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Regulation of COP1 nuclear localization by the COP9 signalosome via direct interaction with CSN1

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SUMMARY

COP1 and COP9 signalosome (CSN) are key regulators of plant light responses and development. Deficiency in either COP1 or CSN causes a constitutive photomorphogenic phenotype. Through coordinated actions of nuclear- and cytoplasmic-localization signals, COP1 can respond to light signals by differentially partitions between nuclear and cytoplasmic compartments. Previous genetic analysis in Arabidopsis indicated that the nuclear localization of COP1 requires CSN, an eight-subunit heteromeric complex. However the mechanism underlying the functional relationship between COP1 and CSN is unknown. We report here that COP1 weakly associates with CSN *in vivo*. Furthermore, we report on the direct interaction involving the coiled-coil domain of COP1 and the N-terminal domain of the CSN1 subunit. In onion epidermal cells, expression of CSN1 can stimulate nuclear localization of GUS-COP1, and the N-terminal domain of CSN1 is necessary and sufficient for this function. Moreover, CSN1-induced COP1 nuclear localization requires the nuclear-localization sequences of COP1, as well as its coiled-coil domain, which contains both the cytoplasmic localization sequences and the CSN1 interacting domain. We also provide genetic evidence that the CSN1 N-terminal domain is specifically required for COP1 nuclear localization in Arabidopsis hypocotyl cells. This study advances our understanding of COP1 localization, and the molecular interactions between COP1 and CSN.

Keywords: CSN1, COP9 signalosome, COP1, nuclear localization, protein interaction.

INTRODUCTION

Light serves as one of the most important growth stimuli for plant development. Through genetic screens for altered light responses in Arabidopsis, a group of genetic loci known as *Constitutively photomorphogenic* or *De-etiolated* have been isolated (Hardtke and Deng, 2000; Serino and Deng, 2003). These genes encode COP1, a RING-finger E3 ubiquitin ligase (Osterlund *et al.*, 2000); the COP10–DDB1–DET1 (CDD) complex component, a DET1 and the COP10 ubiquitin conjugating enzyme variant (Reed and Chory, 1994; Schroeder *et al.*, 2002; Yanagawa *et al.*, 2004); and multiple components of the COP9 signalosome (CSN) complex (Chamovitz *et al.*, 1996). Mutations in each of these genes cause the plants to take on a light-grown morphology constitutively, even when grown in the dark (Chory *et al.*, 1989; Deng *et al.*, 1991; Wei and Deng, 1992; Wei *et al.*, 1994).

COP1 protein contains a RING domain at the N-terminal region, followed by a coiled-coil domain and a WD-40 repeat domain at the C-terminal region (Yi and Deng, 2005). A unique feature of COP1 protein is its ability to respond to environmental light by differentially localizing to nuclear and cytoplasmic compartments. COP1 is enriched in the nucleus in darkness, but upon stimulation by light it shuttles to the cytoplasm in a light-quantity dependent manner (von Arnim and Deng, 1994). It was proposed that COP1 acts inside the nucleus to suppress photomorphogenic development in darkness, whereas light inactivates COP1 activity in part by the translocation of COP1 from the nucleus to the

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cytoplasm (Yi and Deng, 2005). This dynamic process requires coordinated actions of both the nuclear localization signals (NLS; residues 293–314) and the cytoplasmic localization signals (CLS; residues 67–177) of COP1, with the latter overlapping with its coiled-coil domain (Stacey *et al.*, 1999, 2000). Mutations disrupting either sequences of COP1 abolish its light responsiveness, and cripple its function *in vivo* (Stacey *et al.*, 2000). The differential localization of COP1 is highly specific to light signals, because many phytohormones such as cytokinine and brassinosteroids have no effect on COP1 localization (von Arnim *et al.*, 1997). Apart from the identification of NLS and CLS, very little is known about the molecular mechanisms underlying the light-responsive nuclear–cytoplasmic shuttling. The protein factors that mediate COP1 localization have not yet been reported.

The genetic link among different *cop/det* mutants prompted the hypothesis that these genes act collaboratively, or in the same pathway, to regulate the light response of plants (Wei and Deng, 1996). In line with this hypothesis, COP1 was not detected in the cell nucleus in any of the *csn*, *det1* or *cop10* mutants, regardless of whether they were grown in dark or light conditions (Chamovitz *et al.*, 1996; von Arnim *et al.*, 1997). These findings indicate that the CSN and CDD complexes are required for nuclear import or nuclear retention/accumulation of COP1. However, it can be argued that as mutations in CSN/CDD cause constitutive light activation, COP1 would consequently accumulate in the cytoplasm, as expected in a normal light response. In this case, CSN/CDD would not play a direct role in regulating COP1 localization. Interestingly, COP1 protein accumulates at higher levels in *csn* or *cop10* mutants (Wei and Deng, 1992; Wei *et al.*, 1994). However, because of its aberrant localization, COP1 function is presumably disabled in these mutants, despite its higher abundance. Molecular interactions between the COP/DET group of proteins have also been reported. For example, COP10 was shown to interact with COP1 in yeast two-hybrid assays (Suzuki *et al.*, 2002), and the mammalian homolog of COP1 and DET1 were shown to co-immunoprecipitate in independent studies (Wertz *et al.*, 2004; Pick *et al.*, 2007). However, the direct interaction between COP1 and CSN has, to our knowledge, not yet been reported.

The CSN consists of eight subunits, from CSN1 (also known as FUS6, or COP11) to CSN8 (also known as COP9) (Serino and Deng, 2003). The CSN complex exhibits an isopeptidase activity that removes the modification of an ubiquitin-like protein, Rub1 (or Nedd8), from cullin proteins in a process known as derubylation or deneddylation (Lyapina *et al.*, 2001; Schwechheimer *et al.*, 2001; Cope and Deshaies, 2003). As a result, CSN regulates the activity of cullin-based E3 ubiquitin ligases. Aside from this enzymatic activity, CSN also interacts with de-ubiquitinating enzymes and kinases (Bech-Otschir *et al.*, 2002; Zhou *et al.*, 2003). Genetic studies in fission yeast indicate that CSN possesses additional functions beyond derubylation, and that CSN1 and CSN2 can function independently of CSN3, CSN4 or CSN5 (Mundt *et al.*, 2002; Liu *et al.*, 2003; Zhou *et al.*, 2003).

CSN1 contains a large PCI domain located at the C-terminal half of the protein, which is necessary for subunit–subunit interaction and complex assembly (Tsuge *et al.*, 2001; Wang *et al.*, 2002). The N-terminal domain (NTD) of CSN1, on the other hand, does not have a structural role, but is involved in a critical function of CSN that is not yet understood molecularly. In the Arabidopsis *fus6/C231* line, the transgene expressing the CSN1 C-terminal 231-amino-acid (aa) fragment (residues 211–441 aa) rescues the formation of the CSN complex, as well as the derubylation activity, but is unable to rescue the lethality of the null *csn1* (*fus6*) mutant (Wang *et al.*, 2002). These observations underscored the physiological significance of the function associated with CSN1-NTD.

Here, we report the interaction between Arabidopsis CSN and COP1, and the effect of CSN1 on COP1 subcellular localization in plants. We show that CSN1 can mediate COP1 nuclear translocation in onion epidermal cells, and in Arabidopsis hypocotyl cells, and that COP1 interacts with CSN1-NTD through its coiled-coil domain. These findings advance our understanding of the functional link between COP1 and CSN, and provide an insight into the mechanism of nuclear–cytoplasmic localization of COP1.

RESULTS

The specific association between COP1 and CSN *in vivo*

To investigate the functional relationship between CSN and COP1, we first examined the possible association between the two regulators. Two different transgenic Arabidopsis lines were used for the *in vivo* co-immunoprecipitation experiments: *fus6/FS1(3–4)* and *TAPa-COP1*. The *fus6/FS1(3–4)* plants express Flag-tagged CSN1 that has previously been shown to be fully functional in the *csn1* (or *fus6*) null mutant background (Wang *et al.*, 2002). Flag-CSN1 was immunoprecipitated via Flag tag, and was analyzed by western blots using specific antibodies. CSN1 and CSN4 were highly enriched in the immunocomplex, as expected for any of the CSN subunits (Figure 1a). CUL1, which is a known CSN-associated protein, was pulled-down weakly compared with CSN4. Under the same conditions, endogenous COP1 could be detected in Flag-CSN1 precipitates from both dark- and light-grown transgenic plants, and not from wild-type control plants (Figure 1a). This result suggests that CSN1 can associate with COP1 *in vivo*, but that their association appeared slightly weaker than the CSN–CUL1 interaction, and much weaker than the CSN1–CSN4 interaction.

We conducted another co-immunoprecipitation experiment using a *TAPa-COP1* line (Rubio *et al.*, 2005), which

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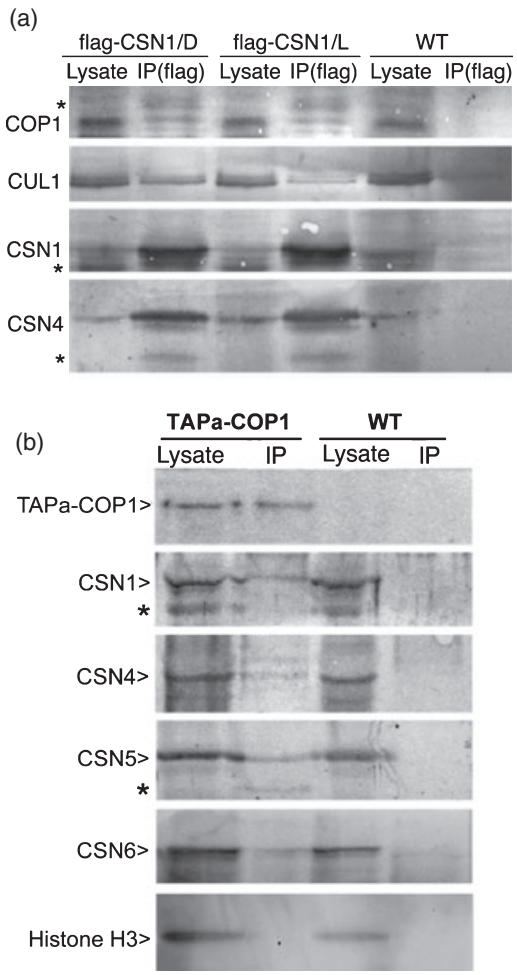

Figure 1. Interaction between COP1 and CSN1.
(a) Co-immunoprecipitation of Flag-CSN1 with endogenous COP1. Protein extracts from 15-day-old light (L)- and dark (D)-grown Flag-CSN1 transgenic [*fus6/FS1*(*3-4*)] and wild-type (WT) Arabidopsis seedlings were incubated with anti-Flag M2 resin to immunoprecipitate Flag-CSN1 (IP). Samples were blotted with the indicated antibodies, including COP1, CUL1, CSN1 and CSN4.
(b) Co-immunoprecipitation of TAPa-COP1 with endogenous CSN. Protein extract from 10-day-old light-grown TAPa-COP1 transgenic and WT Arabidopsis seedlings were incubated with Ni-charged His-Bind resin to pull-down TAPa-COP1 (IP). Samples were blotted with the indicated antibodies, including anti-myc (for the detection of TAPa-COP1), antibodies against representative CSN subunits and anti-Histone H3. *Non-specific bands.

expresses a COP1 fusion protein that is N-terminally tagged with TAPa (protein-A IgG binding domain, the His-tag and the myc-tag epitopes). TAPa-COP1 fusion proteins were isolated from 10-day-old seedlings using the His-Bind resin, and the samples were analyzed by western blot to detect any association with endogenous CSN components (Figure 1b). CSN1 and CSN5 could be clearly detected in TAPa-COP1 precipitates, whereas CSN4 and CSN6 appeared weaker. Under the same conditions, Histone H3 was not detected in the His-Bind pull-down. These CSN proteins were co-precipitated by the His-Bind resin only from TAPa-COP1 transgenic plants, i.e. not from the wild-type control plants, suggesting that the co-precipitation of the CSNs was specific to TAPa-COP1. This result showed an association between CSN and COP1 *in vivo*.

Direct interaction between the N-terminal domain of CSN1 and the coil domain of COP1

To determine whether COP1 and CSN1 can directly interact, we next conducted an *in vitro* binding experiment. Bacteria extracts containing maltose binding protein (MBP) or MBP-COP1 fusion protein (von Arnim and Deng, 1993) were incubated with recombinant His-tagged full-length CSN1 (His-CSN1), or the CSN1 truncations: His-N139 (residues 1–139 of CSN1) and His-C231 (residues 211–441 of CSN1). After MBP pull-down, anti-His tag western blots were performed. MBP-COP1 was found to readily pull-down His-CSN1 and His-N139, but not His-C231 (Figure 2a, lanes 4–6); whereas MBP alone failed to pull-down any of the CSN1 proteins (Figure 2a, lanes 7–9). This result showed that COP1 can directly bind to CSN1 *in vitro*, and that COP1 has a specific affinity with the N-terminal 1–139 aa region of CSN1.

Yeast two-hybrid protein interaction assays were employed to further dissect the interaction domains on both CSN1 and COP1. COP1 was fused to the yeast GAL4 DNA binding domain (DBD fusion), whereas the CSN1 truncations were fused to the GAL4 activation domain (AD fusion) (Figure 2b). Expression of these proteins in yeast was confirmed by western blot (Figure S1). Quantitative measurement of the MEL1 (α-Gal) reporter activity showed that, similarly to the *in vitro* binding data, COP1 selectively interacted with CSN1-N139, but not with CSN1-C231 (Figure 2b).

Next, we performed a deletion analysis of COP1 to determine the domain responsible for binding to CSN1-N139. As illustrated in Figure 2c, a deletion series of COP1 fused to the transcription activation domain (Torii *et al.*, 1998) was paired with the CSN1-N139 DBD fusion (LexA DNA binding domain fusion protein). Quantitative measurement of the lacZ reporter activity revealed that CSN1 N139 interacted with COP1 N-terminal domain N282 (1–282 aa), and had an even stronger reacted with the plain coil domain (128–209 aa) (Figure 2c). In all cases, deletion of the coil domain completely abolished the interaction with CSN1-N139. Expression levels of COP1 truncation fragments in yeast were similar, based on an immunoblot analysis (Figure S1). These experiments showed that the coil domain of COP1 is necessary and sufficient to interact with CSN1-N139 in this assay.

Nuclear localization of CSN1 and its NTD

In an effort to understand the functional implication of COP1-CSN1 interaction, we set out to determine the role of

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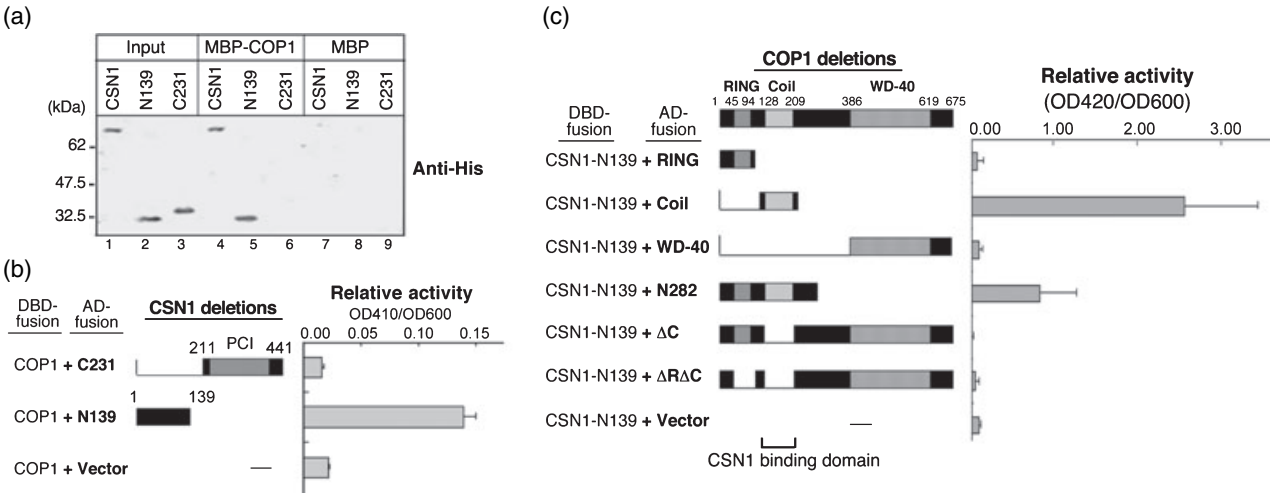

Figure 2. Dissecting CSN1-COP1 interaction domains.
(a) COP1 binds CSN1-NTD *in vitro*. Purified CSN1, N139 and C231 His-tagged fusion proteins were incubated with bacteria extracts containing MBP-COP1 or MBP. Following the pull-down with MBP resin, samples were examined by western blot using anti-His-tag antibody.
(b) COP1 binds CSN1 NTD in a yeast two-hybrid assay. COP1 fused to the GAL4 DNA binding domain (DBD) was tested for interaction with CSN1-N139 or CSN1-C231, both fused to the transcription activation domain (AD). The approximate position of the PCI domain is shaded in gray and marked. The graph on the right indicates the relative α-galactosidase activity, calculated as ratios of OD_{410}/OD_{600}. The data represent the mean value of six independent transformants for each pair. The error bars represent the standard deviations.
(c) Analysis of the interactions between the mutant series of COP1 and CSN1-N139. CSN1-N139 fused to the LexA DBD was tested for interaction with a series of COP1 domain fragments fused to the transcription AD. The relative activities of the lacZ reporter for corresponding pairs are shown in the panel on the right. The data represents the mean value of six independent transformants. The error bars represent the standard deviations.

CSN1 in the COP1 nuclear–cytoplasmic localization pattern. This experiment was motivated by the observation that all of the CSN subunit examined so far is required for the nuclear localization of COP1 (Chamovitz *et al.*, 1996; von Arnim *et al.*, 1997). The transient expression of the GUS-COP1 fusion protein in onion epidermal cells, via particle bombardment, has been successfully used to demonstrate the light-responsive nuclear–cytoplasmic localization of COP1 (von Arnim and Deng, 1994; Wang *et al.*, 1999). We therefore adopted this transient expression system. As previously reported, GUS-COP1 displayed a nuclear enrichment in darkness, compared with in light (24 h of continuous illumination), when expressed alone or when co-bombarded with the empty GFP vector that expresses three tandem copies of GFP (Figure 3Aa–d). To quantify the nuclear–cytoplasmic localization of GUS-COP1, we conducted statistical surveys by determining the percentages of cells exhibiting nuclear-enriched GUS staining (Figure 3B). Cells in which the GUS-COP1 staining was stronger in the nucleus than the cytoplasm were scored as 'nuclear' (Figure 3Aa, c, j). These cells were counted against the total number of cells that displayed GUS staining, including both nuclear-enriched cells and those displaying an evenly distributed GUS staining between the nucleus and the cytoplasm (Figure 3Am), or a stronger cytoplasmic GUS staining (Figure 3Ae, g, p).

Next, we constructed CSN expression vectors including CSN1, CSN4, CSN5 and CSN1 deletion mutants, including N139 (1–139 aa), N211 (1–211 aa) and the C-terminal domain, C231 (211–441 aa). All of the CSN proteins were C-terminally fused to GFP, so that their expression could be monitored by following the GFP signal. To prevent passive nuclear diffusion of the small proteins, we fused two or three tandem copies of GFP to the CSN1 truncation fragments, generating N139-GFP3 (with three GFP repeats), N211-GFP2 (with two GFP repeats) and C231-GFP2 (with two GFP repeats).

To confirm the expression of CSN fusion proteins, the above-described plasmids were bombarded into onion epidermal cells, and GFP imaging was used to detect protein expression after 24 h. As shown in Figures 3A l, o, s and 4B, CSN1-GFP and CSN5-GFP could localize to the nuclei, consistent with previous reports of their animal homologs, whereas CSN4-GFP was predominantly cytoplasmic. In addition, although CSN1-GFP, N211-GFP2 and N139-GFP3 localized predominantly to the nuclei (Figure 4Ac–f), C231-GFP2 formed inclusion bodies dispersed throughout the cytoplasm (Figure 4Ag, h). The statistical analysis of the nuclear–cytoplasmic localization pattern of various CSN1 proteins is summarized in Figure 4B. These results showed that CSN1-NTD can translocate into the nucleus independently of the C-terminal region, whereas the C-terminal fragment seems to form aggregates and stay in the cytoplasm. As CSN1 interacts with other CSN subunits through its central and C-terminal domain, but not via the NTD (Wang *et al.*, 2002), the nuclear translocation of CSN1-NTD

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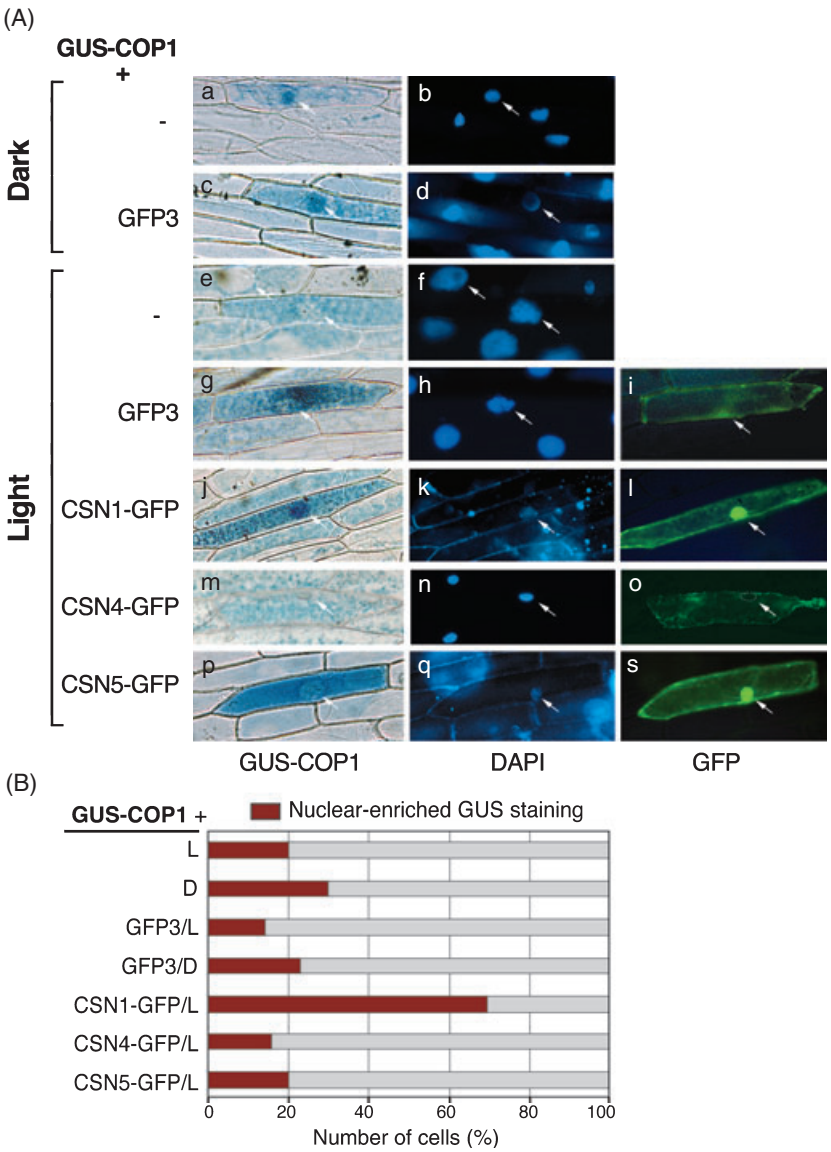

Figure 3. GUS-COP1 localization in onion epidermal cells and the effect of CSN subunits.
GUS-COP1 plasmid was co-bombarded with DNA constructs expressing representative CSN-GFP fusion proteins or the vector alone. The localization of COP1 was determined by GUS staining. Cell nuclei were marked by 4′,6-diamidino-2-phenylindole (DAPI) staining. The expression of CSN was monitored by GFP fluorescence.
(A) Representative images of GUS-COP1 under bright-field microscopy (a, c, e, g, j, m and p), and the corresponding DAPI (b, d, f, h, k, n and q) and GFP signals (i, l, o and s). Samples were incubated for 24 h either in constant darkness or in the light conditions indicated. The nuclei of the stained cells are indicated by arrows.
(a), (b), (e) and (f), GUS-COP1.
(c), (d), (g), (h) and (i), GUS-COP1 + GFP3 vector.
(j)–(l) GUS-COP1 + CSN1-GFP.
(m)–(o) GUS-COP1 + CSN4-GFP.
(p)–(s) GUS-COP1 + CSN5-GFP.
(B) Statistical summary of GUS-COP1 subcellular localization. Cells containing nuclear-enriched GUS staining, such as in panels (a), (c) and (j), were scored as 'nuclear'. Percentages of nuclear-enriched cells among the total cells transformed are shown. A minimum of 200 cells were counted for each sample.

fragments is most likely to be independent of its association with the CSN holocomplex. Notably, the subcellular localization patterns of different CSN1 domain fragments are identical to those of the corresponding truncations of human CSN1 (Tsuge *et al.*, 2001).

CSN1 can promote the nuclear localization of COP1 in onion epidermal cells

To investigate the effect of CSN1 on COP1 localization, CSN1-GFP vectors were co-bombarded with the GUS-COP1 plasmid into onion cells, and COP1 localization was detected by GUS staining (Figures 3A and 4C). When CSN1 was co-expressed with GUS-COP1, the number of cells exhibiting nuclear-enriched GUS staining increased more than threefold, from about 20% or less, to more than 60% under light conditions (Figure 3B, compare CSN1-GFP/L with L). CSN1 also promoted nuclear localization of GUS-COP1 in the dark (Figure S2 and data not shown). Notably, not all of the CSN subunits exhibited such an effect. Co-expression of CSN4 or CSN5 did not affect GUS-COP1 localization (Figure 3A, B). These results indicate that CSN1, but not CSN4 or CSN5, can promote the nuclear localization of COP1.

To determine which domain of CSN1 is able to influence COP1 localization, we co-expressed the above-described CSN1-GFP deletion derivatives together with GUS-COP1 in onion epidermal cells (Figure 4C, D). Both full-length CSN1 and N139 dramatically increased the percentage of cells displaying nuclear COP1-GUS staining, whereas C231 had no effect on COP1 localization (Figure 4). N211-GFP2 behaved identically to N139 in its nuclear localization pattern, and in its effect on GUS-COP1 localization (data

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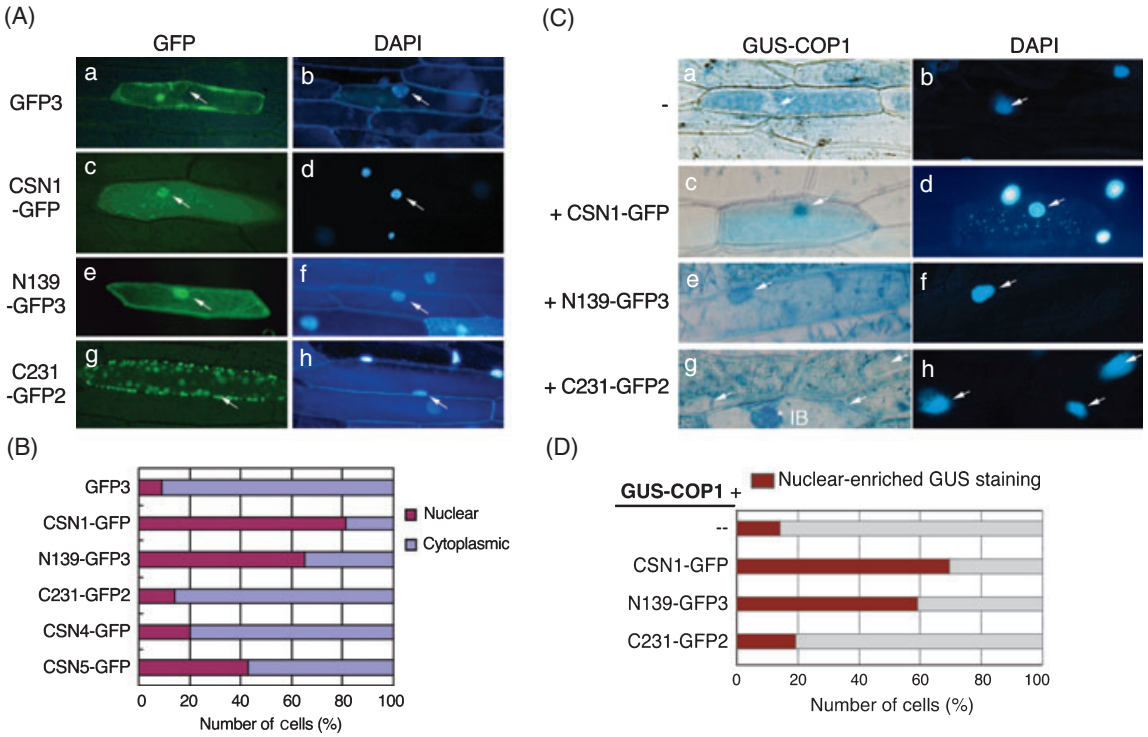

Figure 4. Localization pattern of CSN1 truncation proteins and their effect on GUS-COP1 localization.
(A) Onion epidermal cells showing GFP (a, c, e and g) and the corresponding 4′,6-diamidino-2-phenylindole (DAPI; b, d, f and h) fluorescence. The positions of nuclei are indicated by arrows.
(a) and (b), GFP3: GFP vector containing three copies of GFP.
(c) and (d), CSN1-GFP: full-length CSN1 C-terminally fused to GFP.
(e) and (f), N139-GFP3: CSN1 1–139-aa fragment C-terminally fused to three copies of GFP.
(g) and (h), C231-GFP2: CSN1 211–441-aa fragment C-terminally fused to two copies of GFP.
(B) The percentage of cells showing a nuclear enrichment in the GFP signal was determined. At least 106 GFP-positive cells were counted per sample.
(C) The GUS-COP1 plasmid was co-bombarded with vectors encoding CSN1-GFP, N139-GFP or C231-GFP fusion proteins into onion cells. Samples were incubated under constant white light. Bright-field images of GUS staining (a, c, e and g), along with the corresponding DAPI staining (b, d, f and h), are shown. The positions of nuclei are indicated by arrows. *Inclusion body (IB).
(a) and (b), GUS-COP1.
(c) and (d), GUS-COP1 + CSN1-GFP.
(e) and (f), GUS-COP1 + N139-GFP3.
(g) and (h), GUS-COP1 + C231-GFP2.
(D) Statistical summary of cells with nuclear-enriched GUS staining, calculated as the percentages of total transformed cells. For each sample, at least 200 cells were counted.

not shown). These data showed that CSN1-NTD is necessary and sufficient to facilitate COP1 nuclear localization in onion cells.

CSN1-induced COP1 nuclear localization requires COP1 nuclear localization signals

We reasoned that CSN1 may induce COP1 nuclear localization by modulating the normal nuclear–cytoplasmic translocation pathway, but it is also possible that CSN1 somehow invoked an unrelated mechanism that is physiologically irrelevant. To address this question, we asked whether CSN1-induced nuclear enrichment was still dependent on COP1 NLS and CLS, the two signal elements that normally control the subcellular location of COP1. GUS-COP1(mut1, mut2) (Figure 5a) contains point mutations at the NLS that have been shown to abolish COP1 nuclear import (Stacey *et al.*, 1999, 2000). As previously reported, transient expression of GUS-COP1(mut1, mut2) showed a near background level of nuclear localization, similar to that of the control GFP experiment, regardless of dark or light conditions (Figure S2 and Figure 5b). Importantly, whereas CSN1 or N139 facilitated the nuclear localization of wild-type GUS-COP1, they had no effect on GUS-COP1(mut1, mut2) (Figure 5b). As expected, the COP1 NLS point mutant still maintained the CSN1-binding capacity (Figure 5c). This result clearly indicates that CSN1-mediated COP1 nuclear localization still requires the presence of the original COP1 NLS. It also shows that COP1 does not simply piggyback on CSN1 to translocate into the

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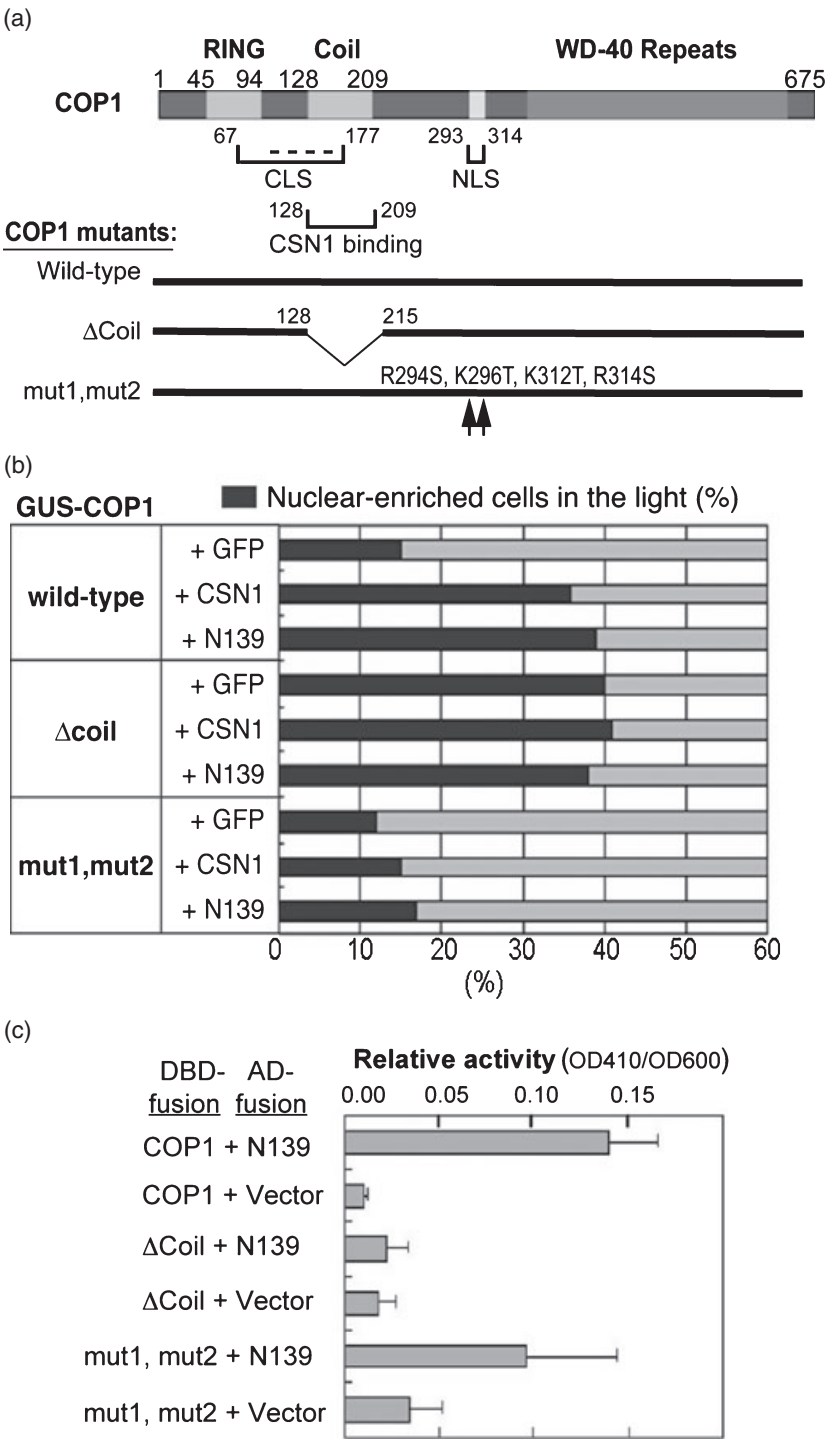

Figure 5. CSN1-induced GUS-COP1 nuclear localization is dependent on COP1 localization signals.
(a) Diagrams of COP1 protein features and the corresponding COP1 mutants used in the experiments. The positions of the RING, coil, cytoplasmic localization signal (CLS), nuclear localization signal (NLS), CSN1-binding and WD-40 repeat domains are indicated. The dashed line indicates the core CLS (105–177 aa).
(b) Statistical summary of the subcellular localization of GUS-COP1 mutants in onion epidermal cells. The plasmids encoding GUS-COP1(wild type), GUS-COP1(Δcoil), or GUS-COP1(mut1, mut2) were co-expressed with GFP vector (+GFP), CSN1-GFP (+CSN1) or N139-GFP3 (+N139) by bombardment. The GUS staining was performed after 24 h of incubation under light. The percentage of cells showing predominantly nuclear GUS staining (nuclear, dark gray) among the total transformed cells is presented in the graph. In this experiment, at least 41 transformed cells per sample were counted.
(c) Interaction of CSN1-N139 with the COP1 NLS/CLS mutants in a yeast two-hybrid assay. The COP1 mutants fused to the GAL4 DNA binding domain (DBD) were tested for interaction with CSN1-N139, fused to the GAL4 activation domain (AD). The graph on the right indicates the relative activity of α-galactosidase reporter expressed as an OD_{410}/OD_{600} ratio. The data represent the mean values of six independent transformants for each pair. The error bars represent the standard deviations.

nucleus, suggesting that the mechanism of NLS-dependent nuclear localization of COP1 involves a specific function of CSN1.

GUS-COP1(Δcoil), which has a deletion of residues 128–215 aa (Figure 5a) (Torii *et al.*, 1998), exhibited a strong nuclear enrichment as a result of the disruption of the core CLS. Stacey *et al.* (1999) defined the full CLS domain as the region from residues 67 to 177 aa, with the core CLS residing in residues 105–177 aa. Evidently, part of the CLS core is deleted in GUS-COP1(Δcoil). Indeed, deletion of the coil domain resulted in an increase of COP1 nuclear localization, to the level similar to that achieved by co-expression of CSN1 or N139 (Figure 5b). Importantly, co-expression of CSN1 or N139 could not further stimulate

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the nuclear enrichment of GUS-COP1(Δcoil) (Figure 5b). This result indicates that CSN1 cannot work additively with the CLS removal to promote COP1 nuclear localization. Unfortunately, the CLS of COP1 overlaps with its CSN1 binding domain (Figure 2c), which were both disrupted in the COP1 Δcoil mutant. Indeed, deletion of the coil domain (Δcoil) abolished the interaction with CSN1-N139 in the yeast-two-hybrid assay (Figure 5c). The expression of these proteins in yeast was confirmed by western blot (Figure S3). Therefore, the failure of CSN1 to modulate GUS-COP1(Δcoil) localization could be caused by the destruction of the CLS, the disruption of the CSN1 interaction, or by both. Together, these results show that CSN1-induced COP1 nuclear localization requires COP1 NLS, and either or both of the intact CLS and CSN1 binding domains.

CSN1-NTD is necessary for COP1 to accumulate in the nucleus in vivo

Our data showed that CSN1-NTD was necessary and sufficient to mediate COP1 nuclear localization when transiently expressed in onion epidermal cells. We want to address the same question *in planta*. However, although CSN1-NTD transgenic Arabidopsis plants can express CSN1 N210 (1–210 aa; Wang *et al.*, 2002) or N139 (1–139 aa; unpublished data, X. Wang) at the mRNA level, these plants failed to express the corresponding truncated protein products. As a result, the gain-of-function effect of CSN1-NTD could not be evaluated *in planta*. On the other hand, we have previously shown that the C-terminal domain of CSN1, C231 (211–441 aa), is not only successfully expressed in the *csn1(fus6)* null mutant background, but can also be correctly integrated with other CSN subunits into a CSN complex ($CSN^{S1\text{-}C231}$) that lacks only the N-terminal domain of CSN1 (Wang *et al.*, 2002). This mutant line (*fus6/C231*) was used to test the loss-of-function effect of CSN1-NTD on COP1 localization *in vivo*.

We genetically crossed the GUS-COP1 transgene into the *fus6/C231* mutant background, and subsequently performed GUS staining on dark- and light-grown seedlings. In dark-grown wild-type seedlings, most hypocotyl cells display GUS staining in the nucleus, whereas the GUS staining in the light-grown hypocotyl cells was diffused throughout the entire cells, and was sometimes present in cytoplasmic inclusion bodies (Figure 6a,b,e,f), as has been previously noted (von Arnim and Deng, 1994; Stacey *et al.*, 1999). In contrast, in *fus6/C231* hypocotyl cells, regardless of whether they were dark- or light-grown, GUS-COP1 exhibited a diffused staining pattern throughout the cells, which was occasionally present in cytoplasmic inclusion bodies (Figure 6c, d, g, h). We carefully examined at least a dozen dark-grown *fus6/C231 GUS-COP1* seedlings, and not a single cell was found that displayed nuclear GUS staining. In fact, the pattern of GUS-COP1 staining in *fus6/C231* was indistinguishable from that in null *csn* mutants, as described in Chamovitz *et al.* (1996). This result demonstrates that CSN1-NTD is specifically required for COP1 to accumulate in the nucleus *in planta*.

DISCUSSION

Genetic studies suggest that Arabidopsis COP1 and CSN function collaboratively to mediate light responses. One important aspect of their functional interaction is the fact that COP1 nuclear localization is dependent on the CSN complex *in vivo*. However, the mechanism by which CSN regulates COP1 localization is not understood. In this report, we demonstrate that CSN1, via its NTD, directly interacts with COP1 at the coiled-coil domain, and stimulates the nuclear localization of COP1 in an onion cell transient expression system. Moverover, CSN1-NTD is required for

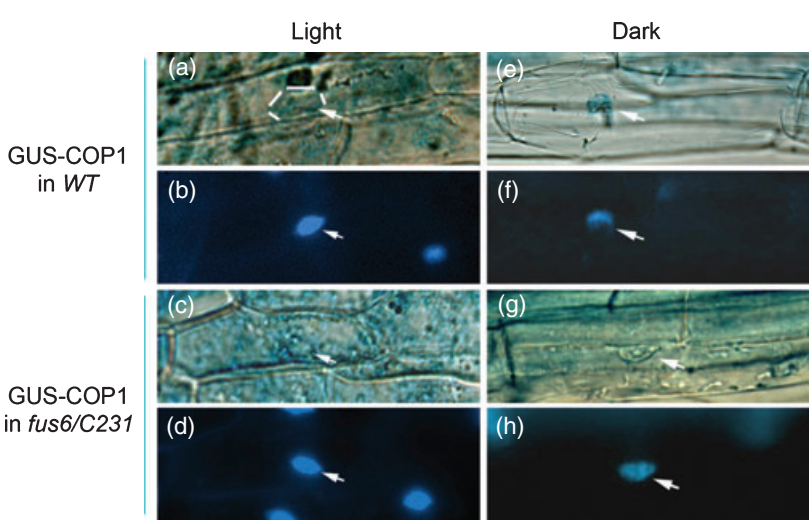

Figure 6. CSN1-NTD is required for the nuclear localization of GUS-COP1 in the hypocotyl cells of Arabidopsis plants.
Seedlings carrying the *GUS-COP1* transgene in the wild-type (a, b, e and f) or the *fus6/C231* mutant (c, d, g and h) backgrounds were grown for 8 days in constant light or dark conditions, as indicated. Seedlings were stained for GUS activity without prior fixation, and were mounted in the presence of 4′,6-diamidino-2-phenylindole (DAPI). Individual cells were photographed in bright field for GUS staining (a, c, e and g), and under fluorescence for DAPI staining of the nuclei (b, d, f, and h). The nuclei are indicated by arrows.

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COP1 nuclear localization in Arabidopsis plants. These findings provide a mechanistic insight into the interrelationship between COP1 and the CSN complex.

COP1 is a RING-type E3 ubiquitin ligase that has a discrete domain structure (Yi and Deng, 2005). Its light-dependent subcellular localization is achieved by coordinated actions of its NLS and CLS elements (Stacey *et al.*, 1999, 2000). We have demonstrated the strict requirement of COP1 NLS in CSN1-induced nuclear enrichment. This result explicitly rules out the possibility that COP1 piggybacks onto CSN1 for nuclear translocation. Residing between the N-terminal RING domain and the C-terminal WD-40 repeat, the coil domain appears to be a focal point of actions that are crucial for COP1 localization and its response to CSN1. The coil domain harbors part of the CLS (Stacey *et al.*, 1999, 2000), and, as shown here, the CSN1 interacting domain. Deletion of the coil domain not only causes COP1 to constitutively localize to the nucleus, but also simultaneously abrogates the effect of CSN1. This observation indicates that CLS and/or CSN binding are critically important in CSN1-facilited COP1 nuclear translocation, or nuclear retention. One hypothesis is that CSN1, through binding to the coil domain, may inhibit COP1 CLS activity, which would result in increased COP1 nuclear localization. In the absence of the coil domain, as in the COP1(Δcoil) mutant, CSN1 cannot bind to COP1, and therefore is unable to manifest its effect. In view of this, it may not be coincident that the CSN1 binding domain overlaps with COP1 CLS. Given that CSN1 is predominantly localized to the nucleus, we favor the idea that CSN1 plays a role in the nuclear retention of COP1, as opposed to nuclear import.

Interestingly, the coil domain is also important for COP1 homodimerization (Torii *et al.*, 1998) and its association with 'COP1 partners', such as the SPA family members (Hoecker and Quail, 2001; Saijo *et al.*, 2003). Thus, CSN1 interaction must be coordinated and regulated in concert with other COP1 interacting proteins, as well as with COP1 dimerization. The dynamic interactions of different regulators, including CSN1, at the coil domain may hold a key to the light-responsive nuclear–cytoplasmic translocation of COP1. It is interesting to note that the nuclear–cytoplasmic localization of human COP1 can be regulated by ATM kinase-mediated phosphorylation, in response to DNA-damaging irradiation (Dornan *et al.*, 2006), and that Arabidopsis ATM has been reported to co-purify with CSN-associated kinase activities (Malec and Chamovitz, 2006). This finding raises the question of whether CSN/CSN1 affects the post-translational modifications of COP1 that are important in its subcellular localization. Clearly, further studies are required to delineate the molecular mechanism of COP1 localization and its regulation by CSN.

Increasing lines of evidence indicate that different CSN subunits have distinct structural and functional characteristics, and that the CSN complex appears to be more dynamic than previously thought (Wei and Deng, 2003; Fukumoto *et al.*, 2005; Gusmaroli *et al.*, 2007). Although null mutations in each of the Arabidopsis CSN subunits cause early lethality, and a characteristic fusca phenotype (purple seedling) (Gusmaroli *et al.*, 2007), partial loss-of-function mutants of *csn1* and *csn5* show different growth phenotypes (Wang *et al.*, 2002, 2003; Gusmaroli *et al.*, 2007), indicating that the functions of CSN subunits are not identical. In this study, we found that CSN1, but not CSN4 or CSN5, can influence the localization pattern of COP1. Unlike CSN1 and CSN5, CSN4 was unable to localize to the nucleus in onion cells, although we cannot exclude the possibility that CSN4-GFP was incorrectly folded. Nevertheless, functional differences in CSN subunits have also been noted in Drosophila and fission yeast (Mundt *et al.*, 2002; Oron *et al.*, 2002). For example, deletion of CSN1 or CSN2, but not of CSN3, CSN4 or CSN5, causes a growth defect (Mundt *et al.*, 2002; Liu *et al.*, 2003). In Arabidopsis, CSN1 stability is strongly dependent on the proper assembly of the CSN holocomplex. Null CSN mutations, such as *csn4/cop8*, *csn5* (*csn5a-1* and *csn5b-1)*, *csn6* (*csn6a-1* and *csn6b-1*), *csn7/fus5* and *csn8/cop9* drastically reduce the cellular abundance of CSN1 to nearly undetectable levels (Staub *et al.*, 1996; Gusmaroli *et al.*, 2007). Given that CSN1-NTD function is essential for the nuclear localization of COP1 *in vivo* (Figure 6), the deficiency of CSN1 in these *csn* mutants may account for, at least in part, the aberrant localization of COP1 (Chamovitz *et al.*, 1996; von Arnim *et al.*, 1997).

In a highly conserved manner, CSN1 central and C-terminal regions are shown to play a role in the structural stability of the CSN complex, whereas the NTD was shown to associate with other important activities (Wei and Deng, 2003). Analysis of an Arabidopsis *fus6/C231* mutant shows that the deletion of CSN1-NTD (1–210 aa) does not jeopardize cullin derubylation (Wang *et al.*, 2002), a major activity of the CSN complex. Nonetheless, the same deletion clearly causes aberrant localization of COP1, as shown in Figure 6 of this study. Thus, the function of CSN in modulating COP1 localization seems to be independent of its derubylation activity.

Human CSN1, via CSN1-NTD (1–196 aa), has been shown to inhibit AP-1 (c-Jun/c-Fos) transactivation (Tsuge *et al.*, 2001). In an independent study, human CSN1 was reported to interact and inhibit inositol 1,3,4-trisphosphate 5/6 kinase, the substrates of which include c-Jun (Wilson *et al.*, 2001). Interestingly, the human homologs of COP1, DET1 and CSN1 have all been shown to negatively regulate c-Jun activity and AP-1 transcription (Tsuge *et al.*, 2001; Bianchi *et al.*, 2003; Wertz *et al.*, 2004; Yi and Deng, 2005). These observations are consistent with the genetic link among CSN, COP1 and DET1 that was originally established in Arabidopsis. Our findings set a foundation for further investigations into the mechanism of COP1 subcellular localization in plants.

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EXPERIMENTAL PROCEDURES

Plant materials and growth conditions

The *Arabidopsis thaliana fus6/C231* and *fus6/FS1(3–4)* lines were in the Wassilewskijce (WS) background (Wang *et al.*, 2002); the *GUS-COP1* line was in the Nossen (No-0) background (von Arnim and Deng, 1994); the *TAPa-COP1* (Rubio *et al.*, 2005) line and the wild-type plants were in Columbia-0 (Col-0) ecotype. Seeds were surface sterilized, sown on MS medium (Sigma-Aldrich, http://www.sigmaaldrich.com) plates containing 3% sucrose, vernalized at 4°C for about 5 days, and then placed in a growth chamber under white light or in darkness for 6–15 days, as indicated.

Plasmid construction

Constructs used in the yeast two-hybrid systems. For the Gal4 system used in Figure 2b, PCR-amplified fragments of *CSN1 N139* were subcloned into the *Sma*I and *Xho*I sites of pGADT7, generating pAD-N139. The *C231* fragment was PCR amplified and inserted into the *BamH*I and *Xho*I sites of pGADT7, generating pAD-C231. The PCR-amplified *COP1* gene was inserted into the *EcoR*I and *Pst*I sites of pGBKT7, giving rise to pBD-COP1. For the constructs used for the LexA yeast two-hybrid system (Figure 2c), the *CSN1 N139* fragment was subcloned into the *EcoR*I and *Xho*I sites of pEG202. The RING, coil, WD-40, N282, ΔC and ΔRΔC fragments of *COP1* were fused at the amino terminus to a transcription activation domain (AD-fusion) in pJG4-5 (Ang *et al.*, 1998; Torii *et al.*, 1998). For the Gal4 system used in Figure 5c, the COP1(Δcoil) fragment from pJG4-5-ΔC (Torii *et al.*, 1998) was inserted into the *BamH*I and *Sac*I sites of pBD-COP1, generating pBD-COP1(Δcoil). The COP1(mut1, mut2) fragment from pRTL2-GUS-COP1(mut1, mut2) (Stacey *et al.*, 1999) was inserted into the *BamH*I and *Sac*I sites of pBD-COP1, generating pBD-COP1(mut1, mut2).

Constructs used in the particle bombardment experiment. The *CSN* genes were fused to *GFP* in *pRTL2-S65TGFP* (von Arnim *et al.*, 1998). To generate C231-GFP2, an *Nco*I-digested C231 PCR fragment was first inserted into the pRTL2 vector, generating pRTL2-C231-S65TGFP. Then it was cut with *Xho*I and *Bgl*II, filled-in, and inserted into the *Xho*I and *Nco*I (filled-in) sites of pRTL2, generating pRTL2-C231-GFP2. To create N139-GFP3, the *Nco*I-digested *CSN1-N139* fragment was subcloned into pRTL2 to generate pRTL2-N139-S65TGFP. Then, the *Bgl*II (filled-in) and *Xba*I fragments of N139-S65TGFP were ligated into the *Nco*I (filled-in) and *Xba*I sites of pRTL2, generating pRTL2-N139-GFP2. The procedure was repeated to generate pRTL2-N139-GFP3. The *CSN4* PCR fragment was inserted into the *Nco*I site of pRTL2, generating pRTL2-S65TGFP-CSN4. The *CSN5* PCR fragment was digested with *BamH*I and *Xba*I, and inserted into the *Bgl*II and *Xba*I sites, generating pRTL2-S65TGFP-CSN5. All of the above clones were confirmed by DNA sequencing. pRTL2-GUS-COP1 (von Arnim and Deng, 1994), pRTL2-S65TGFP (von Arnim *et al.*, 1998) and pRTL2-GUS-COP1(mut1, mut2) (Stacey *et al.*, 1999) have been described previously. The *COP1(Δcoil)* fragment from pJG4-5-ΔC (Torii *et al.*, 1998) was inserted into the *BamH*I and *Sac*I sites of pRTL2-GUS-COP1, generating pRTL2-GUS-COP1(Δcoil).

Constructs for the in vitro *binding experiment.* The PCR-amplified fragment of *CSN1* was subcloned into the *Nco*I and *Xho*I sites of pET-30(a) to generate pET30(a)-CSN1. The *CSN1-N139* fragment derived from pEG-N139 was used to generate pET30(a)-N139. The *CSN1-C231* fragment derived from pAD-C231 was used to generate pET30(a)-C231. The vector pMal-c2X and MBP-COP1 have been described previously (von Arnim *et al.*, 1998).

Yeast two-hybrid assays

Yeast transformation was performed following the manufacturer's manual for Matchmaker (Clontech, http://www.clontech.com). An α-galactosidase (MEL1) quantitative assay was performed according to the Yeast Protocols Handbook (Clontech), with minor modification. Briefly, AH109 clones harboring both pGBK and pGAD were cultured in SD-Leu-Trp medium to an OD_{600} of approximately 1.0. Aliquots of 80 μl of culture supernatant were mixed with 240 μl of assay buffer (0.33 M NaOAc, pH 4.5) containing 33 mM *p*-nitrophenyl α-D-galactopyranoside (PNP-α-Gal; Sigma-Aldrich). The reaction mixes were incubated at 30°C for 3 h before stopping the reaction with the addition of 680 μl of stop buffer (1 M Na_2CO_3). The α-galactosidase activity was measured at 410 nm with a spectrophotometer. For the LexA system, pEG-N139 was transformed into the yeast strain EGY48, whereas the pJG4-5 constructs were transformed in the yeast strain L40. The transformants were mated, and the diploid strains carrying the indicated construct combinations were measured for β-galactosidase activity using *o*-nitrophenyl β-D-galactopyranoside (ONPG; Sigma-Aldrich) as the substrate. Briefly, the diploid strains were cultured until the OD_{600} reached about 0.6. The cells were collected by centrifugation and were then resuspended in 300 μl of Z buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl and 1 mM $MgSO_4$, pH 7.0). Aliquots of 100 μl of the cell suspension were mixed with 700 μl of Z buffer containing 40 mM β—mercaptoethanol, and with 160 μl of 4 mg ml^{-1} ONPG in Z buffer. The reaction mixes were incubated at 30°C for 3 h before stopping the reaction with the addition of 400 μl of stop buffer (1 M Na_2CO_3). After centrifugation, the β-galactosidase activity was measured at 420 nm with a spectrophotometer.

In vitro and *in vivo* protein binding experiments

His-CSN1, His-N139, His-C231, MBP-COP1 and MBP were expressed in BL21(DE3) cells, and extracted by sonication as described in the pET System Manual (Novagen, http://www.merckbiosciences.co.uk/docs/docs/PROT/TBOSS.pdf) and the pMAL Protein Fusion and Purification System Instruction Manual (New England Biolabs, http://www.neb.com). His-CSN1, His-N139 and His-C231 were purified by Ni-charged His-Bind Resin (Novagen), according to the manufacturer's protocol.

For the *in vitro* binding assay, 30 μl of amylose resin was incubated with *Escherichia coli* extracts containing MBP or MBP-COP1 in column buffer [20 mM Tris-HCl, pH 7.4, 200 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulphonyl fluoride (PMSF) and 10 mM β-mercaptoethanol] for 1 h at 4°C. Unbound proteins in the supernatants were discarded, whereas the resins charged with MBP or MBP-COP1 were mixed with purified His-CSN1 (50 μg), His-N139 (60 μg) or His-C231 (50 μg) in a 1-ml reaction volume. The mixtures were incubated for 3 h at 4°C on a rotator. The resins were washed with column buffer, and bound proteins were eluted with10 mM maltose in the column buffer. Eluted proteins were analyzed by western blot using anti-His antibody.

For plant *TAPa-COP1* immunoprecipitation, 10-day-old light-grown *TAPa-COP1* or wild-type seedlings (10 g, fresh weight) grown on MS medium were ground in liquid nitrogen and resuspended in two volumes of extraction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10% glycerol, 1 mM PMSF, 0.1% Nonidet P-40, and 1x complete protease inhibitor cocktail; Roche, http://

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www.roche.com), filtered through four layers of cheesecloth, and centrifuged at 10 000 ***g*** for 15 min at 4°C. After measuring the protein concentrations by Bradford assay, the supernatants containing10 mg of total proteins were loaded into 1 ml of Ni-charged His-Bind Resin (Novagen). The flow-through was reloaded once. The column was then washed with 30 ml of wash buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10% glycerol, 1 mM PMSF and 20 mM imidazole). The proteins were eluted in 10 ml of elution buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM PMSF and 100 mM imidazole), and were captured in 10 fractions of 1 ml each. The fraction with the highest protein content, as determined by UV absorbance at 280 nm, was analyzed by western blot using antibodies against the myc tag (for TAPa-COP1 detection; Cell Signaling Technology, http://www.cellsignal.com), CSN1 (Staub *et al.*, 1996), CSN4 (Serino *et al.*, 1999), CSN5 (Kwok *et al.*, 1998) and CSN6 (Peng *et al.*, 2001), and Histone H3 (Upstate Biotechnology, now part of Millipore, http://www.millipore.com).

For *in vivo* Flag-CSN1 immunoprecipitation, 15-day-old light- or dark-grown *fus6/FS1(3-4)* or wild-type seedlings (1 g, fresh weight) were ground in liquid nitrogen, resuspended in two volumes of extraction buffer, and centrifuged at 13 400 ***g*** for 10 min at 4°C. Protein concentrations in the supernatants were measured by Brandford assay. Aliquots of 50 μl of Anti-Flag M2 beads (Sigma-Aldrich) were mixed with 1 mg of total protein extracts for 3 h at 4°C. The beads were then washed six times with the wash buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM $MgCl_2$, 10% glycerol, 1 mM EDTA, 1 mM PMSF and 1x complete protease inhibitor; Roche), and eluted with 50 μl of 2 × SDS-PAGE sample buffer (125 mM Tris-HCl, pH 6.8, 4% SDS, 20% glycerol and 0.004% bromophenol blue). Proteins were analyzed by western blot using antibodies against COP1 (McNellis *et al.*, 1994), CUL1(Wang *et al.*, 2002), CSN1 and CSN4.

Transient co-expression in onion epidermal cells

The pRTL2–GUS–COP1 plasmid DNA (6 μg) was co-bombarded with pRTL2–S65TGFP–CSN1 (6 μg) or pRTL2–S65TGFP vector (6 μg) into onion epidermal cells, as described by von Arnim and Deng (1994), and Wang *et al.* (1999). After bombardment, the onion cell layers were incubated at 22°C in darkness or continuous white light for 24 h. Samples were stained for GUS activity and mounted in the presence of 1 mg L^{-1} 4′,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich) to visualize the nucleus. Cells containing cytoplasmic or whole-cell GUS staining were counted as 'cytoplasmic', whereas cells displaying obvious nuclear-enriched GUS staining were counted as 'nuclear'.

GUS-COP1 subcellular localization in hypocotyls of Arabidopsis seedlings

Plants carrying the GUS-COP1 transgene in wild-type (von Arnim and Deng, 1994) or *fus6/C231* backgrounds (Wang *et al.*, 2002) were grown on MS plates for 6–10 days in light or dark conditions. GUS staining was carried out using the same procedure described above for GUS histochemical staining. Hypocotyl cells were examined for the GUS staining pattern.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Expression of proteins in the yeast two-hybrid experiments shown in Figure 2.

Figure S2. Subcellular localization of GUS-COP1 mutants in onion epidermal cells.

Figure S3. Expression of proteins in the yeast two-hybrid experiment shown in Figure 5C.

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REFERENCES

Ang, L.H., Chattopadhyay, S., Wei, N., Oyama, T., Okada, K., Batschauer, A. and Deng, X.W. (1998) Molecular interaction between COP1 and HY5 defines a regulatory switch for light control of Arabidopsis development. *Mol. Cell.* **1**, 213–222.

von Arnim, A.G. and Deng, X.W. (1993) Ring finger motif of *Arabidopsis thaliana* COP1 defines a new class of zinc-binding domain. *J. Biol. Chem.*, **268**, 19626–19631.

von Arnim, A.G. and Deng, X.W. (1994) Light inactivation of Arabidopsis photomorphogenic repressor COP1 involves a cell-specific regulation of its nucleocytoplasmic partitioning. *Cell*, **79**, 1035–1045.

von Arnim, A.G., Osterlund, M.T., Kwok, S.F. and Deng, X.W. (1997) Genetic and developmental control of nuclear accumulation of COP1, a repressor of photomorphogenesis in Arabidopsis. *Plant Physiol.* **114**, 779–788.

von Arnim, A.G., Deng, X.W. and Stacey, M.G. (1998) Cloning vectors for the expression of green fluorescent protein fusion proteins in transgenic plants. *Gene*, **221**, 35–43.

Bech-Otschir, D., Seeger, M. and Dubiel, W. (2002) The COP9 signalosome: at the interface between signal transduction and ubiquitin-dependent proteolysis. *J. Cell Sci.* **115**, 467–473.

Bianchi, E., Denti, S., Catena, R., Rossetti, G., Polo, S., Gasparian, S., Putignano, S., Rogge, L. and Pardi, R. (2003) Characterization of human constitutive photomorphogenesis protein 1, a RING finger ubiquitin ligase that interacts with Jun transcription factors and modulates their transcriptional activity. *J. Biol. Chem.* **278**, 19682–19690.

Chamovitz, D.A., Wei, N., Osterlund, M.T., von Arnim, A.G., Staub, J.M., Matsui, M. and Deng, X.W. (1996) The COP9 complex, a novel multisubunit nuclear regulator involved in light control of a plant developmental switch. *Cell*, **86**, 115–121.

Chory, J., Peto, C., Feinbaum, R., Pratt, L. and Ausubel, F. (1989) *Arabidopsis thaliana* mutant that develops as a light-grown plant in the absence of light. *Cell*, **58**, 991–999.

Cope, G.A. and Deshaies, R.J. (2003) COP9 signalosome: a multifunctional regulator of SCF and other cullin-based ubiquitin ligases. *Cell*, **114**, 663–671.

Deng, X.W., Caspar, T. and Quail, P.H. (1991) cop1: a regulatory locus involved in light-controlled development and gene expression in Arabidopsis. *Genes Dev.* **5**, 1172–1182.

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Dornan, D., Shimizu, H., Mah, A., Dudhela, T., Eby, M., O'Rourke, K., Seshagiri, S. and Dixit, V.M. (2006) ATM engages autodegradation of the E3 ubiquitin ligase COP1 after DNA damage. *Science*, **313**, 1122–1126.

Fukumoto, A., Tomoda, K., Kubota, M., Kato, J.Y. and Yoneda-Kato, N. (2005) Small Jab1-containing subcomplex is regulated in an anchorage- and cell cycle-dependent manner, which is abrogated by ras transformation. *FEBS Lett.* **579**, 1047–1054.

Gusmaroli, G., Figueroa, P., Serino, G. and Deng, X.W. (2007) Role of the MPN subunits in COP9 signalosome assembly and activity, and their regulatory interaction with Arabidopsis Cullin3-based E3 ligases. *Plant Cell*, **19**, 564–581.

Hardtke, C.S. and Deng, X.W. (2000) The cell biology of the COP/DET/FUS proteins. Regulating proteolysis in photomorphogenesis and beyond? *Plant Physiol.* **124** 1548–1557.

Hoecker, U. and Quail, P.H. (2001) The phytochrome A-specific signaling intermediate SPA1 interacts directly with COP1, a constitutive repressor of light signaling in Arabidopsis. *J. Biol. Chem.* **276**, 38173–38178.

Kwok, S.F., Solano, R., Tsuge, T., Chamovitz, D.A., Ecker, J.R., Matsui, M. and Deng, X.W. (1998) Arabidopsis homologs of a c-Jun coactivator are present both in monomeric form and in the COP9 complex, and their abundance is differentially affected by the pleiotropic cop/det/fus mutations. *Plant Cell*, **10**, 1779–1790.

Liu, C., Powell, K.A., Mundt, K., Wu, L., Carr, A.M. and Caspari, T. (2003) Cop9/signalosome subunits and Pcu4 regulate ribonucleotide reductase by both checkpoint-dependent and -independent mechanisms. *Genes Dev.* **17**, 1130–1140.

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.

Malec, P. and Chamovitz, D.A. (2006) Characterization and purification of kinase activities against Arabidopsis COP9 signalosome subunit 7. *Isr. J. Chem.* **46**, 239–246.

McNellis, T.W., von Arnim, A.G., Araki, T., Komeda, Y., Misera, S. and Deng, X.W. (1994) Genetic and molecular analysis of an allelic series of cop1 mutants suggests functional roles for the multiple protein domains. *Plant Cell*, **6**, 487–500.

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.

Oron, E., Mannervik, M., Rencus, S., Harari-Steinberg, O., Neuman-Silberberg, S., Segal, D. and Chamovitz, D.A. (2002) COP9 signalosome subunits 4 and 5 regulate multiple pleiotropic pathways in *Drosophila melanogaster*. *Development*, **129**, 4399–4409.

Osterlund, M.T., Hardtke, C.S., Wei, N. and Deng, X.W. (2000) Targeted destabilization of HY5 during light-regulated development of Arabidopsis. *Nature*, **405**, 462–466.

Peng, Z., Serino, G. and Deng, X.W. (2001) Molecular characterization of subunit 6 of the COP9 signalosome and its role in multifaceted developmental processes in Arabidopsis. *Plant Cell*, **13**, 2393–2407.

Pick, E., Lau, O.S., Tsuge, T., Menon, S., Tong, Y., Dohmae, N., Plafker, S.M., Deng, X.W. and Wei, N. (2007) Mammalian DET1 regulates Cul4A activity and forms stable complexes with E2 ubiquitin-conjugating enzymes. *Mol. Cell. Biol.* **27**, 4708–4719.

Reed, J.W. and Chory, J. (1994) Mutational analyses of light-controlled seedling development in Arabidopsis. *Semin. Cell Biol.* **5**, 327–334.

Rubio, V., Shen, Y., Saijo, Y., Liu, Y., Gusmaroli, G., Dinesh-Kumar, S.P. and Deng, X.W. (2005) An alternative tandem affinity purification strategy applied to Arabidopsis protein complex isolation. *Plant J.* **41**, 767–778.

Saijo, Y., Sullivan, J.A., Wang, H., Yang, J., Shen, Y., Rubio, V., Ma, L., Hoecker, U. and Deng, X.W. (2003) The COP1-SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. *Genes Dev.* **17**, 2642–2647.

Schroeder, D.F., Gahrtz, M., Maxwell, B.B., Cook, R.K., Kan, J.M., Alonso, J.M., Ecker, J.R. and Chory, J. (2002) De-etiolated 1 and damaged DNA binding protein 1 interact to regulate Arabidopsis photomorphogenesis. *Curr. Biol.* **12**, 1462–1472.

Schwechheimer, C., Serino, G., Callis, J., Crosby, W.L., Lyapina, S., Deshaies, R.J., Gray, W.M., Estelle, M. and Deng, X.W. (2001) Interactions of the COP9 signalosome with the E3 ubiquitin ligase SCFTIRI in mediating auxin response. *Science*, **292**, 1379–1382.

Serino, G. and Deng, X.W. (2003) The COP9 signalosome: regulating plant development through the control of proteolysis. *Annu. Rev. Plant Biol.* **54**, 165–182.

Serino, G., Tsuge, T., Kwok, S., Matsui, M., Wei, N. and Deng, X.W. (1999) Arabidopsis cop8 and fus4 mutations define the same gene that encodes subunit 4 of the COP9 signalosome. *Plant Cell*, **11**, 1967–1980.

Stacey, M.G., Hicks, S.N. and von Arnim, A.G. (1999) Discrete domains mediate the light-responsive nuclear and cytoplasmic localization of Arabidopsis COP1. *Plant Cell*, **11**, 349–364.

Stacey, M.G., Kopp, O.R., Kim, T.H. and von Arnim, A.G. (2000) Modular domain structure of Arabidopsis COP1. Reconstitution of activity by fragment complementation and mutational analysis of a nuclear localization signal in planta. *Plant Physiol.* **124**, 979–990.

Staub, J.M., Wei, N. and Deng, X.W. (1996) Evidence for FUS6 as a component of the nuclear-localized COP9 complex in Arabidopsis. *Plant Cell*, **8**, 2047–2056.

Suzuki, G., Yanagawa, Y., Kwok, S.F., Matsui, M. and Deng, X.W. (2002) Arabidopsis COP10 is a ubiquitin-conjugating enzyme variant that acts together with COP1 and the COP9 signalosome in repressing photomorphogenesis. *Genes Dev.* **16**, 554–559.

Torii, K.U., McNellis, T.W. and Deng, X.W. (1998) Functional dissection of Arabidopsis COP1 reveals specific roles of its three structural modules in light control of seedling development. *EMBO J.* **17**, 5577–5587.

Tsuge, T., Matsui, M. and Wei, N. (2001) The subunit 1 of the COP9 signalosome suppresses gene expression through its N-terminal domain and incorporates into the complex through the PCI domain. *J. Mol. Biol.* **305**, 1–9.

Wang, H., Kang, D., Deng, X.W. and Wei, N. (1999) Evidence for functional conservation of a mammalian homologue of the light-responsive plant protein COP1. *Curr. Biol.* **9**, 711–714.

Wang, X., Kang, D., Feng, S., Serino, G., Schwechheimer, C. and Wei, N. (2002) CSN1 N-terminal-dependent activity is required for Arabidopsis development but not for Rub1/Nedd8 deconjugation of cullins: a structure-function study of CSN1 subunit of COP9 signalosome. *Mol. Biol. Cell.* **13**, 646–655.

Wang, X., Feng, S., Nakayama, N., Crosby, W.L., Irish, V., Deng, X.-W. and Wei, N. (2003) The COP9 signalosome interacts with SCF UFO and participates in Arabidopsis flower development. *Plant Cell*, **15**, 1071–1082.

Wei, N. and Deng, X.W. (1992) COP9: a new genetic locus involved in light-regulated development and gene expression in arabidopsis. *Plant Cell*, **4**, 1507–1518.

Wei, N. and Deng, X.W. (1996) The role of the COP/DET/FUS genes in light control of arabidopsis seedling development. *Plant Physiol.* **112**, 871–878.

Wei, N. and Deng, X.W. (2003) The COP9 signalosome. *Annu. Rev. Cell Dev. Biol.* **19**, 261–286.

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Wei, N., Kwok, S.F., von Arnim, A.G., Lee, A., McNellis, T.W., Piekos, B. and Deng, X.W. (1994) Arabidopsis COP8, COP10, and COP11 genes are involved in repression of photomorphogenic development in darkness. *Plant Cell*, **6**, 629–643.

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.

Wilson, M.P., Sun, Y., Cao, L. and Majerus, P.W. (2001) Inositol 1,3,4-trisphosphate 5/6-kinase is a protein kinase that phosphorylates the transcription factors c-Jun and ATF-2. *J. Biol. Chem.* **276**, 40998–41004.

Yanagawa, Y., Sullivan, J.A., Komatsu, S. *et al.* (2004) Arabidopsis COP10 forms a complex with DDB1 and DET1 in vivo and enhances the activity of ubiquitin conjugating enzymes. *Genes Dev.* **18**, 2172–2181.

Yi, C. and Deng, X.W. (2005) COP1 – from plant photomorphogenesis to mammalian tumorigenesis. *Trends Cell Biol.* **15**, 618–625.

Zhou, C., Wee, S., Rhee, E., Naumann, M., Dubiel, W. and Wolf, D.A. (2003) Fission yeast COP9/signalosome suppresses cullin activity through recruitment of the deubiquitylating enzyme Ubp12p. *Mol. Cell.* **11**, 927–938.

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