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A Light-Independent Allele of Phytochrome B Faithfully Recapitulates Photomorphogenic Transcriptional Networks

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ABSTRACT Dominant gain-of-function alleles of *Arabidopsis* phytochrome B were recently shown to confer light-independent, constitutive photomorphogenic (*cop*) phenotypes to transgenic plants (Su and Lagarias, 2007). In the present study, comparative transcription profiling experiments were performed to assess whether the pattern of gene expression regulated by these alleles accurately reflects the process of photomorphogenesis in wild-type *Arabidopsis*. Whole-genome transcription profiles of dark-grown *phyAphyB* seedlings expressing the $Y^{276}H$ mutant of phyB (YHB) revealed that YHB reprograms about 13% of the *Arabidopsis* transcriptome in a light-independent manner. The YHB-regulated transcriptome proved qualitatively similar to but quantitatively greater than those of wild-type seedlings grown under 15 or 50 μmol m^{-2} m^{-1} continuous red light (Rc). Among the 2977 genes statistically significant two-fold (SSTF) regulated by YHB in the absence of light include those encoding components of the photosynthetic apparatus, tetrapyrrole/pigment biosynthetic pathways, and early light-responsive signaling factors. Approximately 80% of genes SSTF regulated by Rc were also YHB-regulated. Expression of a notable subset of 346 YHB-regulated genes proved to be strongly attenuated by Rc, indicating compensating regulation by phyC-E and/or other Rc-dependent processes. Since the majority of these 346 genes are regulated by the circadian clock, these results suggest that phyA- and phyB-independent light signaling pathway(s) strongly influence clock output. Together with the unique plastid morphology of dark-grown *YHB* seedlings, these analyses indicate that the YHB mutant induces constitutive photomorphogenesis via faithful reconstruction of phyB signaling pathways in a light-independent fashion.

Key words: light signaling; signal transduction; transcriptome analysis; photomorphogenesis; *Arabidopsis*; phytochrome.

INTRODUCTION

Light sensors perform essential roles throughout the lifecycle of plants to mediate adaptive responses to the changing quality, quantity, duration, and direction of light in the natural environment (Chen et al., 2004; Franklin et al., 2005; Schäfer and Nagy, 2005). Arguably amongst the most important of these are the phytochromes, a family of biliprotein photoreceptors optimized for sensing red and far-red light (Nagy and Schäfer, 2002; Quail, 2002; Rockwell et al., 2006; Bae and Choi, 2008). The five *Arabidopsis* phytochrome genes (*PHYA-E*) encode highly related apoproteins, all of which bind the same linear tetrapyrrole (bilin) chromophore (Sharrock and Quail, 1989). Despite their similar molecular architectures, the modes of photosensory perception by the phyA-E holoproteins are distinct. The phyA photoreceptor is primarily responsible for both very low fluence responses (VLFR) and high irradiance responses to far-red light (FR–HIR), while the phyB–E photoreceptors function as red/far-red (R/FR) photoreversible sensors in the low fluence range (Shinomura et al., 1996; Whitelam and Devlin, 1997; Shinomura et al., 2000). This photosensory diversity enables long-term adaptation to FR-enriched shade environments and confers an adaptive advantage to shade-avoiding plant species that can effectively compete for limited photosynthetically active radiation with their neighbors (Mathews, 2006; Franklin, 2008).

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Although distinct in their mode of light sensing, the five *Arabidopsis* phytochromes share a similar mechanism of action, namely to regulate expression of a distinct and overlapping set of genes following light-activated translocation from the cytoplasm to the nucleus (Nagatani, 2004; Jiao et al., 2007; Kevei et al., 2007). Whole-genome microarray analyses have established that a significant percentage of the *Arabidopsis* genome is regulated by light. To determine which genes are specifically phytochrome-regulated, comparative R- and FR-dependent transcription profiling of wild type and phytochrome-null mutants has been undertaken by a number of laboratories. Quail and colleagues (2007) focused on gene expression during seedling de-etiolation following exposure to R or FR light. Their studies indicated that phyA is wholly responsible for the rapid transcriptional response to FR, that phyA and phyB together regulate nearly all of the early R-responsive genes, and that phyC–E further contribute to sustained regulation of a subclass of R-responsive genes (Tepperman et al., 2001, 2004, 2006). By contrast with seedling de-etiolation analyses, Deng and colleagues examined the effect of sustained light treatment on both *Arabidopsis* and rice transcriptomes (Ma et al., 2001; Wang et al., 2002; Jiao et al., 2005; Ma et al., 2005). Their studies not only fingerprint the process of seedling photomorphogenesis at the transcriptional level, but also represent a useful approach to elucidate the regulatory roles of individual phytochromes and of potential signaling components following prolonged illumination.

Whole-genome profiling analyses have established that phytochromes reprogram the plant transcriptome primarily through a rapid light-dependent regulation of a transcription factor cascade (Jiao et al., 2007; Quail, 2007). Although the precise mechanism of this reprogramming process has not been fully elucidated, it is clear that phytochromes do not effect gene regulation via direct DNA binding. Instead, photoactivated phytochromes interact with a diverse array of signaling molecules to alter gene transcription (Bae and Choi, 2008; Josse et al., 2008). Recent studies indicate that phytochromes target many of these factors for degradation by the 26S proteosome—a process that is preceded by their phosphorylation (Shen et al., 2005; Al-Sady et al., 2006; Shen et al., 2007, 2008). Genetic approaches have identified two major transcriptional networks mediated by phytochromes. One network involves the PIFs, members of the PIF3 family of bHLH transcription factors that regulate genes involved in hormone biosynthesis/perception pathways impacting seed germination, elongation growth, cell division, and photosynthetic pigment biogenesis (Khanna et al., 2004; Al-Sady et al., 2006). The direct interaction between photoactivated phytochromes and PIFs initiates this signaling cascade apparently by targeting both PIFs and phytochrome for degradation (Al-Sady et al., 2008; Leivar et al., 2008). The second network entails reprogramming of protein degradation through suppression of the activity of COP/DET/FUS complexes that target key transcription factors HY5, HFR1, and LAF1 in darkness (Ma et al., 2003). The molecular mechanism of phytochrome-mediated inactivation of COP/DET/FUS factors is not fully understood; however, it appears to involve both direct and indirect pathways (Chen et al., 2004; Jiao et al., 2007).

The process of plant photomorphogenesis involves a complex interplay between multiple light-sensing systems that include multiple regulatory photoreceptors, photosynthetic pigments, and other photoprotective or photodynamic pigments (Schäfer and Nagy, 2005). In order to understand the contribution of specific photosensors to this process, investigators typically use monochromatic irradiation regimes to selectively activate photoreceptors. While monochromatic R irradiation can distinguish between B/UV-absorbing photoreceptors and phytochromes, it is difficult to distinguish the effect of R absorbed by each of the five phytochromes, by porphyrin/chlorin precursors and/or by the photosynthetic apparatus itself. Our recent discovery of a new class of gain-of-function missense alleles of phytochromes, which confer their light-independent activation, represents a valuable tool to assess the regulatory roles of individual phytochromes without exciting other photoreceptor systems (Su and Lagarias, 2007). In the present study, we examine the influence of the constitutively active $Y^{276}H$ missense allele of *Arabidopsis PHYB* (designated as *YHB* throughout) on the *Arabidopsis* transcriptome using Affymetrix ATH1 microarrays. Comparative transcription profiling of wild-type and YHB-expressing transgenic *Arabidopsis* seedlings grown in darkness or in continuous red light (Rc) reveals that YHB faithfully regulates the process of Rc-dependent photomorphogenesis at the genome level. Our investigations also indicate that a significant subset of the YHB-regulated gene complement is suppressed by other Rc-sensing photoreceptor systems, thereby documenting the utility of gain-of-function phytochrome alleles to elucidate the interplay between phytochrome-dependent and phytochrome-independent photomorphogenetic pathways in plants.

RESULTS

YHB-Expressing Seedlings Exhibit Constitutive Photomorphogenesis

We previously reported that expression of *YHB* alleles, either as the CaMV 35S-promoter-driven cDNA or as a native promoter-driven genomic construct, confers a dominant, constitutive photomorphogenic (*cop*) phenotype to dark-grown *Arabidopsis* seedlings (Su and Lagarias, 2007). As shown in Figure 1A and 1B, two independent genomic *YHB*/*phyAphyB* transgenic lines grown in darkness possess short hypocotyls, open apical hook, and expanded cotyledons, in striking contrast with the etiolated phenotype of both L*er* wild type and the *phyAphyB* parent. When grown under continuous red light (Rc), hypocotyl growth inhibition and cotyledon expansion of the *phyAphyB* double mutant are absent, while wild type exhibits hypocotyl elongation inhibition in a fluence rate-dependent manner. Moreover, *YHB* seedlings grown under a high fluence rate of Rc (50 μmol m^{-2} s^{-1})

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