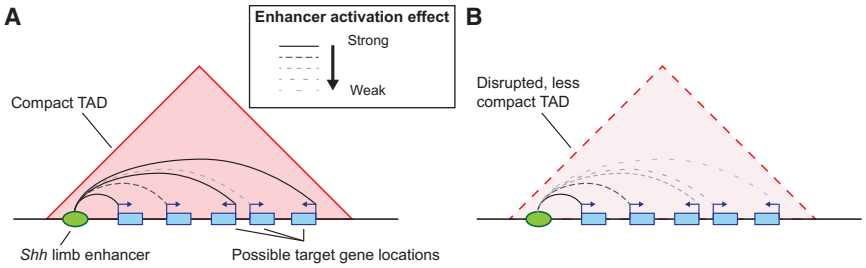

Figure 1. The *Shh* TAD Facilitates Enhancer Activity over Large Genomic Distances
(A) The formation of a compact topological domain (TAD; pink) enables the *Shh* limb enhancer (green) to activate gene expression across very large genomic distances. Although enhancer activity is pervasive throughout the TAD, it is not uniform. Genes (blue) located in certain "cold spots" are less affected by the activity of the enhancer, either due to the specific folding of chromatin within the TAD or due to local chromatin effects. (B) When the surrounding TAD is disrupted and made less compact (e.g., by a genomic inversion encompassing one of the TAD boundaries), the activity of the limb enhancer becomes dependent on the genomic distance between the enhancer and a target gene.

TAD might become activated after TAD disruption.

Symmons et al. (2016) expands an increasing body of evidence pointing toward a key role for genome folding at the level of TADs in enhancer function. However, some inconsistency remains between the reported cell-type invariance of TAD boundaries and the cell-type specificity of developmental enhancers. The role of chromatin folding in developmental gene expression and the selection of target genes by enhancers remain open questions. Solving them will be crucial if we are to understand not only developmental diseases arising from large structural changes to the genome but also the natural phenotypic variation that may result from more subtle sequence differences. These challenges will require a continuing exchange of ideas between functional studies that follow the consequences of specific changes during development and "omics" approaches that interrogate thousands of genomic regions or sequence variants in tandem in specific cell types.

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Cutting Out the Middle Man in Light-Hormone Interactions

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In this issue of *Developmental Cell*, Shi et al. (2016a) show that red-light-activated phytochrome B interacts with transcriptional regulators of ethylene signaling, EIN3/EIL1, triggering their degradation by bringing the F-box proteins EBF1 and 2 to the complex. These findings provide a paradigm for crosstalk between light and hormone signaling pathways.

One of the most dramatic environmental changes that a plant experiences during germination is the transition from growing in a dark underground environment to the bright sun of the soil surface. Prior to emergence, dark-germinated seedlings are under the developmental control of a program called skotomorphogenesis, which guides the young seedlings toward their energy source, the light, while protecting their valuable and delicate meristems: dark-grown seedlings develop

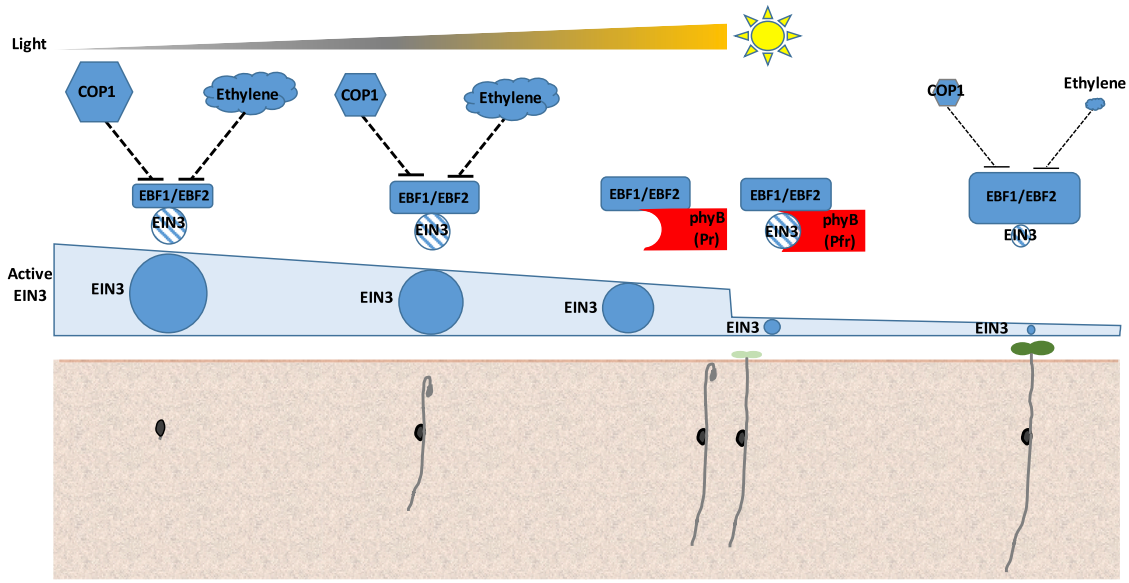

Figure 1. Interactions between Ethylene and Light Signaling during Seedling Emergence from the Soil
Upon emergence from soil, seedlings switch from an ethylene-promoted skotomorphogenetic developmental program to photomorphogenesis. Previous work (Shi et al., 2016b) has shown that ethylene and light, acting via the E3 ubiquitin ligase COP1, converge to regulate the levels of the EBF1 and EBF2 proteins, which negatively regulate ethylene signaling. As the seedlings approach the soil surface, the amount of light perceived increases, resulting in a reduction of COP1 activity and a corresponding increase in the levels of EBF1 and EBF2, leading to degradation of the key ethylene transcription factor EIN3. In addition to this gradual mechanism, the work by Shi et al. (2016a) now shows that high-intensity light exposure at the time of seedling emergence triggers the rapid activation of phytochome (phyB). PhyB binds both EIN3 and the EBFs, eliciting a rapid degradation of EIN3 and a quick shutdown of the repressive activity of ethylene on the photomorphogenic program. The size of the different geometrical shapes represents the abundance of the corresponding proteins, and the size of the cloud similarly reflects the levels of the hormone ethylene. Bars indicate increasing and decreasing levels of light and EIN3, respectively. The striped blue circles represent EIN3 ubiquitination and degradation mediated by EBFs.

long hypocotyls (embryonic stems), and their apices are bent to form apical hooks that enable efficient soil penetration. Once the seedling reaches the soil surface, its growth pattern changes to slow down hypocotyl elongation and to induce apical hook opening and expansion of the cotyledons, a program collectively known as photomorphogenesis. It is then that the different components of the photosynthetic machinery are produced and assembled to enable the capture of the energy of light. The transition from skoto- to photomorphogenesis must be precisely timed because, for example, excess accumulation of photosynthetic precursors such as protochlorophyllide can cause extensive oxidative damage once the seedlings are exposed to light (Reinbothe et al., 1996), whereas a delay in the initiation of this program would deprive young seedlings of the valuable energy source at a critical moment in their development.

The identification and characterization of mutants affected in this transition has uncovered a series of genes that repress or promote photomorphogenesis, including the E3 ubiquitin ligase *CONSTITUTIVE PHOTOMORPHOGENIC1* (*COP1*) (Seo et al., 2003) and the red light photoreceptors *phytochromes* (Quail et al., 1995), respectively. Although these genes have been known for a long time, only recently have we started to understand the molecular mechanisms by which they trigger dramatic morphological and physiological changes. Earlier studies showed that the plant hormone ethylene plays a key role in repressing photomorphogenesis in dark-grown seedlings and preparing the plants for their transition to the light environment (Shi et al., 2016b; Zhong et al., 2009; Zhong et al., 2014). COP1 was found to promote ethylene activity by stimulating the degradation of two negative regulators of the ethylene signaling pathway, EIN3-BINDING F-BOX1 (EBF1) and EBF2. Gradual inactivation of COP1 as the plant approaches the soil surface results in the attenuation of ethylene responses. Shi et al. (2016a) now show that ethylene plays an additional role in modulating the rapid changes that take place upon seeding emergence from the soil. Interestingly, these responses are triggered by the direct binding of the photo-activated light receptor, phytochrome B (phyB), to the transcriptional master regulator of ethylene responses, ETHYLENE INSENSITIVE3 (EIN3).

Upon seedling emergence from the soil, light responses get activated, and ethylene responses get turned off. In fact, Shi et al. (2016a) found that the hook opening and cotyledon expansion phenotypes typically observed under red-light conditions could be effectively counteracted by the activation of the ethylene signaling pathway, suggesting the existence of a mechanism by which ethylene responses are actively repressed at the time of seedling emergence from the soil. A clue for such an active mechanism comes from our understanding of the modes of action of phytochromes and the hormone ethylene themselves. Red light activates phytochromes, promoting their translocation from the cytosol to the nucleus, where they interact with the PHYTOCHROME-INTERACTING FACTOR (PIF) family of transcription factors. These interactions

trigger the degradation of the PIFs and thus a series of transcriptional changes associated with the plant responses to red light (Ni et al., 2014). On the other hand, ethylene responses are initiated by the stabilization and activation of the transcription factors EIN3 and ETHYLENE INSENSITIVE3-LIKE1 (EIL1). This transcriptional induction is, at least in part, mediated by the inactivation of two F-box proteins, EBF1 and EBF2, that, in the absence of ethylene, target EIN3 and EIL1 to ubiquitin-mediated degradation (Guo and Ecker, 2003).

Surprisingly, Shi et al. (2016a) found that EIN3 levels rapidly decline during the first 30 min of seedling exposure to red light, thus questioning the role of the previously reported, inherently slower, COP1-dependent downregulation of EIN3 levels via the gradual accumulation of EBFs under prolonged light-growth conditions (Shi et al., 2016b). Furthermore, although both EBF1 and EBF2 are required for the rapid degradation of EIN3 in response to red light, the accumulation of these two F-box proteins did not significantly change over the course of the light treatment carried out by Shi et al. (2016a). Thus, the observed light-triggered disappearance of EIN3 implied the existence of a different mechanism that enabled the rapid degradation of EIN3 in response to red light. The first critical clue for the mechanism behind this rapid response to red light came from the observation that phyB interacts, both in vitro and in vivo, with EIN3; importantly, this interaction preferentially occurs between the red-light-activated form of phyB and EIN3. In addition, EBF1 and EBF2 were found to also interact with phyB, although in this case with both its active and inactive forms.

Taken together, these results suggest an elegant, mechanistic model in which, upon emergence from the soil, phyB is induced by red light, promoting the phyB-EBF1/2-EIN3 interactions, thus triggering the rapid degradation of EIN3—the transcriptional master regulator of ethylene responses—and thereby turning off the ethylene-mediated repression of photomorphogenesis (Figure 1). Consistent with this model, a short exposure of dark-grown seedlings to red light promoted the interaction between EIN3 and EBFs. In fact, immunoprecipitation experiments, both in vivo and in vitro, showed that EIN3, EBFs, and phyB form a tripartite protein complex, and the formation of this complex is promoted by the red-light-mediated activation of phyB.

The study by Shi et al. (2016a) thus builds on earlier findings implicating phytochrome-ethylene crosstalk in critical aspects of the developmental transition from dark to light, revealing a complex picture of multiple mechanisms of interaction between these two important signals in plants. The surprising discovery that activated phyB functions as the "molecular glue" between the key ethylene transcription factors and their negative regulators opens an exciting possibility of similar, strikingly direct mechanisms functioning in the interaction between light and other hormone signaling pathways.

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