Update on the COP9 Signalosome

The COP9 Signalosome: Its Regulation of Cullin-Based E3 Ubiquitin Ligases and Role in Photomorphogenesis

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Plants are sessile organisms that have evolved an extraordinary phenotypic and developmental plasticity to optimally adapt to the ambient environment. Light is one of the most critical environmental factors, since it is a key signal for regulating a wide range of developmental and physiological processes. The Constitutively Photomorphogenic9 (COP9) signalosome (CSN), originally discovered as an essential complex that regulates light-induced development in Arabidopsis (Arabidopsis thaliana), is a conserved protein complex found among all eukaryotes (Wei and Deng, 1992; Wei et al., 1994, 1998; Chamovitz et al., 1996). This evolutionarily conserved complex has a central role regulating the ubiquitin (Ubq)/26S proteasome pathway and participates in diverse cellular and developmental processes, including cell cycle progression, gene expression, embryogenesis, circadian rhythms, plant responses to light and hormones, and DNA repair (Wei and Deng, 2003; Wolf et al., 2003).

The Ubq-proteasome pathway mediates the degradation of proteins and is the dominant selective protein turnover system in plants (Dreher and Callis, 2007). Ubiquitination and proteolysis by this pathway are achieved by the consecutive activities of three enzymes, E1, E2, and E3. Proteins that are destined for proteasomal degradation are first recognized by E3 Ubq ligases, which subsequently position the substrate for the optimal transfer of Ubq. E2 Ubq-conjugating enzymes, which have been charged with Ubq molecules by E1 Ubq-activating enzymes, provide the activated Ubq, which allows E3 to stimulate the conjugation of Ubq to the substrate. The polyubiquitinated substrates are then degraded by the 26S proteasome (Smalle and Vierstra, 2004). The best-studied biochemical function of CSN is the regulation of the cullin-RING-type E3 ubiquitin ligases (CRLs) by physically interacting with CRLs and removing RUB1

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THE STRUCTURE OF CSN

CSN consists of eight distinct subunits named CSN1 through CSN8, each having a homologous subunit to the Id subcomplex of the 26S proteasome (Deng et al., 2000; Schwechheimer et al., 2001). Each subunit of the CSN complex is composed of one of two conserved domains. Subunits CSN1 to -4, -7, and -8 contain a PCI (for proteasome, COP9, and initiation factor3) domain. Subunits CSN5 and -6 contain an MPN (for Mpr1 and Pad1 N-terminal) domain (Glickman et al., 1998; Hofmann and Bucher, 1998; Wei et al., 1998). Analyses of csn loss-of-function mutants of all eight Arabidopsis subunits indicate that each subunit is structurally required for the formation of the CSN complex. The loss of any of these subunits triggers CSN instability and cellular depletion of the intact CSN complex (Gusmaroli et al., 2007). The PCI domain mediates and stabilizes protein-protein interactions within multisubunit protein complexes and, therefore, is essential for protein complex assembly (Kapelari et al., 2000; Tsuge et al., 2001). Furthermore, PCI subunits are essential for interactions with other MPN subunits and potentially other interacting partners, such as nucleic acids (Dessau et al., 2008). The role for MPN domains can be subdivided into the biochemically active MPN+ and inactive MPN. The MPN+ domain contains the metalloprotease motif, JAMM (for Jab1/MPN/Mov34), in CSN5 and confers the deubiquitylation activity (Cope et al., 2002; Maytal-Kivity et al., 2002; Tran et al., 2003; Ambroggio et al., 2004). CSN6 contains an inactive MPN domain that lacks metal binding and catalytic activity but likely plays a structural role and contributes...
to the binding of CSN to E3 ligases (Lyapina et al., 2001; Choi et al., 2011; Zhao et al., 2011; Zhang et al., 2012).

PCI and MPN domain-containing proteins are also found in two other multisubunit protein complexes: the lid subcomplex of the 26S proteasome and the eukaryotic translation initiation factor eIF3 (Glickman et al., 1998; Kim et al., 2001; Hinnebusch, 2006). While these three protein complexes all have independent biochemical functions, they share homology with each other at several levels. CSN and the proteasome lid share the highest homology, with each subunit of the CSN complex having a paralogous subunit in the lid with a ratio of 6:2 PCI:MPN, with the MPN subunits directly interacting with each other. For example, CSN5 and Rpn11 (for regulatory particle non-ATPas subunit11) are paralogs of each other, with both containing the MPN+ motif. Functionally, Rpn11 harbors the activity center for Ubq deconjugation of the proteasome lid and CSN5 performs RUB1 deconjugation of CRLs, and they share a similar location on the periphery of their respective complexes (Cope et al., 2002; Maytal-Kivity et al., 2002; Verma et al., 2002). Each of these complexes also have interactions directly with each other (Peng et al., 2003), interactions between subunits of the three complexes (Hoareau Alves et al., 2002), or subunits of one complex interact with another complex (Kwok et al., 1999; Yahalom et al., 2001, 2008). Moreover, negative stain-electron microscopy (EM) and single-particle analyses have revealed that CSN, the lid subcomplex, and eIF3 have a common architecture, similar to a palm with partly curved fingers (Bohn et al., 2010; Enchev et al., 2010). This architectural similarity and the marked sequence homology between subunits of the complexes suggest that they have evolved from a common ancestor.

Crystallizing the entire CSN complex has not been reported. To date, two individual subunit crystal structures have been solved for CSN: a structure of CSN7 from Arabidopsis and the MPN domain of CSN6 from *Drosophila melanogaster* (Dessau et al., 2008; Zhang et al., 2012). Analysis of CSN7 revealed that its PCI domain is composed of a helical bundle and winged helix subdomains. Although the PCI domain of CSN7 can interact with CSN1 and CSN8, the PCI domain itself is not sufficient to assemble into the CSN. However, the CSN7 PCI domain plus part of the C-terminal CSN6-binding domain is able to assemble into CSN, indicating that the C-terminal tail of CSN6 is essential for CSN7 function and the integrity of the complex (Fu et al., 2001; Serino et al., 2003; Dessau et al., 2008). Moreover, the C-terminal tail of CSN7 is required for the interaction and regulation of extra-complex proteins (Halimi et al., 2011). Analysis of the MPN domain in CSN6 revealed that the domain dimerizes in vitro and that the residues that make up that dimer face are also highly conserved, indicating that the interface may play an important role for CSN6 function and association. Furthermore, the MPN domain was shown to be an interaction domain, likely regulating CSN5 function or ensuring the correct localization of the substrate during derubylaion (Zhang et al., 2012).

Due to the difficulty in crystallizing large multi-subunit protein complexes, including CSN, a variety of other methods, such as mass spectrometry (MS) and negative stain-EM, have been performed to elucidate the topology and structure of reconstituted and biochemically active human CSN (Sharon et al., 2009; Enchev et al., 2010). The MS approach led to the model that CSN is a 321-kD complex, consisting of two symmetrical modules that are composed of CSN1 to -3, -8, and CSN4 to -7 and are connected by a major link between CSN1 and CSN6. Furthermore, this study proposes that CSN can probably form a variety of subcomplexes, in agreement with other studies that have described “mini-CSN” complexes, suggesting that changes in its subunit composition are likely linked to the ability of CSN to participate in many functional roles in vivo (Sharon et al., 2009). The EM study preliminarily characterized the topology of CSN and revealed that it is highly conserved between the proteasome lid and elf3. Furthermore, the study revealed that CSN contains a central cleft along the two CSN modules, with the same average diameter as the cullin repeats. It is possible that this groove is the binding pocket for CRLs, making RUB1 accessible for derubylatation by CSN5 (Enchev et al., 2010). Further studies need to be completed, since the MS-based approach did not support the interactions reported in the CSN7 structure study and the EM-based approach only allowed for a low-resolution structure that does not elucidate the binding partners in CSN.

**CULLIN-BASED E3 UBQ LIGASES AND THE ESSENTIAL ROLE OF CSN**

CSN is a critical player in many diverse cellular and developmental processes, and to date, the only enzymatic activity attributed to CSN is the removal of RUB1 from CRLs (Wei et al., 2008). In fact, loss of any of the subunits of CSN in Arabidopsis causes the destabilization of CSN, which results in abnormal accumulation of rubylated CRLs (Gusmaroli et al., 2007). CSN5 also exists in the monomeric form in the wild type or *csn* mutants from all organisms studied to date. Although the CSN5 monomer has been implicated independently of the CSN complex in a mammalian system (Wei et al., 2008), its functional relevance in plants remains to be established (Cope et al., 2002; Dohmann et al., 2005, 2008).

The CRL superfamily of E3 Ubq ligases, which are assembled on cullin scaffolding proteins, represent the largest family of Ubq ligases (Smalle and Vierstra, 2004; Hua and Vierstra, 2011). In Arabidopsis, there are 11 cullin proteins, but only CUL1, CUL3a, CUL3b, and CUL4 have been demonstrated to be rubylated in vitro and in vivo (del Pozo and Estelle, 1999; Bostick et al., 2004; Figueroa et al., 2005; Weber et al., 2005; Chen et al., 2006; Hotton and Callis, 2008). Cullins provide the
scaffolding for a small RING (for Really Interesting New Gene)-Box domain protein (RBX1) and a variety of adaptors that are specific for each cullin protein, which are associated with substrate receptors that specifically recognize their corresponding degradation substrates (Fig. 1). In mammalian cells, it is suggested that 20% of all proteasome-mediated degradation is CRL dependent (Soucy et al., 2009). It is likely that this percentage is higher in plant systems, since it has been reported that many hundreds to 1,000 distinct CRLs are expressed in plant species. For example, the CUL1-based family of CRLs has 69 F-box proteins in humans, compared with close to 700 identified F-box proteins in Arabidopsis with the potential to assemble to more than 1,500 distinct CRLs, thus capable of regulating a variety cellular processes (Fig. 1; Hua and Vierstra, 2011).

Since CRLs control multiple cellular processes, it is imperative that CRLs undergo disassembly and reassembly cycles to properly regulate protein degradation. This cyclical process is mediated by RB1 and CAND1 (for CULLIN-ASSOCIATED and NEDDYLATION-DISSOCIATED), which work in concert to regulate CRLs (Fig. 2A). The first modification involves the covalent attachment of RUB1 to the cullin protein by a three-step reaction cascade, similar to ubiquitination (del Pozo and Estelle, 1999; Gray et al., 2002; Dharmasiri et al., 2007). Rubylation stimulates the ubiquitination of substrate proteins by inducing a conformational change to CRLs that results in an open and flexible structure, allowing for the E2 to tilt closer to the substrate-binding pocket (Duda et al., 2008; Saha and Deshaies, 2008). The RUB1 modification is removed by CSN via its deneddylation activity. Meanwhile, CSN associates with one or more deubiquitinating enzymes, such as USP15 (for ubiquitin-specific peptidase15) and UBP12 (ubiquitin-specific processing protease12) in humans and fission yeast, respectively, to remove Ubq molecules that are autocatalytically attached to the CRL substrate adaptors (Hetfeld et al., 2005; Wee et al., 2005).

The second cycle is mediated by the noncovalent binding of CAND1 to unmodified cullin-RBX complexes, which subsequently inhibits CRL assembly and RUB1 activation (Liu et al., 2002; Zheng et al., 2002; Feng et al., 2004). The dissociation of CAND1 from CRLs is promoted upon either the availability or increased levels of the CRL substrate adaptor, which displaces CAND1, allowing CRL rubylation (Bornstein et al., 2006; Schmidt et al., 2009). CRLs would remain active and unable to bind to CAND1 until CSN-mediated derubylation (Fig. 2A). It is interesting that loss of CAND1 in Arabidopsis does not result in a strong phenotype, indicating that CAND1 is not an absolutely essential component in every cullin E3 ligase (Feng et al., 2004). Furthermore, it is not precisely clear how RUB1 globally affects CRL status. Although genetic studies have revealed an essential role for RUB1, a recent pharmacological and genetic study in human cell cultures has shown that prolonged global derubylation does not convert CRLs to cullin-CAND1 complexes; instead, most cullins are bound to adaptor molecules (Xirodimas, 2008; Bennett et al., 2010). In addition, CSN has been shown to directly regulate CRL activity by nonenzymatic mechanisms in vitro and in Neurospora crassa (Fischer et al., 2011; Zhou et al., 2012). These studies suggest that the binding of CSN, the cycle of rubylation/derubylation, and CAND1 work in concert with the abundance of adaptor modules to control the dynamic activities of each specific CRL.

Since CSN is a key regulator of all CRLs, it is commonly questioned how a specific ligase is regulated by CSN without affecting the remaining CRLs. Recently, new insight has been presented on the role of CSN-mediated regulation of CRLs. In CUL4-DAMAGED DNA BINDING PROTEIN1 (DDB1)-RBX1-based E3 ligases (CRL4), CSN applies its inhibition in a JAMM-independent, nonenzymatic fashion by the CSN, with CSN1 to -3 likely playing scaffolding roles. This function is conserved between two DDB1-BINDING WD40 (DWD)-WDxR-DDB1-CUL4 ASSOCIATED FACTOR (DCAF) CRL4s that are required for DNA repair, CRL4DDB2 and CRL4CSA. This inhibition is only relieved when the DWD/WDxR/DCAF binds to its substrate, which causes CSN release and activation of CRL4DCAF (Fig. 2B; Fischer et al., 2011). Taken together, CSN possesses intrinsic derubylation activity, nonenzymatic CRL4 inhibition, and associates with deubiquitinases. All three mechanisms simultaneously cease upon the substrate binding to its respective E3 ligase receptor, although it is still unknown how these mechanisms contribute to CSN-mediated control of CRLs in vivo. It is known that CSN dissociation results in the loss of receptor protection and allows for autoubiquitination of the receptor, causing its subsequent degradation. This could potentially allow for proper

Figure 1. Model of the three CRLs regulated by CSN in Arabidopsis. The numbers of adaptors are based on studies by Farras et al. (2001), Risseeuw et al. (2003), Lee et al. (2008), Zhang et al. (2008), and Hua et al. (2011).
timing of receptor ligase activity following substrate recognition or for the reassembly of new CRLs (Fischer et al., 2011).

REGULATION OF PHOTOMORPHOGENESIS BY CSN AND CRL4

Light provides a variety of critical cues, including quality, intensity, duration, and direction, which have profound effects on plant development. Light-grown seedlings have short hypocotyls and open, expanded cotyledons, while dark-grown seedlings have elongated hypocotyls and closed, unexpanded cotyledons with an apical hook, termed photomorphogenesis (deetiolated) and skotomorphogenesis (etiolated), respectively (Von Arnim and Deng, 1996). The switch between dark- and light-grown development involves genome-wide transcriptional and massive translational changes triggered by the perception of light through five classes of photoreceptors, which subsequently regulates many aspects of plant development (Li et al., 2012; for DET1, DDB1 associated1; Liu et al., 2012).

Genetic screening of Arabidopsis mutants displaying light-grown characteristics in the dark revealed nine pleiotropic and evolutionarily conserved COP/DET/FUS (for COP/Deetiolated/Fusca) genes (Sullivan et al.,...
COP1 is a conserved RING finger E3 Ub ligase that has been shown to directly target photomorphogenesis-promoting proteins for degradation, such as the far-red light receptor phytochrome A (phyA), the red light receptor phytochrome B, and the transcription factors ELOMENDED HYPOCOTYL5 (HY5), HY5-HOMOLOG (HYH), LONG AFTER FAR-RED LIGHT1 (LAF1), and LONG HYPOCOTYL IN FAR-RED1 (HFR1; Yi and Deng, 2005; Jang et al., 2010). COP1 also directly interacts with SPA1, which was first identified as a repressor of phyA (Saijo et al., 2003; Seo et al., 2003). In Arabidopsis, there are three additional SPA1-like proteins, with the quadruple spa mutant displaying a phenotype similar to that of strong cop1 alleles (Laubinger et al., 2004). Furthermore, biochemical analyses demonstrated that the SPA proteins interact with each other to form a heterogeneous complex with a core tetramer of two COP1 proteins and combinations of two SPA proteins in Arabidopsis (Zhu et al., 2008). This finding is consistent with the hypothesis that SPA proteins function synergistically with COP1 in controlling photomorphogenesis. In the dark, COP1 is responsible for the proteasome-mediated degradation of photomorphogenesis-promoting factors such as HY5. In the presence of light, COP1 is repressed, although the complete mechanism underlying COP1 light inactivation is unknown. Light-dependent export of COP1 to the cytoplasm is a slow process, requires long exposure to light (more than 24 h), and is likely a mechanism to suppress COP1 activation under extended light conditions (Lau and Deng, 2012). A mathematical model proposed an alternative two-step mechanism of COP1 inactivation. The two-step process is a rapid inhibition of COP1 caused by a photoreceptor-related inhibitor, which also activates a CRL4 that subsequently causes slow depletion of COP1 (Pokhilko et al., 2011). It should be noted, however, that COP1 is also required for optimal UV-B-induced photomorphogenesis (Oravecz et al., 2006; Favory et al., 2009).

The CDD complex also plays an important role in the light signaling pathway and requires CSN for stabilization, but understanding the molecular role of the CDD has been challenging (Suzuki et al., 2002). The CDD complex binds to CRL4 but differs from the conventional CRL4 substrate receptor, since both DET1 and COP1 lack a DWD or WDxR motif but still bind to DDB1. Interestingly, DET1 contains a conserved HLH-box-like sequence at its N terminus, a domain shown to allow DCAF binding to DDB1’s clam-shaped pocket, indicating that DET1 might use a similar binding mechanism (Biedermann and Hellmann, 2011; Fischer et al., 2011). DET1 has no recognizable domains but has been linked to chromatin regulation and can bind to the unmodified N-terminal tail of histone H2B (Benvenuto et al., 2002). DET1 was recently shown to physically interact with CIRCADIAN CLOCK ASSOCIATED1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), two closely related MYB transcription factors that are core components of the plant circadian clock. Furthermore, CSN also plays a critical role in regulating the circadian rhythms in Drosophila and N. crassa, but by modulating the stability of the F-box proteins (Knowles et al., 2009; Braus et al., 2010; Zhou et al., 2012). DET1 is recruited to the promoter of CCA1/LHY target genes in a CCA1/LHY-dependent manner and acts as a transcriptional corepressor by directly binding to and repressing transcription factor function (Lau et al., 2011). COP10 is a Ubq-conjugating (E2) variant and can directly bind to and enhance the activity of other E2s, an activity likely to be specific to CRL4 for proper ubiquitination of its substrates (Yanagawa et al., 2004; Lau and Deng, 2009). In the light signaling pathway, the CRL4-CDD E3 ligase may repress light-activated genes through its recruitment by light-regulating transcription factors and subsequent chromatin remodeling. Interestingly, the mammalian homolog of the CDD is called the DDD-E2 complex, in which a true E2 enzyme, rather than a COP10-like Ubq-conjugating (E2) variant, forms a stable complex with mammalian DET1 and DDB1 as well as a small protein called DDA1 (for DET1, DDB1 associated1; Pick et al., 2007).

In Arabidopsis, COP-SPA complexes also interact with CRL4 and form a group of E3 ligases distinct from the CRL4-CDD complex (Chen et al., 2010). Although the CDD and COP-SPA complexes have no direct interaction, they both regulate photomorphogenesis (Fig. 3). Genetic studies have shown that these two groups of ligases work in concert to modulate the light regulation of plant development by targeting photomorphogenesis-promoting factors for degradation (Nixdorf and Hoecker, 2010). It is likely that the combination of the transcriptional repression by the CDD complex in conjunction with the COP1-SPA complex-mediated proteolysis of photomorphogenesis-promoting transcription factors could ensure stringent control of photomorphogenic development (Fig. 3; Lau et al., 2011). However, the relationship between CDD and COP1-SPA complexes and the mechanism by which the interactions between the complexes are regulated are still unknown.

An interesting feature of COP1-SPA complexes is that all known degradation substrates of COP1 colocalize with COP1 in speckle-like photobodies (See et al., 2003; Zhu et al., 2008). Photobodies are plant-specific photoreceptor-containing nuclear bodies whose size and number are directly regulated by external light cues; however, the function of photobodies remains to be elucidated. In the dark, COP1 translocates in a CSN-dependent manner and directly interacts with HY5 in the nucleus, resulting in degradation of HY5 by the proteasome. Nuclear translocation of COP1 requires the N-terminal domain of CSN1, but it is uncertain exactly how CSN targets COP1 to the nucleus (Wang et al., 2009). Although it has yet to be demonstrated if the proteasome colocalizes with photobodies in plants, the...
PERSPECTIVE

For plants to cope as sessile organisms, they have become extremely plastic. Increasing evidence demonstrates that the degradation of proteins by the Ubq/26S proteasome is imperative in allowing plants to rapidly adapt to their environment. This is clearly observed by the complexity of the Ubq pathway and the large portion of the Arabidopsis genome that is devoted to factors participating in the Ubq/26S proteasome pathway. Since the discovery of CSN 20 years ago, there have been a variety of studies that have advanced our understanding of its function, but it has left many more unanswered questions. A resounding question has remained, what is the topology of CSN? The recent crystal study on CRL4DCAF-CSN has challenged the classic CSN model and therefore questions exactly how CSN functions in all aspects of CRL regulation and if this mechanism is conserved among all CRLs (Fischer et al., 2011).

Recently, the UV-B photoreceptor, UV RESISTANCE LOCUS8 (UVR8), was identified in Arabidopsis, and it presents a new area of research in photomorphogenesis (Rizzini et al., 2011; Wu et al., 2012). To date, no role has been assigned to CSN in low-fluence UV-B light signaling. It is speculated that CSN plays a major role in regulating UV-B photomorphogenesis, since COP1 and photomorphogenic transcription factors are key regulators in the signaling pathway. Additionally, there are two DCAF proteins that repress UV-B photomorphogenesis and directly bind to UVR8 whose E3 ligase activities remain to be determined (Gruber et al., 2010).

We anticipate that in the near future many exciting and novel insights into CSN function will come to realization by using a combination of structural biology, biochemistry, forward and reverse genetics, proteomics, and genomic approaches to answer some of these questions and uncover new regulatory mechanisms of CSN.

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