The COP9 signalosome: at the interface between signal transduction and ubiquitin-dependent proteolysis

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Summary

Recently the COP9 signalosome (CSN) has become a focus of interest for many researchers, because of its function at the interface between signal transduction and ubiquitin-dependent proteolysis. It is required for the proper progression of the cell cycle in *Schizosaccharomyces pombe* and is essential for development in plants and *Drosophila*. However, its function in mammalian cells remains obscure. Although the CSN shares structural similarities with the 26S proteasome lid complex (LID), its functions seem to be different from that of the LID. A variety of CSN-specific protein-protein interactions have been described in mammalian cells. However, it is currently unclear how many reflect true functions of the complex. Two activities associated with the CSN have been identified so far: a protein kinase and a deneddylase. The CSN-associated kinase phosphorylates transcription factors, which determines their stability towards the ubiquitin system. The associated deneddylase regulates the activity of specific SCF E3 ubiquitin ligases. The CSN thus appears to be a platform connecting signalling with proteolysis.

Key words: COP9 signalosome, 26S proteasome, Kinase activity, Deneddylation

Introduction

The ubiquitin (Ub) system is the most important proteolytic machinery in eukaryotic cells and is involved in the regulation of essential cellular processes such as the cell cycle, signal transduction and antigen processing (Hershko and Ciechanover, 1998). In recent years the control of Ub-dependent proteolysis by upstream factors has become a focus of attention of many researchers. The COP9 signalosome (CSN) is a protein complex that lies at the interface between signal transduction and Ub-dependent proteolysis and appears to control degradation of certain substrates by the Ub system.

Deng and co-workers first identified the CSN in *Arabidopsis* (COP stands for constitutive photomorphogenesis) as a suppressor of light-dependent development (Wei et al., 1994; Wei and Deng, 1999). The complex drew more general interest when it was identified in fission yeast (Mundt et al., 1999) and higher eukaryotes (Wei et al., 1998; Seeger et al., 1998), and isolation of highly purified complex from mammalian cells provided the basis for a detailed characterisation of its subunit composition. The CSN contains eight subunits: CSN1 – CSN8 (Wei et al., 1998; Seeger et al., 1998; Deng et al., 2000). Among these are the thyroid-hormone-receptor-interacting protein TRIP15 (CSN2) and the Jun-activation-domain-binding protein Jab1 (CSN5). The presence of these signalling molecules, together with the early data obtained in *Arabidopsis*, suggested that the CSN has a global role in signal transduction. This idea is supported by the fact that isolated CSN from human erythrocytes exhibits a kinase activity that specifically phosphorylates Jun, IκBα and the NF-κB p50 subunit precursor p105 (Seeger et al., 1998). Recent data indicate that the kinase is associated with the CSN rather than being part of the complex (see below).

Cloning and sequencing of all the CSN subunits revealed another very interesting fact: CSN subunits share significant sequence homology with the eight subunits of the 26S proteasome lid complex (LID) (Glickman et al., 1998; Wei et al., 1998; Seeger et al., 1998; Henke et al., 1999). The 26S proteasome is a large protein complex that selectively degrades cell cycle regulators, transcription factors, enzymes and other essential cellular proteins (Hershko and Ciechanover, 1998) to which Ub has been covalently linked. Attachment of Ub involves a cascade containing ubiquitin-activating (E1) and ubiquitin-conjugating (E2) enzymes and ubiquitin ligases (E3). The 26S complex consists of >30 different subunits. It comprises a proteolytic core (the 20S proteasome) and a 19S regulator, which confers substrate specificity and ATP dependence on the 26S complex (Ferrell et al., 2000). The 19S regulator consists of two subcomplexes: the BASE and the LID (Glickman et al., 1998; Henke et al., 1999). The BASE contains six ATPases that display reversed chaperone activity on substrate proteins (Braun et al., 2000); the function of the LID, by contrast, has not been yet elucidated.

The homology shared by the LID and the CSN subunits mostly encompasses the MPN and PCI domains (Hofmann and Bucher, 1998) – 200 residue motifs at the N- or C-terminus, respectively, of LID components. Each LID subunit can be related to a homologous subunit of the CSN (Henke et al., 1999; Wei and Deng, 1999). The two complexes contain six polypeptides that possess PCI domains and two that possess MPN domains. The PCI domain of CSN1 is required for

incorporation of the subunit into the complex (Tsuge et al., 2001). Interestingly, PCI and MPN domains have also been identified in subunits of the translation initiation factor complex eIF3 (Karniol and Chamovitz, 2000). At the moment the physiological significance of the homology with eIF3 is unclear.

Data from many laboratories demonstrate that there is a functional relationship between the CSN and the Ub pathway, and recent work has implicated the complex in removal of the Ub-like protein NEDD8 from SCF, the E3 ubiquitin ligase responsible for the ubiquitination of cell cycle regulators (Schwechheimer and Deng, 2001). The CSN and the Ub system might thus co-operate in regulating the stability of cell cycle regulators and transcription factors. Is the CSN just another LID that can be integrated into the 26S proteasome to confer different substrate specificity upon it? Or do enzymatic activities associated with the CSN have a broader, regulatory role? These and other questions are discussed in this commentary.

Biological roles of the CSN

Deng and co-workers discovered the CSN in *Arabidopsis* when they characterised mutants of light-dependent development. Morphogenesis of germinating seedlings is light dependent, and light triggers the developmental process called photomorphogenesis. A number of mutations in the *Arabidopsis* COP/DET/FUS loci result in the loss of the CSN complex and in a similar *cop* phenotype, which exhibits signal-independent expression of light-induced genes (Wei and Deng, 1999). Therefore the CSN was hypothesised to be a repressor of photomorphogenesis (Osterlund et al., 1999). The transcription factor HY5 is a positive regulator of light-regulated genes. It is degraded in the dark by the Ub system, and the CSN, as well as the autonomous repressor of photomorphogenesis COP1, is required for this process (Schwechheimer and Deng, 2001). In the dark, COP1 accumulates in the nucleus and prevents HY5 from activating downstream genes (Hardtke et al., 2000). It contains a RING-finger domain typical of a number of E3 Ub ligases. Therefore, it has been suggested that COP1 ubiquitinates HY5 and triggers its degradation by the 26S proteasome (Osterlund et al., 2000). In the light, COP1 is relocated from the nucleus into the cytosol, allowing HY5-mediated photomorphogenesis.

Another link between the CSN and plant development is the degradation of the transcriptional repressor proteins AUX/IAA, which regulate a number of developmental processes. In the presence of the phytohormone auxin the turnover of the AUX/IAA proteins is increased. In several auxin-response mutants the induction of *AUX/IAA* genes is reduced. The same effect has been observed in CSN5 transgenic plants (Schwechheimer et al., 2001). Proper auxin response in *Arabidopsis* is mediated by an E3 Ub ligase SCF (Gray and Estelle, 2000). The CSN interacts with the SCF complex and apparently regulates its activity (Schwechheimer et al., 2001; Schwechheimer and Deng, 2001). Recent data support the idea that the CSN regulates multifaceted developmental processes in *Arabidopsis* through its involvement in the Ub-dependent proteolysis. Reduction-of-function transgenic lines of CSN3 and CSN6 accumulate multiubiquitinated proteins and exhibit diverse developmental defects (Peng et al., 2001a; Peng et al., 2001b).

The CSN is essential for development in invertebrates as shown for *Drosophila melanogaster*. Deletion of genes encoding CSN subunits causes lethality at the late larval or pupal stages (Freilich et al., 1999). Interestingly, in both *Drosophila* and plants that lack components of the CSN there is successful embryogenesis before the death of the organisms during later developmental stages.

Studies on the CSN in fission yeast revealed that the complex has a role in cell cycle regulation. The *S. pombe* CSN1 homologue, Caa1, is required for proper S phase progression. Interestingly, fission yeast cells lacking *csn1*$^{+}$ and *csn2*$^{+}$ display several defects, including slow growth, UV sensitivity and cell elongation, whereas *csn3*-, *csn4*- and *csn5*-null mutants do not show this phenotype (Zhou et al., 2001). Therefore the observed defects may reflect a CSN1/CSN2-specific function.

There are no CSN subunit homologues in prokaryotes, and no proteins homologous to CSN components have been identified in the *S. cerevisiae* genome, except CSN5. Future investigations will show whether the *S. cerevisiae* CSN5 forms a complex resembling the CSN and whether this complex has an impact on the cell cycle.

At the moment, the functions of the CSN in mammalian cells are unclear. Biochemical analysis (see below) indicates that there is a functional cooperation between the CSN and the Ub system in regulating the stability of transcription factors and cell cycle regulators. This implies a participation of the CSN in developmental processes in mammals; these process will have to be elucidated by future research.

The architectures of the COP9 signalosome and the 26S proteasome lid

One might have expected the significant sequence homologies between the CSN and the LID subunits to correlate with a common architecture for the two complexes. Surprisingly, however, an identical architectural plan for the two complexes could not be deduced from recent 2D electron microscopic images of the CSN and the LID (Kapelari et al., 2000). However, the structures of both complexes do have common features. The two particles have similar sizes and show complete asymmetry in their subunit arrangements. Furthermore, the CSN and the LID are characterised by a central groove (Fig. 1a) (Kapelari et al., 2000).

The CSN and LID complexes differ significantly from simple ring-shaped complexes such as the 11S regulator or the rings formed by ATPases of the AAA family (Gray et al., 1994; Peters et al., 1992). In addition, the two complexes appear in different forms, possibly as a consequence of post-translational modifications such as phosphorylation, which would hint at their involvement in signalling pathways. Scaffolding functions for both complexes have been suggested (Kapelari et al., 2000). The CSN might serve as a scaffold for enzymes necessary for substrate ubiquitination such as kinase, deneddylase and E3 Ub ligase. The scaffolding function of the LID might consist in bringing together components necessary for the degradation of Ub conjugates. It is still an open question whether their similar architectures reflect similar functions of the two complexes.

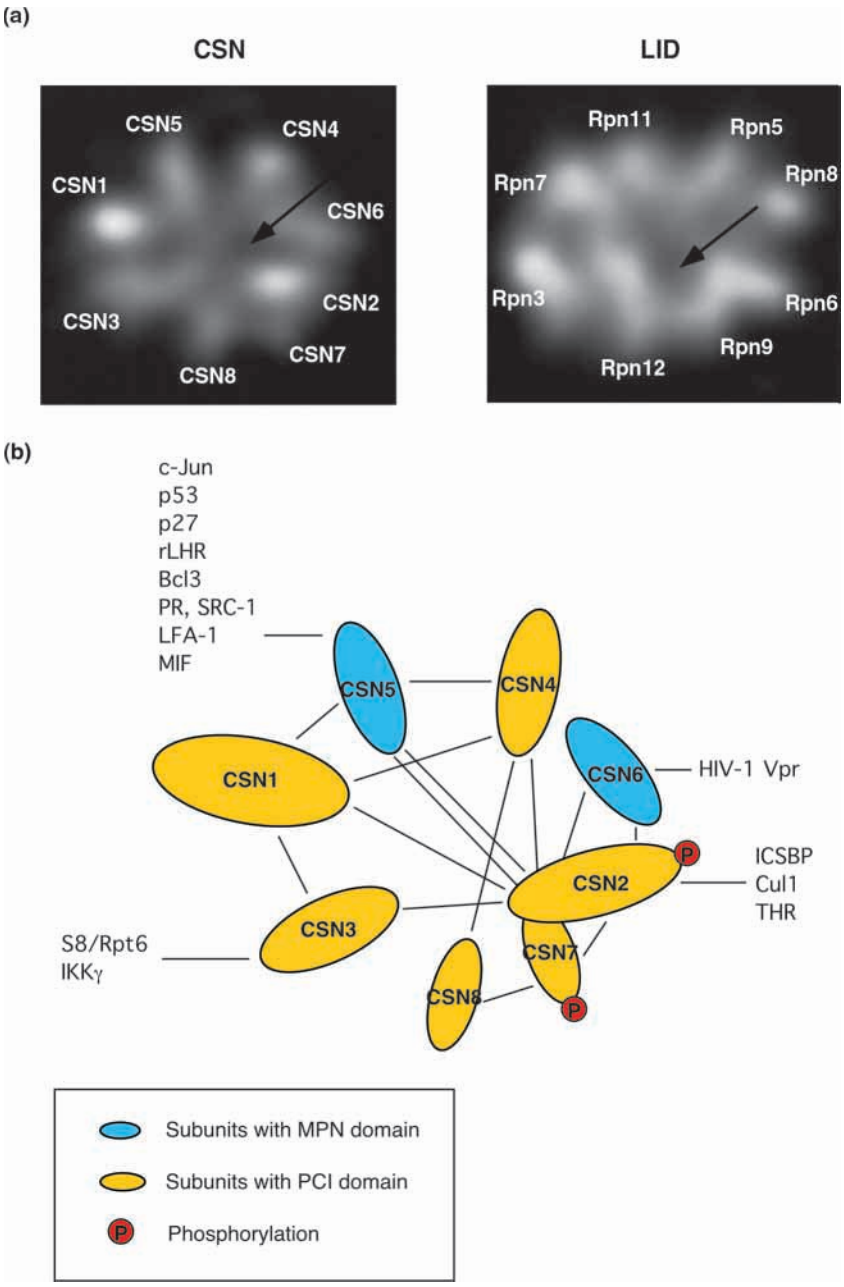

Fig. 1. (a) 2D electron microscopic images of the CSN and the LID. The putative subunit arrangement of the CSN on the basis of the subunit-subunit interaction studies (Kapelari et al., 2000) is indicated. LID subunit-subunit interactions were deduced from CSN data by arranging homologous subunits. The central groove is marked by black arrow. The images were provided by B. Kapelari. (b) CSN interactions with other proteins. The CSN model is based on the electron microscopic image shown in (a) and on subunit-subunit interaction studies summarised recently (Kapelari et al., 2000). Subunits containing MPN domains are blue and those with PCI domains are yellow. The phosphorylation of CSN2 and CSN7 is indicated. All interactions of CSN subunits with other proteins are referenced in Table 1.

COP9 signalosome interactions

Two-hybrid screening and other methods, such as a filter-binding approach and pull-down assays, have revealed a number of CSN subunit-subunit interactions (Freilich et al., 1999; Karniol et al., 1999; Serino et al., 1999; Mundt et al., 1999; Kapelari et al., 2000). By contrast, LID subunit-subunit interactions have not been defined. Fig. 1b shows a model of the CSN complex based on the electron microscopy image shown in Fig. 1a and illustrates the known subunit-subunit interactions. Assuming an analogous arrangement of homologous CSN subunits in the LID, we propose the subunit arrangement of the LID complex shown in Fig. 1a.

A considerable number of proteins might interact with CSN subunits (Table 1; Fig. 1b) – although how physiological these interactions are remains unclear (see below). Surprisingly, most of the interactions involve CSN5 (JAB1), which contains an MPN domain (Hofmann and Bucher, 1998). In the case of $p27^{Kip1}$ the interaction with CSN5 promotes the export of $p27^{Kip1}$ from the nucleus to the cytoplasm and enhances its degradation by the 26S proteasome (Tomoda et al., 1999). Similarly, binding of CSN5 to the intracellular, immature precursor-form of the lutropin/choriogonadotropin (rLHR) triggers its proteolysis (Li et al., 2000), presumably by the 26S proteasome. CSN5 also interacts with the progesterone receptor (PR) and steroid receptor co-activator 1 (SRC-1) and with Bcl3, which leads to stabilisation of the PR-SRC-1 complex and Bcl3-p50 heterodimerisation, respectively. The CSN5-induced stabilisation of these complexes enhances their DNA-binding activity and consequently their ability to stimulate transcription (Chauchereau et al., 2000; Dechend et al., 1999).

Jun is another transcription factor stabilised by interaction with CSN5 (Claret et al., 1996). The activity of the Jun-containing transcription factor AP-1 (activating protein 1) is stimulated by interaction of CSN5 with the integrin LFA-1 (Bianchi et al., 2000) but inhibited by its interaction with the cytokine migration inhibitory factor (MIF) (Kleemann et al., 2000). Moreover, CSN-directed phosphorylation of Jun (see below) also increases AP-1 activity.

There are only a few published examples of interactions involving other subunits of the CSN (Table 1). Of these, binding of HIV-1 Vpr to CSN6 affects cell-cycle-associated signalling (Mahalingam et al., 1998). In addition, CSN3 interacts with IKKγ, a component of the IκB-kinase complex, and appears to regulate NF-κB activity when overexpressed (Hong et al., 2001). A further recently identified interaction between CSN2 and cullin is discussed in more detail below.

Currently, the physiological relevance of many of these interactions is unclear, because they are based on overexpression data or in vitro studies. Whether they occur in the CSN complex rather than just with an overexpressed uncomplexed CSN subunit is thus an open question and particularly relevant given that, at least in *S. pombe*, uncomplexed subunits could not be detected (Zhou et al., 2001).

Table 1. Subunit-non-subunit interactions of the CSN

CSN subunits	Interacting proteins	References
CSN2	ICSBP, interferon consensus sequence binding protein	Cohen et al., 2000
	cullin1 (Cul1), SCF complex core component	Schwechheimer et al., 2001; Lyapina et al., 2001
	THR, thyroid hormone receptor	Lee et al., 1995
CSN3	S8/Rpt6, proteasomal regulatory ATPase	Kwok et al., 1999
	IKKγ, IκB-kinase complex component	Hong et al., 2001
CSN5	c-Jun, transcription factor	Claret et al., 1996
	p53, tumour suppressor	Bech-Otschir et al., 2001
	p27, cyclin-dependent kinase inhibitor	Tomoda et al., 1999
	rLHR, lutropin/choriogonadotropin receptor precursor	Li et al., 2000
	Bcl3, member of the IκB multigene family	Dechend et al., 1999
	PR, progesterone receptor and SRC-1, steroid receptor co-activator	Chauchereau et al., 2000
	LFA-1, integrin	Bianchi et al., 2000
	MIF, cytokine macrophage migration inhibitory factor	Kleemann et al., 2000
CSN6	Vpr, HIV-1 protein	Mahalingam et al., 1998

Although the physiological relevance of all the detected interactions is questionable, the fact that almost all are consistent with a role for the CSN as an interface between signal transduction and ubiquitin-dependent proteolysis is intriguing. It is therefore tempting to speculate that the CSN controls signalling pathways at two points: by interacting with certain receptors and their coactivators and by controlling the stability of different transcription factors or cell cycle regulators.

CSN-directed phosphorylation regulates the degradation of proteins by the ubiquitin system

Recent work indicates that the CSN copurifies with kinase activity. Many of the proteins that interact with the CSN are substrates of this kinase, including c-Jun (Seeger et al., 1998), p53 (Bech-Otschir et al., 2001), ICSBP (Cohen et al., 2000) and p27 (E. W. Wagner and W. Dubiel, unpublished). Others are phosphorylated by the CSN-associated kinase (e.g. IκBα and p105) (Seeger et al., 1998), but the exact binding sites have not yet been determined. Wilson et al., copurified the CSN from calf brain with inositol 1, 3, 4-triphosphate 5/6-kinase, which also phosphorylates c-Jun (Wilson et al., 2001). However, it is too early to decide whether this is the CSN-associated activity. At the moment it is also not clear whether what we call the CSN-associated kinase is a single or multiple kinase.

We identified the CSN-associated kinase in in vitro kinase assays using highly purified CSN and c-Jun as substrate (Seeger et al., 1998). It is unique compared with other kinases and can be efficiently inhibited by curcumin, the yellow pigment in curry (Henke et al., 1999). Analysis of substrate phosphorylation sites shows that the CSN-associated kinase is a Ser/Thr kinase (Seeger et al., 1998; Cohen et al., 2000; Bech-Otschir et al., 2001).

Mapping of Jun CSN-associated kinase-specific phosphorylation sites revealed several serine and threonine residues as putative phosphate acceptors. Among those that are phosphorylated by the CSN-associated kinase are Ser63 and Ser73 (Seeger et al., 1998), residues that are also targets of the JNK (Karin et al., 1997). Jun is, at least in part, regulated through control of its stability towards the Ub system. Phosphorylation of Ser63 and Ser73 prevents Jun ubiquitination and, therefore, its degradation by the 26S proteasome (Musti et al., 1997). There is a direct correlation between the amount of CSN, Jun stability and AP-1 activity in cells; overexpression of CSN2 dramatically increases AP-1 transactivation activity (Naumann et al., 1999). Under these conditions, a high percentage of the overexpressed protein integrates into the cellular CSN complex, and an increase in the amount of cellular CSN is detected probably because of de novo assembly of the complex. Increased cellular Jun concentration is probably responsible for the elevated AP-1 activity. Since JNK is inactive under these conditions, we have proposed the existence of a CSN-directed Jun signalling pathway (Naumann et al., 1999).

The CSN-associated kinase is constitutively active in red blood cells, HeLa, HL-60 and MCF-7 cells (Seeger et al., 1998; Bech-Otschir et al., 2001), whereas JNK is inactive and must be activated by a MAP kinase pathway (Karin et al., 1997). Interestingly, however, there is crosstalk between JNK- and CSN-dependent Jun signalling. Transient expression of CSN1 inhibits JNK1 and consequently Jun-dependent signalling (Spain et al., 1996). The N-terminal domain of CSN1 seems to be responsible for the repression activity (Tsuge et al., 2001). Thus the CSN-directed pathway might suppress the JNK pathway and perhaps vice versa.

Another transcription factor regulated by the CSN is the tumour suppressor p53. In this case, CSN-directed phosphorylation increases the binding of p53 to the Ub ligase Mdm2 and thereby targets the protein to degradation by the Ub system (Bech-Otschir et al., 2001). In common with Jun, p53 binds to CSN5. The two transcription factors might therefore compete for interaction with the complex, and indeed CSN-directed p53 phosphorylation can be inhibited by Jun in vitro (Pollmann et al., 2001). The CSN specifically phosphorylates Ser149, Thr150 and Thr155 in the DNA-binding domain of p53. Mutational analysis indicates that Thr155 is the most important for p53 stability. Constitutive CSN-directed phosphorylation of Thr155 seems to keep p53 in a conformation that has high affinity for the Ub ligase Mdm2, and, therefore, promotes degradation of the tumour suppressor by the Ub/26S proteasome pathway (Bech-Otschir et al., 2001) (Fig. 2). Under normal growth conditions, continuous CSN-dependent phosphorylation appears to cause its degradation by the Ub system and keeps the tumour suppressor at low levels. At the same time, Jun, and perhaps specific AP-1 forms, are stabilised, supporting normal cell growth and differentiation.

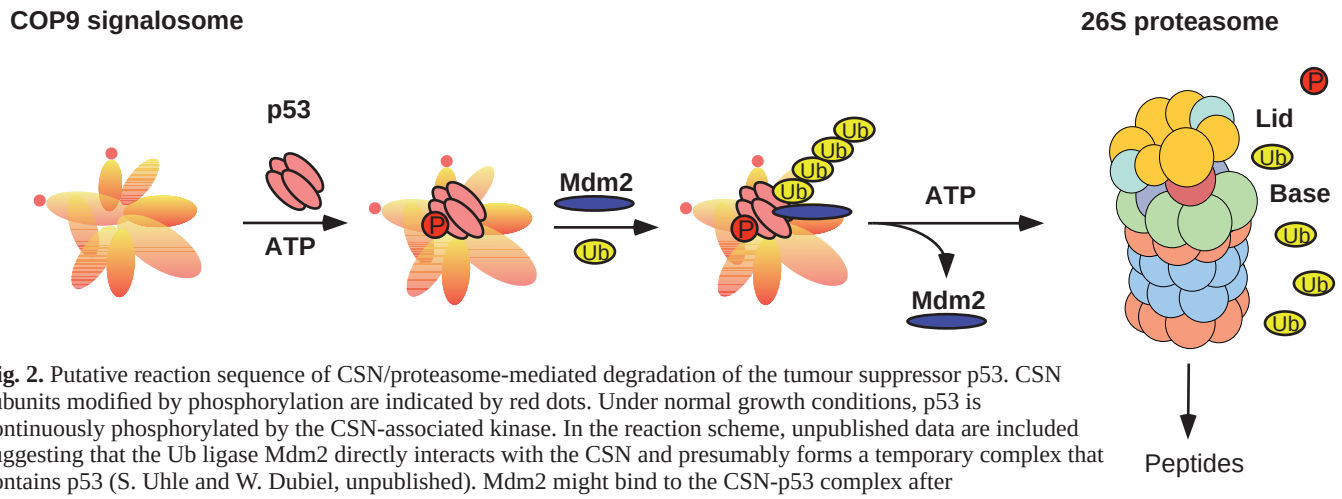

Fig. 2. Putative reaction sequence of CSN/proteasome-mediated degradation of the tumour suppressor p53. CSN subunits modified by phosphorylation are indicated by red dots. Under normal growth conditions, p53 is continuously phosphorylated by the CSN-associated kinase. In the reaction scheme, unpublished data are included suggesting that the Ub ligase Mdm2 directly interacts with the CSN and presumably forms a temporary complex that contains p53 (S. Uhle and W. Dubiel, unpublished). Mdm2 might bind to the CSN-p53 complex after phosphorylation and ubiquitinate the tumour suppressor. At the moment, it is unclear how the p53-Ub conjugates are delivered to the 26S proteasome. The 19S regulator of the 26S proteasome consists of the LID and the BASE. In the figure, it is attached to only one side of the 20S proteasome, the proteolytic machinery.

The c-Jun/p53 balance, controlled, at least in part, by the CSN/Ub system interrelationship is disturbed in many tumour cells. For example, in cervix carcinoma cells, p53 degradation is accelerated by the papilloma virus protein E6, which activates the p53 Ub ligase E6-AP (Scheffner et al., 1993). The consequence is increased Jun levels, which induce enhanced VEGF (vascular endothelial growth factor) production in these cells (Pollmann et al., 2001).

The COP9 signalosome is involved in cullin modification by NEDD8

Recently Lyapina et al. discovered that the CSN is associated with components of the SCF E3 Ub ligase (Lyapina et al., 2001), pointing to another link between the CSN and the Ub/26S proteasome system. The CSN and SCF E3 ligase can be co-precipitated from human and fission yeast cells. Moreover, yeast two-hybrid studies and experiments in *Arabidopsis* have revealed that the SCF core component cullin 1 (Cul1) associates with CSN2 and CSN6 and the RING-domain SCF core component HRT1 interacts with CSN1 and CSN6 (Schwechheimer et al., 2001).

SCF belongs to a family of cullin-containing E3 complexes that also contain a RING-domain protein, the adapter Skp1 and a substrate-binding F-box protein (Deshaies, 1999; Tyers and Jorgensen, 2000). The cullin is covalently modified with the ubiquitin-like protein NEDD8 (Lammer et al., 1998; Liakopoulos et al., 1998; Osaka et al., 2000). In concert with an E2 Ub-conjugating enzyme, SCF promotes the phosphorylation-dependent ubiquitination and subsequent degradation of various substrates including $p27^{Kip1}$ and IκBα. Recently Kawakami et al. showed that NEDD8 modification of Cul1 enhances recruitment of the E2 Ub-conjugating enzyme to the SCF complex (Kawakami et al., 2001). It is now believed that repeated cycles of neddylation and deneddylation are required for the maintenance of SCF E3 Ub ligase activity (Schwechheimer and Deng, 2001).

Studies in fission yeast and in *Arabidopsis* have revealed that the CSN has a role in the removal of NEDD8 from cullin (Lyapina et al., 2001; Schwechheimer et al., 2001; Zhou et al., 2001). CSN-deficient *S. pombe* cells accumulate 'neddylated' cullins and display a significant increase in the related ubiquitination activity. The CSN might thus be associated with a deneddylase activity. Because the effect on SCF-dependent ubiquitination is more dramatic than the accumulation of neddylated cullins, Zhou et al. have suggested that other – as yet unknown – CSN functions contribute to inhibition of SCF (Zhou et al., 2001).

In *Arabidopsis*, downregulation of CSN5 causes not only an accumulation of neddylated cullin but also stabilisation of the putative SCF substrate PSIIA6 (Schwechheimer et al., 2001). However, although *S. pombe* cells lacking *csn1*$^+$ also show an accumulation of neddylated cullin, the stability of the SCF substrate Rum1 is not altered (Lyapina et al., 2001). It would be interesting to find out whether these cells accumulate ubiquitinated Rum1, which would indicate that downstream events such as recognition or degradation by the 26S proteasome are rate limiting.

The CSN-dependent deneddylating activity is sensitive to the alkylating agent N-ethylmaleimide, and Lyapina et al. have therefore suggested that cleavage of NEDD8 conjugates is performed by a cysteine protease similar to those known to cleave ubiquitin- or SUMO-protein conjugates (Lyapina et al., 2001). CSN5 is the only COP9 signalosome subunit that harbours a conserved cysteine residue. However, replacement of this cysteine residue by alanine does not affect the deneddylase activity in these mutants, and recombinant CSN5 failed to cleave NEDD8-cullin conjugates (Zhou et al., 2001). Therefore, it is unlikely that deneddylase activity can be assigned to CSN subunits. Instead, it might be an associated isopeptidase, such as USP21, which has been shown to cleave ubiquitin- and NEDD8-conjugated proteins (Gong et al., 2000). Zhou et al. observed that deletion of *csn1*$^+$ affects deneddylation to an extent similar to that of deletion of *csn3*$^+$, *csn4*$^+$ or *csn5*$^+$, implying that the integrity of the CSN complex is crucial for the deneddylation process (Zhou et al., 2001). Taken together, the available data suggest that CSN promotes deneddylation of cullins by an as yet unknown mechanism. This might regulate the SCF ubiquitination activity, although other CSN functions might also contribute to this effect.

Conclusions and Perspectives

The CSN interacts with a number of transcription factors, receptors and cell cycle regulators, regulating their stability towards the Ub/26S proteasome system.

Although the CSN and LID share significant structural similarities, their functions seem to be different. Neither protein-protein interactions nor associated activities described for the CSN have been identified for the LID. The CSN appears to be a platform connecting signalling, perhaps through phosphorylation and deneddylation, with degradation by the 26S proteasome.

Future work must identify the CSN-associated enzymatic activities and the exact mechanisms that regulate the stability of CSN-interacting proteins.

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