

Modulation of the subcellular localization of key factors is a common mechanism of developmental regulation. One of the best-characterized examples is the REL family of transcription factors, found in both invertebrates and vertebrates¹⁻³. In this case, regulation of nuclear localization is vital in the control of development. By specifically regulating the availability of the nuclear localization sequence (NLS) of a protein, or the protein itself, to the nuclear-import machinery, the subcellular localization and thus activity of specific developmental regulators can be controlled.

In plants, differential subcellular localization of several key regulators seems to play an important role in control of light-dependent development. For example, light signals initiate the nuclear import of phytochrome B (Ref. 4) and specific transcription factors^{5,6}. By contrast, light triggers a reduction in the nuclear abundance of COP1, a repressor of photomorphogenic development⁷. This review summarizes recent developments in understanding regulation of the subcellular localization of COP1 and how this relates to its developmental function.

Arabidopsis COP1 is a repressor of photomorphogenic development

As illustrated in Fig. 1, wild-type *Arabidopsis* seedling development follows two contrasting patterns, skotomorphogenesis (or etiolation) in darkness and photomorphogenesis in the light. Dark-grown (skotomorphogenic) seedlings are characterized by elongated hypocotyls and closed cotyledons on an apical hook, whereas photomorphogenic seedlings have short hypocotyls and expanded green cotyledons. COP1 was identified initially through recessive loss-of-function mutations in *Arabidopsis* that display a constitutively photomorphogenic phenotype regardless of the light environment⁸. The constitutively photomorphogenic phenotype and recessive nature of *cop1* mutations indicate that COP1 acts as a negative regulator, or a light-inactivated repressor, of photomorphogenesis. Overexpression of full-length COP1 results in quantitative hyper-suppression of photomorphogenic development⁹, which substantiates the genetic model and suggests that COP1 is a regulatory step in mediating the repression of photomorphogenic development. Those overexpression studies, together with mutational epistasis studies, are consistent with the notion that COP1 acts downstream of multiple photoreceptors^{9,10}.

Nuclear localization of COP1 is regulated by light

To investigate the cellular basis of COP1 function, a translational fusion between COP1 and the β -glucuronidase reporter (GUS) was expressed in *Arabidopsis* seedlings, and its subcellular localization examined⁷. In the hypocotyl cells of young seedlings, GUS-COP1 accumulates in the nucleus to a high level when seedlings are grown in the dark (Fig. 2). When they are grown in the light, the nuclear abundance of GUS-COP1 is reduced greatly. As this transgene can functionally rescue the defects of *cop1* mutations, and the observed localization patterns are also seen when it is expressed in a null *cop1* mutant background, it is reasonable to conclude

The role of COP1 in repression of *Arabidopsis* photomorphogenic development

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Photomorphogenic development in Arabidopsis is regulated by the key repressor COP1, which interacts with specific transcription factors in the nucleus to modulate their activities. In the dark, COP1 accumulates in the nucleus and represses photomorphogenic development. Light diminishes the nuclear accumulation of COP1 and abrogates its repressor activity. A number of cellular components are involved in light-dependent nucleocytoplasmic partitioning of COP1, including the multisubunit COP9 complexes and at least three well-characterized photoreceptors. This review discusses current understanding of the mechanisms of COP1 action.

that GUS-COP1 localization patterns reflect those of endogenous COP1. However, the total cellular content of COP1 is not noticeably affected by the light environment¹¹, thus, light-dependent depletion of COP1 from the nucleus is accompanied presumably by an increase in cytosolic COP1. The GUS-COP1 localization pattern suggests that COP1 acts in the nucleus to suppress photomorphogenic development in darkness, and the light-dependent inactivation of COP1 correlates with its reduced abundance in the nucleus. However, it is unlikely that the depletion of COP1 from the nucleus is the sole mechanism of COP1 inactivation. The redistribution of GUS-COP1 within the cell is too slow to account for the rapid changes observed in some COP1-mediated molecular events¹². Therefore, an early event must be responsible for the initial light-dependent inactivation of COP1, and the depletion of COP1 from the nucleus probably serves to maintain longterm COP1 inactivity.

The role of photoreceptors in regulating COP1 nuclear localization

An analysis of the role of specific photoreceptors has provided insight into how light regulates the nucleocytoplasmic partitioning of COP1. In

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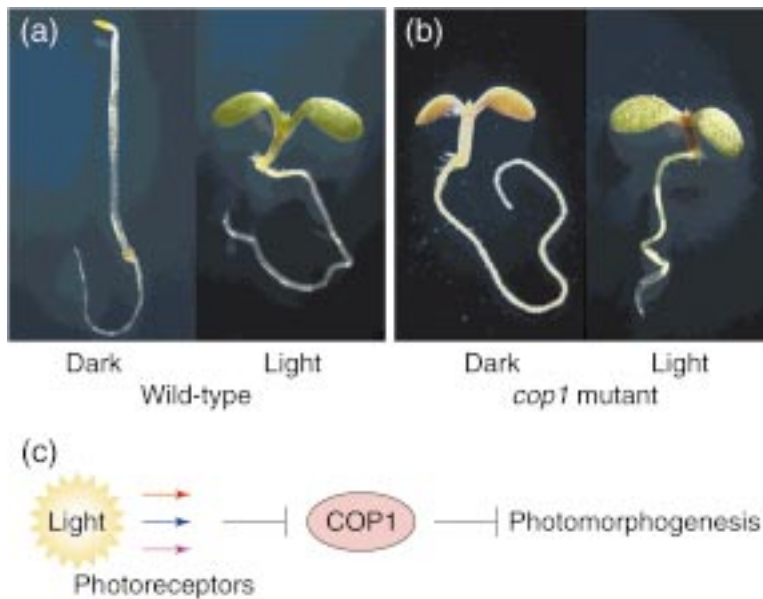


FIGURE 1

Arabidopsis COP1 acts as a light-inactivatable repressor of photomorphogenic development. (a) Wild-type *Arabidopsis* seedlings display a skotomorphogenic (etiolated) phenotype in the dark and a photomorphogenic phenotype in the light. (b) A loss-of-function mutation in the *COP1* gene results in constitutively photomorphogenic development in the dark. (c) A genetic model indicating that COP1 is a repressor of photomorphogenic development, whereas light signals, perceived by photoreceptors, abrogate this repressive action.

Arabidopsis, multiple complementing photoreceptors modulate seedling development by sensing distinct wavelengths of light. The three best-characterized of these, phyA, phyB and CRY1, play primary roles in mediating far-red-, red- and blue-light-dependent inhibition of hypocotyl elongation during seedling development, respectively^{13–15}. To examine the roles of these photoreceptors in the control of COP1 nucleocytoplasmic partitioning, the *GUS–COP1* transgene was introduced into null mutant and gain-of-function overexpression backgrounds of the photoreceptors¹⁶. Compared with wild-type seedlings, the abundance of *GUS–COP1* in the hypocotyl nuclei of each of the null mutants increased when the seedlings were grown under the wavelengths of light corresponding to that detected by each photoreceptor. This result led to the conclusion that phyA, phyB and CRY1 are responsible for initiating the signal-transduction pathway that regulates the abundance of COP1 in the nucleus, each seeming to be primarily responsible for a distinct wavelength of light.

It is not currently known whether light-initiated signals block nuclear import of COP1, activate its nuclear export, accelerate its degradation specifically within the nucleus, or a combination of these possibilities. The consistent overall cellular abundance of COP1 between light- and dark-grown seedlings suggests that selective degradation *per se* is unlikely to be a means of regulation. However, a combination of selective degradation in the nucleus and an over-accumulation in the cytosol would achieve the observed COP1 repartitioning in response to light. The identification of a cytoskeleton-localized *Arabidopsis* protein, CIP1, which can interact

specifically with COP1, might suggest a cytoplasmic anchoring mechanism for regulating the nucleocytoplasmic partitioning of COP1¹⁷. The presence of a typical bipartite NLS suggests that COP1 nuclear import is mediated by the standard nuclear-transport machinery. Interestingly, COP1 also seems to have a novel cytoplasmic-localization sequence (CLS), which can direct the cytoplasmic localization of a heterologous nuclear-localized protein¹⁸. Although it is not clear whether the CLS acts as a cytoplasmic-retention signal or a nuclear-export signal, light could be used to regulate the balance between the effects of the CLS and the NLS.

The subcellular localization of CRY1 is not known, and phyA is thought to be localized in the cytoplasm. However, although phyB is cytoplasmic in the dark, light triggers its nuclear import⁴. The distinct subcellular localization patterns of phyA and phyB suggest that they regulate the nuclear abundance of COP1 in different ways. If it is exclusively cytoplasmic, phyA would require both cytoplasmic and nuclear intermediates to relay light signals to COP1 in the nucleus, but phyB could influence COP1 directly or through only nuclear intermediates. It is also possible that phyA inhibits the nuclear import of cytoplasmic COP1, whereas phyB primarily regulates the nuclear export or degradation of COP1 within the nucleus. Clearly, further studies are required to identify the actual mechanisms.

A group of pleiotropic COP/DET/FUS genes are required for nuclear accumulation of COP1

In addition to the multiple photoreceptors responsible for initiating the signalling cascade(s) that leads to the depletion of COP1 from the nucleus, multiple downstream regulators are required to maintain the nuclear localization of COP1 in the darkness. Besides *COP1*, mutations at ten other loci result in constitutively photomorphogenic phenotypes (*COP1*, *DET1*, *FUS4–FUS6*, *COP8–COP10*, *FUS11*, *FUS12*, *COP16*)¹⁹. Collectively known as the pleiotropic *COP/DET/FUS* genes, the similar mutant phenotypes of these genes suggest that they might function cooperatively to perform a role that is essential for the activity of COP1. Indeed, an examination of *GUS–COP1* in the *cop/det/fus* mutants revealed that none of them shows an accumulation of *GUS–COP1* in the hypocotyl nuclei when seedlings are grown in the dark^{12,20}. This suggests that the wild-type gene products of these loci have roles in localizing COP1 to the nucleus. It further suggests that light could regulate the nuclear abundance of COP1 by modulating the activity of the *COP/DET/FUS* proteins.

One model is that the majority of the *COP/DET/FUS* proteins are part of a large protein complex^{19,20}. If this complex plays a role in ensuring the proper accumulation of COP1 in the dark, this could account for the similar phenotypes seen among *cop1* mutants and mutants of all complex-subunit genes. Indeed, many of the *COP/DET/FUS* proteins are part of a multisubunit complex (the COP9 complex or COP9 signalosome), which is localized primarily within the nucleus and consists of eight core subunits²¹. The molecular characterization of the COP9

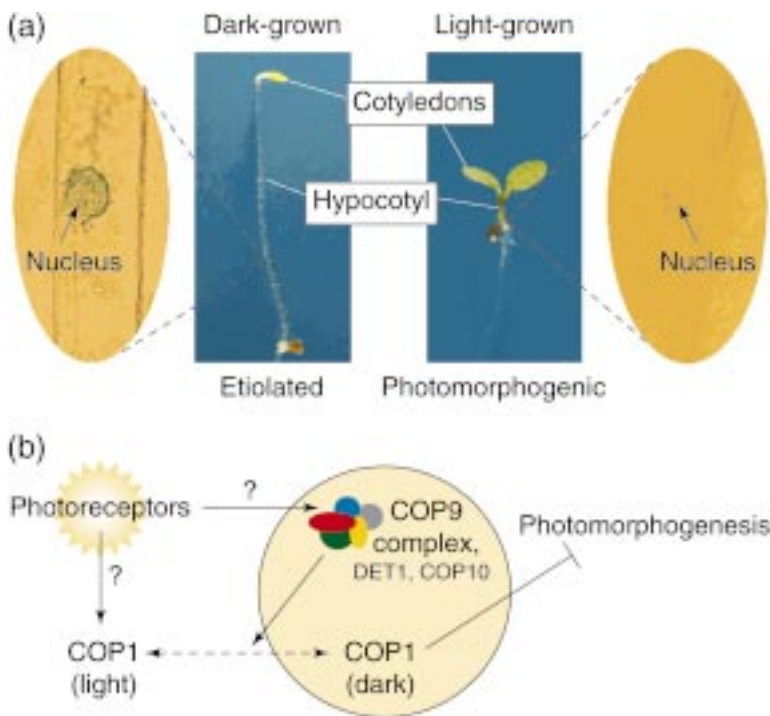


FIGURE 2

Light regulates the nucleocytoplasmic partitioning of COP1 in *Arabidopsis* hypocotyl cells. (a) The distinct patterns of seedling development correlate with the abundance of GUS–COP1 in hypocotyl nuclei; GUS–COP1 is abundant in the nucleus in the dark but is undetectable in the nucleus in the light. Note that the GUS–COP1 fusion protein in the cytosol tends to form inclusion bodies (not in the pictured area) rather than a uniform distribution. The GUS–COP1 transgene was integrated stably into the genome. Labels indicate the cotyledons (embryonic leaf) and the hypocotyls (stem-like structures below the cotyledons). (b) A proposed cellular model for how the COP9 complex, photoreceptors and other cellular factors (DET1, COP10) regulate nucleocytoplasmic partitioning of COP1. In the dark, the COP/DET/FUS gene products promote the nuclear accumulation of COP1. In the light, activation of photoreceptors results in the reduction of COP1 in the nucleus. This could be achieved through cytoplasmic factors, nuclear factors (COP/DET/FUS gene products) or a combination of the two. Note that the repartitioning of COP1 between the cytosol and the nucleus might or might not involve the active nuclear export of COP1. Arrows only indicate the flow of information and do not imply direct interactions or the nature (negative or positive) of the effect.

complex has revealed a striking feature. All eight subunits exhibit sequence similarity to distinct non-ATPase subunits of the 19S regulatory particle of the 26S proteasome²¹. The 19S regulatory particle consists of two subcomplexes, one of which, the lid complex, comprises the eight non-ATPase subunits²². This similarity between the COP9 complex and the proteasome lid subcomplex could suggest that proteasome-mediated protein degradation is involved in regulation of COP1 localization. It is conceivable that the COP9 complex protects COP1 from proteasome degradation in the dark, and light inactivates the COP9 complex and therefore results in an accelerated degradation of COP1 in the nucleus. It will be important to evaluate this possibility further and investigate the mechanism involved.

COP1 acts as a master regulator of specific transcription factors within the nucleus

The first nuclear target identified for COP1 was the bZIP-type transcription factor HY5, characterized as a positive regulator of photomorphogenic development and as a suppressor of *cop1* mutations^{10,23,24}. HY5 binds to a light-responsive promoter element, the G-box, and is essential for activating light-inducible genes containing this G-box in their promoters^{25,26}. Yeast two-hybrid assays and *in vitro* binding assays confirmed the direct interaction between COP1 and HY5. Transient colocalization studies in living plant cells provided evidence for the *in vivo* interaction between COP1 and HY5²⁶. Furthermore, when coexpressed in onion epidermal cells, COP1 and HY5 colocalize to specific nuclear

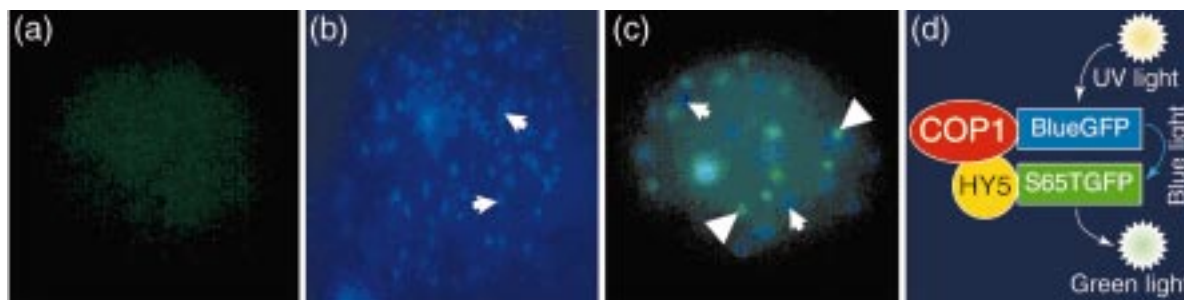


FIGURE 3

Physical association of COP1 and HY5 in living plant cells visualized by fluorescence resonance energy transfer (FRET). S65TGFP and blueGFP are two versions of green fluorescent protein (GFP) that have optimal excitations in blue and UV light and maximal emissions for green and blue light, respectively³⁶ (d). Under UV excitation, the uniform green nuclear localization of S65TGFP–HY5 is barely visible (a, c) owing to inefficient excitation by UV light. The blueGFP–COP1 clearly localizes to specific foci (or speckles, indicated by arrows) within the nucleus, as visualized by the blue fluorescence under UV excitation (b)²⁷. The nucleus of a typical onion epidermal cell coexpressing S65TGFP–HY5 and blueGFP–COP1 under UV excitation (c) reveals not only blue fluorescent speckles (as indicated by the arrows) but also bright green speckles (indicated by triangles) on a faint green background. The green speckles in (c) represent the recruitment of HY5 to the COP1 foci, where FRET occurs between S65TGFP–HY5 and blueGFP–COP1 through their close proximity. As diagrammed in (d), under the FRET condition, energy that would be emitted as the blue fluorescence by blueGFP–COP1 is transferred directly to S65TGFP–HY5 and emitted as green fluorescence. The presence of some blue speckles in (c) suggests that, in those foci, either HY5 is absent or is not in the appropriate conformation relative to COP1 to allow FRET.

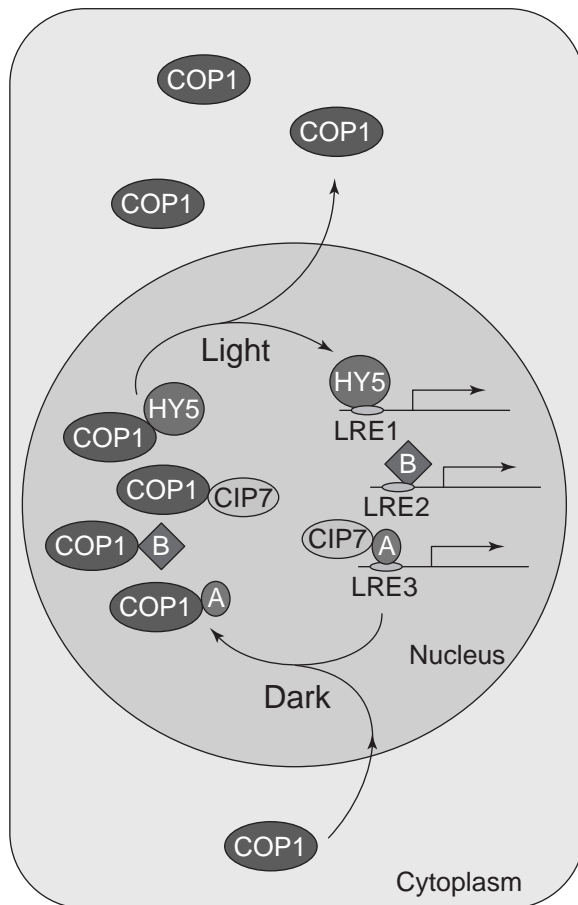


FIGURE 4

A working model illustrating the effect of COP1 on specific transcription factors in order to control the expression of light-regulated genes. LREs represent light-responsive promoter elements. It is proposed that COP1 interacts with CIP7, HY5 and other as-yet-unidentified transcription factors (A and B) in the dark to prevent these transcription factors from activating the expression of the target genes. When the nuclear abundance of COP1 is reduced in the light, these transcription factors become activated and act together to induce gene expression through specific LREs, leading to photomorphogenic development.

foci and allow fluorescence resonance energy transfer (FRET) between them, which shows that they are in close contact (Fig. 3). The interaction between COP1 and HY5 seems to negatively regulate HY5 activity, as HY5 deletion mutants that have disrupted COP1 interactions are hyperactive²⁶. Recent genetic evidence has suggested that HY5 also interacts with DET1, one of the COP/DET/FUS gene products that is not part of the COP9 complex²⁷. This could suggest a coordinated effort between COP1 and DET1 in the repression of positive regulators of photomorphogenesis.

More recent results suggest that, once in the nucleus, COP1 interacts with multiple transcription factors to regulate gene expression and thus suppress photomorphogenic development. This is supported by the identification of the transcriptional regulator CIP7 as a COP1-interactive protein. CIP7 localizes to the nucleus, contains transcriptional-activation activity and its expression is upregulated in the light²⁸. CIP7 antisense lines display reduced

expression levels of multiple light-inducible genes but have no effect on hypocotyl elongation²⁸. These data suggest that CIP7 is a positive regulator of light-regulated gene expression and a possible downstream target of COP1. It is possible that, in the dark, nuclear-localized COP1 binds to CIP7 and inhibits its transcriptional-activation activity.

Taken together, the available data support the model for COP1-mediated repression of photomorphogenic development shown in Fig. 4. The COP/DET/FUS gene products are essential for accumulation of COP1 in the nucleus, but, once in the nucleus, COP1 interacts with and inhibits the activity of multiple positive regulators of photomorphogenesis. Light signals, detected and transduced by multiple photoreceptors, deplete the nuclear abundance of COP1 and thus relieve its inhibitory effect on the transcription factors that initiate photomorphogenic development. The pleiotropic nature of *cop1* mutants suggests that COP1 represses the activities of multiple transcription factors and is not restricted to CIP7 and HY5, the only two reported so far.

The functional roles of the structural motifs of COP1

As shown in Fig. 5, in addition to the typical bipartite NLS and the newly defined CLS¹⁸ (see above), COP1 possesses three other recognizable structural motifs: an N-terminal RING-finger, a coiled-coil domain and a C-terminal WD-40 repeat region^{11,29}. All three domains are involved characteristically in protein-protein interactions³⁰⁻³². The results of a systematic dissection of the functional roles of the COP1 domains are summarized in Fig. 5^{18,33,34}. The NLS or CLS of COP1 can each act independently of light to target heterologous proteins to the nucleus or cytosol¹⁸. Deletion of the RING-finger or the coiled-coil domains does not affect the light-regulated localization of COP1³⁴. However, deletion of the RING-finger and the coiled-coil domain results in the constitutive nuclear localization of COP1³⁴, possibly owing to a disruption of the CLS, which is flanked by the two motifs¹⁸. COP1 acts primarily as a homodimer and probably dimerizes through the coiled-coil domain – although the RING-finger domain does seem to play a minor role in this self-association too³³. Currently, it is not clear how light regulates the relative activity of the CLS and NLS of COP1, although the N-terminal half of the protein, which includes all those motifs, is sufficient for the light-regulated nuclear localization of COP1¹⁸.

As overexpression of any COP1 mutant containing intact WD-40 repeats and an NLS results in skotomorphogenic-like phenotypes^{18,34}, the C-terminal WD-40 domain appears to function as an autonomous repressor of photomorphogenesis. This repression can be accounted for, at least in part, by the direct interaction between the WD-40 domain and HY5. Deletion of the coiled-coil domain inhibits severely the COP1-HY5 interaction²⁷, so COP1 self-association appears to be a prerequisite for interaction between the WD-40 repeats and HY5. Interestingly, a heterologous self-association domain, such as the GUS protein, can replace the

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deleted coiled-coil of COP1 and restore the interaction with HY5³⁴. As WD-40 repeats have the capacity to interact with multiple partners³², it is unlikely that HY5 is the only transcription factor that interacts with COP1 through this domain.

Perspectives and implications

Research in the past few years has established COP1 as a key contributor to the light-mediated control of *Arabidopsis* seedling development. Light-dependent regulation of COP1 nucleocytoplasmic partitioning plays a crucial role in this developmental process. Although it has been established that the photoreceptors and the pleiotropic COP/DET/FUS proteins are involved in regulating the activity of COP1, the molecular mechanisms underlying this process remain largely unknown. Clearly, further studies are required to understand the cellular and biochemical mechanisms.

COP1 and the other known pleiotropic COP/DET/FUS proteins are highly conserved in all multicellular eukaryotes^{35,37}. For example, mammals, and possibly all multicellular organisms, possess a very similar COP9 complex (now called the COP9 signalosome)³⁷. The similarities between subunits of the *Arabidopsis* and human complexes range from 35 to 70% amino acid identity. This striking similarity might suggest a fundamental and conserved cellular role for this group of proteins, which, in plants, has been adapted for mediating light control of gene expression and development. It is also possible that their role in light regulation is only part of their overall function in plants. In fact, a multifaceted role of the COP9 signalosome in mammalian cell-signalling processes is hinted at by its associated novel kinase activity. In addition, several subunits are involved in regulating multiple cell-signalling pathways and in cell-cycle progression³⁷. The well-established cellular and biochemical methodologies that are available with mammalian cells will complement the molecular-genetic analysis in *Arabidopsis*. Future complementary and comparative research in mammalian and plant systems might prove to be highly informative in understanding the function of *Arabidopsis* COP1 and its relation to other pleiotropic COP/DET/FUS proteins. It will be of great interest to see how this highly conserved regulatory system has been utilized in organisms as diverse as *Arabidopsis* and humans and to reveal the biochemical and molecular basis responsible for its cellular and developmental function.

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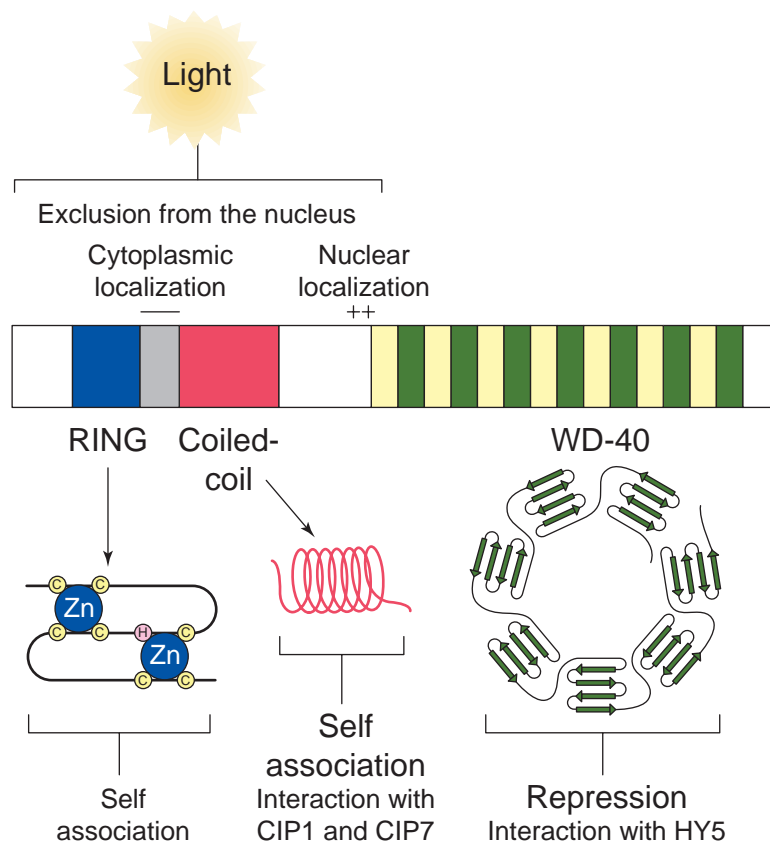


FIGURE 5

A working model for the specific roles of COP1 structural motifs in the light control of *Arabidopsis* seedling development. Besides the nuclear-localization sequence (NLS) and the cytoplasmic-localization sequence (CLS), the other three motifs of COP1 are essential for its activity in repressing photomorphogenic development, and each plays a specific role. Light signals, transduced through signalling intermediate(s), diminish the nuclear abundance of COP1 by acting through the N-terminal half of the protein. A combination of the RING-finger (minor role) and the coiled-coil (major role) domains is necessary for COP1 dimerization, a prerequisite for COP1 and HY5 interaction. The WD-40 repeats are responsible for interacting with HY5, a transcription factor whose activity is inhibited by COP1. The coiled-coil domain is responsible for interacting with CIP1 and CIP7.

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Special-interest subgroups at the ASCB

The annual meeting of the American Society for Cell Biology (ASCB) is a large and diverse gathering. At last year's meeting, there were over 8000 attendees, and the topics discussed covered many areas of cell biology. It would be impossible to cover the entire meeting within a trends in CELL BIOLOGY report, so instead we are focusing on an aspect of it that provided some of the most interesting and fruitful discussions. On Saturday afternoon, before the main symposia began, there were 11 special-interest subgroup meetings. The atmosphere at these meetings was informal, and they encouraged open and frank discussion of data and issues. This report provides a brief summary of the discussions at seven of the special-interest subgroup meetings.*

*The American Society for Cell Biology 38th Annual Meeting, San Francisco, USA; 12–16 December, 1998.

Program chair:
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Regulation of actin dynamics by ADF/cofilins *in vivo* and *in vitro*

Organizers: S. Ono, Emory University, USA; A. McGough, Baylor College of Medicine, USA; and J. R. Bamburg, Colorado State University, USA.

The actin-depolymerizing factor (ADF)/cofilin (abbreviated AC) family of proteins is responsible for the rapid turnover of actin filaments *in vivo*. In this symposium, several speakers (A. Weeds, Cambridge, UK; M-F. Carlier, Gif-sur-Yvette, France; L. Blanchoin, San Diego, USA; J. Condeelis, New York, USA; S. Maciver, Edinburgh, UK) addressed the issues of filament severing and dynamics. AC has a weak but noncatalytic severing activity, but it is not known whether severing is active or passive. AC increases the actin off-rate from the pointed end of filaments 20–30 fold. A. McGough (Houston, USA) reported that the degree of twist imparted to an actin filament by cooperative binding of AC depends on the source of AC: mutants with greater depolymerizing activity induce a larger twist. J. Condeelis showed that recombinant ACs are not

as active in severing as native proteins, and AC proteins sever filaments at the leading edge of migrating cells. The enhanced nucleation that results from severing is inhibited by Lim kinase 1 (LK1). K. Mizuno (Fukuoka, Japan) described how activation of LK1 by rac1 inhibits the activity of AC by phosphorylation. LK1 is found preferentially in nerve tissue, but LK2, which also phosphorylates ACs, is distributed more widely. The pH regulation of ACs *in vivo* not involving changes in phosphorylation was described by B. Bernstein (Fort Collins, USA).

K. Okada (Chiba, Japan) showed that actin-interacting protein 1 (AIP1) binds preferentially to AC-saturated actin filaments (1 AIP1/2 AC-actins) and enhances filament severing. A. Rodal (Berkeley, USA) showed that deletion of AIP1 in yeast has no effect on growth phenotype but is synthetically lethal with nonlethal cofilin mutants. H. Aizawa (Tokyo, Japan) showed effects of AIP1 on actin filament length in *Dictyostelium*. S. Almo (New York, USA) found that some essential surface residues on ACs do not interact with actin, suggesting that there is another important interacting protein, which could be AIP1.

A number of speakers addressed abnormalities that occur in processes requiring the ordered addition or removal of actin filaments when AC is absent. Abnormal ring canals and cuticular ridges in bristles of *Drosophila* were discussed by K. Gunsalus (Piscataway, USA). S. Ono (Atlanta, USA) described abnormalities in body-wall muscle development in *Caenorhabditis elegans* accompanying mutations in the *unc60* gene. This gene gives two isoforms by alternative splicing – *unc60A*, essential for any development, and *unc60B*, essential for muscle development. T. Obinata (Chiba, Japan) reviewed the role of AC in development of striated muscle and presented evidence for a non-phosphorylation-dependent regulation of AC in myotubes. B. Molitoris (Indianapolis, USA) reported that AC and actin are major urinary proteins following ischemic kidney damage. ACs are dephosphorylated rapidly during ischemia, making them prime candidates for mediating actin cytoskeletal changes during renal failure. Thus, an emerging theme is that AC proteins play essential functions in actin-based organization in normal development and in many pathological conditions.