FEBS Letters 585 (2011) 2818–2825

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

Detecting UV-lesions in the genome: The modular CRL4 ubiquitin ligase does it best!

Andrea Scrima, Eric S. Fischer, Gondichatnahalli M. Lingaraju, Kerstin Böhm, Simone Cavadini, Nicolas H. Thomä *

Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, CH-4058 Basel, Switzerland

ARTICLE INFO

Article history:
Received 31 March 2011
Revised 25 April 2011
Accepted 27 April 2011
Available online 6 May 2011

Edited by Ashok Venkitaraman and Wilhelm Just

Keywords:
Genome stability
CUL4
CDT2
DDB2
CSA

ABSTRACT

The DDB1–DDB2–CUL4–RBX1 complex serves as the primary detection device for UV-induced lesions in the genome. It simultaneously functions as a CUL4 type E3 ubiquitin ligase. We review the current understanding of this dual function ubiquitin ligase and damage detection complex. The DDB2 damage binding module is merely one of a large family of possible DDB1–CUL4 associated factors (DCAF), most of which are substrate receptors for other DDB1–CUL4 complexes. DDB2 and the Cockayne-syndrome A protein (CSA) function in nucleotide excision repair, whereas the remaining receptors operate in a wide range of other biological pathways. We will examine the modular architecture of DDB1–CUL4 in complex with DDB2, CSA and CDT2 focusing on shared architectural, targeting and regulatory principles.

1. Repair of UV-induced DNA lesions is facilitated by CUL4 type E3 ubiquitin transferases

The nucleotide excision repair (NER) pathway safeguards the genome against bulky DNA adducts and UV-light induced pyrimidine dimers [1–4]. If left unrepaired, these lesions interfere with the progression of transcription [5] and replication [6], requiring extensive post replicative repair. It only very recently emerged that NER requires targeted ubiquitination events in vivo [7]. The two major CUL4 E3 ubiquitin ligase substrate receptors in NER are the Cockayne-syndrome protein A (CSA) and the damage DNA binding protein 2 (DDB2), both of which are connected to the ligase through the DDB1 adaptor subunit. Proteomic studies have revealed that the remainder of the CUL4-DDB1 RING LIGASE (CRL4) family comprises more than fifty different substrate receptor complexes. Additionally, two closely related CUL4 human paralogs, CUL4A and CUL4B have been identified which differ mainly in a large N-terminal extension present only in CUL4B [8]. By examining three of the best characterized CRL4 ubiquitin E3 ligases, all of which function in the UV-response to damage ($CRL4^{DDB2}$, $CRL4^{CSA}$ and $CRL4^{CDT2}$), we will highlight common architectural principles and detail our current understanding of CRL4 targeting and regulation.

2. Nucleotide excision repair, an overview

NER consists of two branches that differ in the mechanism of lesion detection: in transcription coupled repair (TCR) damaged DNA bases initiate NER through RNA polymerase II stalling [9,10]; in global genome repair (GGR) the non-transcribed genome is continuously interrogated for DNA damage through specialized surveillance protein complexes including XPC-RAD23 [11,12] and DDB1–DDB2 [2]. TCR and GGR are thought to ultimately converge into a common pathway comprising: (i) local scanning for the lesion and duplex unwinding by the 10 subunit TFIIH complex [7,13], (ii) 5′ incision through the XPF-ERCC1 endonuclease [14], (iii) initiation of DNA gap re-synthesis and 3′ incision catalyzed by XPG, (iv) removal of a 24–32 bp damage containing oligonucleotide, (v) and nick ligation. Overall more than 30 polypeptides are involved in this process resulting in error free repair [15]. NER generally proceeds in a rapid fashion and does not interfere with cell cycle progression. Checkpoint activation is typically only triggered once the damage is considered too extensive to be repaired [16]. Mutations in NER components result in a number of rare autosomal recessive diseases including Xeroderma pigmentosum (XP), Cockayne syndrome (CS & XP-CS), UV-sensitive syndrome ($UV^{S}S$) and Trichothiodystrophie (TTD) [3,9,17,18]. These types of DNA repair defects are frequently associated with various forms of UV-sensitivity, neurological and development complications, and in case of XP pronounced skin cancer predisposition.

* Corresponding author.
E-mail address: nicolas.thoma@fmi.ch (N.H. Thomä).

0014-5793
doi:10.1016/j.febslet.2011.04.064

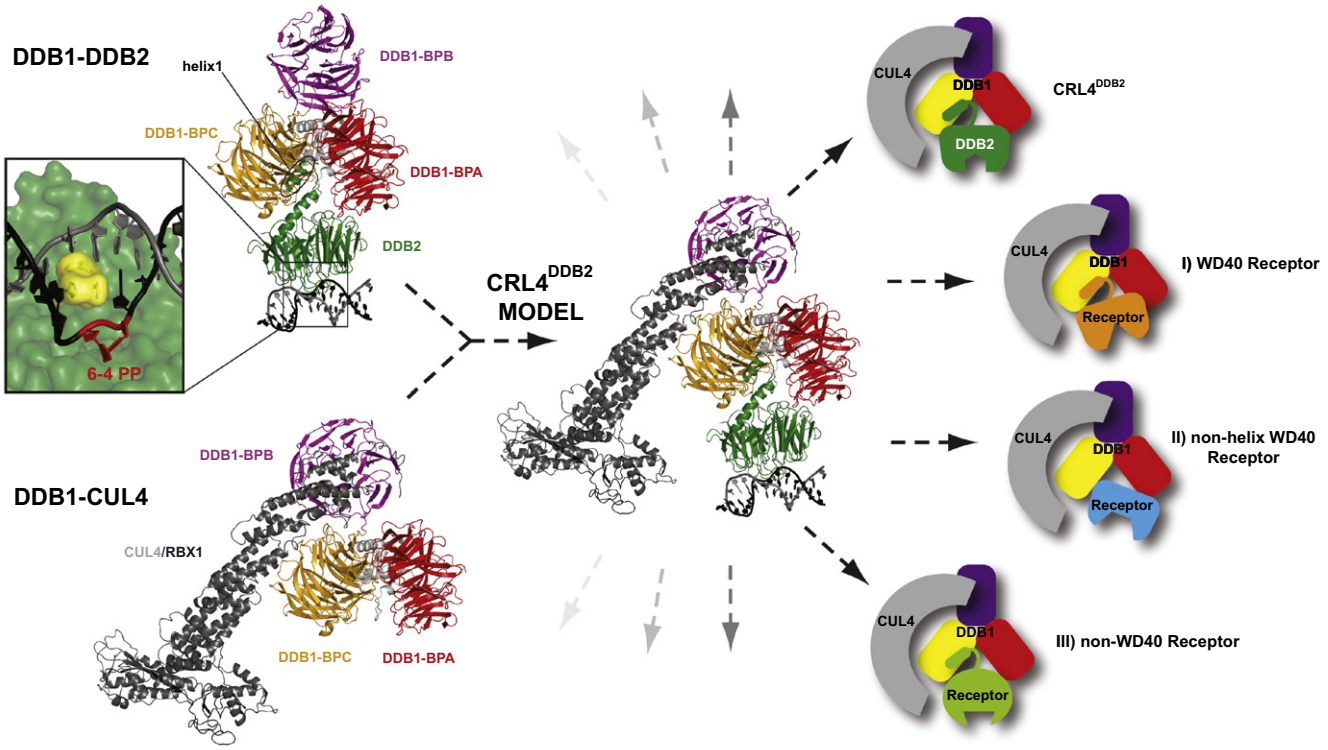

Fig. 1. Modular architecture of CRL4^{DCAF} complexes. Tentative model of the CRL4^{DDB2} complex by overlaying the DDB1–DDB2 complex bound to a 6-4PP containing oligonucleotide [30] and the DDB1-CUL4/RBX1 complex [33], assuming no overall conformational changes. The architecture of DDB1-DDB2 serves as a structural archetype for complex formation between WD40-type DCAF receptor proteins and DDB1. The overall assembly of CRL4^{DCAF} complexes and putative modes of association of (I) helix-loop-helix containing WD40-type DCAF receptors (as seen in DDB1–DDB2), (II) hypothetical assembly of non-helix WD40 DCAFs and (III) helix containing non-WD40 DCAFs (as seen in DDB1-SV5V) are depicted. Inlet panel: Structural details of UV-lesion recognition. The DDB2 (green) FQH-hairpin 'finger' (yellow) inserts into the damaged DNA duplex (grey and black) and concomitantly extrudes the 6-4 PP lesion (red) into a hydrophobic surface pocket.

3. First responders: the CRL4^{DDB2} ligase in pyrimidine dimer detection in vivo

UV-light transforms adjacent pyrimidine bases into covalent photo-dimers. The majority of these cross-links are cyclobutane-pyrimidine dimers (CPD), and to a lesser extent 6-4 pyrimidine–pyrimidone photoproducts (6-4PP) [19,20]. Detecting pyrimidine dimers within a large genome poses an exquisite challenge. The DDB1–DDB2 complex plays an important role in the initial pyrimidine dimer recognition in vivo [21–25]. Within the DDB1–DDB2 complex, the DDB2 subunit is found mutated in patients belonging to Xeroderma pigmentosum complementation group E (XP-E) [26]. Cells lacking DDB2 are substantially impaired in the repair of CPDs [27–29]. Recent X-ray crystallographic studies provided the molecular mechanism of high affinity and specificity 6-4PP recognition by DDB1–DDB2 (Fig. 1) [30]. DDB1–DDB2 largely comprises four WD40 propeller domains. The 127 kDa DDB1 protein contains three WD40-domains (BPA, BPB & BPC) [31]. The sides of the DDB1–BPA and BPC propeller domains are facing each other at an angle of ~60° and form the binding cavity for DDB2 [30]. DDB2 binding to DDB1 is mediated by the N-terminal helix-loop-helix motif preceding the DDB2 WD40 propeller [30,32]. The DDB1–DDB2 complex binds damage containing DNA duplexes exclusively through the DDB2 WD40 propeller domain. The DDB1–BPB domain, which is located on the opposing face of the DDB1–DDB2 module, provides the attachment site for the cullin4 ubiquitin ligase subunit [33] (Fig. 1). In vivo, DDB1–DDB2 exists in complex with both CUL4A-RBX1 and CUL4B-RBX1 paralogs [34–37]. Architecturally, DDB1 acts as an adaptor linking the E3 ubiquitin ligase (CUL4) to the UV-damage detection module (DDB2). The protein complex specialized for the recognition of pyrimidine dimers in human cells thus doubles as an E3 ubiquitin ligase complex.

4. The mechanism of pyrimidine dimer recognition: showing DNA damage the damage recognition finger

DDB2 utilizes a conserved tri-peptide Phe-Gln-His (FQH) hairpin to interrogate the duplex for damage. This hairpin forms a surprisingly rigid unit that inserts into the minor groove, at the lesion, in a finger like fashion. Concomitant with insertion of the damage recognition finger, the lesion is flipped out and stabilized in a hydrophobic pocket present at the DDB2 surface (Fig. 1). This pocket serves to restrict the size of the modification accommodated, biasing DDB2 towards photo-dimer recognition and preventing larger base adducts from being bound. Co-crystallisation of DDB1–DDB2 with a single-nucleotide abasic site (AP) lesion, embedded in a duplex, revealed an almost identical dual base pair flip of the abasic site and the adjacent 3′ undamaged base. This is surprising, as unlike in the case of 6-4PP, only one base is damaged in the AP containing duplex. The damage recognition finger, which spans exactly two nucleotides, therefore inherently triggers

a di-nucleotide flip upon insertion. This flip is independent of whether the adjacent 3′ base is modified or not [30]. Contrary to the common notion, DDB2 does not appear to recognize the 'helix distortion of the lesions' per se, as the DDB2 bound DNA conformation differs significantly from those of damaged duplexes free in solution. DDB2s therefore rather tests whether the damage recognition 'finger' can be inserted into the duplex, assessing the DNA conformation around the damage, and examines whether the DNA can fit to the rigid DDB2 binding 'mold'. The structural basis of high affinity CPD recognition, the biological role of the DDB2 in which it excels above all other known human damage detection factors, currently remains elusive.

5. UV-lesion detection in chromatin: the missing link

The challenging task of detecting UV-lesions within vast genomes is further compounded by the presence of chromatin. In chromatin, the DNA is wrapped around an octamer of core histones, with additional linker histones implicated in further compaction. In vivo, the position of the photo-dimers respective to the nucleosome core particle depends on the kind of lesion present: the strongly duplex distorting 6-4PP is largely found randomly localized in nucleosomes and linkers [38], while the highly mutagenic and difficult to detect CPD clusters are found in surface exposed regions of nucleosome [39]. How then are these lesions detected and repaired within nucleosomes? While the global genome repair branch of NER can be effectively reconstituted on naked DNA, the presence of nucleosomes was clearly inhibitory for NER repair in vitro [40]. NER inhibition occurred on multiple levels including damage recognition [41,42]. Nucleosome remodeling complexes can function as a principal means to remove nucleosomes, providing NER with a DNA substrate that more closely resembles the naked DNA. Several chromatin remodeling complexes have been implicated in NER. Cells in which chromatin remodelers Ino80 [43] and SWI/SNF (Brg1) [44,45] have been deleted become UV-sensitive. While remodelers offer a potential solution to facilitate downstream repair processes, they are unlikely to provide a means to directly find the damage. How then is damage being read out in chromatin? Recent studies focusing on nucleosome dynamics in the presence and absence of damage indicated that the DNA around the octamer core unwraps leaving proteins sufficient time (and room) to gain access to the lesion [46,47]. In vivo, DDB2 localizes to chromatin in a UV-dependent manner [48,49] and remains associated with mono-nucleosomes upon treatment with micrococcal nuclease [35]. DDB1–DDB2 is therefore a likely candidate for recognizing pyrimidine dimers embedded in nucleosome core particles. The direct interactions between NER damage detection factors including DDB1–DDB2, XPC/Rad4 and chromatin remodelers has been described [43,45,50] offering a principal means to recruit remodelers and facilitate NER in an otherwise repressive chromatin environment.

6. DDB1–DDB2 mediated histone ubiquitination surrounding the sites of damage

Additional evidence implicating DDB1–DDB2–CUL4–RBX1 ($CRL4^{DDB2}$) in damage recognition in chromatin came from studies reporting $CRL4^{DDB2}$ dependent ubiquitination of histones H2A, H3 and H4 in response to UV-irradiation [51–53]. This histone ubiquitination response appeared to be largely mono-ubiquitination. In vitro, CUL4 mediated ubiquitination gives rise to poly-ubiquitin chains. Whether a de-ubiquitination enzyme, a specific E2 transferase, or a regulatory protein serves to restrict the extent of ubiquitination in vivo, is currently not known. $CRL4^{DDB2}$ mediated ubiquitination of histones is likely to be local and restricted to the immediate ~100 Å vicinity of the lesion [30,54]. In vitro, histone ubiquitination by $CRL4^{DDB2}$ altered the stability of the nucleosome core driving partial histone eviction [53]. Histone ubiquitination with concomitant destabilisation of neighbouring nucleosomes thus offers an additional mechanism to evict histones and drive assembly of the NER machinery in a chromatin environment (Fig. 2) [30,55]. Mono-ubiquitination of histones could, in addition, function as a recruitment signal for additional auxiliary repair factors (see below).

7. Ubiquitination overseeing damage handover from DDB2 to XPC

$CRL4^{DDB2}$ ubiquitination has been implicated in resolving one of the central conundrums of NER damage recognition [25]: while DDB1–DDB2 has the highest affinity for UV-induced photo-dimers in vitro, and appears to be the first protein complex at the lesion in vivo [23,24,41], it is dispensable in vitro [15]. XPC, in contrast binds 6-4PP with two orders of magnitude lower affinity than DDB2 [25,56,57] and has no discernable affinity for CPD, yet is essential for NER both in vitro and in cells. As DDB1–DDB2 is required for efficient XPC recruitment to chromatin [23], the question arises as to how damage is handed over from DDB2 to XPC. Recent work demonstrated that $CRL4^{DDB2}$ targets XPC, as well as DDB2 for ubiquitination in a UV-dependent fashion [25]. Poly-ubiquitination of DDB2 ablates DNA binding by DDB1–DDB2 and results in proteasome mediated DDB2 degradation [58,59]. Poly-ubiquitination of XPC, on the other hand, does not appear to affect DNA damage binding [25] and XPC is protected from immediate proteasomal degradation, likely through association with RAD23 [60–62]. This and further studies [63] place DDB1–DDB2 in the recruitment of XPC to the sites of damage in chromatin, with a subsequent hand-over of the lesion from DDB2 to XPC in an ubiquitin dependent manner [7,30]. XPC thereby emerges as an indispensable core component of NER, which in vivo does, however, require assistance from DDB1–DDB2 in finding specific lesions. The exact molecular nature of the damage handover complex from DDB2 to XPC remains elusive. The damage probing hairpin of both DDB2 (hsDDB2: ^{334}FQH^{336}) and XPC (hsXPC: ^{799}FHGGYS^{804}) cannot simultaneously engage with the lesion damage due to large steric clashes [30,64]. Three, mutually non-exclusive possibilities for such a hand-over complex have been considered: (i) XPC binding, via the TGD domain, to the undamaged duplex 3′ of the lesion with DDB2 engaging the pyrimidine dimer; (ii) XPC attaching to DDB2 through protein–protein interaction [25], and (iii) in a more indirect fashion XPC recruitment by ubiquitination (for example of the histone or DDB2) as the RAD23 subunit also carries a UBA domain [65]. In the latter mechanism, in particular, $CRL4^{DDB2}$ could recruit XPC to lesions such as CPDs, for which XPC has no measurable affinity by itself, yet is required for repair in vivo. Interpretation of the role of CRL4 mediated ubiquitination in NER has been complicated [66], also by the finding that mice carrying a *Cul4a* deletion in skin cells are less likely to develop UV-induced skin cancers and appear more repair proficient [67]. As CUL4A is involved in many different cellular pathways regulated in response to UV, such as the $CRL4^{CDT2}$-p21 controlled by UV cell cycle checkpoint, it is currently unclear which CRL4 substrate receptor and pathway is responsible for the mouse cancer phenotype.

8. A related DDB1-CSA-CUL4 ligase in Cockayne syndrome

A general feature of DDB1–CUL4 cullin E3 ligases is their modularity (Fig. 1). Largely through proteomic studies a number of DDB1–CUL4 associated proteins have been identified [33,68–70,99,116]. The family of these proteins is known as DCAFs

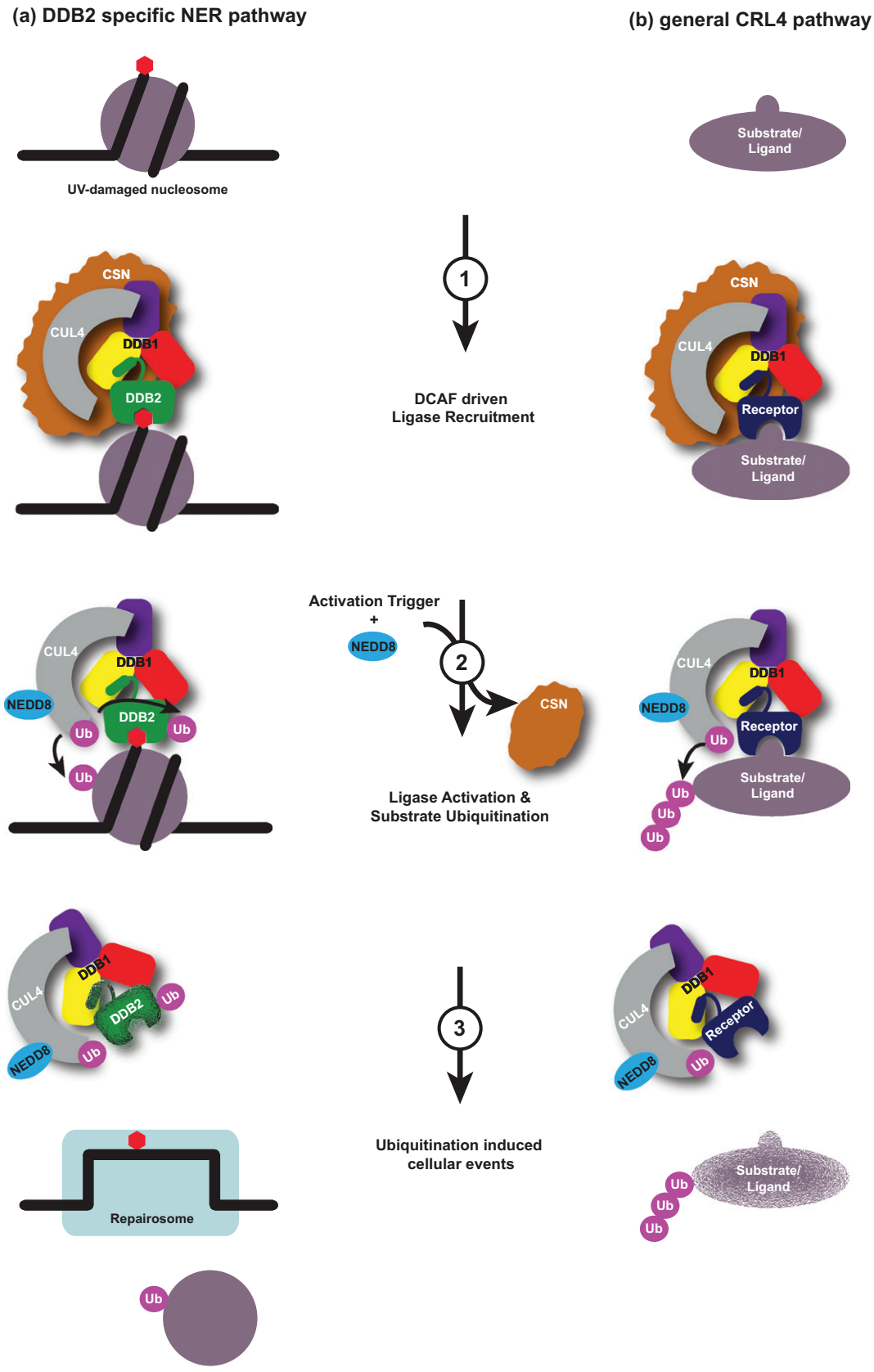

Fig. 2. Regulation of CRL4^{DCAF} complexes. a) DDB2 specific NER pathway: (1) Recruitment of inactive CSN-CRL4^{DDB2} to the nucleosome embedded lesion. (2) Ubiquitin ligase activation by CSN release and CUL4 neddylation; subsequent ubiquitination of nearby proteins, including DDB2 auto-ubiquitination, XPC and histones. (3) Ubiquitination induced eviction of the histone octamer facilitating recruitment of the NER-repairosome and initiation of damage repair. Proteasomal degradation of ubiquitinated DDB2 is implicated in CRL4^{DDB2} release and damage handover to downstream NER factors. (b) General model for CRL4^{DCAF} regulation: (1) Recruitment of inactive CSN-CRL4^{DCAF} through DCAF receptor proteins. (2) Release of CSN with subsequent CUL4 neddylation and ligase activation resulting in substrate ubiquitination. (3) Pathway specific induction of coordinated cellular events, such as proteasomal degradation of the substrate protein, relocalisation or recruitment of interaction partners.

(DDB1-CUL4-associated-factor) [33], DWD-proteins (DDB1-binding and WD40-repeat) [69] or CDW-proteins (CUL4- and DDB1-associated WDR proteins) [70]. These DCAFs fall in two potential categories: substrate receptors recruiting CRL4 complexes and regulators of CUL4 function. DDB2 thereby is one out of more than fifty possible substrate receptors described. DDB2 recruits the ligase to the sites of damage, whereas the majority of these CUL4-DDB1 ligase receptors, including CSA [71], are likely to recognize protein epitopes, or posttranslational modifications of proteins. In a 'plug and play' fashion these receptors can be exchanged, customizing CRL4 substrate specificity to different pathways (Fig. 1).

9. The role of the Cockayne syndrome A protein (CSA) in transcription coupled repair

We will first examine the Cockayne syndrome A $CRL4^{CSA}$ ligase complex involved in transcription coupled repair of damages located on the actively transcribed strand [4,9,72–74]. Cockayne syndrome is a rare autosomal disease with mutations in two proteins CSA [75] and CSB [76]. The hallmarks of Cockayne syndrome include developmental defects, photosensitivity, segmental premature aging and mental retardation [3,77]. While CSA is integrated in a $CRL4^{CSA}$ ubiquitin ligase complex [35], CSB functions as SWI/SNF chromatin remodeler [78]. CSA and CSB both appear intertwined with the general transcription machinery [13]. Mutations in CSA and CSB are indistinguishable on the patient level arguing for a common pathway. Upon RNA polymerase II (RNAPII) stalling, both CSA and CSB are required for repair and transcription restart. CSB has been implicated in the remodeling of stalled RNAPII complexes to which it binds tightly in the presence of damage [79,80]. CSA is translocated to the nucleus, in a CSB-dependent manner, and co-localizes with CSB at sites of stalled RNAPII [81]. Arrival of CSA is required for recruitment of HMGN1 (high mobility group nucleosome-binding domain-containing protein 1), XAB2 (XPA-binding protein 2) and transcript elongation factor TFIIS [82].

Five lines of evidence suggest the involvement of the ubiquitin proteasome system in TCR: (i) CSA is constitutively found in complex with DDB1 and CUL4 [35], (ii) ubiquitination of CSB was observed under high UV conditions, with proteasome dependent CSB degradation during later stages of TCR [71], (iii) UV-dependent CSB degradation is absent in cells lacking CSA but can be restored by exogenous CSA, and (iv) the DDB1-CSA-CUL4A ligase was capable of CSB ubiquitination in vitro; (v) finally, CSB has a ubiquitin binding (UBA) domain which was found essential for CSB function in TCR [83]. The structure and architecture of the $CRL4^{CSA}$ complex is currently unknown. The current data is consistent with CSB functioning as a $CRL4^{CSA}$ recruitment and ubiquitination substrate [71]. Other, as yet unidentified targets cannot be ruled out at present, however. While the $CRL4^{DDB2}$ and $CRL4^{CSA}$ ligase complexes differ substantially in respect to their function, there are interesting mechanistic parallels: the respective main targets of $CRL4^{DDB2}$ and $CRL4^{CSA}$, XPC-RAD23 and CSB both carry UBA domains believed to play important regulatory roles in NER. What these UBA domains recognise and what functional consequences UBA target binding has, remains an active area of research.

10. The role of DDB1-CDT2-CUL4 in genomic stability and beyond

DDB1-CDT2-CUL4 ($CRL4^{CDT2}$), a third essential CUL4 ligase, oversees the S/G2 cell cycle transitions through degradation of the replication licensing factor CDT1 [84–86]. Additional human targets include the cell cycle regulator p21 [87,88] and the histone methyltransferase SET8/Pr-SET7 [89–92]. Degradation of these substrates proceeds in a DNA replication and UV-dependent manner. In *Schizosaccharomyces pombe* $CRL4^{Cdt2}$ has been implicated in the ubiquitination of Spd1, a ribonucleotide reductase inhibitor [93] and Epe1 [94], which assists in the sculpting of heterochromatic boundaries. In most cases examined, CDT2 has been shown to interact with its substrates through a conserved, PIP (PCNA-interacting peptide) box containing degron motif in a manner dependent on the proliferation cell nuclear antigen (PCNA) [86,95–100]. The requirement of two polypeptides (PCNA + PIP containing target) for substrate recognition is intriguing, and might suggest that CDT2 uses PCNA as an additional level of proofreading in proper substrate selection.

11. CSN functions as a master regulator of cullin type E3 ligases

The COP9 signalosome (CSN) has been reported to play a central role in the regulation of all cullin E3 ligases (Fig. 2) [35,101]. Similar to other cullin-RING E3 ligases [102], the CUL4 E3 ligase is activated through attachment of NEDD8, a small ubiquitin-like modifier [35,54]. The removal of NEDD8 from cullins is catalyzed through the metallo-isopeptidase activity of the COP9 signalosome (CSN) [103,104]. CSN is an eight subunit, ~350 kDa protein complex conserved in all eukaryotes [35,105–107]. CSN shares significant sequence and structural homology with the components of the 19S proteosome lid. The first three-dimensional EM structure of the CSN complex at 25 Å resolution shows a central cleft along with two CSN segments corresponding to CSN1/2/3/8 and CSN4/5/6/7 [108–110]. The exact mode of cullin binding to CSN is currently not known.

12. CSN a master regulator of ubiquitin ligase, challenges for regulation

Detailed understanding of CSN regulation is complicated by the observed discrepancies between biochemical and genetic properties of the complex. Gene deletion studies, for example, demonstrated that *S. pombe csn1*$^-$ and *csn2*$^-$ strains are sensitive to UV and ionizing radiation, along with a slow DNA replication phenotype [111,112]. The catalytic *csn5* deletion mutant, in contrast, did not display this pronounced phenotype [111], suggesting that the CSN function extends beyond catalytic cullin de-neddylation through the CSN5 isopeptidase activity. Intriguingly, despite being a master regulator of all cullins, CSN appears nevertheless able to differentially regulate CRL4 ligases in response to a common stimulus, such as UV: in the absence of UV-damage, CSN is associated with the un-neddylated $CRL4^{DDB2}$ and $CRL4^{CSA}$ complexes. Upon UV-damage, CSN dissociates from $CRL4^{DDB2}$ [35,49] allowing its neddylation. At later time points, CSN de-neddylates and re-associates with the $CRL4^{DDB2}$ complex [113]. In TCR, on the other hand, $CRL4^{CSA}$ complexed to CSN rapidly locates to the damage site upon UV-irradiation [35], and only dissociates at a much later time point. The PCNA-dependent ubiquitin-mediated proteolysis of CDT1 by $CRL4^{CDT2}$ for example also proceeds in response to UV, similarly mediated by CSN [100,114]. The open question currently is whether CSN can selectively regulate defined CRL4-substrate receptor complexes in response to a given stimulus, while not interfering with the remainder of cullin-CSN complexes, and if so how it does achieve this kind of mechanistic specificity? Specialized CSN release CRL4 factors may exist regulating CSN release in response to cellular signals [49].

13. General principles of DDB1-DCAF-CUL4 architecture and regulation

What general architectural, targeting and regulatory principles can we deduce from these CRL4 complexes?

13.1. Architecture

All structurally characterized DDB1–DCAF complexes [32] utilize a helical motif in binding DDB1. This motif structurally resembles DDB2 helix1. Helix1 equivalents, although only moderately conserved, have been identified in the protein sequence of several DCAF proteins and are referred to as DDB-box (devoted to DDB1 binding) [115]. A number of WD40 containing DCAFs, however, do not appear to contain recognizable helical domains. It is currently unclear if those helices simply escape detection or whether fundamentally different DDB1 binding modes exist (Fig. 1). When comparing different proteomic studies identifying DCAFs, a common set of about 20 WD40 containing DCAFs, have been consistently identified [33,68–70,99,116], most of those appear to have helical motifs that could be used for DDB1 binding. Currently, there is no direct evidence for DDB1 binding mediated by WDXR motifs equivalent to the WDXR motif present in DDB2. As seen in the DDB1–DDB2 structure, The WDXR motif is not part of the DDB1 interface or the DNA binding interface [30]. Yet mutation of the WDXR motif (R273H) in DDB2 gives rise to a mutant protein that is no longer able to bind to DNA damage, likely due to local unfolding of the propeller [57]. By analogy, it should thus be considered that mutation of the corresponding WDXR in other DCAFs could also indirectly ablate DDB1 binding through interference with WD40 folding, leaving the possibility that WDXR is not necessarily part of the DDB1-DCAF interface. Additional DDB1-DCAF structures are required to resolve this issue.

13.2. WD40 containing and non-WD40 containing DCAFs

The majority of DCAFs comprise WD40 propeller domains. We propose that those DCAFs who have helical elements preceding the WD40 propeller bind DDB1 in a manner resembling the DDB1–DDB2 complex. Other proteins have been classified as DCAFs that do not contain WD40 propeller domains. They often do have helical elements, nevertheless, and likely bind DDB1 using those motifs (Fig. 1). Their mode of DDB1 attachment is likely to be equivalent to that seen in the SV5V-DDB1 [31] and DDB2 (helix1)-DDB1 structures.

13.3. Substrate recognition and ubiquitination

In case of WD40 containing DCAFs, the WD40 propeller is used for recruiting the CRL4 complex to the ubiquitination target. The ligand binding site of this WD40 propeller is expected to be located at the narrow face of the WD40 propeller cone, pointing away from DDB1 [30] (Fig. 1). This ligand, which recruits the CRL4 complex via the WD40 of the substrate receptor, however, does not necessarily have to be the target that undergoes ubiquitination (see DDB2). The ligase is likely to be able to span distances up to 100 Å. The recruiting ligand and the ubiquitination target might therefore also be separate proteins/ligands (Fig. 2).

13.4. Regulation

As outlined above, the CRL4 family is likely to be under the control of the signalosome (Fig. 2). Substrate binding to the WD40 DCAF could also be regulated through post-translational modification, as is commonly observed in the CUL1 family of targets [117]. For example, substrates might require phosphorylation prior to $CRL4^{DCAF}$ binding [118], with phosphorylation being the key determinant for binding/regulation. As is already evident in case of $CRL4^{CDT2}$, more complicated substrate binding schemes appear in operation, ensuring tight regulation of the ligase function. Additionally, a number of DDB1 binding proteins have been identified, for example DET1 [119] and DDA1 [116], which might have a regulatory role rather than serving as a substrate receptor.

Within the large CUL4 family [36], the $CRL4^{DDB2}$ ligase is currently the best understood representative in respect to its structure, function and regulation. While DDB2 recognizes damaged DNA as a recruiting substrate, the majority of the remaining receptors likely recognizes protein epitopes (or posttranslational modification thereof). More work will be needed to define what these epitopes are and how ubiquitination is regulated in these $CRL4^{DCAF}$ ligases. Furthermore, we will need to understand the functional role of the plethora of DDB1-CUL4 associated factors, which do not function as substrate receptors. Knowledge of CRL4 targeting and regulation is expected to significantly improve our understanding of the various biological pathways these proteins operate in.

Acknowledgements

A.S. was supported by an EMBO LTF and Ambizione by the Swiss National Science Foundation. Work in the laboratory is supported by the Novartis Research Foundation, an ERC young investigator grant and grants by the Swiss National Science Foundation and OncoSuisse.

References

[1] Bergink, S., Jaspers, N.G.J. and Vermeulen, W. (2007) Regulation of UV-induced DNA damage response by ubiquitylation. DNA Repair (Amsterdam) 6, 1231–1242.
[2] Wittschieben, B. and Wood, R. (2003) DDB complexities. DNA Repair (Amsterdam) 2, 1065–1069.
[3] Hoeijmakers, J.H. (2009) DNA damage, aging, and cancer. N. Engl. J. Med. 361, 1475–1485.
[4] Friedberg, E.C. (2001) How nucleotide excision repair protects against cancer. Nat. Rev. Cancer 1, 22–33.
[5] Brueckner, F., Hennecke, U., Carell, T. and Cramer, P. (2007) CPD damage recognition by transcribing RNA polymerase II. Science 315, 859–862.
[6] Lopes, M., Foiani, M. and Sogo, J.M. (2006) Multiple mechanisms control chromosome integrity after replication fork uncoupling and restart at irreparable UV lesions. Mol. Cell 21, 15–27.
[7] Sugasawa, K. (2010) Regulation of damage recognition in mammalian global genomic nucleotide excision repair. Mutat. Res. 685 (1–2), 29–37.
[8] Kerzendorfer C, Hart L, Colnaghi R, Carpenter G, Alcantara D, Outwin E, Carr AM & O'Driscoll M (2011) CUL4B-deficiency in humans: Understanding the clinical consequences of impaired Cullin 4-RING E3 ubiquitin ligase function. *Mech. Ageing Dev.*, in press, doi:10.1016/j.mad.2011.02.003.
[9] Hanawalt, P.C. and Spivak, G. (2008) Transcription-coupled DNA repair: two decades of progress and surprises. Nat. Rev. Mol. Cell Biol. 9, 958–970.
[10] Fousteri, M. and Mullenders, L.H.F. (2008) Transcription-coupled nucleotide excision repair in mammalian cells: molecular mechanisms and biological effects. Cell Res. 18, 73–84.
[11] Sugasawa, K. (2008) XPC: its product and biological roles. Adv. Exp. Med. Biol. 637, 47–56.
[12] Clement, F.C., Camenisch, U., Fei, J., Kaczmarek, N., Mathieu, N. and Naegeli, H. (2010) Dynamic two-stage mechanism of versatile DNA damage recognition by xeroderma pigmentosum group C protein. Mutat. Res. 685, 21–28.
[13] Lainé, J.-P. and Egly, J.-M. (2006) When transcription and repair meet: a complex system. Trends Genet. 22, 430–436.
[14] Staresincic, L., Fagbemi, A.F., Enzlin, J.H., Gourdin, A.M., Wijgers, N., Dunand-Sauthier, I., Giglia-Mari, G., Clarkson, S.G., Vermeulen, W. and Schärer, O.D. (2009) Coordination of dual incision and repair synthesis in human nucleotide excision repair. EMBO J. 28, 1111–1120.
[15] Aboussekhra, A., Biggerstaff, M., Shivji, M.K., Vilpo, J.A., Moncollin, V., Podust, V.N., Protić, M., Hübscher, U., Egly, J.M. and Wood, R.D. (1995) Mammalian DNA nucleotide excision repair reconstituted with purified protein components. Cell 80, 859–868.
[16] Giannattasio, M., Follonier, C., Tourrière, H., Puddu, F., Lazzaro, F., Pasero, P., Lopes, M., Plevani, P. and Muzi-Falconi, M. (2010) Exo1 competes with repair synthesis, converts NER intermediates to long ssDNA gaps, and promotes checkpoint activation. Molecular Cell 40, 50–62.
[17] Rapić-Otrin, V., Navazza, V., Nardo, T., Botta, E., McLenigan, M., Bisi, D.C., Levine, A.S. and Stefanini, M. (2003) True XP group E patients have a defective UV-damaged DNA binding protein complex and mutations in DDB2 which reveal the functional domains of its p48 product. Hum. Mol. Genet. 12, 1507–1522.
[18] Gillet, L.C.J. and Schärer, O.D. (2006) Molecular mechanisms of mammalian global genome nucleotide excision repair. Chem. Rev. 106, 253–276.

[19] Ravanat, J.L., Douki, T. and Cadet, J. (2001) Direct and indirect effects of UV radiation on DNA and its components. J. Photochem. Photobiol. B, Biol. 63, 88–102.

[20] Cadet, J., Voituriez, L., Grand, A., Hruska, F.E., Vigny, P. and Kan, L.S. (1985) Recent aspects of the photochemistry of nucleic acids and related model compounds. Biochimie 67, 277–292.

[21] Chu, G. and Chang, E. (1988) Xeroderma pigmentosum group E cells lack a nuclear factor that binds to damaged DNA. Science 242, 564–567.

[22] Keeney, S., Chang, G.J. and Linn, S. (1993) Characterization of a human DNA damage binding protein implicated in xeroderma pigmentosum E. J Biol Chem 268, 21293–21300.

[23] Fitch, M., Nakajima, S., Yasui, A. and Ford, J. (2003) In vivo recruitment of XPC to UV-induced cyclobutane pyrimidine dimers by the DDB2 gene product. J. Biol. Chem. 278, 46906–46910.

[24] Moser, J., Volker, M., Kool, H., Alekseev, S., Vrieling, H., Yasui, A., van Zeeland, A. and Mullenders, L. (2005) The UV-damaged DNA binding protein mediates efficient targeting of the nucleotide excision repair complex to UV-induced photo lesions. DNA Repair (Amsterdam) 4, 571–582.

[25] Sugasawa, K., Okuda, Y., Saijo, M., Nishi, R., Matsuda, N., Chu, G., Mori, T., Iwai, S., Tanaka, K., Tanaka, K., et al. (2005) UV-induced ubiquitylation of XPC protein mediated by UV–DDB–ubiquitin ligase complex. Cell 121, 387–400.

[26] Tang, J. and Chu, G. (2002) Xeroderma pigmentosum complementation group E and UV-damaged DNA-binding protein. DNA Repair (Amsterdam) 1, 601–616.

[27] Hwang, B.J., Ford, J.M., Hanawalt, P.C. and Chu, G. (1999) Expression of the p48 xeroderma pigmentosum gene is p53-dependent and is involved in global genomic repair. Proc. Natl. Acad. Sci. U.S.A. 96, 424–428.

[28] Bohr, V.A., Smith, C.A., Okumoto, D.S. and Hanawalt, P.C. (1985) DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. Cell 40, 359–369.

[29] Tang, J.Y., Hwang, B.J., Ford, J.M., Hanawalt, P.C. and Chu, G. (2000) Xeroderma pigmentosum p48 gene enhances global genomic repair and suppresses UV-induced mutagenesis. Mol Cell 5, 737–744.

[30] Scrima, A., Konickova, R., Czyzewski, B.K., Kawasaki, Y., Jeffrey, P.D., Groisman, R., Nakatani, Y., Iwai, S., Pavletich, N.P. and Thoma, N.H. (2008) Structural basis of UV DNA-damage recognition by the DDB1–DDB2 complex. Cell 135, 1213–1223.

[31] Li, T., Chen, X., Garbutt, K.C., Zhou, P. and Zheng, N. (2006) Structure of DDB1 in complex with a paramyxovirus V protein: viral hijack of a propeller cluster in ubiquitin ligase. Cell 124, 105–117.

[32] Li, T., Robert, E.I., van Breugel, P.C., Strubin, M. and Zheng, N. (2010) A promiscuous alpha-helical motif anchors viral hijackers and substrate receptors to the CUL4-DDB1 ubiquitin ligase machinery. Nat Struct Mol Biol 17, 105–111.

[33] Angers, S., Li, T., Yi, X., MacCoss, M.J., Moon, R.T. and Zheng, N. (2006) Molecular architecture and assembly of the DDB1-CUL4A ubiquitin ligase machinery. Nature 443, 590–593.

[34] Alekseev, S., Luijsterburg, M.S., Pines, A., Geverts, B., Mari, P.O., Giglia-Mari, G., Lans, H., Houtsmuller, A.B., Mullenders, L.H., Hoeijmakers, J.H., et al. (2008) Cellular concentrations of DDB2 regulate dynamic binding of DDB1 at UV-induced DNA damage. Mol. Cell Biol. 28, 7402–7413.

[35] Groisman, R., Polanowska, J., Kuraoka, I., Sawada, J., Saijo, M., Drapkin, R., Kisselev, A., Tanaka, K. and Nakatani, Y. (2003) 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.

[36] Jackson, S. and Xiong, Y. (2009) CRL4s: the CUL4-RING E3 ubiquitin ligases. Trends Biochem. Sci. 34, 562–570.

[37] Shiyanov, P., Nag, A. and Raychaudhuri, P. (1999) Cullin 4A associates with the UV-damaged DNA-binding protein DDB. J. Biol. Chem. 274, 35309–35312.

[38] Gale, J.M. and Smerdon, M.J. (1990) UV induced (6-4) photoproducts are distributed differently than cyclobutane dimers in nucleosomes. Photochem. Photobiol. 51, 411–417.

[39] Gale, J.M., Nissen, K.A. and Smerdon, M.J. (1987) UV-induced formation of pyrimidine dimers in nucleosome core DNA is strongly modulated with a period of 10.3 bases. Proc. Natl. Acad. Sci. U.S.A. 84, 6644–6648.

[40] Gong, F., Kwon, Y. and Smerdon, M.J. (2005) Nucleotide excision repair in chromatin and the right of entry. DNA Repair (Amsterdam) 4, 884–896.

[41] Yasuda, T., Sugasawa, K., Shimizu, Y., Iwai, S., Shiomi, T. and Hanaoka, F. (2005) Nucleosomal structure of undamaged DNA regions suppresses the non-specific DNA binding of the XPC complex. DNA Repair (Amsterdam) 4, 389–395.

[42] Thoma, F. (2005) Repair of UV lesions in nucleosomes–intrinsic properties and remodeling. DNA Repair (Amsterdam) 4, 855–869.

[43] Jiang, Y., Wang, X., Bao, S., Guo, R., Johnson, D.G., Shen, X. and Li, L. (2010) INO80 chromatin remodeling complex promotes the removal of UV lesions by the nucleotide excision repair pathway. Proc. Natl. Acad. Sci. U.S.A. A107, 17274–17279.

[44] Zhang, L., Zhang, Q., Jones, K., Patel, M. and Gong, F. (2009) The chromatin remodeling factor BRG1 stimulates nucleotide excision repair by facilitating recruitment of XPC to sites of DNA damage. Cell Cycle 8 (23), 3953–3959.

[45] Zhao, Q., Wang, Q.E., Ray, A., Wani, G., Han, C., Milum, K. and Wani, A.A. (2009) Modulation of nucleotide excision repair by mammalian SWI/SNF chromatin-remodeling complex. J. Biol. Chem. 284, 30424–30432.

[46] Duan, M.-R. and Smerdon, M.J. (2010) UV damage in DNA promotes nucleosome unwrapping. J. Biol. Chem. 285, 26295–26303.

[47] Li, G., Levitus, M., Bustamante, C. and Widom, J. (2005) Rapid spontaneous accessibility of nucleosomal DNA. Nat. Struct. Mol. Biol. 12, 46–53.

[48] Otrin, V.R., McLenigan, M., Takao, M., Levine, A.S. and Protic, M. (1997) Translocation of a UV-damaged DNA binding protein into a tight association with chromatin after treatment of mammalian cells with UV light. J. Cell Sci. 110, 1159–1168. Pt 10.

[49] Takedachi, A., Saijo, M. and Tanaka, K. (2010) DDB2 complex-mediated ubiquitylation around DNA damage is oppositely regulated by XPC and Ku and contributes to the recruitment of XPA. Mol. Cell Biol. 30, 2708–2723.

[50] Gong, F., Fahy, D. and Smerdon, M.J. (2006) Rad4-Rad23 interaction with SWI/SNF links ATP-dependent chromatin remodeling with nucleotide excision repair. Nat. Struct. Mol. Biol. 13, 902–907.

[51] Guerrero-Santoro, J., Kapetanaki, M., Hsieh, C., Gorbachinsky, I., Levine, A. and Rapic-Otrin, V. (2008) The cullin 4B-based UV-damaged DNA-binding protein ligase binds to UV-damaged chromatin and ubiquitinates histone H2A. Cancer Res. 68, 5014–5022.

[52] Kapetanaki, M.G., Guerrero-Santoro, J., Bisi, D.C., Hsieh, C.L., Rapić-Otrin, V. and Levine, A.S. (2006) The DDB1-CUL4ADDB2 ubiquitin ligase is deficient in xeroderma pigmentosum group E and targets histone H2A at UV-damaged DNA sites. Proc. Natl. Acad. Sci. U.S.A. 103, 2588–2593.

[53] Wang, H., Zhai, L., Xu, J., Joo, H., Jackson, S., Erdjument-Bromage, H., Tempst, P., Xiong, Y. and Zhang, Y. (2006) Histone H3 and H4 ubiquitylation by the CUL4-DDB-ROC1 ubiquitin ligase facilitates cellular response to DNA damage. Mol. Cell 22, 383–394.

[54] Duda, D., Borg, L., Scott, D., Hunt, H., Hammel, M. and Schulman, B. (2008) Structural insights into NEDD8 activation of cullin-RING ligases: conformational control of conjugation. Cell 134, 995–1006.

[55] Sugasawa, K. (2009) UV-DDB: a molecular machine linking DNA repair with ubiquitination. DNA Repair (Amsterdam) 8, 969–972.

[56] Batty, D., Rapic'-Otrin, V., Levine, A. and Wood, R. (2000) Stable binding of human XPC complex to irradiated DNA confers strong discrimination for damaged sites. J. Mol. Biol. 300, 275–290.

[57] Wittschieben, B., Iwai, S. and Wood, R. (2005) DDB1-DDB2 (xeroderma pigmentosum group E) protein complex recognizes a cyclobutane pyrimidine dimer, mismatches, apurinic/apyrimidinic sites, and compound lesions in DNA. J. Biol. Chem. 280, 39982–39989.

[58] Chen, X., Zhang, Y., Douglas, L. and Zhou, P. (2001) UV-damaged DNA-binding proteins are targets of CUL-4A-mediated ubiquitination and degradation. J. Biol. Chem. 276, 48175–48182.

[59] Nag, A., Bondar, T., Shiv, S. and Raychaudhuri, P. (2001) The xeroderma pigmentosum group E gene product DDB2 is a specific target of cullin 4A in mammalian cells. Mol. Cell Biol. 21, 6738–6747.

[60] Ortolan, T.G., Chen, L., Tongaonkar, P. and Madura, K. (2004) Rad23 stabilizes Rad4 from degradation by the Ub/proteasome pathway. Nucleic Acids Res. 32, 6490–6500.

[61] Schauber, C., Chen, L., Tongaonkar, P., Vega, I., Lambertson, D., Potts, W. and Madura, K. (1998) Rad23 links DNA repair to the ubiquitin/proteasome pathway. Nature 391, 715–718.

[62] Heessen, S., Masucci, M.G. and Dantuma, N.P. (2005) The UBA2 domain functions as an intrinsic stabilization signal that protects Rad23 from proteasomal degradation. Mol. Cell 18, 225–235.

[63] Nishi, R., Alekseev, S., Dinant, C., Hoogstraten, D., Houtsmuller, A., Hoeijmakers, J., Vermeulen, W., Hanaoka, F. and Sugasawa, K. (2009) UV-DDB-dependent regulation of nucleotide excision repair kinetics in living cells. DNA Repair (Amsterdam) 8 (6), 767–776.

[64] Min, J. and Pavletich, N. (2007) Recognition of DNA damage by the Rad4 nucleotide excision repair protein. Nature 449, 570–575.

[65] Ryu, K.-S., Lee, K.-J., Bae, S.-H., Kim, B.-K., Kim, K.-A. and Choi, B.-S. (2003) Binding surface mapping of intra- and interdomain interactions among hHR23B, ubiquitin, and polyubiquitin binding site 2 of S5a. J. Biol. Chem. 278, 36621–36627.

[66] Chen, X., Zhang, J., Lee, J., Lin, P.S., Ford, J.M., Zheng, N. and Zhou, P. (2006) A kinase-independent function of c-Abl in promoting proteolytic destruction of damaged DNA binding proteins. Mol. Cell 22, 489–499.

[67] Liu, L., Lee, S., Zhang, J., Peters, S.B., Hannah, J., Zhang, Y., Yin, Y., Koff, A., Ma, L. and Zhou, P. (2009) CUL4A abrogation augments DNA damage response and protection against skin carcinogenesis. Mol. Cell 34, 451–460.

[68] Bennett, E.J., Rush, J., Gygi, S.P. and Harper, J.W. (2010) Dynamics of cullin-RING ubiquitin ligase network revealed by systematic quantitative proteomics. Cell 143, 951–965.

[69] He, Y.J., McCall, C.M., Hu, J., Zeng, Y. and Xiong, Y. (2006) DDB1 functions as a linker to recruit receptor WD40 proteins to CUL4-ROC1 ubiquitin ligases. Genes Dev. 20, 2949–2954.

[70] Higa, L.A., Wu, M., Ye, T., Kobayashi, R., Sun, H. and Zhang, H. (2006) CUL4-DDB1 ubiquitin ligase interacts with multiple WD40-repeat proteins and regulates histone methylation. Nat. Cell Biol. 8, 1277–1283.

[71] Groisman, R., Kuraoka, I., Chevallier, O., Gaye, N., Magnaldo, T., Tanaka, K., Kisselev, A.F., Harel-Bellan, A. and Nakatani, Y. (2006) 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.

[72] Svejstrup, J.Q. (2007) Contending with transcriptional arrest during RNAPII transcript elongation. Trends Biochem. Sci. 32, 165–171.

[73] van Hoffen, A., Natarajan, A.T., Mayne, L.V., van Zeeland, A.A., Mullenders, L.H. and Venema, J. (1993) Deficient repair of the transcribed strand of active genes in Cockayne's syndrome cells. Nucleic Acids Res. 21, 5890–5895.

[74] Venema, J., Mullenders, L.H., Natarajan, A.T., van Zeeland, A.A. and Mayne, L.V. (1990) The genetic defect in Cockayne syndrome is associated with a defect in repair of UV-induced DNA damage in transcriptionally active DNA. Proc. Natl. Acad. Sci. U.S.A. A87, 4707–4711.
[75] Henning, K.A., Li, L., Iyer, N., McDaniel, L.D., Reagan, M.S., Legerski, R., Schultz, R.A., Stefanini, M., Lehmann, A.R., Mayne, L.V., et al. (1995) The Cockayne syndrome group A gene encodes a WD repeat protein that interacts with CSB protein and a subunit of RNA polymerase II TFIIH. Cell 82, 555–564.
[76] Troelstra, C., van Gool, A., de Wit, J., Vermeulen, W., Bootsma, D. and Hoeijmakers, J.H. (1992) ERCC6, a member of a subfamily of putative helicases, is involved in Cockayne's syndrome and preferential repair of active genes. Cell 71, 939–953.
[77] Cleaver, J.E., Lam, E.T. and Revet, I. (2009) Disorders of nucleotide excision repair: the genetic and molecular basis of heterogeneity. Nat. Rev. Genet. 10, 756–768.
[78] Troelstra, C., Odijk, H., de Wit, J., Westerveld, A., Thompson, L.H., Bootsma, D. and Hoeijmakers, J.H. (1990) Molecular cloning of the human DNA excision repair gene ERCC-6. Mol. Cell Biol. 10, 5806–5813.
[79] van Gool, A.J., Citterio, E., Rademakers, S., van Os, R., Vermeulen, W., Constantinou, A., Egly, J.M., Bootsma, D. and Hoeijmakers, J.H. (1997) The Cockayne syndrome B protein, involved in transcription-coupled DNA repair, resides in an RNA polymerase II-containing complex. EMBO J. 16, 5955–5965.
[80] Citterio, E., Van Den Boom, V., Schnitzler, G., Kanaar, R., Bonte, E., Kingston, R.E., Hoeijmakers, J.H. and Vermeulen, W. (2000) ATP-dependent chromatin remodeling by the Cockayne syndrome B DNA repair-transcription-coupling factor. Mol. Cell Biol. 20, 7643–7653.
[81] Kamiuchi, S., Saijo, M., Citterio, E., de Jager, M., Hoeijmakers, J.H. and Tanaka, K. (2002) Translocation of Cockayne syndrome group A protein to the nuclear matrix: possible relevance to transcription-coupled DNA repair. Proc. Natl. Acad. Sci. U.S.A. A99, 201–206.
[82] Fousteri, M., Vermeulen, W., van Zeeland, A.A. and Mullenders, L.H. (2006) Cockayne syndrome A and B proteins differentially regulate recruitment of chromatin remodeling and repair factors to stalled RNA polymerase II in vivo. Mol. Cell 23, 471–482.
[83] Anindya, R., Mari, P.O., Kristensen, U., Kool, H., Giglia-Mari, G., Mullenders, L.H., Fousteri, M., Vermeulen, W., Egly, J.M. and Svejstrup, J.Q. (2010) A ubiquitin-binding domain in Cockayne syndrome B required for transcription-coupled nucleotide excision repair. Mol. Cell 38, 637–648.
[84] Arias, E.E. and Walter, J.C. (2005) Replication-dependent destruction of Cdt1 limits DNA replication to a single round per cell cycle in Xenopus egg extracts. Genes Dev. 19, 114–126.
[85] Zhong, W., Feng, H., Santiago, F.E. and Kipreos, E.T. (2003) CUL-4 ubiquitin ligase maintains genome stability by restraining DNA-replication licensing. Nature 423, 885–889.
[86] Nishitani, H., Sugimoto, N., Roukos, V., Nakanishi, Y., Saijo, M., Obuse, C., Tsurimoto, T., Nakayama, K.I., Nakayama, K., Fujita, M., et al. (2006) Two E3 ubiquitin ligases, SCF-Skp2 and DDB1-Cul4, target human Cdt1 for proteolysis. EMBO J. 25, 1126–1136.
[87] Nishitani, H., Shiomi, Y., Iida, H., Michishita, M., Takami, T. and Tsurimoto, T. (2008) CDK inhibitor p21 is degraded by a proliferating cell nuclear antigen-coupled Cul4-DDB1Cdt2 pathway during S phase and after UV irradiation. J. Biol. Chem. 283, 29045–29052.
[88] Abbas, T., Sivaprasad, U., Terai, K., Amador, V., Pagano, M. and Dutta, A. (2008) PCNA-dependent regulation of p21 ubiquitylation and degradation via the CRL4Cdt2 ubiquitin ligase complex. Genes Dev. 22, 2496–2506.
[89] Abbas, T., Shibata, E., Park, J., Jha, S., Karnani, N. and Dutta, A. (2010) CRL4 (Cdt2) regulates cell proliferation and histone gene expression by targeting PR-Set7/Set8 for degradation. Mol. Cell 40, 9–21.
[90] Centore, R.C., Havens, C.G., Manning, A.L., Li, J.-M., Flynn, R.L., Tse, A., Jin, J., Dyson, N.J., Walter, J.C. and Zou, L. (2010) CRL4 (Cdt2)-mediated destruction of the histone methyltransferase Set8 prevents premature chromatin compaction in S phase. Mol. Cell 40, 22–33.
[91] Oda, H., Hübner, M.R., Beck, D.B., Vermeulen, M., Hurwitz, J., Spector, D.L. and Reinberg, D. (2010) Regulation of the histone H4 monomethylase PR-Set7 by CRL4(Cdt2)-mediated PCNA-dependent degradation during DNA damage. Mol. Cell 40, 364–376.
[92] Jørgensen, S., Eskildsen, M., Fugger, K., Hansen, L., Larsen, M.S.Y., Kousholt, A.N., Syljuåsen, R.G., Trelle, M.B., Jensen, O.N., Helin, K., et al. (2011) SET8 is degraded via PCNA-coupled CRL4(CDT2) ubiquitylation in S phase and after UV irradiation. J. Cell Biol. 192, 43–54.
[93] Liu, C., Poitelea, M., Watson, A., Yoshida, S.H., Shimoda, C., Holmberg, C., Nielsen, O. and Carr, A.M. (2005) Transactivation of *Schizosaccharomyces pombe* cdt2+ stimulates a Pcu4-Ddb1-CSN ubiquitin ligase. EMBO J. 24, 3940–3951.
[94] Braun, S., Garcia, J.F., Rowley, M., Rougemaille, M., Shankar, S. and Madhani, H.D. (2011) The Cul4-Ddb1(Cdt)^2 ubiquitin ligase inhibits invasion of a boundary-associated antisilencing factor into heterochromatin. Cell 144, 41–54.
[95] Arias, E.E. and Walter, J.C. (2006) PCNA functions as a molecular platform to trigger Cdt1 destruction and prevent re-replication. Nat. Cell Biol. 8, 84–90.
[96] Senga, T., Sivaprasad, U., Zhu, W., Park, J.H., Arias, E.E., Walter, J.C. and Dutta, A. (2006) PCNA is a cofactor for Cdt1 degradation by CUL4/DDB1-mediated N-terminal ubiquitination. J. Biol. Chem. 281, 6246–6252.
[97] Havens, C.G. and Walter, J.C. (2009) Docking of a specialized PIP Box onto chromatin-bound PCNA creates a degron for the ubiquitin ligase CRL4Cdt2. Mol. Cell 35, 93–104.
[98] Hu, J. and Xiong, Y. (2006) An evolutionarily conserved function of proliferating cell nuclear antigen for Cdt1 degradation by the Cul4-Ddb1 ubiquitin ligase in response to DNA damage. J. Biol. Chem. 281, 3753–3756.
[99] Jin, J., Arias, E.E., Chen, J., Harper, J.W. and Walter, J.C. (2006) A family of diverse Cul4-Ddb1-interacting proteins includes Cdt2, which is required for S phase destruction of the replication factor Cdt1. Mol. Cell 23, 709–721.
[100] Higa, L.A., Banks, D., Wu, M., Kobayashi, R., Sun, H. and Zhang, H. (2006) L2DTL/CDT2 interacts with the CUL4/DDB1 complex and PCNA and regulates CDT1 proteolysis in response to DNA damage. Cell Cycle 5, 1675–1680.
[101] Hotton, S.K. and Callis, J. (2008) Regulation of cullin RING ligases. Annu. Rev. Plant Biol. 59, 467–489.
[102] Chiba, T. and Tanaka, K. (2004) Cullin-based ubiquitin ligase and its control by NEDD8-conjugating system. Curr. Protein Pept. Sci. 5, 177–184.
[103] Cope, G.A., Suh, G.S., Aravind, L., Schwarz, S.E., Zipursky, S.L., Koonin, E.V. and Deshaies, R.J. (2002) Role of predicted metalloprotease motif of Jab1/Csn5 in cleavage of Nedd8 from Cul1. Science 298, 608–611.
[104] Lyapina, S., Cope, G., Shevchenko, A., Serino, G., Tsuge, T., Zhou, C., Wolf, D.A., Wei, N., Shevchenko, A. and Deshaies, R.J. (2001) Promotion of NEDD-CUL1 conjugate cleavage by COP9 signalosome. Science 292, 1382–1385.
[105] Wei, N., Serino, G. and Deng, X.W. (2008) The COP9 signalosome: more than a protease. Trends Biochem. Sci. 33, 592–600.
[106] Pintard, L., Kurz, T., Glaser, S., Willis, J.H., Peter, M. and Bowerman, B. (2003) Neddylation and deneddylation of CUL-3 is required to target MEI-1/Katanin for degradation at the meiosis-to-mitosis transition in *C. elegans*. Curr. Biol. 13, 911–921.
[107] Schwechheimer, C. and Deng, X.W. (2001) COP9 signalosome revisited: a novel mediator of protein degradation. Trends Cell. Biol. 11, 420–426.
[108] Kapelari, B., Bech-Otschir, D., Hegerl, R., Schade, R., Dumdey, R. and Dubiel, W. (2000) Electron microscopy and subunit-subunit interaction studies reveal a first architecture of COP9 signalosome. J. Mol. Biol. 300, 1169–1178.
[109] Enchev, R.I., Schreiber, A., Beuron, F. and Morris, E.P. (2010) Structural insights into the COP9 signalosome and its common architecture with the 26S proteasome lid and eIF3. Structure 18, 518–527.
[110] Sharon, M., Mao, H., Boeri Erba, E., Stephens, E., Zheng, N. and Robinson, C.V. (2009) Symmetrical modularity of the COP9 signalosome complex suggests its multifunctionality. Structure 17, 31–40.
[111] Mundt, K.E., Liu, C. and Carr, A.M. (2002) Deletion mutants in COP9/signalosome subunits in fission yeast *Schizosaccharomyces pombe* display distinct phenotypes. Mol. Biol. Cell 13, 493–502.
[112] Mundt, K.E., Porte, J., Murray, J.M., Brikos, C., Christensen, P.U., Caspari, T., Hagan, I.M., Millar, J.B., Simanis, V., Hofmann, K., et al. (1999) The COP9/signalosome complex is conserved in fission yeast and has a role in S phase. Current Biol.: CB 9, 1427–1430.
[113] Luijsterburg, M.S., Goedhart, J., Moser, J., Kool, H., Geverts, B., Houtsmuller, A.B., Mullenders, L.H.F., Vermeulen, W. and van Driel, R. (2007) Dynamic in vivo interaction of DDB2 E3 ubiquitin ligase with UV-damaged DNA is independent of damage-recognition protein XPC. J. Cell Sci. 120, 2706–2716.
[114] Higa, L.A., Mihaylov, I.S., Banks, D.P., Zheng, J. and Zhang, H. (2003) Radiation-mediated proteolysis of CDT1 by CUL4-ROC1 and CSN complexes constitutes a new checkpoint. Nat. Cell Biol. 5, 1008–1015.
[115] Fukumoto, Y., Dohmae, N. and Hanaoka, F. (2008) *Schizosaccharomyces pombe* Ddb1 recruits substrate-specific adaptor proteins through a novel protein motif, the DDB-box. Mol. Cell. Biol. 28, 6746–6756.
[116] Olma, M.H., Roy, M., Le Bihan, T., Sumara, I., Maerki, S., Larsen, B., Quadroni, M., Peter, M., Tyers, M. and Pintard, L. (2009) An interaction network of the mammalian COP9 signalosome identifies Dda1 as a core subunit of multiple Cul4-based E3 ligases. J. Cell Sci. 122, 1035–1044.
[117] Duda, D.M., Scott, D.C., Calabrese, M.F., Zimmerman, E.S., Zheng, N. and Schulman, B.A. (2011) Structural regulation of cullin-RING ubiquitin ligase complexes. Curr. Opin. Struct. Biol. 21 (2), 257–264.
[118] Choe, K.P., Przybysz, A.J. and Strange, K. (2009) The WD40 repeat protein WDR-23 functions with the CUL4/DDB1 ubiquitin ligase to regulate nuclear abundance and activity of SKN-1 in *Caenorhabditis elegans*. Mol. Cell Biol. 29, 2704–2715.
[119] Wertz, I.E., O'Rourke, K.M., Zhang, Z., Dornan, D., Arnott, D., Deshaies, R.J. and Dixit, V.M. (2004) Human De-etiolated-1 regulates c-Jun by assembling a CUL4A ubiquitin ligase. Science 303, 1371–1374.