

COP1 – from plant photomorphogenesis to mammalian tumorigenesis

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The COP1 (constitutive photomorphogenic 1) protein, comprising RING finger, coiled-coil and WD40 domains, is conserved in both higher plants and vertebrates. In plants, COP1 acts as an E3 ubiquitin ligase to repress light signaling by targeting photoreceptors and downstream transcription factors for ubiquitylation and degradation. The activity of COP1 in plant cells correlates with its cytoplasmic and nuclear partitioning according to dark or light conditions. In addition, various signaling molecules have been shown to directly interact with COP1 and modulate its activity. Recently, scientists have begun to probe the function and regulation of COP1 in mammalian systems. Initial studies have pointed at possible roles for mammalian COP1 in tumorigenesis and the stress response through regulating the activities of p53 and c-Jun.

Introduction

The ultimate energy source for virtually all life on earth is sunlight, but only plants and some bacteria are able to directly absorb sunlight and convert it into bio-usable chemical energy. Because of their absolute dependency on light, plants have developed sophisticated mechanisms to sense light conditions and adjust their developmental programs accordingly. So far, three classes of photoreceptors (phytochromes, cryptochromes and phototropins) have been molecularly defined in plants, each detecting light of a specific range of wavelengths [1–3]. Upon light absorption, these photoreceptors activate multiple distinct, yet partially overlapping, signal-transduction cascades, resulting in the specific activation of light-inducible genes. Many components of the light signaling cascades have been uncovered over the years, mostly by genetic approaches [4–7]. Among them, *COP1* was the one of the first cloned and is one of the most extensively studied [8,9]. It was established early on that *COP1* functions as an essential negative regulator of light-mediated plant development, evidenced by *cop1* mutant seedlings undergoing photomorphogenic development (Box 1) even in the absence of light, and *cop1* null alleles never surviving to adult stage [8,10]. The concept that *COP1* acts as a central switch in light signal transduction was further corroborated by a cDNA microarray study, which demonstrated that the majority of light-controlled genome expression is attributable to *COP1* activity [11].

COP1 also exists in non-plant multicellular organisms, although its function is less understood [12]. Among vertebrates, *COP1* is well conserved between fish, amphibians, birds and mammals. By contrast, the *COP1* gene has not yet been identified in the nearly completely sequenced *Drosophila* or *Caenorhabditis elegans* genomes, but it is present in the mosquito genome. It is apparent that, unlike plants, animals do not undergo photomorphogenesis. Several initial studies have implied that animal *COP1* might have adopted roles in tumorigenesis and the stress response, although its biochemical activities and some of the signaling elements it interacts with appear to be conserved between plants and animals [13–17].

The *COP1* protein comprises three recognizable domains: a RING-finger motif, followed by a coiled-coil domain and seven WD40 repeats, all of which have been implicated in mediating the interaction of *COP1* with other proteins and/or its self-dimerization [13,14,18–30] (Figure 1). *COP1* functionality is highly modular, as demonstrated in *Arabidopsis* [31]. Introduction of an N-terminal fragment of *Arabidopsis* *COP1* (*AtCOP1*) containing the RING-finger and coiled-coil domains into a *cop1* null allele rescued its lethal phenotype, indicating that the *AtCOP1* N-terminal region alone is able to sustain a basal function during development [31]. The *AtCOP1* C-terminal WD40 domain, by contrast, led to repression of photomorphogenesis when expressed in a wild-type background but failed to complement a *cop1* loss-of-function allele [31]. Expression of two separate polypeptides representing these two *AtCOP1* functional modules can partially reconstitute *AtCOP1* activity *in vivo* [31]. *COP1* contains nuclear import and export signals, and, in plants, its subcellular localization is regulated by light [12,13,32] (Figure 1). This review highlights the latest developments in our comprehension of *COP1* biochemical activities (primarily its role in mediating protein ubiquitylation), and considers how *COP1* function is modulated through nucleocytoplasmic translocation and by other interacting factors in both plant and mammalian cells.

COP1 functions as an E3 ubiquitin ligase

AtCOP1 targets photomorphogenesis-promoting transcription factors for ubiquitylation and degradation in the dark

The notion that *AtCOP1* represses photomorphogenesis by promoting the degradation of positive light signaling

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Box 1. Photomorphogenic development

Arabidopsis seeds germinating under the soil without sunlight go through a form of development called skotomorphogenesis (or etiolation), characterized by long stems (hypocotyls), closed, unexpanded leaves (cotyledons) protected by an apical hook and lack of pigmentation. In an etiolated *Arabidopsis* seedling, the hypocotyl elongates and pushes the folded cotyledons upward through the soil. When a seedling emerges from the soil and is exposed to light, development switches from a program of skotomorphogenesis to photomorphogenesis (de-etiolation): seedlings slow down hypocotyl elongation, and the cotyledons open up, turn green and enlarge to optimize the absorption of light.

regulators was first introduced in a study attempting to characterize the regulation of HY5, a bZIP transcription factor that, in response to light, activates a subset of genes that set *Arabidopsis* seedlings onto the course leading to photomorphogenic development [33]. In this study, the HY5 protein was found to accumulate to a much higher level in light-grown seedlings and, upon light-to-dark transition, is degraded through proteasome-mediated proteolysis [33], a process that usually requires the targeted proteins first to be modified by a chain of ubiquitin. Protein ubiquitylation in general requires a specific E3 ubiquitin ligase, which can be a single protein or a multicomponent protein complex. An E3 typically functions by recruiting ubiquitin-conjugating enzymes (E2s) through a RING-finger motif and the substrate through another protein-protein interaction domain. COP1 was immediately suspected to be the HY5 E3 as COP1, a RING-finger protein and negative regulator of HY5, had been previously shown to directly interact and colocalize with HY5 to subnuclear speckles in living plant cells [34,35]. This hypothesis was further strengthened by the observations that HY5 degradation during light-to-dark transitions is impaired in *cop1* mutant seedlings, transgenic seedlings expressing *HY5* with point mutations at the *HY5* COP1-interacting motif or in

COP1 mutants with point mutations in the COP1 WD40 domain abolishing HY5 interaction [20,33]. Moreover, HY5 becomes stabilized in white light when the COP1 protein is excluded from the nucleus [33]. AtCOP1 was later confirmed to possess intrinsic E3 activity and to ubiquitylate HY5 *in vitro* [36]. Interestingly, another bZIP transcription factor, HYH, which heterodimerizes with HY5 and appears to function primarily in blue light signaling, also binds to the COP1 WD40 domain and is degraded in a COP1-dependent manner under conditions of darkness [24].

COP1 ubiquitylation targets in *Arabidopsis* are not just limited to bZIP family transcription factors. For example, LAF1, a myb transcription factor and positive regulator of phytochrome A (phyA)-mediated far-red light signaling, and HFR1, a bHLH transcription factor that is involved in both far-red and blue-light signaling, have also been identified as physiological substrates for AtCOP1 [29,30,37,38]. Thus, COP1 functions as a master switch in the dark to shut down photomorphogenesis by destroying transcription factors that activate specific light responses (Figure 2).

AtCOP1 is responsible for the rapid degradation of light-labile photoreceptors after exposure to light

Recently, it was suggested that COP1 also plays an active role in fine-tuning light activation [27]. phyA and cryptochrome 2 (*cry2*), two photoreceptors specific for far-red and blue light, respectively, become rapidly activated upon illumination and are important for the initiation of light signaling. However, both photoreceptors are extremely unstable under light and are quickly degraded by the proteasome when exposed to continuous light [39]. Recent studies demonstrated that COP1 directly interacts with these light-labile photoreceptors and targets them for ubiquitylation and degradation, presumably preventing over-activation of the light-signaling pathways [27,40]

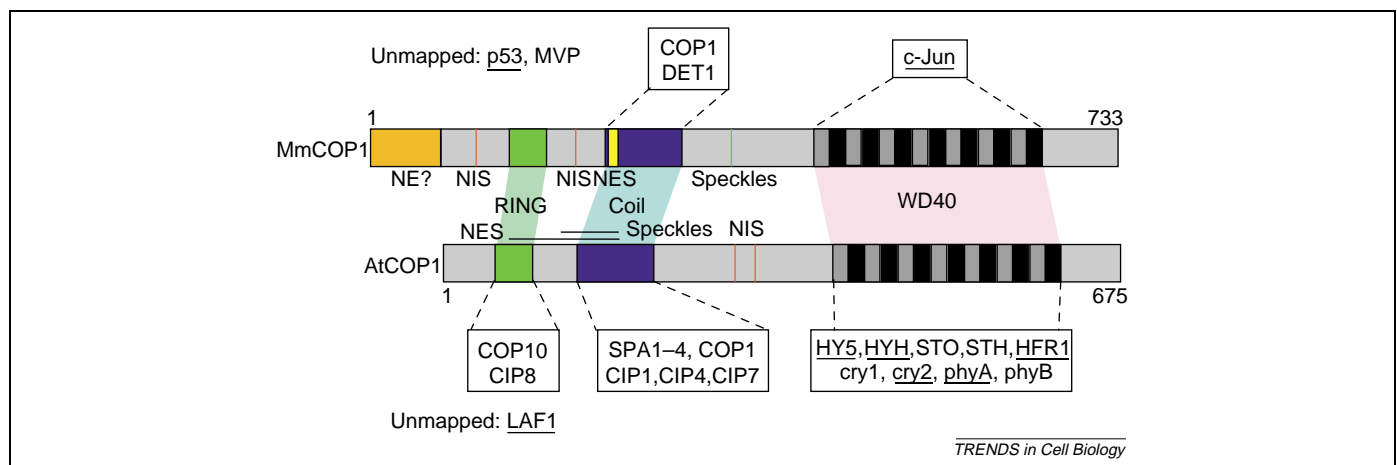


Figure 1. COP1 structural domains and interacting proteins. Both plant and mammalian COP1 proteins contain three structural domains: a RING finger, followed by a coiled-coil domain and seven WD40 repeats at the C-terminus. Compared with plant COP1, mammalian COP1 has an N-terminal extension that might be responsible for targeting COP1 to the nuclear envelope (NE) [12]. COP1 shuttles between the nucleus and the cytoplasm and forms subnuclear speckles in both plants and mammals. As indicated in the diagram, MmCOP1 and AtCOP1 utilize different nuclear import and nuclear export signals (NIS and NES) located at distinct regions for their nucleocytoplasmic shuttling and subnuclear localizations [12,48,49,51]. Several COP1-interacting partners have been identified. The AtCOP1 RING finger interacts with CIP8 and COP10 [25,28,65]. COP1 utilizes the coiled-coil domain for self-dimerization in both plants and mammals [13]. In addition, the AtCOP1 coiled-coil domain interacts with the SPA proteins (SPA1–SPA4), CIP1, CIP4 and CIP7 [19,22,26,63,64], while the coiled-coil domain of mammalian COP1 has been implicated in binding to DET1 [14]. COP1 interacts through its WD40 repeats with HY5, HYH, STO, STH, HFR1, *cry1*, *cry2*, phyA and phyB in plants and with c-Jun in mammals [13,20,21,23,24,27,29,34]. Other COP1-interacting factors whose interacting domains have not been mapped include LAF1 in *Arabidopsis*, and p53 and the major vault protein MVP in mammals [15,17,37].

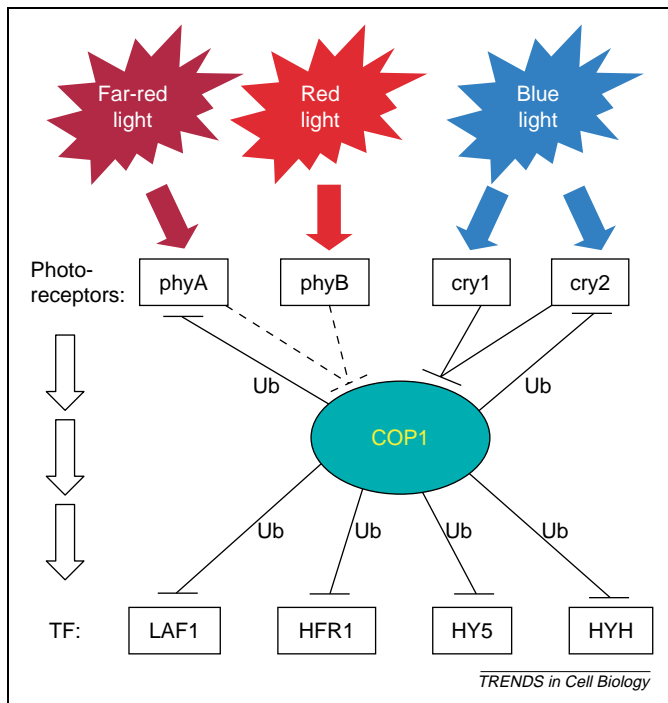


Figure 2. COP1 functions as a central switch in light control of *Arabidopsis* seedling development. *Arabidopsis* detects various colors of light through different photoreceptors, which initiate distinct, yet partially overlapping, signaling pathways, leading to the activation of photomorphogenesis by promoting the activation of transcription factors. AtCOP1 represses photomorphogenic development by directly interacting with and targeting some photoreceptors (phyA and cry2) and their downstream transcription factors (HY5, HYH, HFR1 and LAF1) for ubiquitylation and degradation [24,27,29,30,33,36–38,40]. Through direct interactions with COP1, the cryptochromes cry1 and cry2 negatively regulate COP1 activity by currently unknown mechanism [21,23,55,56]. It is currently not known whether phyA or phyB can negatively regulate COP1 in a manner similar to that of cry1 and cry2 (indicated by the dashed lines).

(Figure 2; V. Rubio and X.W. Deng, unpublished). COP1 also physically interacts with other, more stable, photoreceptors, including phytochrome B (phyB) and cryptochrome 1 (cry1), but does not appear to affect their stabilities [21,23]. Conceivably, COP1 might regulate these photoreceptors by affecting their interactions with downstream signaling targets. An interesting study recently showed that COP1 acts positively in red light signaling mediated by phyB [41], suggesting that COP1 might play opposing roles under light and dark conditions.

Based on the current evidence, the major scheme that AtCOP1 employs to repress light signaling is to target photomorphogenesis-promoting transcription factors (HY5, HYH, LAF1 and HFR1) and the photoreceptors themselves (phyA and cry2) for ubiquitylation and proteolysis (Figure 2). The physiological relevance of AtCOP1 E3 activity is corroborated by the newly identified *cop1^{eid6}* allele, which, similar to other weak *cop1* alleles, displays hypersensitivity under light conditions and leads to dwarf stature and early flowering at the adult stage [42]. The *cop1^{eid6}* allele carries a single mutation at a conserved histidine site within its RING finger, which is expected to severely compromise the integrity of the RING structure and consequently COP1 E3 activity. Intriguingly, unlike other *cop1* alleles, *cop1^{eid6}* mutant seedlings do not exhibit a constitutive photomorphogenic phenotype in the dark [42]. Further studies are required to

understand whether this apparent lack of phenotype in the dark is due to the retention of partial E3 activities of the mutant COP1 protein.

COP1 might also regulate light signal transduction through mechanisms other than protein degradation. For example, AtCOP1 was recently demonstrated to be required for the nuclear accumulation of the bHLH transcription factor PIF3 in darkness but not for its rapid degradation under red and far-red light [43].

COP1 mediates the ubiquitylation and degradation of c-Jun and p53 in mammalian cells

Initial characterizations suggested that mammalian COP1, like its plant counterpart, is involved in ubiquitylation and is itself a substrate of its own ubiquitylation activity [12,13]. So far, two COP1 ubiquitylation substrates have been identified in mammals: c-Jun and p53 [14,15]. COP1-mediated p53 degradation is an important regulatory mechanism for p53 function in the cell. Overexpression of COP1, not its RING-finger mutant, inhibited p53-dependent apoptosis, whereas siRNA-ablation of COP1 led to p53 accumulation and cell-cycle arrest [15]. Overexpression of COP1 also counteracted p53 activation and subsequent cell-cycle arrest induced by MLF1, a newly identified upstream activator of p53 [44]. Importantly, COP1 has been found to be overexpressed in a large percentage of breast and ovarian adenocarcinoma, and, among the tumors that retain wild-type p53 gene, COP1 expression correlates with reduction of p53 protein levels and attenuation of the downstream target gene *p21* [16].

While the intrinsic COP1 E3 activity is sufficient for the ubiquitylation and degradation of p53, COP1 appears to require the cooperation of other factors in mediating ubiquitylation of c-Jun (Figure 3). It has been suggested that mammalian COP1 functions as an adaptor protein recruiting c-Jun to a E3 complex possibly containing DET1, DDB1, cullin4A and Roc1, through direct interaction with DET1 (Figure 3). Interestingly, similar to COP1, the *Arabidopsis* DET1 homolog belongs to the COP/DET/FUS loci, whose mutant seedlings all display the so-called *cop* phenotype (Box 2). In addition, a transient association between a DET1-containing complex and COP1 has been reported in *Arabidopsis* [28]. However, a direct interaction between plant COP1 and DET1 has not been demonstrated. Mammalian COP1 binds to c-Jun through a conserved motif shared by plant bZIP family COP1 substrates HY5 and HYH [13]. Mammalian COP1 also represses c-Jun-mediated AP-1 transcription without affecting c-Jun protein levels [13,17].

The function of COP1 is modulated by nucleocytoplasmic translocation

In cells performing photosynthesis, the subcellular distribution of AtCOP1 is adjusted according to light conditions [45]. In darkness, AtCOP1 is mainly localized to the nucleus, where it presumably targets photomorphogenesis-promoting transcription factors, such as HY5, HYH, LAF1 and HFR1, for ubiquitylation and degradation, thereby repressing the expression of photomorphogenesis genes. When cells are exposed to light, there is a drastic

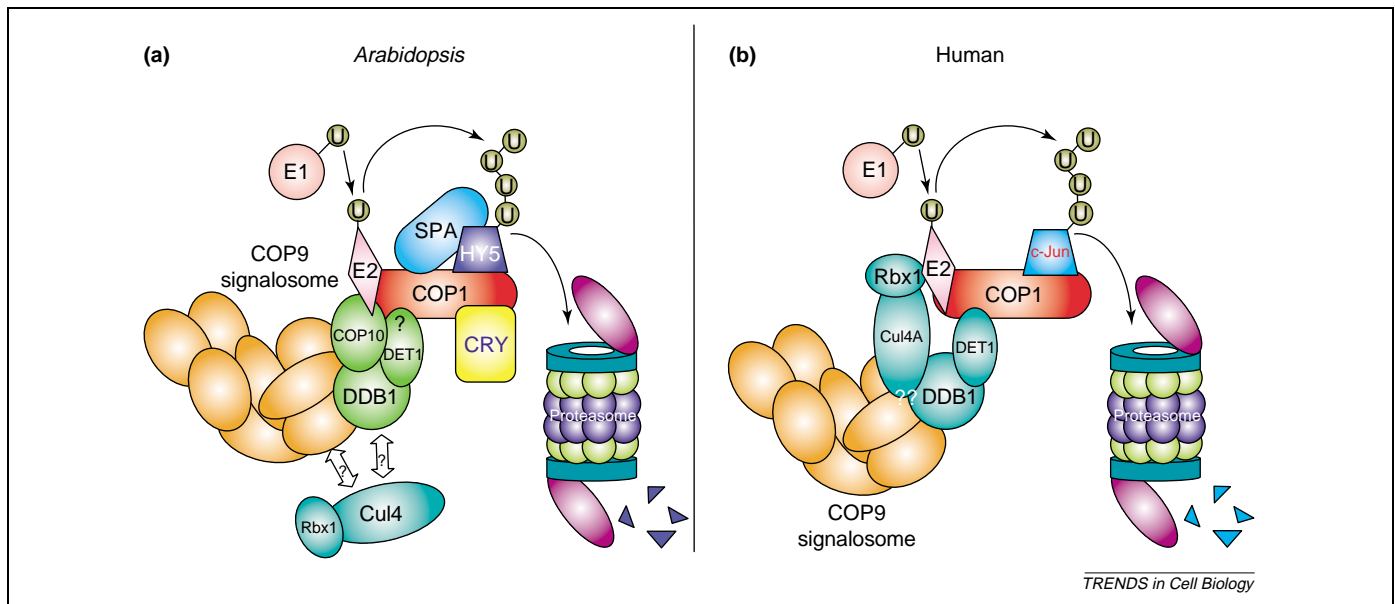


Figure 3. COP/DET/FUS proteins function collaboratively in mediating protein ubiquitylation. The majority of the COP/DET/FUS proteins are conserved in both plants and mammals. COP1 is able to target the bZIP transcription factors HY5 (in *Arabidopsis*) and c-Jun (in human) for ubiquitylation and proteasome-mediated degradation [14,36]. In *Arabidopsis* (a), COP10, DET1 and DDB1 form a stable protein complex, called the CDD complex [28]. In particular, COP10 has been shown to interact with E2 proteins, the COP1 RING finger and the COP9 signalosome [25,28]. It is not known whether the CDD complex is conserved in mammals (b). In human cells, the existence of a putative E3 complex comprising Cul4A, DDB1, Rbx1 and DET1 has been suggested [14]. COP1 was proposed to function as a substrate adaptor, linking c-Jun to the Cul4A–DDB1–Rbx1–DET1 E3 complex through direct interaction with DET1 [14]. Direct interaction between COP1 and DET1 has not been demonstrated in *Arabidopsis*. Cul4A and Rbx1 are present in *Arabidopsis* as well; however, it is not known whether they form E3 complexes with DDB1 and DET1. The COP9 signalosome has been copurified with Cul4A–DDB1–Rbx1-containing E3 complexes from mammalian cells [62]. Nevertheless, whether it associates with the Cul4A–DDB1–Rbx1–DET1 complex is not known. In *Arabidopsis*, COP1 activity requires the SPA proteins and is negatively regulated by the cryptochromes [19,21,23,26,54–56]. The direct interaction between COP1 and the cryptochromes appears to be plant specific, despite the fact that both proteins are conserved in animals [23].

reduction in AtCOP1 levels in the nucleus, allowing nuclear-localized transcription factors to re-accumulate. These transcription factors, in turn, activate the transcription of downstream genes required for plants to undergo photomorphogenesis. COP1 nuclear abundance change is a relatively slow process (up to 24 h) [46]. According to a recent study, the early-response genes in light signaling start to accumulate within the first hour of light exposure [47], suggesting that other mechanisms, such as direct repression of COP1 activity by the cryptochromes (discussed in detail below), must operate to quickly turn off COP1 activity after exposure to light. Nonetheless, nuclear–cytoplasmic repartitioning still serves as an important means of regulating AtCOP1 activity, as demonstrated by a series of mutagenesis studies [31,48,49]. When the conserved leucine residues within the AtCOP1 nuclear export signal were mutated, mutant *AtCOP1* protein showed augmented nuclear localization, and corresponding transgenic plants became hypo-sensitive to light [48,49]. By contrast, transgenic plants expressing *AtCOP1* with a mutated nuclear import signal displayed constitutive photomorphogenic phenotypes at the seedling stage and were severely dwarf in stature at the adult stage [31,48].

Within the nucleus, AtCOP1 has been found to form subnuclear speckles with HY5, HYH, LAF1, ABI5, HFR1 and phyA, most of which, with the exception of ABI5, are validated COP1 ubiquitylation substrates and are involved in light signaling [24,27,29,30,34,37,38,50]. The sequence that targets AtCOP1 to subnuclear speckles has been mapped to a region overlapping the coiled-coil domain [51] (Figure 1). Nevertheless, the WD40 domain, through which

AtCOP1 interacts with the majority of its substrates, also seems crucial for speckle formation [51]. Deletion of the entire WD40 domain decreases the fraction of cells showing subnuclear speckles, whereas several mutations at the WD40 domain also abolish the subnuclear speckles [51]. Importantly, *Atcop1* homozygous mutant alleles containing the same mutations show constitutive photomorphogenic phenotypes at the seedling stage and are adult lethal [52]. Therefore, these subnuclear speckles formed by AtCOP1 and its substrates, at which AtCOP1-mediated substrate ubiquitylation probably takes place, might be required for normal *Arabidopsis* development. However, to date, all the evidence that COP1 subnuclear speckles are functionally important has been correlative. A more definite understanding of the physiological significance of these speckles and what signals regulate their assembly and disassembly requires comprehensive mutagenesis combined with transgenic studies, as well as biochemical characterization of speckle compositions.

Similar to AtCOP1, mammalian COP1 also shuttles between the nucleus and the cytoplasm and forms

Box 2. The cop phenotype

The pleiotropic *cop/det/fus* (constitutive photomorphogenic/de-etiolated/fusca) mutants, all of which display light-grown phenotypes with short hypocotyls and open cotyledons even under continuous darkness, were mapped to a dozen distinctive loci in the *Arabidopsis* genome. The constitutive photomorphogenic phenotypes (*cop*) of these loss-of-function mutants indicate that they function as negative regulators of light signaling. Genetic studies have placed these gene products downstream of multiple photoreceptors [61].

subnuclear speckles [12,13]. However, despite their high homology and conserved biochemical activities, mammalian COP1 utilizes nuclear import and export signals completely distinct from those of AtCOP1, probably owing to the different signaling pathways that plant and mammalian COP1 proteins are respectively involved in [12] (Figure 1). Furthermore, a fraction of the mammalian COP1 proteins are targeted to the nuclear envelope through an N-terminal domain that is absent in AtCOP1 [12] (Figure 1). The functional significance of the mammalian COP1 nuclear envelope targeting has not been investigated. Also not known is what extracellular and/or intracellular signal(s) control COP1 subcellular localization in mammalian cells. Given the possible involvement of mammalian COP1 in the stress response through regulating c-Jun and p53, it would certainly be interesting to test the possible effects of c-Jun or p53 upstream regulators on COP1 subcellular localization.

COP1 regulatory factors

COP1 forms stable high-molecular-mass protein complexes in both mammalian and plant cells [12,36]. Scientists in the field have identified, mainly through yeast two-hybrid screens, genetic interaction studies and protein complex purifications, numerous factors that interact with COP1 (Figure 1), several of which have been proven to be important physiological regulators of COP1 activities.

SPA proteins: COP1 regulators mediating phytochrome signaling

Among the COP1-interacting partners in *Arabidopsis* are members of a small family of four phytochrome-specific negative regulators called the SPA proteins, whose first member was initially identified as 'suppressor of phyA' [53]. All the SPA proteins contain an N-terminal kinase-like domain, followed by a coiled-coil domain and seven WD40 repeats at the carboxyl-terminus. Interestingly, the WD40 domains of the SPA proteins are highly homologous to the COP1 WD40 domain, and the SPA proteins physically interact with COP1 through their mutual coiled-coil domains [26,54] (Figure 1; Figure 3). While the quadruple *spa* mutants exhibit strong *cop* phenotypes almost indistinguishable from *cop1* mutants, single, double and triple *spa* mutants show no or restricted phenotypes only under certain light conditions and developmental stages, suggesting that the SPA proteins modulate COP1 activity in a partially overlapping fashion [26,54].

How exactly SPA proteins regulate COP1 activity remains an open question. The highly conserved WD40 domains shared by COP1 and the SPA proteins, the presence of SPA1 in COP1 subnuclear speckles [37], together with evidence that the SPA1 WD40 domain also binds to HY5 [36], suggest that the SPA proteins might enhance COP1 activity by helping it to recruit substrates. Nevertheless, this does not rule out the possibility that the SPA proteins might be required for the nuclear accumulation of COP1. Alternatively, the SPA proteins might be essential for the stability of COP1 complexes [36]. The SPA proteins could also control COP1 activity by modifying

COP1 (e.g. phosphorylation, especially as the SPA proteins contain kinase-like domains). Clearly, further research is required to address these possibilities. Given that COP1 and SPA1 cofractionate in large complexes in *Arabidopsis* seedlings [36], purification of the SPA-COP1 complex(es) should shed some light on this question. SPA genes are not conserved in animals, consistent with the fact that the phytochrome system and photomorphogenic development are not employed by animals.

Activated cryptochromes directly turn off COP1 activity

Physical interactions between AtCOP1 and several major photoreceptors, including phyA, phyB, cry1 and cry2, have been recently reported [21,23,27] (Figure 1). As described above, AtCOP1 desensitizes both phyA and cry2 activation in response to illumination by targeting them for proteasome-mediated proteolysis. The interaction between COP1 and phyB has only been shown in yeast two-hybrid assays, and the physiological significance of this interaction is unclear [23]. The cryptochromes cry1 and cry2, however, have been shown to negatively regulate COP1 activity in response to light mainly through the direct association between the cryptochrome C-terminal (CCT) domains and the COP1 WD40 domain [21,23,55,56] (Figure 1; Figure 2; Figure 4). Transgenic plants expressing wild-type CCT1, CCT2 or full-length cry1 fused with β -glucuronidase reporter (GUS), but not those with point mutations at the CCT domains that disrupt COP1 interactions, phenocopy weak *cop1* mutants [23,55]. The functionality of the cryptochromes also requires homodimerization mediated by the cryptochrome N-terminal (CNT) domains [55] (Figure 4). Light-induced phosphorylation likely represents a key step in cryptochrome activation, considering that both cry1 and cry2 become rapidly phosphorylated upon irradiation with blue

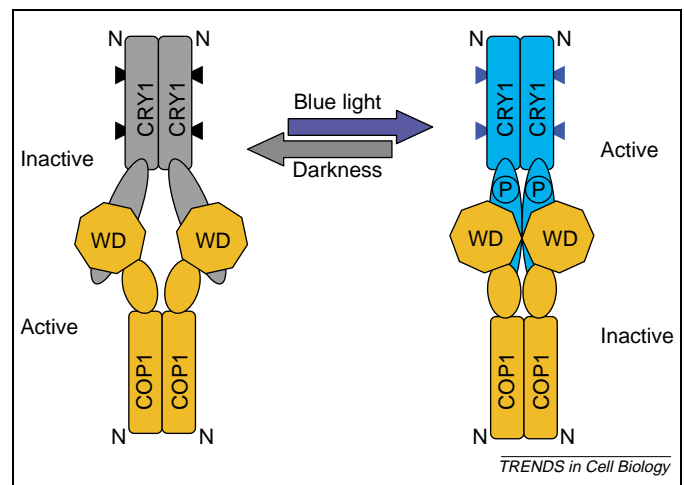


Figure 4. Cryptochromes inhibit COP1 activity under blue light. In this working model, cryptochromes homodimerize through their N-terminal light-absorbing domains (CNTs) and bind to the COP1 WD40 domains through their C-terminal domains (CCTs) [21,23,55]. In darkness, the cryptochrome homodimers are in an inactive state, allowing COP1 to repress light signaling; however, after the absorption of blue light, the cryptochromes undergo electron-transfer reactions at the N-terminal domains, which lead to conformational changes of the C-terminal domains that possibly cause intermolecular autophosphorylation and ultimate activation of the cryptochromes [58,59]. Activated cryptochromes inhibit COP1 activity through currently undefined mechanisms. Triangles represent the chromophore attachment sites in each of the cryptochromes.

light, mutant *cry1* proteins failing to undergo blue-light-induced phosphorylation are inactive, and constitutively active GUS–CCT2 is phosphorylated in both light- and dark-grown plants [40,57]. The phosphorylation and activation of the cryptochromes upon light absorption require an initial electron-transfer step within the CNT domain and a subsequent conformational change of the CCT domain [58,59] (Figure 4). Substitution of two conserved tryptophans required for light-induced electron transfer in the *cry1* protein resulted in significant reduction of light-activated *cry1* autophosphorylation *in vitro* and of its photoreceptor function *in vivo* [58].

Taking all these findings into consideration, we propose that the cryptochromes probably function through the following mechanism (Figure 4): in darkness, the cryptochrome homodimers, even though constitutively associated with COP1, are in an inactive state; after the absorption of blue light, the cryptochromes undergo electron-transfer reactions at the N-terminal domains, which leads to conformational changes of the C-terminal domains that possibly cause intermolecular autophosphorylation and ultimate activation of the cryptochromes. This rapid phosphorylation-based cryptochrome activation and resulting COP1 deactivation might represent, at least in blue light responses, the 'fast-acting' COP1 deactivation mechanism (versus the slow nuclear exclusion mechanism) that has long eluded researchers in this field [32].

Questions still remain about how exactly phosphorylated cryptochromes inhibit COP1 activity. Another area that requires further investigation is whether the phytochromes utilize a similar mechanism to deactivate COP1 under red and far-red light. Also worth mentioning is that, similar to COP1 and in contrast to phytochromes, cryptochromes have been identified in animals. However, the C-terminal sequence important for COP1 interaction in *Arabidopsis* is not conserved in animal cryptochromes. Thus, it is not clear whether the COP1–cryptochrome interaction is conserved in animals. As could be expected, mammalian cryptochromes and COP1 failed to show interaction in the same yeast two-hybrid assay that demonstrated direct interactions of their *Arabidopsis* counterparts [23].

COP/DET/FUS: indistinguishable phenotypes, related functions and direct interactions

COP1 belongs to the COP/DET/FUS loci identified through genetic screens in *Arabidopsis* that searched for mutant seedlings that displayed constitutive photomorphogenic phenotypes [60]. Except for COP1, the rest of the COP/DET/FUS loci encode polypeptides that have been shown to form two large protein complexes: the COP9 signalosome (CSN) and the CDD complex [61] (Figure 3). The eight-subunit COP9 signalosome, conserved in both plants and animals, associates with multiple cullin-containing E3 ubiquitin ligase complexes and regulates their E3 activities by removing the essential ubiquitin-like RUB/Nedd8 modification from the cullins [61]. The CDD (COP10–DDB1–DET1) complex, so far only reported in plants, is able to enhance E2 ubiquitin-conjugating activity *in vitro* [28]. The mammalian homologs of DDB1

and DET1 have also been shown to form complexes with one another [14]. COP10, the third component of the CDD complex and a ubiquitin-conjugating enzyme variant, has not been identified in animals. Therefore, it is unknown whether mammalian cells contain similar complexes. As mentioned above, DDB1 and DET1 have been shown to form an E3 complex with cullin 4A, Roc1 and COP1 [14] (Figure 3). As all components of this E3 complex have homologs in *Arabidopsis*, it would be very interesting to confirm whether a similar E3 complex exists in *Arabidopsis* and whether the CDD complex is part of a large E3 complex. To date, little is known about the relationship between these COP/DET/FUS proteins, despite the strikingly similar phenotypes exhibited by their mutants in *Arabidopsis* and their common involvement in ubiquitylation. An earlier study revealed that mutations at other *cop/det/fus* loci abolish the nuclear accumulation of the COP1 protein in the dark [46]. Later studies showed that the stability and integrity of the CDD complex but not of the COP1 complex are affected by mutations at CSN subunits, whereas *COP10* mutations lead to a size shift of the COP1 complex [25,36]. A very recent study in mammalian cells showed that siRNA-knockdown of CSN subunit 3 (CSN3) results in accumulation of the COP1 protein, suggesting that, at least in mammals, CSN might negatively regulate COP1 activity by facilitating degradation of that protein [44]. In *Arabidopsis*, COP10 was shown to bind to various E2 proteins, the COP1 RING finger and CSN, while direct COP1–DET1 interaction was recently implied in mammals [14,25,28] (Figure 3). The discovery of DET1 and COP1 as part of a cullin-containing complex provides a possible functional link between these two proteins and the CSN, particularly as the CSN has been repeatedly shown to interact with and regulate cullin-containing E3 complexes, including Cul4A–DDB1–Rbx1-containing E3 complexes [61,62] (Figure 3).

Other potential COP1 regulators

Yeast two-hybrid screens against specific domains of the AtCOP1 protein have fished out several AtCOP1-interacting proteins (CIPs), including CIP1, CIP4, CIP7, CIP8, STO and STH [20,22,63–65]. None of the CIP proteins is conserved in animals, suggesting that they might regulate plant-specific aspects of AtCOP1 function. Among the CIPs, the most interesting is probably CIP8, a small RING protein whose RING-H2 domain binds to the COP1 RING finger [65]. *In vitro*, CIP8 is a functional E3, able to ubiquitinate both itself and HY5 [66]. The authors of that study suggested that CIP8 might collaborate with COP1 in promoting HY5 ubiquitylation in the dark [66].

In mammalian cells, major vault protein (MVP), the main component of a ribonucleoprotein organelle called the vault, was recently identified as a major cytoplasmic COP1-interacting protein [17]. MVP, also known as lung resistance protein (LRP), was found to interact specifically and cooperate with COP1 under unstressed conditions to suppress c-Jun-mediated AP-1 transcription, thereby preventing cells from undergoing the stress response [17]. MVP is probably an animal-specific COP1 regulator as no plant MVP homolog has been reported in plants with fully sequenced genomes.

Concluding remarks

As summarized in this review, much progress has been made in recent years in efforts to dissect COP1 function and regulation. For future research, one important direction is to uncover the compositions of the COP1 complexes. The evidence implies that COP1 might form distinct complexes under different physiological conditions. Characterization of these COP1 complexes will help us understand how COP1 confers specific regulatory activities towards particular signaling pathways according to external conditions. Another question that has long puzzled researchers in this field is how the COP/DET/FUS proteins function in concert at the biochemical level. Doubtless they work closely together during *Arabidopsis* seedling development. This intimate relationship is most likely also shared by their mammalian homologs. Further dissection of the biochemical interplays between the COP/DET/FUS proteins in the animal system might help solve this mystery. A recent study showed that COP1 is overexpressed in certain breast and ovarian adenocarcinomas [16], suggesting that COP1 might be involved in tumorigenesis, possibly through its regulation of p53, c-Jun and probably other unidentified factors. However, a more definitive understanding of COP1 biological function in mammals certainly requires additional research, including the generation of animal *cop1* knockout models.

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