The Cell Biology of the COP/DET/FUS Proteins. Regulating Proteolysis in Photomorphogenesis and Beyond?[1]

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Plants as sessile organisms have evolved a great deal of developmental plasticity to optimally respond to their immediate environment. Because light is one of the most important cues for plant growth, mechanisms to respond to light conditions are highly elaborated. In particular, the transition from dark-grown (skotomorphogenic) to light-grown (photomorphogenic) development (Fig. 1) in Arabidopsis is steered by a complex molecular network. This network senses the intensity and quality of light and transduces the light signal to downstream effectors that govern the physiological changes that will eventually result in photomorphogenesis.

Numerous loci involved in this process have been identified over the last several years by genetic screens (Fig. 2). They include upstream signaling components, like the photoreceptors (for review, see Batschauer, 1998), and intermediate factors transducing the signal to downstream regulators such as *EID1*, *FHY1*, *FHY3*, *FIN2*, *SPA1*, *FAR1*, *PAT1*, *FIN219*, *RSF1*, or *HFR1* (Whitelam et al., 1993; Soh et al., 1998; Hoecker et al., 1999; Hudson et al., 1999; Bolle et al., 2000; Büche et al., 2000; Fairchild et al., 2000; Fankhauser and Chory, 2000; Hsieh et al., 2000). The downstream components integrate the light signals from the various photoreceptors and bring about the changes in metabolism and gene expression that eventually lead to photomorphogenesis. More downstream effectors that directly interact with photoreceptors have recently been identified by protein-to-protein interaction approaches (Ni et al., 1998; Choi et al., 1999; Fankhauser et al., 1999). Also, several downstream components identified by their mutant phenotype are negative regulators of photomorphogenesis. They constitute a genetic bottleneck that represses the onset of photomorphogenesis in darkness. Excellent reviews on the signal transduction from photoreceptors to downstream regulators have been published recently (Deng and Quail, 1999; Casal, 2000; Nagy and Schäfer, 2000; Neff et al., 2000). In this update we will thus focus on current progress in the dissection of the molecular function of negative regulators of photomorphogenesis, in particular those of the pleiotropic constitutive photomorphogenic class.

A BRIEF SUMMARY OF THE *COP/DET/FUS* LOCI

Several loci acting as negative regulators of photomorphogenesis have been isolated from genetic screens that sought to identify mutants that display characteristics of light-grown seedlings in complete darkness. They were named constitutive photomorphogenic (*COP*; Deng et al., 1991) or de-etiolated (*DET*; Chory et al., 1989). A number of these loci turned out to be identical to previously isolated *fusca* (*FUS*) mutants (Misera et al., 1994), which were named after their purple seed color resulting from anthocyanin accumulation. Thus this group of genes is collectively referred to as the pleiotropic *COP/DET/FUS* loci of Arabidopsis (Wei and Deng, 1999).

Mutants in the 11 pleiotropic *COP/DET/FUS* loci display a photomorphogenic phenotype in complete darkness (Fig. 1). This phenotype is not only characterized by morphological changes such as open, expanded cotyledons, suppression of hypocotyl growth, and anthocyanin accumulation, but also corresponding changes in subcellular features such as chloroplast differentiation and de-repressed expression of normally light-induced genes. The mutants in all 11 *COP/DET/FUS* loci are recessive and can be generally grouped into two classes: mutations in eight loci, which lack the COP9 signalosome (Chamovitz et al., 1996; Karniol et al., 1999; Serino et al., 1999; Deng et al., 2000) and mutations in three loci that do not (Fig. 2). It is interesting that all *cop/det/fus* mutants lacking the signalosome are lethal at the seedling stage. Although this is also true for severe alleles of the non-signalosome mutants *cop1*, *cop10*, and *det1*, their weak alleles are viable (Chory et al., 1989; Kwok et al., 1996; Vogel et al., 1998). Thus the COP9 signalosome seems to have a more general role in Arabidopsis development. This update will focus on the most recent advances, whereas earlier progress can be found in several previous reviews (Wei and Deng,

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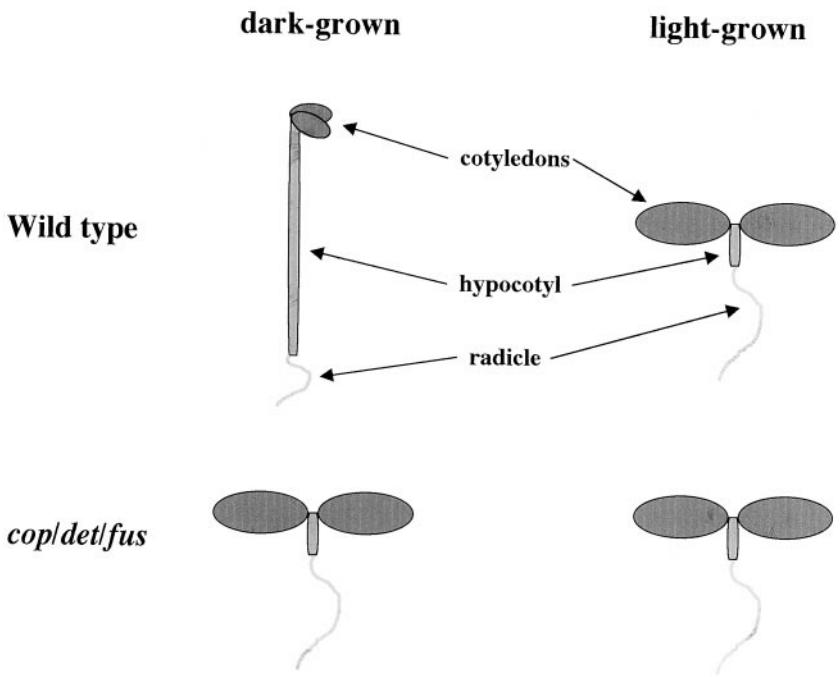

Figure 1. Schematic presentation of the light control of seedling development and the role of *COP/DET/FUS* loci. Wild-type seedlings grown in the light display photomorphogenic development, i.e. a short hypocotyl and green expanded cotyledons. In complete darkness, wild-type seedlings develop skotomorphogenically, characterized by a highly elongated hypocotyl and small, closed, undifferentiated cotyledons. In contrast, constitutive photomorphogenic seedlings (*cop/det/fus* mutants) are hyperphotomorphogenic in the light and display a light-grown morphology in darkness.

1996, 1999; Osterlund et al., 1999; Schwechheimer and Deng, 2000).

COP1-HY5 INTERACTION SUGGESTS A CONCEPTUAL ROLE OF COP1 IN PROTEIN DEGRADATION

Recent progress in understanding the molecular basis of *COP/DET/FUS* gene action has come from a better understanding of the nature of the interaction between the COP1 and HY5 (*long hypocotyl 5*) (Koornneef et al., 1980) proteins. So far, *HY5* is the only genetically characterized positive regulator of photomorphogenesis that acts downstream of the bottleneck comprised by the *COP/DET/FUS* loci. *HY5* encodes a bZIP transcription factor that binds to the G-box in the promoters of light-inducible genes such as *RBCS1A* or *CHS1*, and thereby plays a vital role in their activation during seedling morphogenesis (Oyama et al., 1997; Ang et al., 1998; Chattopadhyay et al., 1998).

The *hy5* mutant has been re-isolated as an extragenic suppressor of *cop1*, and molecular analysis has confirmed that both gene products also interact physically (Ang and Deng, 1994; Ang et al., 1998). HY5 is a constitutively nuclear localized protein, whereas COP1 is excluded from the nucleus in the light, but nuclear localized in darkness (von Arnim and Deng, 1994). Thus an early working hypothesis suggested that the physical interaction between COP1 and HY5 could only take place in darkness and should negatively regulate HY5 activity (Ang et al., 1998). The molecular basis for the negative regulation remained elusive, however, and had to await a more detailed characterization of the HY5 protein.

Analysis of HY5 in Arabidopsis seedling development indicated highly elevated HY5 protein levels in light-grown seedlings as opposed to dark-grown seedlings (Osterlund et al., 2000a, 2000b). HY5 accumulates to about 20-fold within 15 h after shifting seedlings from darkness into light, and this accumulation is reversible by the opposite light shift. This finding did not correspond, however, to HY5 mRNA levels, which show only a 2- to 3-fold difference between dark and light. In transgenic lines ectopically overexpressing HY5, HY5 protein levels are independent from endogenous *HY5* mRNA levels. It is striking that the same light-dark dependence of HY5 abundance was observed again. By uncoupling any effects on mRNA translation from protein stability by the application of a protein synthesis inhibitor, enhanced degradation of HY5 in darkness could be established as the primary determinant for HY5 abundance.

Analysis of HY5 levels in *cop1* mutant backgrounds showed that HY5 abundance in dark-grown *cop1* mutant seedlings is similar to the levels in light-grown seedlings. This finding by itself could be considered a consequence of the light-grown morphology of *cop1* mutants in the dark rather than one of its causes. However, the accumulation dynamics of HY5 argues against the former. A steep rise in HY5 abundance upon illumination of skotomorphogenic seedlings precedes any striking changes in seedling morphology other than apical hook opening. This suggests that photomorphogenesis is at least in part promoted by elevated HY5 activity, in line with the *hy5* mutant phenotype. Thus *cop1* mutants seem to be unable to degrade HY5 in the dark. Moreover, a truncated *HY5* transgene that lacks the COP1 interaction domain is not subject to differential degradation in the dark any more. Since this observation coincides with the nuclear localization of COP1 only in the dark, the re-

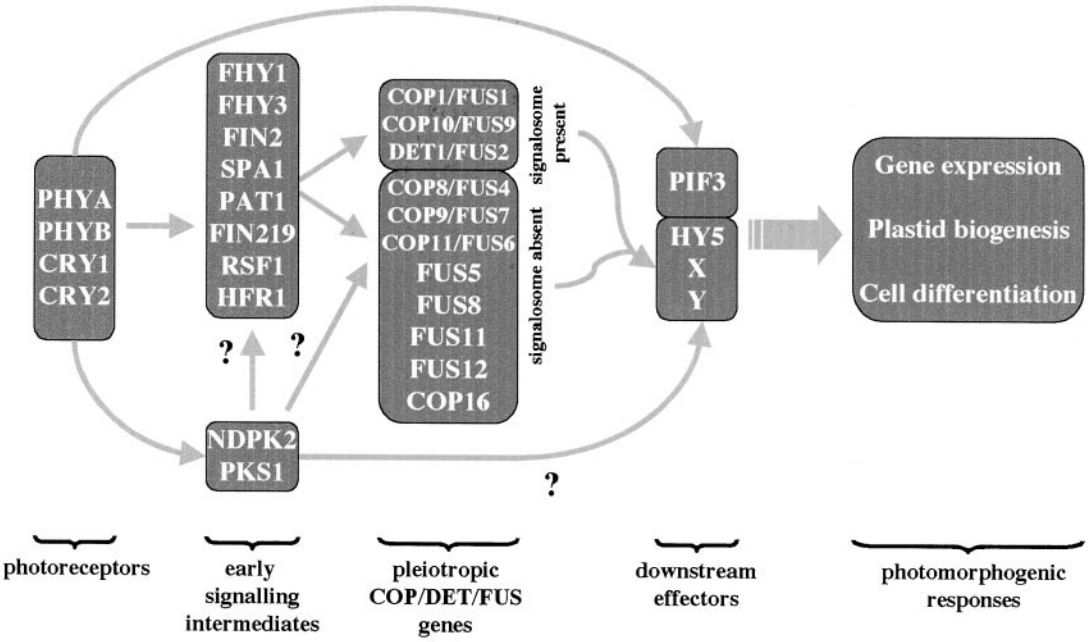

Figure 2. Flow diagram of light signal transduction of Arabidopsis. Only a portion of the known components is listed. Light is perceived by the different photoreceptors that sense light of particular wavelengths. The light signal is then transduced by early response signaling intermediates to the pleiotropic *COP/DET/FUS* loci. The *COP/DET/FUS* genes integrate the various light signals and modulate the activity of downstream effectors such as HY5 and additional unknown factors (X and Y). These factors govern the changes in metabolism and gene expression that will eventually result in the photomorphogenic responses. Early response mechanisms are additionally activated directly by the phytochromes. These include modulation of downstream transcriptional effectors such as PIF3. PIF3 directly promotes light-induced gene expression, bypassing any intermediate signaling input from the pleiotropic *COP/DET/FUS* genes. Also, substrates for phytochrome-associated kinase activity such as NDPK2 and PKS1 may directly influence downstream effectors or modulate the activity of early signaling components and pleiotropic *COP/DET/FUS* genes.

sults suggest that interaction between COP1 and HY5 results in targeted degradation of the HY5 protein.

HY5 ACTIVITY IS ALSO REGULATED BY PHOSPHORYLATION

Recent results indicate that regulation of HY5 activity involves a multitude of events. Not only does COP1 control HY5 abundance by targeted degradation in the dark, but this interaction is also modulated by phosphorylation of HY5 (Hardtke et al., 2000; Fig. 3). A phosphorylation site for a casein kinase II (CKII) activity is present within the COP1 interaction domain of HY5 and very likely a target for this type of kinase. The kinase activity is itself subject to light regulation, since dark-grown seedlings contain elevated kinase activity toward HY5 as compared with light-grown seedlings. It is interesting that CKII has been implicated in regulating a number of other transcription factors involved in light-regulated gene expression (Klimczak et al., 1992, 1995; Sugano et al., 1998). Thus CKII regulation seems to be a recurring theme in the modulation of light-regulated, as well as circadian, gene expression (Ciceri et al., 1997; Lee et al., 1999; Sugano et al., 1999).

In the case of HY5, phosphorylation of the COP1-binding domain on the one hand seems to influence HY5 stability, since unphosphorylated HY5 interacts better with COP1 and is a preferred substrate for degradation. On the other hand, unphosphorylated HY5 is also physiologically more active. This is evidenced by transgenic rescue of a *hy5* null mutant with a transgene whose gene product cannot be phosphorylated in the COP1 interaction domain. In these transgenic lines a slightly hyperphotomorphogenic phenotype, as well as a delay in lateral root outgrowth, is observed, which is essentially an overcompensation of *hy5* mutant traits. The overcompensated root phenotype by a HY5, which is a better substrate for COP1-mediated degradation represents a paradox, since COP1 is constitutively nuclear localized in root cells even in the light (von Arnim and Deng, 1994). However, it must be considered that the hyperphotomorphogenic phenotype might result in an altered carbon-to-nitrogen ratio due to elevated photosynthesis and thus a reduction in lateral root growth as a secondary effect. In any case, in the context of photomorphogenesis the physiologically more active unphosphorylated HY5 seems to be the prime target for COP1. Thus in darkness unphosphorylated HY5 gets degraded, whereas elevated CKII activity ensures the maintenance of a small pool of less active phosphorylated HY5, which can be rapidly ac-

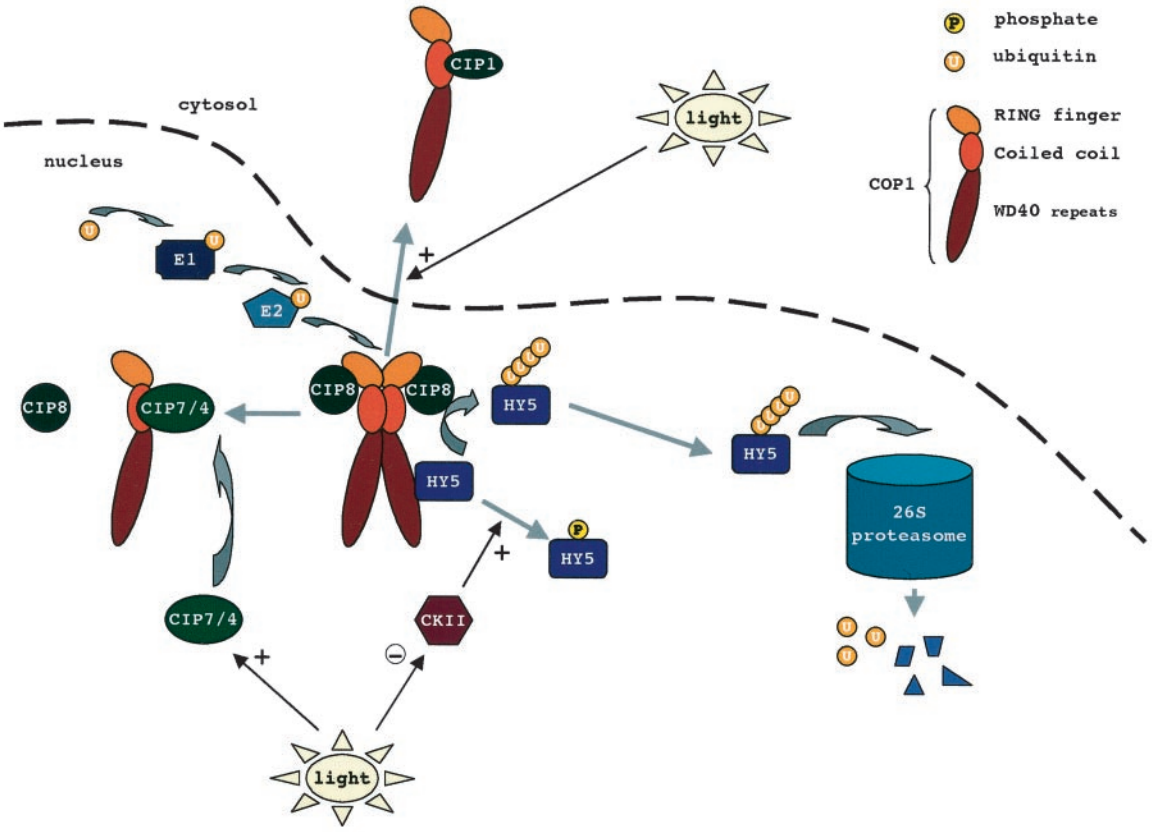

Figure 3. A working model for COP1 action in Arabidopsis photomorphogenesis. It is assumed that COP1 acts as part of an E3 ubiquitin ligase complex. Ubiquitin is activated in the cell by E1 ubiquitin-activating enzymes, which pass the ubiquitin to E2 ubiquitin conjugating enzymes. The E2 enzyme in turn associates with an E3 ubiquitin ligase that directs the transfer of the ubiquitin to target proteins. In its active form, COP1 is associated with other proteins in an E3 ligase complex. Within the E3 complex COP1 provides specificity of the ubiquitination machinery toward selected substrates involved in photomorphogenesis such as the HY5 transcription factor. The contact between COP1 and its substrates is mediated via its WD40 repeat domain. The other functional domains of COP1 process distinct signaling inputs from a number of CIP. The CIP8 protein is incorporated into the E3 complex by interaction with the RING-finger domain of COP1 and drives E2-dependent ubiquitin chain assembly on the target proteins recruited by COP1. In darkness this results in targeting of HY5 for degradation by the 26S proteasome. Upon illumination COP1 might be inactivated by an early response mechanism mediated by CIP7 and/or CIP4 (CIP7/4), nuclear factors whose transcription is highly inducible by light. CIP7/4 act by interacting with the coiled coil domain of COP1, which is necessary for COP1 homodimerization. Because dimerization is necessary for COP1 activity, its disruption results in a stabilization of HY5. Long-term inactivation of COP1 is triggered by light through nuclear exclusion of COP1 protein. Anchoring of COP1 in the cytoplasm might be mediated by CIP1, a cytoskeleton-associated protein, which also interacts with the COP1 coiled coil domain. Moreover, the COP1 substrates may be under control of additional inputs themselves. In the case of HY5, phosphorylation within its COP1-binding domain by a CKII activity results in a physiologically less-active protein. This phosphorylated HY5 is at the same time less susceptible to degradation, since its interaction with COP1 is impaired. This mechanism ensures the maintenance of a small pool of HY5 in darkness. In the light this pool gets rapidly activated due to the inhibition of the CKII activity by light. In combination with the light-induced reduction of COP1 activity this results in an accumulation of more active unphosphorylated HY5 in the light, which promotes the onset of photomorphogenesis.

tivated upon illumination. The combination of regulated HY5 abundance and HY5 phosphorylation thus further extends the HY5 activity differential between dark- and light-grown seedlings.

COP1 TARGETS HY5 FOR DEGRADATION VIA THE 26S PROTEASOME

A hint toward the specific role of COP1 in its interaction with HY5 comes from the finding that HY5 degradation can be blocked by proteasome inhibitors in vitro (Osterlund et al., 2000a). Thus the proteasome pathway seems likely to be responsible for HY5 degradation. Proteins targeted for degradation by the proteasome pathway are in general marked by the covalent attachment of ubiquitin. Ubiquitin is a highly conserved 8-kD protein found in all eukaryotes examined to date and gets attached to a Lys in target proteins via its C terminus. Additional ubiquitin molecules are subsequently linked to an internal Lys in the ubiquitin itself, eventually generating a chain of interconnected ubiquitin molecules on one or more lysines in the target protein. A protein marked this

way is recognized as a substrate by the 26S proteasome, a multiprotein complex that eventually chops the target protein into small peptides.

Attachment of ubiquitin moieties to target proteins is mediated by a system of three enzymes (Fig. 3). In a first step, so-called E1 enzymes activate ubiquitin molecules, which then are transferred to E2 ubiquitin conjugating enzymes. The ubiquitin is eventually transferred from the E2 enzyme to the target protein and to achieve this, the E2 enzyme in numerous cases teams up with a so-called E3 ubiquitin ligase. Most E3 ubiquitin ligases are multisubunit protein complexes and critical for the ubiquitin tagging of substrates since they provide substrate specificity to the reaction by selecting the appropriate target proteins in vivo. Within this scenario COP1 would occupy a place as an integral part of an E3 ligase and mediate interaction between the ubiquitin ligase machinery and specific target proteins. The presence of a RING-finger domain in its N terminus and a number of WD40 repeats in its C terminus is consistent with such a role. Although the N terminus might be required for assembly into the E3 complex, the WD40 repeat domain could serve as an interaction surface for target proteins (see below).

FITTING IN COP1 INTERACTING PROTEINS (CIP)

Apart from HY5, a number of proteins interacting with COP1 have been isolated by the application of two-hybrid interaction screening and far-western analysis. These proteins can generally be classified according to where the interaction with COP1 takes place. Although some proteins seem to interact with domains (RING-finger or coiled coil) in the N terminus of COP1, others seem to interact with the WD40 repeat domain of COP1 (Fig. 3).

A protein interacting with the coiled coil domain in the N terminus of COP1 is the CIP1 (Matsui et al., 1995). CIP1 does not have primary sequence features pointing out any functionally defined domains; however, it seems to have the capacity to form a coiled coil structure itself. More revealing, CIP1 seems to be a cytosolic protein associated with the cytoskeleton and thus might be involved in light-induced nuclear depletion of COP1 by anchoring it in the cytoplasm.

Another protein interacting with the coiled coil domain of COP1 is CIP7 (Yamamoto et al., 1998). Unlike CIP1, CIP7 is a nuclear protein and contains coiled coil domains that mediate interaction with COP1. Although CIP7 possesses some transcription activation potential, it is not clear whether CIP7 indeed functions as a transcriptional regulator. *CIP7* mRNA is hardly detected in the dark, but highly induced by light. Since the kinetics of COP1 nuclear exclusion upon exposure to light are clearly lagging behind the corresponding photomorphogenic changes in the seedling, an early inactivation mechanism for COP1 distinct from nuclear exclusion has been suggested (von Arnim et al., 1997). It is interesting that proper function of COP1 requires its homodimerization, which is also a prerequisite for COP1-HY5 interaction (Torii et al., 1998). Further, COP1 homodimerization is mediated through its coiled coil domain. Thus disruption of COP1 dimerization through occupation of this domain by CIP7 could be a means of rapid COP1 inactivation in response to a light stimulus (Fig. 3). A similar role could be envisioned for the CIP4 protein, which also interacts with the coiled coil domain in COP1 (Yamamoto et al., 2000) and could mediate distinct signaling inputs not covered by CIP7. However, the transactivation potential of CIP4 is much higher than that of CIP7, and an alternative or additional function of CIP4 in transcription control should not be ruled out.

An additional N-terminal interactor of COP1 is CIP8, a RING-finger protein that specifically interacts with the RING finger of COP1 (Torii et al., 1999). Clues as to the function of this interaction are provided by recent findings on the role of RING-finger proteins in the ubiquitination machinery. The RING-finger protein Rbx1 has recently been established as a component of the prototypic SCF E3 ubiquitin ligase complex (Kamura et al., 1999; Seol et al., 1999; Skowyra et al., 1999). Moreover, several RING-finger proteins have been implicated in aiding E2 conjugating enzyme dependent ubiquitination (Lorick et al., 1999). This might also be the case for CIP8 (C.S. Hardtke, H. Okamoto, and X.W. Deng, unpublished data). Thus CIP8 might possibly function as part of an E3 ubiquitin ligase complex that includes COP1 (Fig. 3).

The C-terminal interacting proteins contact COP1 via its WD40 repeat domain. Apart from HY5, a number of other factors that specifically interact with the WD40 repeat domain of COP1 have been identified in yeast two-hybrid screens (Holm and Deng, 1999; M. Holm and X.W. Deng, unpublished data). These possibly represent additional targets for COP1. Thus the WD40 repeat domain could serve as an interaction surface for various substrates specifically selected by COP1 for proteasome-mediated degradation. WD40 repeat domain components are likely responsible for substrate selection in a number of prototypical E3 ubiquitin ligases such as the SCF or APC complexes (Deshaies, 1999).

POSSIBLE ROLES OF THE COP9 SIGNALOSOME IN PHOTOMORPHOGENESIS

How does the COP9 signalosome fit into this scheme? In non-plant eukaryotes from *Schizosaccharomyces pombe* to humans, the COP9 signalosome has been implicated in a number of cellular processes. Individual subunits are, for example, involved in the progression of the cell cycle (Mundt et al., 1999; Tomoda et al., 1999), AP-1 transcription (Claret et al., 1996), and thyroid hormone reception (Lee et al., 1995). It is interesting that no mutants for signalosome subunits have been found in vertebrates to date

and the only mutant in invertebrates is a late larval stage lethal in *Drosophila* (Freilich et al., 1999). Thus the COP9 signalosome might not only be essential for the post-juvenile development of Arabidopsis, but also of other multicellular organisms. Given the multitude of responses the signalosome influences in animal systems, it seems likely that it also takes part in numerous regulatory events in Arabidopsis. In particular, the signalosome subunits AJH1 and AJH2 of Arabidopsis (Kwok et al., 1998), which are homologs of the mammalian transcriptional co-activator Jab1, and FUS5 (Karniol et al., 1999) are also present as monomers in vivo, unlike other signalosome subunits. These monomers might, for instance, be involved in processes other than photomorphogenesis. However, in the absence of viable mutants these speculations are hard to verify. It is obvious that dominant-negative transgenic interference with the COP9 signalosome by ectopic overexpression of one of its subunits could evoke a photomorphogenic response by default, as observed for AJH1/2 (Kwok et al., 1998). The reason might be that a constitutive photomorphogenic phenotype is the earliest manifestation of a lack of signalosome activity. Thus the construction of genetic mosaics or interference with signalosome activity by the application of inducible transgenes will be necessary to decipher the function of the signalosome at post-seedling stages of Arabidopsis development.

Just like *cop1*, all other pleiotropic *cop/det/fus* mutants are unable to degrade HY5 in darkness (Osterlund et al., 2000a). This result might reflect an indirect consequence of the absence of COP1 from the nucleus since COP1 is localized in the cytosol in all the COP9 signalosome mutants even in the dark. It has been suggested that the signalosome could be involved directly or indirectly in the regulation of the nuclear import/export machinery responsible for light-dependent shuttling of COP1 (Chamovitz et al., 1996). On the other hand, cytosolic COP1 in the dark could also be considered a consequence of the photomorphogenic development in these mutants, originally triggered by an inability to degrade positive regulators of photomorphogenesis such as HY5. However, until an experimental system to manipulate nucleocytoplasmic COP1 shuttling is available, neither hypothesis can be tested experimentally for the time being.

THE COP9 SIGNALOSOME IS STRUCTURALLY SIMILAR TO THE LID SUBCOMPLEX OF THE 19S REGULATORY PARTICLE OF THE 26S PROTEASOME

A different line of reasoning implicating the COP9 signalosome in regulated protein degradation comes from studies in other eukaryotic species. The mammalian signalosome was initially purified biochemically from pig spleen (Wei and Deng, 1998). At the same time the complex was also encountered during an attempt to characterize novel components of the 26S proteasome isolated from human blood cells (Seeger et al., 1998). Homologs of the COP9 signalosome have been found in all multicellular organisms examined so far, and additionally in fission yeast, but not in budding yeast. Nevertheless, although *Saccharomyces cerevisiae* does not possess a COP9 signalosome, an important clue toward its function is based on the structure of the *S. cerevisiae* 26S proteasome. The 26S proteasome consists of a 20S catalytic core complex and a 19S regulatory complex (Fig. 4). The regulatory particle seems to be involved in substrate recognition, selecting the proteins to be eventually taken up into the catalytic core for degradation. In *S. cerevisiae* the regulatory complex can be divided further into a base subcomplex and a lid subcomplex (Glickman et al., 1998). Given the evolutionary conservation of the 26S proteasome, this structure seems likely to be true for other eukaryotes as well. It is striking that the lid subcomplex of the regulatory particle shows significant sequence and structural similarity to the COP9 signalosome (Fig. 4A). Both complexes are composed of eight subunits and the subunits show homology to each other in a one-on-one fashion (Wei et al., 1998; Wei and Deng, 1999).

These data suggest the attractive working hypothesis that the COP9 signalosome is involved in degradation of a selected set of substrate proteins, possibly by functioning as an alternative lid subcomplex of the 19S regulatory particle (Fig. 4B). If this were indeed the case one could expect that it might process a specific subset of proteasome substrates delivered by a number of distinct E3 ligase complexes. An E3 ligase complex involving COP1 could be one of them, and it might be an essential E3 ligase in early Arabidopsis development. This would again explain the constitutive photomorphogenic phenotype of the signalosome mutants as the earliest manifestation of a lack of signalosome activity. In these *cop/det/fus* mutants, at the same or later stages in development, inputs from more E3 ligase systems would not be executed, and the combination of these defects could result in the disruption of essential cellular processes. This could explain why so far only lethal alleles of signalosome mutants have been found in Arabidopsis.

The above outlined interpretation would suggest that the substrates directly interact with the COP9 signalosome. No interactions between HY5 and the signalosome have been identified to date; however, this might be due to technical limitations. For instance, yeast two-hybrid assays might not reveal these interactions since the integral structure of the signalosome could be required rather than an individual subunit. Moreover, association in vivo could be hard to detect due to the likely transient nature of the interaction. Nevertheless, direct contact between E3 ligase complexes and the 19S regulatory particle of the proteasome have been found in vivo in *S. cerevisiae* (Xie and Varshavsky, 2000) and it will be

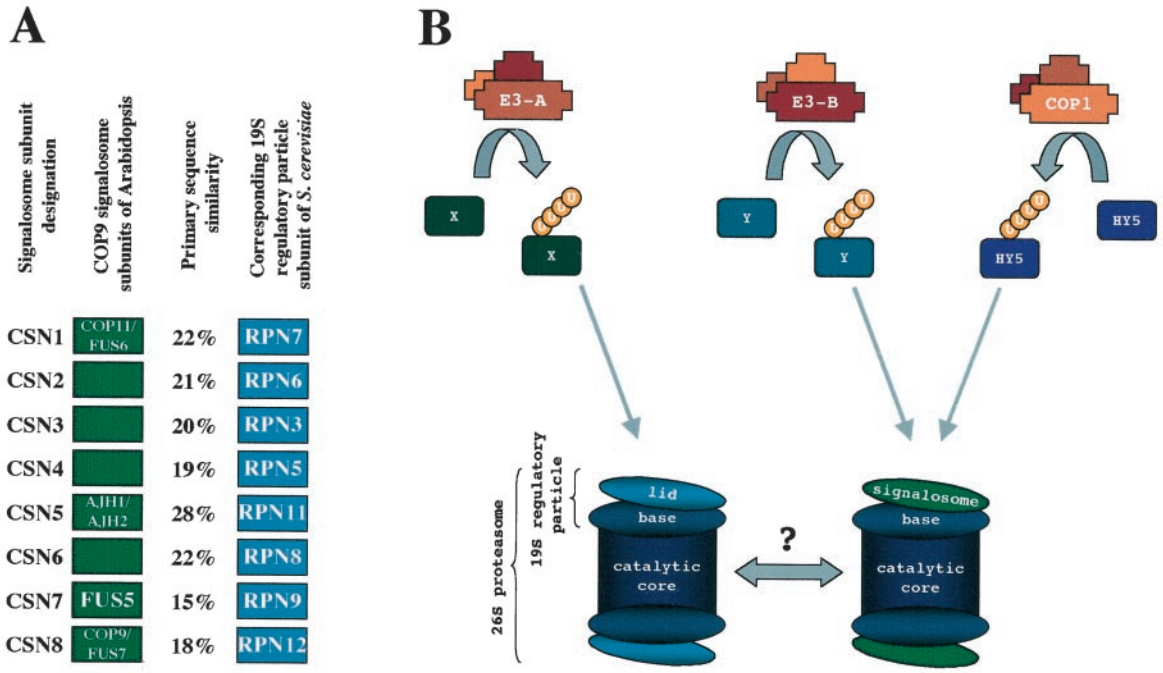

Figure 4. Hypothetical role of the COP9 signalosome in protein degradation. A, Comparison of the Arabidopsis COP9 signalosome and the 19S proteasome lid subcomplex of *S. cerevisiae*. Signalosome subunits and their corresponding counterparts in the 19S regulatory particle are opposite one another and their primary sequence similarity is indicated. Previous names of loci or genes corresponding to signalosome subunits are indicated if applicable. The revised nomenclature for the signalosome is indicated on the left (CSN1–CSN8). B, Model for the hypothetical role of the signalosome as an alternative lid subcomplex in the 19S regulatory particle of the 26S proteasome. The regular proteasome processes substrates that are selected by the lid subcomplex. The lid specifically recognizes substrates ubiquitinated by a set of E3 ubiquitin ligases such as an unknown E3-A, which targets the factor X for degradation. In an alternative scenario, the lid is replaced by the signalosome. The signalosome recognizes a different subset of proteasome substrates targeted for degradation by a different subset of E3 ligases such as a factor Y ubiquitinated by a ligase E3-B. In this subset of E3 ligases, a ligase involving COP1 is included, which targets HY5 for degradation (see Fig. 3). Note that the specificity of the lid and the signalosome could also be provided by direct association with the specific E3 ligase complexes, rather than substrate recognition (not indicated).

interesting to determine if similar interactions can be established for the COP9 signalosome.

THE ROLE OF COP10 AND DET1 IN THE COP1-MEDIATED DEGRADATION OF HY5

Just as in *cop1* and the COP9 signalosome mutants, COP1 is also localized in the cytosol in the dark in *cop10* or *det1* seedlings. Thus both of these loci could also be involved in the nucleocytoplasmic shuttling of COP1, although DET1 or COP10 are not components of the COP9 signalosome (Chamovitz et al., 1996). Nevertheless, other scenarios can be envisioned. *hy5* has also been isolated as an extragenic suppressor of *det1* (Pepper and Chory, 1997). Similar to the interaction of *hy5* and *cop1*, this genetic interaction seems to be allele-specific. However, whereas the *hy5* mutation has no significant influence on the de-repressed gene expression in a *cop1* background, at least *CHS* transcription is reduced to wild-type levels in a *hy5/det1* double mutant. DET1 encodes a nuclear protein of unknown biochemical function to date (Pepper et al., 1994), but it is conceivable that it might be a critical component involved in light-induced gene expression, possibly acting on genes simultaneously under control of HY5. It is clear that a more detailed analysis of the mechanistic nature of the interaction between DET1 and HY5 is needed to address this issue. Also, the role of *COP10* within the context of HY5 degradation remains enigmatic. Although the *cop10* mutant behaves like any other pleiotropic *cop/det/fus* mutant with respect to HY5 accumulation, for *cop10*, just like for *det1*, it is not clear whether it is primarily involved in HY5 degradation. Cloning of the *COP10* gene will hopefully solve this question.

It is interesting that the *HP2* locus of tomato has been shown to encode a *DET1* homolog (Mustilli et al., 1999). Although the *hp2* mutants are hypersensitive to light, they display hardly any morphological phenotype in darkness as compared with Arabidopsis *det1*. Since there seems to be only one *DET1* gene in tomato, redundancy has been ruled out as a possible explanation for this difference. Rather, it has been suggested that *DET1* and *HP2* act as amplifiers of phytochrome signaling, whose quantitative contribution might be different in the two species. How-

ever, it is also possible that the available *hp2* mutants are simply weak mutations.

NEW QUESTIONS AND OUTLOOK

Although a clearer idea about the molecular basis of the function of the *COP/DET/FUS* genes is emerging from recent progress, a number of questions arise from the current data. For instance, the role of DET1 in the above outlined scheme remains obscure. Also, some earlier observations remain to be integrated into the larger picture. For example, why is a *cop1* mutant allele that still expresses an N-terminal fragment, but lacks all of the WD40 repeat domain less severe and viable, whereas a point mutation in the WD40 repeat domain can result in lethality (McNellis et al., 1996; Stoop-Myer et al., 1999)? A better understanding of the structural biology of COP1 within an E3 ligase context might resolve this issue.

Concerning the signalosome, caution must be observed. Although there is a structural similarity to the proteasome lid based on subunit number and primary sequence, recent results indicate that the three-dimensional structure of the signalosome complex seems to be rather distinct from the proteasome lid (Kapelari et al., 2000). Thus whereas a function for the signalosome as an alternative proteasome lid is a tempting working hypothesis, alternative functions should still be considered.

More clues toward a better comprehension of the topic might be expected from the cloning of the remaining pleiotropic *COP/DET/FUS* genes. However, beyond those loci one might ask whether additional genes involved in the system mutate to a different phenotype, for instance due to embryonic lethality, or have not been found yet for other reasons, like genetic redundancy.

The nature of the biochemistry of all hypothesized activities for the pleiotropic COP/DET/FUS proteins is far from clear. In particular, the requirements for the reconstitution of their biochemical activities in vitro might not reflect their more complex mode of action in vivo. For instance, recent reports on E3 ligase function indicate that the RING-finger protein component of the multisubunit APC E3 ligase complex is sufficient to mediate E2-dependent ubiquitination in vitro (Gmachl et al., 2000). Thus CIP8 or even the RING finger in COP1 would already satisfy these requirements.

It is clear that more cell biological and biochemical data are needed to advance our understanding of the mechanisms involved in the photomorphogenic switch. Nevertheless, the recent progress in understanding the COP1-HY5 interaction can be seen as a conceptual breakthrough. Using the COP1-HY5 showcase as a tool and building on the combined results from analyses of the COP9 signalosome will hopefully facilitate future experimental design and lead into further insights.

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