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Does EID1 Aid the Fine-Tuning of Phytochrome A Signal Transduction in Arabidopsis?

The field of plant photobiology has achieved great progress since the early 1990s, when genetic analyses using the model plant Arabidopsis began to be applied to this area of study. One of the characteristics of plants is their developmental plasticity, which defines a difference between plants and animals. Their plasticity is provided by their finely regulated perception of environmental signals, particularly to light. Plant photoreceptors provide plants with the means to sense the quality and quantity of these light signals. The most well-studied photoreceptors that allow plants to measure the quality of light are blue/UV-A (320 to 500 nm) light receptors, termed cryptochromes and phototropins, and the red and far-red (600 to 750 nm) light receptors, the phytochromes. Arabidopsis photoreceptor mutants have been isolated mostly by their insensitivity to certain wavelengths of light, which leads to an elongated hypocotyl phenotype in these conditions. Defects in cryptochrome 1 and 2 result in insensitivity to blue light; likewise, phytochrome B (phyB)- and phytochrome A (phyA)-deficient mutants are insensitive to red and far-red light, respectively (Neff et al., 2000).

Downstream components of phyA signal transduction have been identified by two complementary approaches. One of these is a yeast two-hybrid screen using phyA as the bait. These screens have identified phyA-interacting polypeptides such as PIF3, PKS1, and NDPK2 (Ni et al., 1998; Choi et al., 1999; Fankhauser et al., 1999). In addition, recessive mutations that result in an elongated hypocotyl phenotype under far-red light have been identified in loci other than phyA, including *fhy1*, *fhy3*, *far1*, *fin2*, *pat1*, *fin219*, *hfr1/rep1/rfs1*, and *raf6* (Whitelam et al., 1993; Hudson et al., 1999; Soh et al., 1998, 2000; Bolle et al., 2000; Fairchild et al., 2000; Hsieh et al., 2000; Spiegelman et al., 2000; Moller et al., 2001). Some of these loci have now been characterized at the molecular level. Transient localization assays suggest that PKS1, PAT1, and FIN219 are localized in the cytoplasm, whereas PIF3, FAR1, and HFR1/REP1/RFS1 are localized preferentially to the nucleus. Significantly, PIF3 and HFR1/REP1/RFS1 are related proteins that contain a basic helix-loop-helix (bHLH) domain and that are likely to constitute a family of transcription factors that directly mediate light activation of gene expression. This has been shown for PIF3 (Martinez-Garcia et al., 2000).

A mechanism for the fine-tuning of phytochrome A signaling in plants might have been determined recently through the elegant identification of a new genetic locus, *EID1*, involved in light signaling. The *eid1* mutant was isolated by screening an ethyl methanesulfonate–mutagenized population harboring the Arabidopsis *phyB-5* mutation (Buche et al., 2000). It was known that alternating red and far-red light exposure causes a decrease in the phyA-dependent high irradiance response. Buche et al. (2000) took advantage of this phenomenon and screened for mutants that overcome the red light effect. The mutant seedlings were hypersensitive to hourly red and far-red light pulses and showed a relatively short hypocotyl phenotype. A similar response can be achieved using low fluence far-red light, because the level of phyA-Pfr is relatively lower under weak far-red light. Using this approach, Dieterle et al. (2001) isolated additional alleles of *eid1* by screening the hyperphotomorphogenic mutant seedlings under low fluence far-red and red light conditions. Several T-DNA–tagged alleles were isolated in these screens, which facilitated the molecular cloning of the *EID1* locus (Dieterle et al., 2001).

The *EID1* gene was found to encode an open reading frame of 1008 bp with no intron. The deduced amino acid sequence revealed an N-terminal F-box domain followed by a leucine zipper motif, an acidic domain, and a stretch of basic domain, which is a putative nuclear localization sequence. Missense mutations were identified within the F-box domain as well as in the leucine zipper domain through all stretches of the EID1 sequence in the *eid1* mutants, suggesting that all of these domains play crucial roles in EID1 protein function. The F-box domain–containing proteins generally have been regarded as a substrate binding subunit in the SCF-type E3 ubiquitin ligases. For example, the plant E3 ligase SCF^{TIR1} is composed of a number of subunits including an F-box subunit, designated TIR1, that binds the substrate proteins that are to be ubiquitinated (Gray et al., 1999).

Using yeast two-hybrid screens, Dieterle et al. (2001) identified two proteins, ASK1 and ASK2 (Arabidopsis SKP1-like proteins), that interacted with the full-length EID1 polypeptide. ASK1 and ASK2 were identified previously as components of Arabidopsis SCF-type E3 ubiquitin ligases (Gray et al., 1999). The F-box domain of EID1 was shown to be essential for its interaction with ASK1 and ASK2. First, a point mutation in the F-box or its deletion disrupted the interaction in the two-hybrid assay.

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Second, similarly mutated proteins fused to glutathione *S*-transferase failed to interact with ASKs in in vitro pulldown assays. Evidence that the F-box domain interacts with ASK proteins strongly supports the claim that EID1 is an E3 ligase.

Although it is not proven that EID1 can interact specifically with known phyA signaling components, the identification of EID1 as a component of E3 ligase helps us to understand the fine-tuning of light perception in plants. In many cases, F-box proteins interact differentially with distinct phosphorylated forms of their target proteins. Phytochromes are autophosphorylated upon light activation (Yeh and Lagarias, 1998). Thus, it is possible that phyA itself is a target of EID1. However, the kinetics of phyA degradation in *eid1* mutants is indistinguishable from that of wild type, which argues against this notion. An attractive possibility discussed by Dieterle et al. (2001) is that phytochrome acts to phosphorylate target proteins, such as transcription factors specifically involved in the phyA pathway, which could then be substrates for the ligase.

To provide initial evidence supporting this notion, genetic interaction studies between the *eid1* mutant and far-red light–insensitive mutants such as *far1* (Hudson et al., 1999) and *hfr1*/*rep1*/*rsf1* (Fairchild et al., 2000; Soh et al., 2000; Spiegelman et al., 2000) would be necessary. Those mutations define nuclear intermediates of phyA signaling and likely candidates for EID1-targeted degradation. In addition, the in vivo protein stability of nucleus-localized phyA signaling components such as FAR1 and HFR1/REP1/RSF1 could be tested in *eid1* mutants using antibodies raised against these proteins. The HFR1 protein will be particularly interesting because it has similarity to, and was found to interact with, the bHLH protein PIF3 (Fairchild et al., 2000). It was further shown that PIF3 alone, or in combination with HFR1, interacts preferentially with the Pfr forms of phyA and phyB. Dieterle et al. (2001) speculated that EID1 might work specifically to eliminate phyA signaling intermediates at low Pfr under weak light. Screening of suppressor mutations of the *eid1* mutant will help to identify the component(s) involved in this regulatory pathway.

Dieterle et al. (2001) suggested that EID1 functions to measure the photon fluence rate perceived by the plant, and they provide a possible mechanism for this function. It is well known that phyA mediates a biphasic response composed of very low fluence response (VLFR) and high irradiance response components. The authors propose that EID1 might regulate the VLFR. If EID1 negatively regulates the phyA Pfr-dependent pathway, the *eid1 phyB* double mutant would be predicted to show sensitivity to red light. As predicted, the action spectrum data taken from the *eid1 phyB* double mutant showed sensitivity to a lower fluence red light spectrum (~670 nm), whereas the phyB mutant alone had no sensitivity to that range. These data support the notion that phyA-Pfr is responsible for the red VLFR and that EID1 functions to negatively regulate the VLFR. The presence of this negative regulation indicates that the phyA-Pfr signaling pathway could be fine-tuned according to environmental and developmental cues. One potential role of EID1 could be to raise the threshold of plant responsiveness to the photon signals.

Previous work from Peter Quail's group (Hoecker et al., 1998, 1999) led to the identification of another negative regulator of phyA signal transduction. In this case, the *phyA-105* mutant allele was used to screen for extragenic suppressor mutations and a protein containing a WD-40 repeat was identified. This repeat was first identified as a heterotrimeric G-protein β-subunit (Neer et al., 1994), but some proteins containing this repeat are components of E3 ligases (Tyers and Willems, 1999). The repeat comprises a "seven-propeller" structure, and the top surface of the propeller structure allows these proteins to interact with potential target proteins. In the case of E3 ligases, these would be the target proteins for ubiquitin ligation.

The accumulating evidence of developmental regulation in all organisms indicates that fine-tuning of signal transduction often is achieved by synergistic regulation of activators and negative regulators specific for these signal activators. This negative regulation often is accomplished by eliminating the active component through regulated protein degradation. This is illustrated in another case in the light signaling pathway in higher plants. Recently, HY5, a bHLH protein and a positive regulator of photomorphogenic development, was found to be critically regulated by light through proteasome-mediated degradation. It was demonstrated that HY5 was targeted specifically by a putative E3 ligase, COP1, in the nucleus (Osterlund et al., 2000). COP1 protein contains WD-40 repeats and was identified originally by a mutant screen that has identified a number of *cop*/*det*/*fus* mutants (*constitutively photomorphogenic*, *deetiolated*, *fusca*). HY5 binds to a G-box motif found in the promoter region of a number of light-regulated genes, such as chalcone synthase and the ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit gene *RBCS1A*, and was shown to be critical for their light-activated expression (Chattopadhyay et al., 1998). Thus, the availability of HY5 protein, regulated by COP1, might be a rate-limiting factor for photomorphogenesis. The *hy5* mutations suppress the *cop1* mutant phenotype. EID1, as a component of an E3 ligase for phyA VLFR-specific factors, may function in a similar manner except that it is upstream and phyA specific. Interestingly, the suppressor screen of the *cop1-6* mutant also identified FIN219, a possible component mediating the phyA regulation of COP1 (Hsieh et al., 2000).

Screens of constitutively photomorphogenic mutants also have identified a multiple subunit protein complex called the COP9 signalosome (Wei and Deng, 1999). This complex has been found in most multicellular organisms and is homologous with the 26S proteasome lid subcomplex. Schwechheimer et al. (2001) recently reported the exciting development that the Arabidopsis COP9 signalosome interacts physically with an SCF^{TIR1} E3 ligase in vivo and that this interaction is mediated by direct contact with the core subunits AtRBX1 and AtCUL1 of the SCF-type E3 ligases. It is known that RBX1 and CUL1 are core components of multiple SCF-type E3 ubiquitin ligases that differ in their F-box domain subunits (Deshaies, 1999). Thus, it is likely that EID1, an SCF-type E3 ligase, also is subjected to interaction and regulation by the COP9 signalosome.

In summary, the molecular identification of EID1 has added to the accumulating evidence supporting an important role for protein degradation in photomorphogenesis. The EID1 research suggests a possible mechanism for how plants sense the quantity of light they receive, most likely by raising the threshold of the phyA response pathway.

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