Review

COP9 signalosome function in the DDR

Ronny Hannß, Wolfgang Dubiel*

Department of General, Visceral, Vascular and Thoracic Surgery, Division of Molecular Biology, Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany

ARTICLE INFO

Article history:
Received 17 March 2011
Revised 11 April 2011
Accepted 12 April 2011
Available online 16 April 2011

Edited by Ashok Venkitaraman and Wilhelm Just

Keywords:
COP9 signalosome
Nedd8
Cullin-RING ubiquitin ligase
Nucleotide excision repair
Checkpoint control

ABSTRACT

The COP9 signalosome (CSN) is a platform for protein communication in eukaryotic cells. It has an intrinsic metalloprotease that removes the ubiquitin (Ub)-like protein Nedd8 from cullins. CSN-mediated deneddylation regulates culling-RING Ub ligases (CRLs) and controls ubiquitination of proteins involved in DNA damage response (DDR). CSN forms complexes with CRLs containing cullin 4 (CRL4s) which act on chromatin playing crucial roles in DNA repair, checkpoint control and chromatin remodeling. Furthermore, via associated kinases the CSN controls the stability of DDR effectors such as p53 and p27 and thereby the DDR outcome. DDR is a protection against cancer and deregulation of CSN function causes cancer making it an attractive pharmacological target. Here we review current knowledge on CSN function in DDR.

© 2011 Federation of European Biochemical Societies. Published by Elsevier B.V.

Available online 16 April 2011

Accepted 12 April 2011

Received 17 March 2011

1. Introduction

The COP9 signalosome was discovered in 1996 as a negative regulator of Constitutive Photomorphogenesis (COP) in plants [1]. Two years later the CSN was independently rediscovered in human red blood cells [2] and in pig spleen [3] and it became clear that the complex function is beyond the regulation of light-dependent reactions in plants. In the past the CSN was identified in all eukaryotic cells studied including Saccharomyces cerevisiae [4,5], Schizosaccharomyces pombe [6], Aspergillus nidulans [7], Caenorhabditis elegans [8] and Drosophila melanogaster [9]. Already in 1998 it was demonstrated that CSN subunits possess significant sequence homologies with subunits of the 26S proteasome lid subcomplex [2,10,11]. In addition, similarities with the translation initiation complex 3 (eIF3) were identified [12]. The three complexes (CSN, lid and eIF3) are composed of (Proteasome, COP9 signalosome, Initiation factor eIF3) PCI-domain and (MOV34, Pad1N-terminal) MPN-domain subunits and most likely possess a common ancestor during evolution [13]. In recent years they were summarized as “Zomes” complexes.

According to the unified nomenclature the typically eight CSN subunits were designated as CSN1 to CSN8 [14]. Each subunit was identified as a binding surface of many different interacting regulatory proteins (for recent review see [15]). Whether all of these interactions are specific and exhibit biological relevance has to be elucidated in the future. In the course of this review we will characterize a number of specific CSN interactions. Knockouts of CSN subunits genes in yeast are viable (for review see [16]). In A. nidulans knockouts are viable but can induce a DDR phenotype [17]. In contrast, knockouts of any of the eight CSN subunits in mice are lethal at an early stage of embryonic development [18–21]. These data reflect the increasing significance gained by the CSN during evolution of multi-cellular organisms. The biological function of the CSN is mostly determined by regulating CRLs, the largest family of E3s of the Ub proteasome system (UPS) [22,23]. CRLs are responsible for the poly-ubiquitination and subsequent degradation of many regulatory proteins of cell cycle progression, signal transduction and development (for review see [15,16,24]). Interestingly, the first hint that the CSN is involved in DDR was the observation that S. pombe Csn1 and Csn2 mutants displayed a delay in S phase progression and were hypersensitive to UV- and γ-irradiation [6]. In contrast, deletions of Csn4 and Csn5 behaved like wild type (wt) [25]. Because all cells either lacking Csn1, Csn2, Csn4 or Csn5 showed the same cullin 1 (Cul1)/Pcu1 hyper-neddylation phenotype, a distinct function of these subunits besides deneddylation of Cul1/Pcu1 was indicated [25]. Later a defect in Spd1p degradation, an inhibitor of ribonucleotide reductase, was shown to be the reason for the S. pombe phenotypes, because the enzyme is especially needed in S phase and for DNA repair [26]. Interestingly, Spd1p is degraded by a CRL using cullin 4 (Cul4) as a scaffold (CRL4). Why this depends on CSN1 and CSN2 but not on CSN3 and CSN4 is still not clear. In contrast to S. pombe, there is no delay in S phase in the budding yeast S. cerevisiae after CSN* Corresponding author. Fax: +49 30 450522928.
E-mail address: wolfgang.dubiel@charite.de (W. Dubiel).
subunit deletion nor are mutants hypersensitive to UV [4]. However, homologs of CSN1 or CSN2 are not clearly identifiable in these species [4] and levels of overall sequence identity between subunits from budding yeast and those from other organisms are extremely low [27]. On the other hand, the CSN is involved in DDR in *A. nidulans* [17] and in *D. melanogaster* [28]. It could be unequivocally shown that deletion of *Csn4* or *Csn5* in *D. melanogaster* led to hypersensitivity to methylmethane sulfonate, a DNA methylating agent, suggesting a role of the CSN in DDR in insects. Groisman et al. have shown that the CSN differentially regulates CRLs involved in the repair process in mammalian cells [29]. Through control of a special subset of CRLs the CSN expands its functional importance on chromatin remodeling and DNA repair. This review focuses on recent discoveries regarding the role of the CSN in DDR.

2. Multiple faces of the CSN

2.1. The architecture of the CSN in different species

There are variations of CSN subunit composition in different species responsible for structure and function of the complex. The *S. cerevisiae* complex consists of Rtr1, a real CSN5 homolog, and of additional four subunits (CSN9, CSN10, CSN11 and Csi1) possessing few similarities to subunits in higher eukaryotes [5]. Recently it has been shown that the proteasomal subunit Rpn5 has a dual function in the 26S proteasome and in the CSN [30]. Another situation has been described for *S. pombe*. The fission yeast CSN is composed of CSN1, CSN2, CSN3, CSN4, CSN5 and CSN7a, whereas CSN7b is a subunit of the elf3 complex. Subunits 6 and 8 are missing in *S. pombe* [31]. In *C. elegans* the CSN shares one subunit with elf3 complex, which is called CIF-1 [32]. In *A. thaliana* CSN5 and CSN6 are each encoded by two very homologous genes, *Csn5a* and *Csn5b* or *Csn6a* and *Csn6b* [31,33]. In mammalian cells CSN7 occurs as CSN7a and CSN7b most likely as components of different CSN complexes [34]. Recent systematic proteomic analysis of the mammalian CSN demonstrated that CSN7b is not part of CSN7a-containing complexes [35] indicating that CSN7a and CSN7b are indeed components of different CSN complexes.

The architecture of the human CSN was first studied by electron microscopy (EM) [36]. EM images of the human CSN and lid revealed similar architectures. The two particles occurred in different classes indicating structural diversity most likely due to changes in subunit composition (CSN7a or CSN7b), association of various proteins (for example USP15 or kinases) and/or CSN subunit modifications presumably by phosphorylation. Using CSN EM images and known subunit–subunit interactions a first model of the CSN was created [36], see Fig. 1. Recently a new model has been proposed based on an emerging mass spectrometry approach [37]. In this study Sharon et al. found that the structure of the complex is arranged by two symmetrical clusters/modules, CSN1/2/3/8 and CSN4/5/6/7, connected by a single link via CSN1–CSN6. Within each cluster the two most conserved subunits, CSN2 and CSN5, occupy a peripheral position. The model also revealed remarkable structural similarities with the 19S lid [37]. Another three-dimensional structure of the CSN was proposed based on negative stain EM and single particle analysis. The comparison with the lid and the elf3 complexes revealed a conserved architecture implying similar assembly pathways [38]. Unfortunately, there exist neither cryo-EM data nor crystal structure of the CSN complex. There are no structural data on CSN-based supercomplexes.

2.2. Is the CSN just a deneddylase?

CSN-mediated deneddylase located to CSN5 but requiring the entire CSN complex is probably the most prominent biochemical activity of the CSN. The metalloprotease JAMM/MPN+ motif of CSN5 possesses the His-X-His-X$_{10}$-Asp consensus sequence (where X indicates any residue) accompanied by an upstream conserved Glu [39]. The CSN5 paralog subunit of the 26S proteasome lid is S13/Rpn11, which is a debiquitinating enzyme and essential for 26S proteasome functioning [40–43]. The JAMM/MPN+ motif is a typical Zn$^{2+}$-binding metalloprotease domain, which can be blocked by Zn$^{2+}$ chelators such as o-phenanthroline. The CSN specifically removes Ned8 from isopeptide bonds. It does not cleave Ned8-AMC or Ned8 linear extensions indicating that the CSN is unable to process pre-Ned8 [44]. The deneddylating activity of the CSN can be knocked out by point mutations in the JAMM/MPN+ motif [29,39]. A conditional knockout of *Csn5* in T cells inhibited cell cycle and led to an increase of of -catenin [45]. Interestingly, CSN5 is overexpressed in many tumor cells [46,47].

Cullins are the major substrates of CSN-mediated deneddylation. In mammals Cul1, 2, 3, 4A, 4B, 5, 7 bind to one of the RING domain proteins (Rbx1 or Rbx2) [48] at the C-terminus that mediates E2 interaction. Substrate-specificity is orchestrated by different modules that bind to the C-terminus of cullins. Skp1 and an F-box (FBX) or FBW8 protein assemble into Cul1 and Cul7-based E3 ligases, respectively. Elongin C/B and VHL-box or SOCS-box assemble into Cul2/5 and Cul3-based E3 ligase assembly with Bric-a-brac, Tramtrack, and Broad (BTB) domain proteins representing both adaptor and substrate receptors. Cul4A/B associate with the substrate receptor DDB1 and bind to Cul4-associated factors (DCAFs) [49,50]. Systematic proteomic analysis of the mammalian CSN revealed the actual diversity of CSN–CRL interactions [35,51]. Given the fact that CRLs possess modular structures a large number of complexes can be formed specifically targeting thousands of substrates.

CRLs are post-translationally modified with Ned8 [22]. Neddylation of cullins leads to a conformational change not only promoting recruitment of Ub-charged E2 but bringing the substrate and the E2 in close proximity that facilitates Ub transfer [52,53]. The ligase is inactivated by CSN-mediated deneddylation [54,55], which, on the other hand, is essential to prevent degradation/instability of CRL compounds, in particular, of substrate recognition subunits [56–59]. Non-neddylated cullins can bind to the cullin associated neddylation dissociated protein 1 (CAND1/TIP120A) preventing substrate adaptor/receptor association and blocking the neddylation site [60]. CAND1 seems to promote a substrate receptor exchange and the binding of substrate receptor subunits with low affinity for Cul1 [59]. Therefore, CSN-mediated deneddylation also facilitates the CRL reassembly pathway via CAND1. Knockout experiments in mice suggest that in addition to deneddylation other functions are carried out by the CSN.

**CSN-mediated phosphorylation** has been first described in 1998 [2]. The CSN is associated with a variety of proteins including the protein kinases CK2 and PKD [61], Akt [62] as well as inositol 1,3,4-triphosphate 5/6 kinase [63]. The kinases specifically modify substrates of the UPS and determine their stability [64]. For example, CK2 phosphorylates p53 at Thr155 or modifies p27Kip which targets the tumor suppressors to degradation by the UPS [62,65]. Just recently a CRL1 has been identified which ubiquitinates p53 upon CSN-mediated modification. The responsible CRL1 uses the Kelch domain-containing F-box protein JFK [66]. On the other hand, CSN-mediated phosphorylation of c-Jun stabilizes the transcription factor toward the UPS [61]. The same holds true for the microtubule end-binding protein 1 (EB1), which is also a substrate of the UPS [67]. The transcription factor c-Jun is responsible for the induction of the vascular endothelial growth factor (VEGF) in tumor cells [68], the most important pro-angiogenic regulators in mammals. CSN associated kinase inhibitors including curcumin and a number of curcumin-like compounds [69] stabilize the tumor suppressor p53 and induce UPS-dependent degradation of

---

c-Jun [61,65], which blocks tumor angiogenesis [70]. CSN subunits can be phosphorylated by associated [36,71] or non-associated kinases. Recently the phosphorylation of CSN1 by the glycogen synthase kinase 3β (GSK3β) has been demonstrated being important for the formation of the CSN-based supercomplex with the β-catenin destruction complex [72]. In connection with the DNA-damage-triggered checkpoint control a direct binding of Ataxia-telangiectasia mutated (ATM) kinase with the CSN has been observed as a result of a double DNA strand breaks followed by phosphorylation of CSN3 [73].

CSN-mediated deubiquitination has been first described by Groisman et al. [29]. These authors showed that the CSN has most likely two deubiquitinating enzyme (DUB) activities: one that removes Ub from Cul4A and one activity that depolymerizes Ub chains. The Ub-specific protease 15 (USP15, the S. pombe homolog is called Ubp12) has been identified as the enzyme that can cleave Ub chains [74,75]. Since CSN complexes were able to cleave Ub from Cul4A, it was concluded that the CSN5 metalloprotease might be responsible for this activity. USP15 is localized to the nucleus as well as to the cytoplasm and its nuclear import needs functional CSN [74]. It is a cysteine protease with the typical catalytic triad Cys, His and Asp and belongs to the family of DUBs. In addition it has a zinc (Zn)-finger motif, which is necessary for the binding and cleavage of Ub chains. The cysteine residue 783 of the Zn-finger was mutated to alanine and the recombinant mutant USP15-C783A was unable to cleave Ub lysine 48 chains, although it removed linear-linked Ub from GFP [75]. USP15 protects proteins against autoubiquitination and degradation [74,75]. It has been shown that components of CRLs such as Bbt protein or the RING-protein Rbx1 [59,75] are protected by USP15. In S. pombe the Bbt3p is strongly destabilized in ubp12/USP15 deleted cells [76]. In addition, components of signaling pathways such as 18x8x [77] and the adenomatous polyposis coli (APC) protein [72] are stabilized by USP15. Although few functions of USP15 have been described, its biological significance remained obscure.

Since the CSN is not only a deneddylase but also a kinase complex and a DUB it fulfills multiple jobs which might differ from species to species or from cell to cell. As a regulator of the UPS the CSN is involved in many cellular processes such as cell cycle, signal transduction and development (for recent reviews see [15,16,24,78]).

3. The role of the CSN in the DDR

Cells are constantly threatened by DNA damage due to various exogenous and endogenous stresses, e.g. chemicals, reactive oxygen species, UV or ionizing radiation (IR). These damages have to be repaired to assure survival of the cell and proper transmission of the genetic information to the offspring. Therefore a complex of signal transduction pathways and cellular responses evolved, designated as DDR, to counteract DNA damage (extensively reviewed in [79]). Importantly, the outcome of this response can be a delay or arrest in cell cycle (checkpoint activation), transcriptional activation, senescence, apoptosis or ‘mitotic catastrophe’ [80] depending on the context (see Fig. 1). Thus, DDR has to be tightly regulated and the CSN seems to be an important factor.

3.1. CRL4s and the CSN

Although not all CRL4 ligases are associated with chromatin [48], an amazing hallmark of most CRL4s is that they concentrate their work on chromatin. They play crucial roles in DNA repair, checkpoint control and chromatin remodeling [81,82]. Interestingly, S. cerevisiae has no Cul4 ortholog but its Cul8 assembles to Cul4-like complexes [48]. Accordingly, Cul8 knockout mutants are slower in growth and sensitive to fork arrest due to alkylation, accumulate DNA damage and lose genomic integrity [83,84]. In higher eukaryotes the substrate specificity of CRL4s is determined by DCAFs (see Fig. 2), which usually have WD40 repeats and bind DDB1 [49]. There are more than 90 predicted DCAFs [85]. However, it has to be clarified whether all DCAFs mediate substrate ubiquitination.

Studies have shown that the CRL4 interacting with the substrate receptor DDB2 (CRL4aDB2) also targets ubiquitination of histones contributing to the ‘histone code’ similarly like acetylation or methylation. The post-translational modification of histones...
regulates gene expression or makes the DNA accessible for certain factors. At sites of damaged DNA CRL4<sup>DDDB2</sup> mediates H2A ubiquitination [86]. Histones H3 and H4 are also ubiquitinated in response to DNA damage facilitating the recruitment of DNA repair proteins (XPC) [87]. Skin specific disruption of Cul4A in mice led to increased resistance to UV-induced carcinogenesis and accumulation of both DNA damage sensors, DDB2 and XPC, as well as of p21, the DNA damage checkpoint effector [88]. This results in enhanced global genome (GG)-nuclear excision repair (NER, GG-NER) activity and reinforces UV-responsive G1 DNA damage checkpoint.

3.2. The CSN controls DDR, the impact on nucleotide excision repair

Severe alterations in DNA structure like single strand breaks (SSBs) or double strand breaks (DSBs) bring cells in acute danger. DSBs are recognized by the Ku protein and repaired by non-homologous end-joining (NHEJ) or by the Ku-independent microhomology-mediated end-joining (MMEJ) mechanism. Another possibility to repair DSBs is via homologous recombination (HR) which depends on homologous sequences, requires sister chromatids as template and therefore only operates in S and G2 of the cell cycle. HR is also used to restart stalled replication forks and to repair intrastrand crosslinks. UV mainly produces cyclobutane pyrimidine dimers (CPDs) and (6-4) pyrimidine-pyrimidone (6-4PPs) photodadducts that are repaired by NER. There are two pathways for NER that work either on the whole genome called GG-NER [89] or the rapid transcription coupled repair (TC-NER) that only repairs actively transcribed DNA strands. These two NER pathways mainly differ in the manner how DNA lesions are sensed [90]. During the last decade it could be shown that the CSN controls GG-NER as well as the TC-NER pathway. There are various hereditary diseases that involve defects in NER: xeroderma pigmentosum (XP) and cockayne syndrome (CS). Both XP and CS are characterized by photosensitivity but only XP is associated to increased incidence of sunlight induced skin cancer. Importantly, mainly CS patients, but also patients with other defects in NER display developmental and neurological aberrations. Remarkably, despite the importance of NER neurons show attenuated NER activity and other terminally differentiated tissues [91,92]. In neuronal diseases (e.g. Alzhei-

Fig. 2. The CSN interacts with different CRL4 complexes involved in DDR. At the moment the exact mechanism regulating the binding of the CSN to one CRL4 and then its reassembly into a supercomplex with another CRL4 is not clear. Perhaps the DDR kinases ATM and ATR are involved in the process by direct phosphorylation of CSN subunits and/or of CRL4 components.

reflecting mutations in excision repair proteins. The DDB2 functions as the damage sensor. DDB2 effectively recognizes and binds DNA lesions, e.g. CPDs and with highest affinity to 6-4PPs [94,95]. DDB2 is part of a CRL4A. In unperturbed cells the CRL4<sup>DDDB2</sup> is associated with CSN stabilizing the CRL4<sup>DDDB2</sup> by neddylation. After UV irradiation DDB2 rapidly binds to the lesion and unwinds the DNA inducing a strong kink [96,97]. Now chromatin bound CRL4<sup>DDDB2</sup> is no longer associated with the CSN and facilitates ubiquitination. Knockdown of CSN5 results in the reduction of unscheduled DNA synthesis suggesting that the CSN is a positive regulator of NER activity [29]. The CRL4<sup>DDDB2</sup> ligase also mediates histone ubiquitination around damage sites in vitro [90] promoting recruitment of another NER protein, XPA [90], probably to loosen the chromatin [87]. Interestingly, in vitro studies have shown that DDB2 facilitates its autoubiquitination [98] that lowers its affinity to lesions [99] and initiates its degradation after UV exposure [99,100]. Thus, the ligase complex obviously helps to make the chromatin accessible for NER factors and takes charge of its own degradation. Recent studies have shown that Ku protein inhibits extensive DDB2 ubiquitination around damage sites [90]. Together with XPA the heterotrimeric replication protein A (RPA, binding ssDNA) and XPC bind to the site of the lesion. XPC preferentially recognizes 6-4PPs and is also ubiquitinated by the CRL4<sup>DDDB2</sup> complex. In contrast to DDB2 it increases affinity for DNA and does not promote its own degradation [99]. However, Cul4A null MEFs accumulate both DDB2, XPC and p21 DNA damage sensors [101]. Moreover, another study showed that esophageal degradation of XPC presumably mediated via CRL4A [102]. XPA, RPA and XPC then recruit the transcription factor TFIH containing two helicases, XPD and XBP that unwind the DNA. The damaged strand is incised up- and downstream from the lesion and the DNA gap is filled by recruitment of proliferating cell nuclear antigen (PCNA) and subsequent binding of replicative polymerases (Polδ, Polɛ). In a last step the original duplex is restored by ligation.

Interestingly, Cul4A null MEFs not only accumulate DDB2 and XPC DNA damage sensors, but display dramatically enhanced GG-NER activity in CPD and 6-4PP removal and reinforce UV-responsive G1 DNA damage checkpoint [101]. Microinjection of the purified DDB2 protein complex into XP-E cells that are defective in DDB2 completely restored GG-NER [29] as determined by UV-induced unscheduled DNA synthesis mainly reflecting GG-NER [103].

DNA repair via TC-NER depends on CSA, CSB and XBA2, which are dispensable for GG-NER. The DNA lesion is sensed by stalled/ blocked RNA polymerase II (RNAPII) subsequently recruiting NER enzymes to the site of damage. Thus, DDB1, DDB2 and XPC are dispensable for TC-NER. Groisman et al. could show that the CSN also controls TC-NER [29]. As for DDB2 they demonstrated that CSA forms an active CRL4A using DDB1 as adapter module. In contrast to CRL4<sup>DDDB2</sup> the CRL4<sup>CSA</sup> complex is constitutively associated with chromatin but bound to RNAPII in an UV-dependent manner. After UV irradiation the hyperphosphorylated RNAPII (Ilo) is rapidly ubiquitinated [104,105] in wt cells but fails to be ubiquitinated in CSA or CSB defective cells. Regardless of UV, the CSA complex co-purifies with RNAPII and accumulates on the hyperphosphorylated form of RNAPII (Ilo), although ubiquitination has not been observed under the conditions used in this study [29]. In HeLa cells, CRL4<sup>CSA</sup> targets CSB for ubiquitination and degradation several hours after UV irradiation suggesting a model in which the CRL4<sup>CSA</sup> complex probably preventing ligase activity. However, no neddylated CRL4<sup>CSA</sup> complex was observed, neither before UV
irradiation where CSN is not associated with this explicit complex, nor after UV irradiation.

Noteworthy, NER seems to be tightly controlled by c-Abl, a non-receptor tyrosine kinase activated by e.g. IR but not UV irradiation, which destabilizes DDB2 independently of its kinase activity [107]. Overexpression of c-Abl leads to phosphorylation of DDB2 inhibiting its lesion binding activity suggesting a mechanism preventing DDB2 from interference with other repair pathways [108]. Furthermore, c-Abl, together with other factors, seems to regulate the CRL4A complex by reducing the Cul4A CAND1 interaction [107]. The mechanism behind this observation is still obscure.

3.3. The role of the CSN in checkpoint control

Checkpoints are surveillance mechanisms that ascertain the fidelity of the genome before progression in cell cycle. In response to DNA damage the cell cycle is either slowed down or completely arrested. Only after DNA repair the cell may regain the ability to exit the arrest in a process called checkpoint recovery. In mammalian cells there are two checkpoint signaling pathways each activated depending on the damage occurred. The damages are sensed by proteins, e.g. RPA (binding to single stranded DNA), Mre11-Rad50-Nbs1 (MRN) complex recognizing DSBs. Subsequently, sensor kinases (phosphoinositide 3-kinase related kinases, PIKKs) are activated. UV-induced damage or replication fork stress due to chemicals lead to activation of the sensor kinase ATR and subsequent activation of checkpoint kinase 1 (Chk1). Phosphorylation of Chk1 depends on the Rad9-Rad1-Hus1 (9-1-1) complex that is loaded on DNA. DSBs, e.g. caused by IR, induce ATM autophosphorylation and subsequently activate Chk2. Chk1 and Chk2 represent signal transducing kinases that have many substrates and affect the activity of a myriad of effector proteins, e.g. p53, Cdc25 and Cdc45. Phosphorylation of these proteins eventually inhibits the Cdk2 and Cdc2, that resemble key cyclins in cell cycle progression resulting in G1/S and G2/M arrest, respectively. Unlike ATM, ATR and Chk1 are suggested to be dispensable for the cell cycle due to the fact that ATR- or Chk1-deficient mice die at early embryonic stage.

There is evidence that CSN5 interacts with the 9-1-1 complex, a PCNA-like complex that is loaded on DNA after genotoxic damage. Comimmunoprecipitation analyses reveal a direct interaction of the CSN with the Rad1 [109]. In the same study the authors could show that CSN5 overexpression leads to proteasomal degradation of the 9-1-1 complex, although the stability of the 9-1-1 complex is not altered by inhibition of CSN-associated kinases [109]. Because the PCNA-like 9-1-1 complex is needed for Chk1 phosphorylation, CSN5/jab1 suppresses the checkpoint signaling activation and DNA synthesis recovery after replication stress [109].

The CRL4A\textsuperscript{CDD2} is probably the best studied CRL4 ligase and has been shown to ubiquitinate the DNA repair licensing factor CDT1 constituting a new checkpoint [110]. CDT1 is a licensing factor that assembles on replication origins at the end of mitosis and in G1 [110] to license DNA for replication. Aberrant CDT1 activity leads to re-replication and polyploid nuclei. In S phase or after UV-induced DNA damage CRL4\textsuperscript{CDD2} binds, in a PCNA-dependent manner, to chromatin or to the DNA lesions, respectively, and mediates CDT1 degradation [111]. Unexpectedly, Higa et al. could show that CDT2 binds to Cul4 as well as to DDB1 and regulates CDT1 proteolysis in response to DNA damage [111]. The CSN or at least single subunits have been shown to be associated with CRL4\textsuperscript{CDD2}. Knockdown of either CSN5 or CSN2 did not alter CDT1 proteolysis after DNA damage [111]. Higa et al. hypothesize that the de neddylyating enzyme 1 (DEN1) may be involved in CDT1 proteolysis.

Recently, the CRL4\textsuperscript{CDD2} also has been shown to target PR-Set7/ Set8 for proteasomal degradation [112]. Set8 methylates histone 4 on lysine 20 (H4K20) resulting in chromatin silencing and compaction, important for chromosome segregation in mitosis [113]. Noteworthy, Set8 degradation is mediated by two other ligases: CRL1\textsuperscript{Skp2} and anaphase promoting complex/cyclosome (APC/C\textsuperscript{Cdh1}) [114]. The latter has not yet been shown to be modified by Nedd8 but it is also affected by the CSN [115]. Notably, CRL4\textsuperscript{CDD2} also targets p21 for degradation [116,117]. Interestingly, all proteins ubiquitinated by CRL4\textsuperscript{CDD2} require PCNA and contain an PCNA interacting motif (PIP) that is required for CRL4\textsuperscript{CDD2} mediated degradation [48]. This in concert with the fact that CDT2 binds to Cul4 suggest that the CRL4\textsuperscript{CDD2} ligase differs from other CRLs. PCNA has to be seen as part of the recognition module of CRL4\textsuperscript{CDD2}.

The tumor suppressor protein p53 is a key player in DDR as it is stabilized and activated in response to DNA damage. The CSN has direct impact on the tumor suppressor [65,66]. Induction of p53 leads to upregulation of the kinase inhibitor p21 which binds to Cdk2 thereby inhibiting its activity. Thus, elevated p21 levels cause G1/S arrest and allow time for the DNA repair prior replication of the genome [118]. Interestingly, p21 also inhibits replication by binding to PCNA contributing to S-phase checkpoint. The degradation of p21 is regulated by APC/C\textsuperscript{Cdc20}, CRL1\textsuperscript{Jab1} and CRL4\textsuperscript{CDD2} ligases [116,117,119] and an Ub independent pathway was also reported [120].

After completion of DNA repair checkpoint recovery leads to regain of the ability to exit the arrest. Checkpoint recovery involves UPS-mediated degradation of Cdc25A, clapsin, a checkpoint mediator, and the mitosis inhibiting kinase Wee1 by CRL1\textsuperscript{A-TRCT} [80], which is also regulated by the CSN. Interestingly, in a large-scale proteomic analysis of proteins phosphorylated in response to DNA damage induced by \gamma-irradiation CSN1, CSN3 and CSN7a were identified as putative substrates for the ATM and ATR kinases [121], which supports data published by Shiloh [73]. In summary, at least at the effector level of DDR the CSN fundamentally modulates the outcome as it influences most of the key players (e.g. p21, p27 and p53).

3.4. Manipulation of the CSN in the DDR: possible clinical applications

DDR is thought to be a barrier for tumor development. Hence, many tumors display defects in the DDR. These cells do not efficiently repair their genome and, therefore, are sensitive to chemicals that induce DNA damage, which may lead to senescence or apoptosis. Induction of apoptosis in tumor cells is the aim of therapy. An important effector of DDR is the tumor suppressor p53. Elevation of p53 suppresses tumor growth and induces apoptosis. Curcumin and emodin have been shown previously in our laboratory to inhibit the CSN associated kinases [69] making tumor cells more prone to apoptosis [70]. However, this approach is only meaningful in tumors expressing wt p53. Moreover, the DDB2 promotor harbors p53 binding sites and elevated p53 levels would lead to higher DDB2 levels. Interestingly, DDB2 supports apoptosis by regulating the levels of p21 [122].

It has been proposed that prevention of replication lesion repair could specifically kill cancer cells [123]. Such a strategy could even be more promising applied together with DNA damaging agents or radiation therapy. In fact, several inhibitors that target DNA repair pathways have been developed and are in clinical trials [123]. Knockdown of CSN5 has been shown to cause defects in DNA repair [29]. Studies from CSN5 knockout MEFs have shown that neddylation function is important for proliferation. Csns null cells that are reconstituted with a CSN5 neddylation defective mutant arrest in cell cycle at multiple stages [124]. Hence, blocking the neddylation function of the CSN could be beneficial in cancer therapy.

Due to the fact that lack of CRL4A complexes leads to elevated levels of DDB2 at higher DNA GG-NER rate, pharmacological
inhibition of Cul4A-DDB1 has been proposed to be attractive for preventing UV- and carcinogen-induced cancer [101]. Small molecules inhibiting the interaction of CSN5 with the 9-1-1 complex may lead to checkpoint activation after induction of replication stress and may be beneficial for cancer prevention. Many viruses induce DDR, which can either be beneficial for viral replication or an obstacle to outcome. Furthermore, divergent viruses (paramyxovirus, herpesvirus, lentivirus and hepadnavirus) are known to hijack or target CRL4s suggesting importance in evasion of immune defense and viral propagation. Several viruses seem to disrupt CRL4s or hijacking them to target host cell proteins to facilitate viral replication probably by causing an extended S phase [48]. Thus, manipulating the CRL4–CSN interaction may be beneficial for antiviral therapy.

4. Conclusion

The CSN is not just a deneddylase. CSN-mediated deneddylation, phosphorylation and deubiquitination affect multiple events of DDR. Through regulating the activity of CRL ligases it has been shown to be unequivocally involved in NER. Furthermore, via phosphorylation the CSN directly controls the stability of many important proteins acting as effectors within the DDR. Thus, the CSN most likely regulates the DDR outcome, whether the cell goes into apoptosis or becomes senescent or recovers from damage.

In DDR the CSN may act as a platform arranging protein communication. This is illustrated by the fact that after UV irradiation the CSN dissociates from CRL4A-DDB1 and subsequently forms a new supercomplex by associating with CRL4A-DDB1. How this selective reassembly and the formation of CSN-based supercomplexes are regulated might be a main question in DDR research.

Another important question that has been raised is the feature of CRL4A-DDB1 to cause mono-ubiquitination of histones but polyubiquitination of other proteins.

Since many tumors display defects in DDR and the CSN has a pivotal role in the process, manipulation of CSN activities will be attractive for future tumor therapy.

Acknowledgements

We thank Tilo Schmaler for critical reading of the manuscript. This work was funded by the Priority Program 1365 of the Deutsche Forschungsgemeinschaft.

References


