUL4 and ROC1 (CRL4$^{CSA}$) (Left panel). Upon dissociation of the CSN, the CRL4$^{CSA}$ complex is activated, resulting in ubiquitylation and proteasomal degradation of CSB. This ubiquitylation is counteracted by the DUB USP7, which is recruited to the stalled RNA polymerase by UVSSA, to stabilize CSB during the TC-NER reaction. In addition to the CRL4$^{CSA}$ complex, also other proteins are involved in the ubiquitylation of TC-NER proteins. UVSSA mediates ubiquitylation of stalled RNA pol II, the function of this event is unknown, but it does not result in proteasomal degradation of RNA pol II. Furthermore, BRCA1–BARD1 is described to ubiquitylate CSB and RNA pol II. If TC-NER fails to repair the damage, stalled RNA pol II can be degraded "as a last resort" to remove RNA pol II from the lesion (right panel). This pathway is initiated by NEDD4, which promotes K63-dependent ubiquitylation of the large subunit RPB1 of RNA pol II. These K63-linked chains are trimmed down to a mono-ubiquitylated form of RNA pol II, which is a substrate for Elongin ABC–Cul5–RBX2. This E3 ligase complex induces K48-linked ubiquitylation of RPB1, which is recognized by the p97, resulting in the extraction of the RPB1 subunit from the stalled RNA pol II, which subsequently leads to its degradation by the 26S proteasome.

UV-independent manner [18,19]. Depletion of USP7 resulted in a similar TC-NER deficiency as seen for UVSSA knockdown. In the absence of UVSSA or USP7 a strong increase in the proteasomal degradation of CSB was observed in response to UV. One of the key functions of UVSSA during TC-NER is to recruit the pleiotropic DUB USP7, which has multiple roles both in as well as outside the DNA damage response [56], to TC-NER complexes and thereby counteracting CSB ubiquitylation. Most probably this de-ubiquitylation results in an increased window of opportunity for CSB to assemble the TC-NER complexes before CSB is degraded. Together UVSSA, USP7, CSA and BRCA1 tightly regulate the fate of CSB and thereby the completion of TC-NER (Fig. 2, left side).

### RNA polymerase II

UVSSA encodes two conserved, poorly characterized domains: a C-terminal DUF2043 domain and an N-terminal Vps27–Hrs–STAM (VHS) domain both required for functional TC-NER. Like the UBA domain of CSB, the VHS domain was suggested to interact with ubiquitylated proteins. Indeed it was shown that UVSSA interacts with ubiquitylated RPB1 (the large subunit of the RNA pol II complex) [55]. While the VHS domain is crucial for its function in TC-NER, the exact biological relevance of the ubiquitin binding capacity of UVSSA remains to be discovered. Finally, while UVSSA together with USP7 plays an important role in the de-ubiquitylation of CSB, UVSSA is also important for the ubiquitylation of RPB1. In response to UV, an UVSSA-dependent ubiquitylation of RPB1 was observed, which interestingly does not target it for proteasomal degradation. Not only the precise function of this ubiquitylation event is unknown, but also the molecular mechanism how UVSSA can be responsible for this ubiquitylation needs to be uncovered.

When TC-NER cannot be executed successfully, for example due to mutations in CSA or CSB proteins, lesion-stalled RNA Pol II cannot be properly resolved. It was shown that when lesion-stalled RNA pol II cannot be efficiently processed by TC-NER, RPB1 gets poly-ubiquitylated and degraded [57]. Several ubiquitin E3 ligases were proposed to be involved in the ubiquitylation of RPB1, including CSA [58], BRCA1–BARD1 [59] and NEDD4 [60]. In CS-A and CS-B cells ubiquitylation of RPB1 is remarkably reduced [58]. Since CSA is part of an ubiquitin E3 complex, it was suggested that CSA ubiquitylates RNA pol II. However, more recently, it was shown that this defect in UV-induced RNA pol II ubiquitylation and degradation in CS-A and CS-B cells are most likely indirect and can be explained by the absence of transcription recovery in these cells [60]. In addition, it was shown that the BRCA1–BARD1 complex interacts with RNA pol II [59]. Over-expression of the BRCA1–BARD1 complex stimulated UV-induced ubiquitylation of the hyperphosphorylated form of RPB1, which results in its degradation [61]. Also RPB8, a common subunit in all three RNA polymerases, was shown to be ubiquitylated in response to UV by BRCA1–BARD1 [62]. Interestingly, ubiquitylation of RPB8 does not result in proteasomal degradation, but is implicated in UV survival. Cells expressing an ubiquitin-resistant form of RPB8 were extremely UV-sensitive, which might suggest that the ubiquitylation of RPB8 is an important step in RNA pol II disassembly or dissociation from the transcription blocking lesion. However, Anindya et al. [60] found out that only knockdown of NEDD4, but not of BRCA1, significantly reduced the RPB1-ubiquitylation and subsequent proteasomal degradation. NEDD4 co-immunoprecipitates with RNA pol II in a UV-dependent manner and is able to ubiquitylate RNA pol II in vitro [60]. Interestingly, NEDD4 is only capable of catalyzing mono- or K63 poly-ubiquitylation, which does not target the substrate protein for proteasomal degradation. This suggests that another E3 ligase must be involved. It was shown that the Elongin ABC–Cul5–RBX2 complex can form K48-linked chains on RNA pol II, however only when RPB1 is already ubiquitylated by NEDD4 [63]. In S. cerevisiae the DUB, Ubp2, is associated to Rsp5, the yeast homolog of NEDD4 and trims the K63-linked ubiquitin chains down until a mono-ubiquitin modification remains on RPB1 [63]. The mammalian ortholog of Ubp2 is currently unknown. This concerted action of the E3 ligase NEDD4 an unknown DUB and the Elongin ABC–Cul5–RBX2 complex to modify RPB1 represents a nice example of ubiquitin chain editing (Fig. 2, right side). Elongating RNA pol II is very stably associated to the DNA template even in the presence of UV-induced ubiquitylation [57]. Interestingly, in yeast cells deficient for the ATPase CDC48 K48-linked ubiquitylated RNA pol II accumulates in response to UV, as it is not degraded anymore [64]. Whether the VCP/p97 ubiquitin segregase, the mammalian ortholog of CDC48, is involved in the extraction and degradation of RPB1 in currently unknown; see for more details on the role of p97 (Dantuma et al. this issue). The balance between NEDD4, the Elongin ABC–Cul5–RBX2 complex and de-ubiquitylating enzymes determines the fate of RNA pol II, as soon the K48-linked chain becomes long enough it is recognized by VCP/p97 and stripped from the elongating complex resulting in its degradation by the proteasome. This degradation pathway of RBP1 is more common in TC-NER deficient cells, suggesting that TC-NER is the preferred pathway to deal with lesion-stalled RNA pol II and that degradation and removal of RNA pol II is an important pathway of "last resort" to overcome the highly cytotoxic blocked transcription complexes [57,58].

### Concluding remarks

In this review we summarize the multiple ubiquitylation events implicated in regulating the different damage recognition steps of NER. Ubiquitin modifications are not only important for regulating protein–protein interactions, facilitating the transition through the different NER reaction-steps, but appeared also to function in clearing repair factors from chromatin when their activity is no longer needed or when repair fails or is aborted. How can this high level and complex ubiquitin-dependent regulation of particularly GG-NER and TC-NER initiating proteins be rationalized? One obvious possibility is that NER initiation needs to be carefully regulated to prevent unwanted NER-mediated excision on non-damaged DNA. For this reason the NER reaction itself also has an important damage verification step, which is performed by the joint action of TFIIH and XPA [1]. Non-true NER lesions are not further processed and the formed NER pre-incision complex needs to be disassembled. Ubiquitin might play a crucial role in this reversible nature of the damage sensing complexes. In addition, the different ubiquitylation events during TC-NER seem to play a crucial role in pathway choice; either the reaction will proceed through the "conservative" TC-NER pathway, or when TC-NER failed a "destructive" pathway has to take over to remove the stalled RNA pol II from the DNA. It is expected that with the still advancing isolation procedures for

ubiquitylated proteins and peptides, improved quantitative proteomics and data analysis tools, new ubiquitylation targets and factors involved in NER will be identified [65]. This will give important new insights in the complex regulation of NER. Furthermore, these proteomics approaches might uncover more examples of the interplay between different PTMs on NER factors, as have been observed for ubiquitin with PAR on DDB2 [45] and with SUMO on XPC [49,51]. The current challenge in the field is not only to identify different PTMs, but mainly how these collaborate at each separable step in the repair reaction and how these PTMs facilitate proper repair. Emerging evidence suggests that the "Readers" of modified proteins, which contain specific PTM binding motifs, are important drivers of this process. A nice example is the recognition of SUMOylated XPC by the STUbL RNF111. Furthermore, it will be interesting to study which proteins are recognized by the Ub-binding domains of RAD23B, CSB and UVSSA and how these proteins are involved in repair?

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