

Analysis of far-red light-regulated genome expression profiles of phytochrome A pathway mutants in *Arabidopsis*

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Summary

Phytochrome A (phyA) is the primary photoreceptor responsible for various far-red (FR) light-mediated responses. Previous studies have identified multiple phyA signaling mutants, including both positive and negative regulators of the phyA-mediated responses. How these defined intermediates act to mediate FR light responses is largely unknown. Here a cDNA microarray was used to examine effects of those mutations on the far-red light control of genome expression. Clustering analysis of the genome expression profiles supports the notion that phyA signaling may entail a network with multiple paths, controlling overlapping yet distinct sets of gene expression. FHY1, FAR1 and FHY3 most likely act upstream in the phyA signaling network, close to the phyA photoreceptor itself. FIN219, SPA1 and REP1 most likely act somewhere more downstream in the network and control the expression of smaller sets of genes. Further, this study also provides genomics evidence for the partial functional redundancy between FAR1 and FHY3. These two homologous proteins control the expression of a largely overlapping set of genes, and likely act closely together in the phyA-mediated FR light responses.

Keywords: *Arabidopsis*, phytochrome A, clustering analysis, genome expression profile, microarray.

Introduction

Higher plants are highly plastic in their development in response to environmental stimuli. Light is one of the most important environmental factors that govern plant growth and development. Besides providing an energy source for plants via photosynthesis, it also provides informational cues to control the developmental patterns of plants (Deng and Quail, 1999; Neff *et al.*, 2000). Dark-grown seedlings undergo a skotomorphogenic developmental program, whereas light-grown seedlings develop photomorphogenically (Wang and Deng, 2002b).

Plants detect their light environment through a network of photoreceptors (Neff *et al.*, 2000; Quail, 2002a,b). In *Arabidopsis*, phyA is the primary photoreceptor mediating the high-irradiance response (HIR) to continuous FR light (FRc), including inhibition of hypocotyl elongation, opening of apical hook, expansion of cotyledons, accumulation of

anthocyanin and FRc pre-conditioned blocking of greening (Nagatani *et al.*, 1993; Quail *et al.*, 1995; Whitelam *et al.*, 1993). In addition, phyA is also the photoreceptor responsible for the very low fluence response (VLFR, Yanovsky *et al.*, 1997). Genetic analyses have led to the identification and subsequent molecular characterization of a number of phyA signaling intermediates (Hudson, 2000). Several positive regulators have been defined, including both cytosolic and nuclear proteins. For example, LAF6 is a plastid-localized ATP-binding-cassette protein involved in coordinating intercompartmental communication between plastids and the nucleus (Møller *et al.*, 2001). PAT1 and FIN219 are cytoplasmic proteins (Bolle *et al.*, 2000; Hsieh *et al.*, 2000), whereas FHY1, FHY3, FAR1, HFR1/REP1 and LAF1 are nucleus-localized factors (Ballesteros *et al.*, 2001; Desnos *et al.*, 2001; Fairchild *et al.*, 2000; Hudson *et al.*,

1999; Soh *et al.*, 2000; Wang and Deng, 2002a). LAF1 is a MYB-type transcription activator, whereas HFR1/REP1 is a bHLH-type transcription factor. Two negative regulators, SPA1 and EID1, have also been defined and shown to be nuclear-localized factors (Dieterle *et al.*, 2001; Hoecker *et al.*, 1998, 1999). EID1 is a novel F-box protein, probably involved in ubiquitin-dependent proteolysis. At present, the way phyA transduce the light signal through these signaling components and the way these signaling intermediates interact in the signaling process are largely unknown.

It is generally assumed that the regulation of plant growth and development by light is largely achieved by regulated expression of light-responsive plastid and nuclear genes (Chattopadhyay *et al.*, 1998; Fankhauser and Chory, 1997; Kuno and Furuya, 2000; Kuno *et al.*, 2000; Puente *et al.*, 1996; Quail, 2002a,b; Terzaghi and Cashmore, 1995). Traditional approaches have revealed up to 100 individual genes whose expression is regulated by light. Recently, DNA microarray technology has been applied to investigate genome-wide gene expression profiles during light control of plant development (Harmer *et al.*, 2000; Ma *et al.*, 2001, 2002; Schaffer *et al.*, 2001; Tepperman *et al.*, 2001). These studies suggested that plant photomorphogenesis involves a regulated expression of up to 30% of the genes in the *Arabidopsis* genome (*Arabidopsis* genome initiative (AGI), 2000; Ma *et al.*, 2001), and this massive change in gene expression is likely the result of a transcriptional cascade (Tepperman *et al.*, 2001). For FR light regulation of gene expression in *Arabidopsis*, phyA is translocated into the nucleus from the cytosol upon its photoactivation and regulate a master set of transcription factor genes by direct targeting of light signals to their promoters (Martínez-García *et al.*, 2000; Nagy and Schäfer, 2002; Nagy *et al.*, 2000; Tepperman *et al.*, 2001). The diversity of these regulated transcription factors suggests extensive and immediate branching of the phyA signaling network which further amplifies and diversifies the spectrum of downstream genes that are potentially the targets of these factors (Tepperman *et al.*, 2001).

DNA microarray technology has also been exploited as a powerful tool to characterize the molecular phenotypes of mutants (Perez-Amador *et al.*, 2001) and to probe the intra- and interspecies variations in genome expression patterns (Enard *et al.*, 2002). Information derived from such studies provides unique clues to the characterization of possible relationships among genes or evolutionarily related species. To further dissect the phyA signaling network, here we use a cDNA microarray to conduct a comparative genome expression profiling study of various *Arabidopsis* phyA signaling mutants and perform clustering analysis to assess their functional relationship in the phyA signaling pathway. This study supports the notion that the phyA signaling network entails intersecting branches, rather than a simple linear pathway. Further, this study reveals new

insights regarding the relationship of the genetically defined phyA signaling loci in mediating FR light responses in *Arabidopsis*.

Results and discussion

Four wild-type ecotypes show similar but not identical genome expression profiles under FR light

As available phyA signaling mutants were derived from multiple ecotypes, we first examined FR light-controlled genome expression profiles for four wild-type ecotypes (COL, Ler, No-0, and RLD) of *Arabidopsis*. Phenotypically, these wild-type seedlings have a similar phenotype grown under FR light, including short hypocotyls, open and expanded cotyledons, and accumulation of anthocyanin in the upper portions of hypocotyls (Figure 1a). We utilized the Yale 9.2K EST microarray that represents 6126 unique expressed genes (Ma *et al.*, 2001, 2002) to profile the genome expression. Examination of the expression ratios of the genes in the microarray between FR light versus dark (D)-grown wild-type seedlings revealed that FR light regulates the expression of large portions of genes in these four different ecotypes in a quantitative similar fashion. Overall, there are 1083, 825, 971, and 998 out of 6126 genes display two-fold or more differential expression in the COL, Ler, No-0 and RLD ecotypes.

The gene expression profiles in these four different ecotypes were further compared through cluster analysis. All of the processed data are subjected to the self-organizing map algorithm followed by complete linkage hierarchical clustering of both genes and experiments, using Cluster/TreeView (Eisen *et al.*, 1998). Only the genes with two-fold or higher differential expression in at least one experimental condition were selected and analyzed. Among a total of 1615 genes analyzed in Figure 1(b) (left panel), the vast majority of the genes show qualitatively similar expression (as defined by similar color of variable intensity) by FR light in any of these four ecotypes. However, the genome expression patterns of these four ecotypes are clearly not identical from each other and considerable differences do exist among these four ecotypes. Certain genes (about 5% of the 1615 genes shown in Figure 1b, left panel) even display opposite regulation, being upregulated in some ecotypes and downregulated in other ecotypes in response to FR light. Particularly, the Ler ecotype seems to have a significant number of genes regulated differently from the other three ecotypes by FR light (Figure 1b, left panel). Accordingly, clustering analysis revealed that Ler is most distinct from the other three ecotypes. RLD and No-0 are most closely related to each other, whereas COL represents an intermediate type between Ler and RLD (Figure 1c).

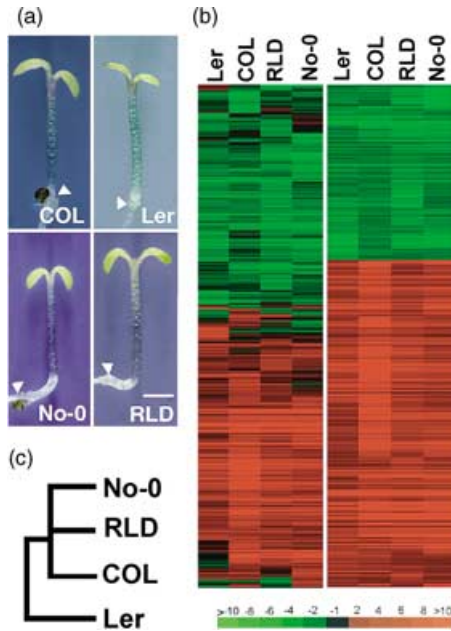


Figure 1. Comparison of genome expression of four ecotypes of *Arabidopsis* (COL, Ler, RLD, No-0) in response to FR light and clustering analysis of their relationships.

(a) Morphological comparison of 5-day-old FR-grown *Arabidopsis* seedlings of the four ecotypes. All seedlings are photographed at the same magnification and the scale bar represents approximately 1 mm. The triangle indicates the junction of hypocotyl and root.

(b) The left panel shows a hierarchical clustering display of expression ratios of the four sample pairs used in this study. The four sample pairs are FR- versus dark-grown wild-type *Arabidopsis* seedlings of ecotypes Ler, COL, RLD and No-0. Only those genes that exhibited two-fold or higher differential expression in at least one sample pair among the four tested were included for comparison. There are 1615 genes included in this cluster (see supplementary data at <http://plantgenomics.biology.yale.edu/> for more information). The right panel shows a hierarchical clustering display of expression ratios of the core group FR light-regulated genes (total 696 genes) identified in all four ecotypes. All genes have 1.5-fold or higher differential expression in all four ecotypes. The color scale bar at the bottom shows the folds of gene regulation.

(c) A dendrogram indicating the relationship among these sample pairs across all the genes included in the first cluster analysis. RLD and No-0 are most close to each other, and they are more close to COL than to the Ler ecotype.

Thus, the result from this genome expression profiling study is in general consistent with other studies exploiting the ecotype variations in light sensitivity of *Arabidopsis* using quantitative loci mapping (Borevitz *et al.*, 2002; Maloof *et al.*, 2000, 2001). The differences in the genome expression profiles among these ecotypes are likely a reflection of their different light sensitivities at the genome expression level. The underlying reasons for such variations could be manifold. For example, duplications, deletions or other types of mutations in the genes, promoter changes, changes in the levels of transcription factors, or epigenetic regulation. In fact, the dynamics (such as dark reversion of the far-red light-absorbing form (Pfr) to the red light-absorbing form (Pr) of phytochrome A molecule itself

has been demonstrated to be variable in different *Arabidopsis* ecotypes (Eichenberg *et al.*, 2000). Such variations may provide adaptation advantages for these ecotypes in their respective natural environments.

A common set of genes are similarly regulated by FR light in all ecotypes

To minimize the ecotype variations on analyzing gene expression pattern and to provide a common basis for comparing the expression profiles of different phyA signaling mutants, we selected a group of genes induced or repressed 1.5-fold or higher in all four ecotypes examined. This group of genes was defined as the 'core group' of FR light-regulated genes (total 696 genes, 453 genes upregulated, 243 genes downregulated, Figure 1b, right half). The 1.5-fold cut-off was used for this purpose based on a recent report that the minimum detectable fold change for differential expression is lower than 1.5-fold and depending on data quality (Yue *et al.*, 2001). Further, the percentage of

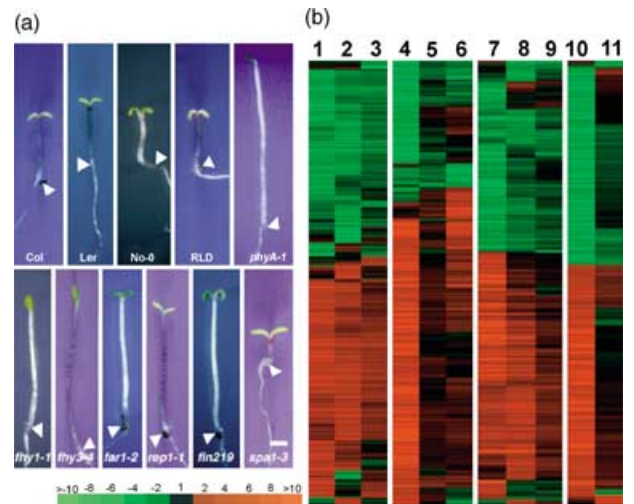


Figure 2. Clustering analysis of various phyA signaling mutants in different ecotype backgrounds.

(a) Morphological comparison of 5-day-old FR-grown *Arabidopsis* seedlings of the seven phyA signaling mutants comparing to their corresponding wild-type ecotype seedlings. All seedlings are photographed at the same magnification and the scale bar represents approximately 1 mm. The triangle indicates the junction of hypocotyl and root.

(b) Hierarchical clustering displays of expression ratios of seven phyA signaling mutants comparing to their corresponding ecotype wild-type seedlings. 1: Ler/FR versus Ler/D; 2: Ler/FR versus phyA-1/FR; 3: Ler/FR versus fhy1-1/FR; 4: COL/FR versus COL/D; 5: COL/FR versus fin219/FR; 6: COL/FR versus rep1-1/FR; 7: No-0/FR versus No-0/D; 8: No-0/FR versus fhy3-4/FR; 9: No-0/FR versus far1-2/FR; 10: RLD/FR versus RLD/D; 11: spa1-3/FR versus RLD/FR. Only those genes that exhibited two-fold or higher differential expression in at least one sample pair among the four tested were included for comparison. There are 1109 genes included in the first cluster, 1115 genes included in the second cluster, 1144 genes included in the third cluster, and 1028 genes included in the fourth cluster (see supplementary data at <http://plantgenomics.biology.yale.edu/> for more information). The color scale bar at the bottom shows the folds of gene regulation.

non-reproducible clones decreased to less than 20% after four replicates when the 1.5 cut-off value was used (Perez-Amador *et al.*, 2001). Finally, a 1.5-fold cut-off used here, instead of commonly used two-fold cut-off, will provide a better coverage of the known *phyA* and FR light-regulated genes (Ma *et al.*, 2001).

Among this selected core group of FR light-regulated genes (for a complete list of these genes, see supplementary data at <http://plantgenomics.biology.yale.edu/>), a large percentage of them have been reported in previous studies as light or *phyA*-regulated genes (Ma *et al.*, 2001, 2002; Tepperman *et al.*, 2001). These genes are involved in a wide range of cellular and biochemical functions, ranging from DNA replication, transcription, translation, metabolism, protein degradation, plant defense, and developmental regulation (Ma *et al.*, 2001, 2002; Tepperman *et al.*, 2001).

A summary of the *phyA* signaling mutants used for this work

A number of *phyA* signaling mutants have been identified using genetic approach, including both positive and negative regulators of the *phyA* signaling pathway (Table 1). Among the molecularly characterized ones, some of them are nuclear localized, whereas some others are localized in the cytosol. The biochemical functions for most of these signaling intermediates are largely unknown and it is not clear how they relate to each other and act to mediate FR light responses and genome expression. Phenotype wise, loss-of-function mutants (presumably null mutant alleles) of a number of positively acting *phyA* signaling components all exhibit partial defects with different spectra and strength in *phyA* signaling (Figure 2a). For example, the *fhy3-1* and *fhy1-1* mutants display similar defects in various FRc responses, including inhibition of hypocotyl growth, apical hook and cotyledon opening, anthocyanin accumu-

lation, and FRc pre-conditioned blocking of greening (Barnes *et al.*, 1996a, 1996b; Wang and Deng, 2002a; Yanovsky *et al.*, 2000). *fin219* and *far1-2* differ from the above mutants in that cotyledon opening and expansion as well as the FRc pre-conditioned greening block are not affected, though they are defective in hypocotyl elongation and anthocyanin accumulation (Hsieh *et al.*, 2000; Hudson *et al.*, 1999; Figure 2a). HFR1/REP1 primarily affects the elongation and geotropic response of hypocotyl, whereas other FRc responses including anthocyanin accumulation, FRc pre-conditioned block of greening, are unaffected in this mutant (Fairchild *et al.*, 2000; Soh *et al.*, 2000; Figure 2a). Therefore, it is apparent that these signaling components control overlapping yet not identical sets of FR light-mediated responses.

Genome expression profile analysis of positive regulator mutants in *phyA* signaling

We used the cDNA microarray technology as a tool to reveal the genome expression profiles of these mutants (Table 1) under FR light. As shown in Figure 2(b) and previously reported (Ma *et al.*, 2001), the gene expression patterns are quite similar between the experimental pairs Ler/FR versus Ler/D and Ler/FR versus *phyA-1*/FR. This indicates that *phyA* plays a major role in FR light regulation of gene expression, because the absence of *phyA* abolishes most of the FR-light triggered gene expression pattern changes (Figure 2b, lanes 1 and 2). Comparison of the genome expression profile of the *fhy1-1* mutant (Figure 2b, lane 3) with the *phyA-1* mutant and their corresponding wild-type (ecotype Ler) reveals that the *fhy1-1* mutation also abolishes most of the FR light and *phyA*-regulated gene expression patterns. However, *fhy1-1* affects most of the genes' expression to a less extent. This result suggests that FHY1 may act early in the *phyA* signaling pathway, and

Table 1 *phyA* signaling mutants and transgenic lines used in this study

Mutants/ transgenic plants	Ecotype background	Molecular lesion or transgene	Reference
<i>phyA-1</i>	Ler	Structural rearrangement	Whitelam <i>et al.</i> (1993)
<i>phyA-211</i>	COL	ND*	Reed <i>et al.</i> (1994)
<i>fhy1-1</i>	Ler	E112 to ETR, then STOP	Desnos <i>et al.</i> (2001)
<i>fhy3-1</i>	COL	R91 to STOP	Wang and Deng (2002a)
<i>fhy3-4</i>	No-0	W501 to STOP	Wang and Deng (2002a)
<i>far1-2</i>	No-0	W419 to STOP	Hudson <i>et al.</i> (1999)
<i>rep1-1</i>	COL	T-DNA insertion in the promoter	Soh <i>et al.</i> (2000)
<i>fin219</i>	COL	Epigenetic mutation	Hsieh <i>et al.</i> (2000)
<i>spa1-3</i>	RLD	Q414 to STOP	Hoecker <i>et al.</i> (1999)
<i>far1-2</i> FHY3	<i>far1-2</i>	FHY3	Wang and Deng (2002a)
<i>fhy3-1</i> FAR1	<i>fhy3-1</i>	FAR1	Wang and Deng (2002a)
FHY3CT	COL	FHY3C473-839	Wang and Deng (2002a)

*ND: not determined.

plays a major role in mediating FR light-regulated gene expression. This is consistent with previous findings that *fhy1-1* mutants are defective in both FR light-mediated HIR and VLFR responses (Barnes *et al.*, 1996a; Whitlam *et al.*, 1993; Yanovsky *et al.*, 2000). The lower fold of gene regulation by FHY1 could be due to a partial functional redundancy with other gene(s) in the *Arabidopsis* genome (Desnos *et al.*, 2001).

We also compared the gene expression patterns of FR light-grown *fin219* and *rep1-1* mutants with their corresponding wild-type seedlings (COL ecotype, Figure 2b, lanes 4–6), and compared *far1-2* and *fhy3-4* mutants with their corresponding wild-type seedlings (ecotype No-0 Figure 2b, lanes 7–9). All these mutations compromise the expression patterns of large percentages of genes regulated by FR light, although the folds of differential expression ratio are usually smaller than FR light regulation in wild type. This is particularly true for *fin219*, *rep1-1*, and *far1-2*. This partial effect suggests that each of these genes only contribute partially to FR light control of gene expression. The expression profile of No-0/FR versus *fhy3-4*/FR is close to that of No-0/FR versus No-0/D, suggesting that FHY3 play a more prominent role in mediating FR light-regulated gene expression.

Overall, these data provides genomic evidence to support the notion that these mutants are defective in FR responses and is consistent with the phenotypic defects of these mutants in FR light-mediated inhibition of hypocotyl elongation and/or promotion of cotyledon opening (Figure 2a). Further, it suggests that different signaling components contribute with different degrees, toward the ultimate gene expression levels controlled by FR light. It should also be pointed out that in each mutant examined, there are small groups of genes appear to be regulated in an opposite manner from the FR light treatment of the wild-type seedlings. The reason for the contrasting regulation is not clear. One possibility, however, might be the loss or compromise of negative feedback regulation of these components on the signaling pathway, as discussed in a previous study (Wang and Deng, 2002a).

Genome expression profile analysis of a negative regulator mutant in phyA signaling

SPA1 is a negative regulator of phyA signaling, and its mutants are hypersensitive to FR light and display much shortened hypocotyls and increased anthocyanin accumulation in the upper portion of hypocotyls (Figure 2a). Genome profile analysis revealed that the *spa1-3* mutation affects a very small group of genes to a significant level, if a two-fold cut-off value was used. However, the expression of a large number of genes is affected by this mutation below the two-fold cut-off value (Figure 2b, lanes 10 and 11).

A genomic view of the regulatory hierarchy of these phyA signaling mutant loci

To compare the relative effects of all above examined mutants on FR light-regulated gene expression, we conducted a clustering analysis of the genome expression profiles of these mutants using the defined core group of FR light-regulated genes to minimize the ecotype effects (Figure 3a). Among this group of FR light-regulated genes (453 upregulated and 243 downregulated), large fractions of them display compromised expression patterns in the *phyA-1*, *fhy3-4* and *fhy1-1* mutants, although the folds of differential expression ratio are usually smaller than FR light regulation in wild type. On the contrast, the numbers of genes affected in the *far1-2*, *spa1-3*, *rep1-1* and *fin219* mutants are much smaller (Figure 3b). Further, large percentages of the genes affected in the *phyA-1* mutant are similarly affected in the *fhy3-4* and *fhy1-1* mutants, and much smaller portions of the genes affected in *phyA-1* are affected in the *far1-2*, *spa1-3*, *rep1-1*, and *fin219* mutants. It should be pointed out that the low number of genes with differential expression ratio reaching the 1.5 cut-off value in the *far1-2* mutant is somewhat unexpected and most likely due to the partially functional redundancy between FAR1 and FHY3 (see sections below for more details). However, clustering analysis shows that the profiles of the experimental pairs Ler/FR versus *fhy1*/FR, No-0/FR versus *fhy3-4*/FR and No-0/FR versus *far1-2*/FR are similar to that of Ler/FR versus *phyA-1*/FR and to each other; whereas the profiles of COL/FR versus *rep1-1*/FR, *spa1-3*/FR versus RLD/FR and COL/FR versus *fin219*/FR are quite distinct from that of Ler/FR versus *phyA-1*/FR and from each other (Figure 3a,c). Particularly interesting, the *rep1-1* mutant displays a genome expression profile significantly different from those of other mutants, with a large number of genes even displaying an opposite regulation (induced versus repressed). This result suggests that REP1, an atypical bHLH protein, functions in phyA signaling pathway to positively regulate some phyA-regulated genes, but also participates in negative regulation of some other phyA-regulated genes. Such information is unique and readily revealed by microarray study, but is not assessable from traditional genetic studies, which only examine a limited number of visible phenotypes.

In summary, available data support a view that these phyA signaling intermediates controlling overlapping but not identical sets of FR light-regulated gene expression, which could account for a molecular basis for the observed overlapping yet distinct sets of morphological defects displayed by these mutants in response to FR light. Further, FHY1, FAR1 and FHY3 likely to act upstream in the signaling pathway, close to the action of phyA photo-receptor. SPA1, REP1 and FIN219 have much limited roles in regulating FR light responsive genes, and they may act

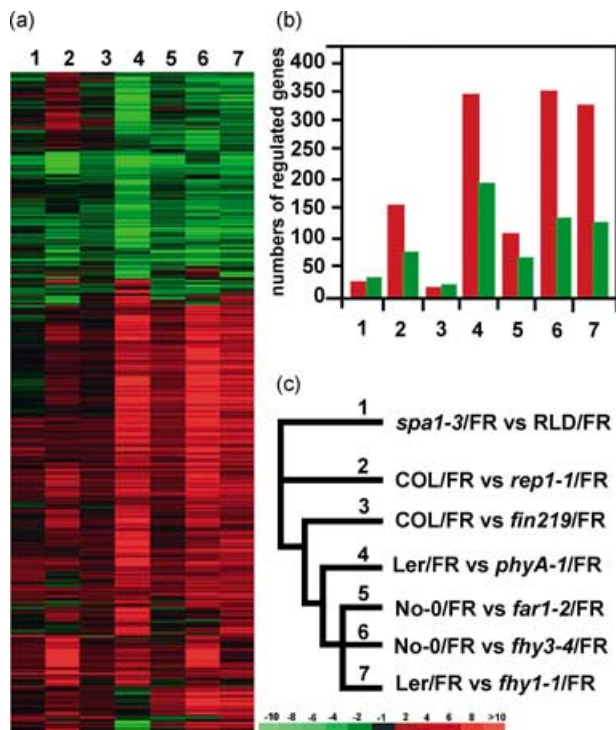


Figure 3. Clustering analysis of seven *phyA* signaling intermediates using the core group of FR light responsive genes.

(a) Hierarchical clustering displays of expression ratios of seven *phyA* signaling mutants based on the core group of FR light-regulated genes. All experimental pairs are FR-grown mutants and their corresponding ecotype seedlings. 1: *spa1-3*/FR versus RLD/FR; 2: COL/FR versus *rep1-1*/FR; 3: COL/FR versus *fin219*/FR; 4: Ler/FR versus *phyA-1*/FR; 5: No-0/FR versus *far1-2*/FR; 6: No-0/FR versus *fhy3-4*/FR; 7: Ler/FR versus *fhy1-1*/FR. Only those genes that exhibited 1.5-fold or higher differential expression in at least one sample pair among the seven tested were included for comparison. There are 656 genes included in this cluster (see supplementary data at <http://plantgenomics.biology.yale.edu/> for more information). The color scale bar at the bottom shows the folds of gene regulation.

(b) Number of genes displaying 1.5 or higher differential expression ratio in the seven *phyA* signaling mutants examined here. The experimental pairs are indicated at the bottom in the same order as (a).

(c) A dendrogram includes the relationship among these sample pairs across all the genes included in this cluster analysis.

more downstream either as effector (such as REP1) or modulator (such as SPA1 and FIN219) of the main pathways.

Genome profile analysis of double mutants

We have previously shown that double mutants *fhy3-1 far1-2*, *fhy3-1 fhy1-1* and *far1-2 fhy1-1* all display more elongated hypocotyls than their parental strains, whereas the *fhy3-1 spa1-3* double mutant has a hypocotyl of intermediate length (Wang and Deng, 2002a; Figure 4a), suggesting that

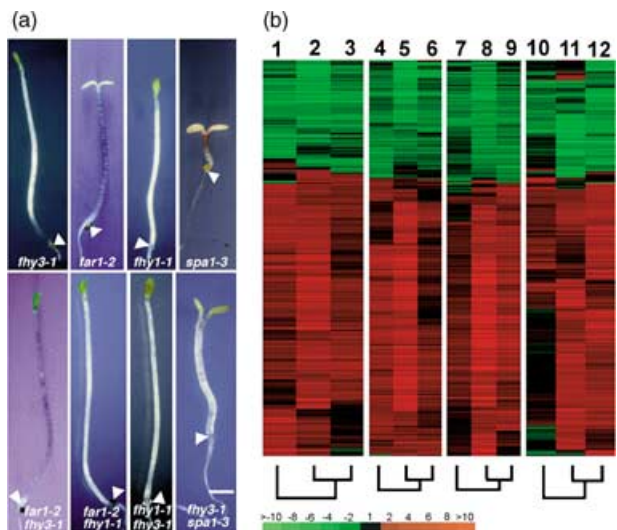


Figure 4. Clustering analysis of four double mutants comparing to their parental single mutants.

(a) Morphological comparison of 5-day-old FR-grown *Arabidopsis* seedlings of the four *phyA* signaling single mutants and four double mutant combinations. All seedlings are photographed at the same magnification and the scale bar represents approximately 1 mm. The triangle indicates the junction of hypocotyl and root.

(b) Hierarchical clustering displays of expression ratios of four double mutants comparing to their parental single mutants, based on the core group of FR light responsive genes. 1: COL/FR versus *fhy3-1*/FR; 2: COL/FR versus *fhy3-1 far1-2*/FR; 3: No-0/FR versus *far1-2*/FR; 4: Ler/FR versus *fhy1-1*/FR; 5: COL/FR versus *fhy3-1 fhy1-1*/FR; 6: COL/FR versus *fhy3-1*/FR; 7: No-0/FR versus *far1-2*/FR; 8: No-0/FR versus *far1-2 fhy1-1*/FR; 9: Ler/FR versus *fhy1-1*/FR; 10: *spa1-3*/FR versus RLD/FR; 11: COL/FR versus *fhy3-1 spa1-3*/FR; 12: COL/FR versus *fhy3-1*/FR. Only those genes that exhibited 1.5-fold or higher differential expression in at least one sample pair among the three tested were included for comparison. There are 461 genes included in the first cluster, 618 genes included in the second cluster, 603 genes in the third cluster, and 406 genes included in the fourth cluster (see supplementary data at <http://plantgenomics.biology.yale.edu/> for more information). The dendrograms at the bottom indicate the relationships among these sample pairs across all the genes included in their respective clustering analysis. The color scale bar at the bottom shows the folds of gene regulation.

there is no clear epistatic relationships among their gene products in mediating FR light responses.

Comparison of the genome expression profiles of these double mutants with those of their respective parental mutants further substantiates such a notion. Among the core group of genes regulated by FR light (total 696 genes, 453 upregulated, 243 downregulated), there are 327 and 130 genes are up- and downregulated, respectively, in the *fhy1-1* mutant, 188 and 83 genes up- and downregulated in the *fhy3-1* mutant, 111 and 70 genes up- and downregulated in the *far1-2* mutant. There are significant increases in the numbers of genes reaching the same 1.5 cut-off value, with 392 and 159 genes up- and downregulated in the *far1-2 fhy1-1* double mutant, 266 and 104 genes up- and downregulated in the *far1-2 fhy3-1* double mutants; 400 and 138 genes up- and downregulated in the *fhy3-1 fhy1-1* double

mutant. Further, clustering analysis reveals that the folds of regulation (either induction or repression) are clearly enhanced in these double mutants comparing to their respective parental strains (Figure 4b, lanes 1–9). These data support the claim that there is no simple down-/upstream relationship among these phyA signaling components.

The genome expression profile of the COL/FR versus *spa1-3/fhy3-1*/FR is distinct from that of either parental strain and is largely a mixture of the patterns of both parents, although it is more similar to that of the *fhy3-1* mutant (Figure 4b, lanes 10–12). This result confirms that there is no simple epistatic relationship between FHY3 and SPA1, and further suggests that SPA1 may function as a modulator to affect FR light-regulated gene expression.

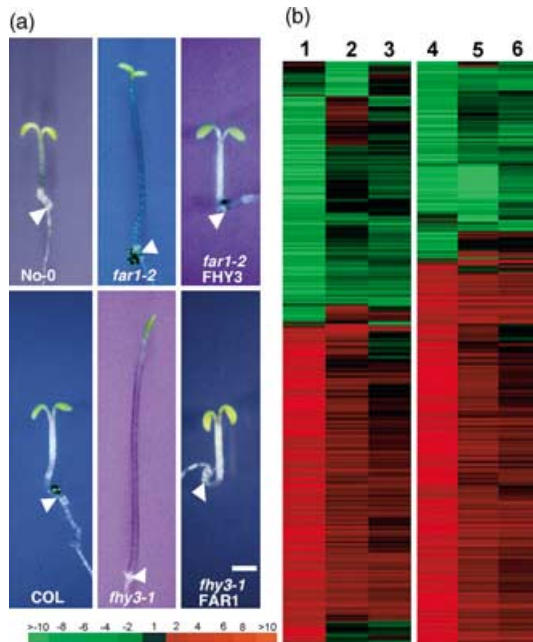


Figure 5. Clustering analysis of FAR1 and FHY3 overexpression strains. (a) Morphological comparison of 5-day-old FR-grown *Arabidopsis* seedlings of the *far1-2*, *fhy3-1* single mutants, two representative suppression lines and their respective wild-type seedlings. *far1-2 FHY3*: a transgenic line overexpressing FHY3 in the *far1-2* mutant background; *fhy3-1 FAR1*: a transgenic line overexpressing FAR1 in the *fhy3-1* mutant background. All seedlings are photographed at the same magnification and the scale bar represents approximately 1 mm. The triangle indicates the junction of hypocotyl and root. (b) Hierarchical clustering displays of expression ratios of the *far1-2 FHY3* and *fhy3-1 FAR1* transgenic seedlings comparing to their respective parental mutant strain. 1: *No-0*/FR versus *No-0*/D; 2: *far1-2 FHY3*/FR versus *far1-2*/FR; 3: *No-0*/FR versus *far1-2*/FR; 4: *COL*/FR versus *COL*/D; 5: *fhy3-1 FAR1*/FR versus *fhy3-1*/FR; 6: *COL*/FR versus *fhy3-1*/FR. Only those genes that exhibited two-fold or higher differential expression in at least one sample pair among the three tested for each clustering analysis were included for comparison. There are 1032 genes included in the first cluster, and 1097 genes in the second cluster (see supplementary data at <http://plantgenomics.biology.yale.edu> for more information). The color scale bar at the bottom shows the folds of gene regulation.

Genomic evidence for the partial function redundancy between FAR1 and FHY3

FAR1 and *FHY3* encode two homologous proteins belonging to a gene family of 14 members in the *Arabidopsis* genome (Hudson *et al.*, 1999; Wang and Deng, 2002a). Further, overexpression of FAR1 or FHY3 can suppress the phenotype of each other's loss-of-function mutations (Wang and Deng, 2002a; Figure 5a). To provide a genomics explanation for such a phenotypic observation, we examined the effects of overexpressing FAR1 and FHY3 on the genome expression profiles in each other's mutant background. As shown in Figure 5(b), The genome expression profile of the experimental pair *far1-2 FHY3*/FR versus

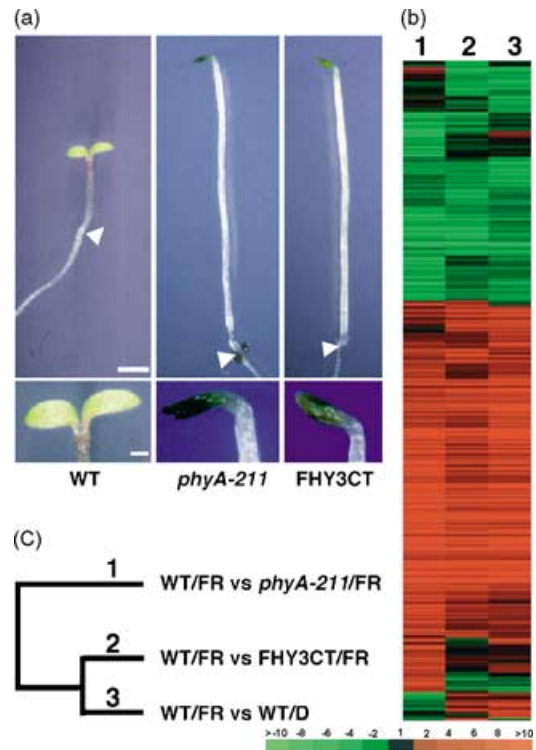


Figure 6. Clustering analysis of the *phyA-211* mutant and the FHY3CT transgenic line. (a) Morphological comparison of 5-day-old FR-grown *Arabidopsis* seedlings of *phyA* mutant, FHY3CT transgenic line and the corresponding WT (ecotype COL). The lower portion shows the close-up views of the corresponding cotyledons of the seedlings shown on the up portion. All seedlings are photographed at the same magnification and the scale bar represents approximately 1 mm. The triangle indicates the junction of hypocotyl and root. (b) Hierarchical clustering displays of expression ratios of *phyA* mutant and the FHY3CT transgenic seedlings. 1: *COL*/FR versus *phyA-211*/FR; 2: *COL*/FR versus FHY3CT/FR; 3: *COL*/FR versus *COL*/D. Only those genes that exhibited two-fold or higher differential expression in at least one sample pair among the three tested for each cluster analysis were included for comparison. There are 1547 genes included in this cluster (see supplementary data at <http://plantgenomics.biology.yale.edu> for more information). The color scale bar at the bottom shows the folds of gene regulation. (c) A dendrogram indicating the relationship among these sample pairs across all the genes included in the clustering analysis.

far1-2/FR is highly similar to that of No-0/FR versus *far1-2*/FR (Figure 5b, lanes 2 and 3); and the genome expression profile of the experimental pair *fhy3-1*FAR1/FR versus *fhy3-1*/FR is quite similar to that of COL/FR versus *fhy3-1*/FR (Figure 5b, lanes 5 and 6), suggesting that the transgenes FHY3 and FAR1 essentially restore the genome expression profiles abolished by the *far1-2* and *fhy3-1* mutations, respectively. This result supports the functional redundancy between FAR1 and FHY3 in mediating FR light-regulated gene expression.

Genomic evidence suggests that the FHY3-containing complex functions as a key node in the phyA signaling network

Previously, it has been shown that overexpression of partial fragments of FHY3 in a wild-type background causes reduced sensitivity to FR light in a dosage-dependent manner. Most strikingly, *Arabidopsis* seedlings homozygous for the transgene overexpressing the C-terminal portion of FHY3 (amino acids 473–839, FHY3CT), which contains a Coiled-coil domain, display an apparent complete loss of FRc responses essentially identical to the *phyA* null mutants (Wang and Deng 2002a; Figure 6a).

To examine whether the dominant negative phenotype results from similar effect on genome expression as the *phyA* mutations, the gene expression profile caused by overexpression of FHY3CT in the wild-type background (COL ecotype) was analyzed and compared to that of a strong *phyA* allele in the same ecotype background, *phy-211* (Reed *et al.*, 1994). As shown in Figure 6(a), the gene expression pattern of the experimental pair COL/FR versus FHY3CT/FR is quite similar to those of COL/FR versus COL/D and COL/FR versus *phyA-211*/FR, much more than any *phyA* signaling single or double mutants examined (Figures 3 and 4). In fact, clustering analysis of these experimental pairs revealed that the genome expression profile of COL/FR versus FHY3CT/FR is more similar to that of COL/FR versus COL/D than to that of COL/FR versus *phyA-211*/FR (Figure 6c).

Our results have two implications. First, it supports the notion that the C-terminal fragment of FHY3 may interact with other intermediates of *phyA* signaling and that non-productive binding of this truncated FHY3 protein with its interactive partners could interfere with their functions in a dominant-negative fashion. This is consistent with the finding that FHY3 and FAR1 could directly interact with each other (Wang and Deng, 2002a). Second, the fact that the genome expression profile of COL/FR versus FHY3CT/FR is more similar to that of COL/FR versus COL/D than to that of COL/FR versus *phyA-211*/FR (Figure 6c) suggests that other photoreceptors (such as phytochromes B to E or cryptochromes) may be also involved in regulating some FR light responsive gene expression to a small degree. In

this case, the FHY3CT transgene could block or interfere with their signaling processes as well. Regardless, available data support the notion that FHY3 constitutes a key node in a regulatory network mediating FR responses.

Conclusions

In this study we used microarray technology and genome expression profile analysis to examine the functional relationship of the genetically defined *phyA* signaling intermediates. This genomic approach offers a number of new insights over traditional molecular genetics in term of determining the genetic interactions (epistasis relationships) among multiple loci involved in *phyA* signaling. First, this cDNA microarray allows us to simultaneously examine the light-regulated expression of 6126 genes included in this array. In a sense, each of these gene expression patterns can be regarded as a molecular marker. Therefore, analysis of all their expression patterns as a whole among the distinct *phyA* signaling mutant strains provides a holistic view of the relationships for those defined loci. Thus, the derived conclusion should be more accurate than that based on the analysis of a limited number of visible phenotypes. Second, we demonstrated that it is feasible to define a core group of FR light-regulated genes that are similarly regulated in all four ecotypes examined. This group of genes can be used as the basis to conduct a comparative analysis of the genome expression profiles of the genetically defined *phyA* signaling mutants derived from these ecotypes, despite the significant variations among the FR-regulated genome expression profiles among the ecotypes used. This could become a general mean to minimize the effects of ecotype variations on the mutant phenotypes. Third, this genomic approach is particularly useful to discriminate the functions of individual members of gene family, such as FHY3 and FAR1, which may have partial redundant function and is difficult to dissect using traditional genetic approach.

Comparative genome expression profiling study of various *Arabidopsis* *phyA* signaling mutants reveals that FHY1, FHY3, and FAR1 control large numbers of FR light-regulated gene expression, whereas FIN219, SPA1, and REP1 control the expression of smaller sets of genes. This provides genomic evidence for the notion that FHY1, FAR1 and FHY3 represent upstream branch components in the *phyA* signaling network, and FIN219, SPA1 and REP1 function more downstream either as effectors or modulators of the network. All these signaling components contribute to the control of FR light-regulated gene expression to different degrees, and they affect overlapping yet distinct sets of genes. These data are in good agreement with traditional phenotype studies that these mutants display similar, yet not identical defects in various FR light-mediated responses. Analysis of the genome expression profiles of

a number of double mutants further substantiates the notion that phyA signaling entails a complex network of intersecting branches. There is no simple upstream/downstream relationships among these phyA signaling components. Further, this study provides a genomic support for the suppression of *far1* and *fhy3* mutant phenotypes by overexpressing each other's homologous protein, FHY3 or FAR1, respectively. Further, our studies also provide a genomic basis for the dominant negative effect of overexpressing FHY3CT on phyA signaling pathway, thus providing evidence for its critical role in phyA signaling network.

Experimental procedures

Plant materials

Four wild-type *Arabidopsis* ecotypes (COL, Ler, RLD, No-0), nine phyA single signaling mutants (*phyA-1*, *phyA-211*, *fhy1-1*, *fhy3-1*, *fhy3-4*, *far1-2*, *fin219*, *rep1-1*, and *spa1-3*), four double mutants (*fhy3-1 far1-2*, *fhy3-1 fhy1-1*, *far1-2 fhy1-1*, and *fhy3-1 spa1-3*), and three transgenic lines (*far1-2FHY3*, *fhy3-1FAR1* and FHY3CT) were included in this study (Hoecker *et al.*, 1999; Hsieh *et al.*, 2000; Hudson *et al.*, 1999; Reed *et al.*, 1994; Soh *et al.*, 2000; Wang and Deng, 2002a; Whitelam *et al.*, 1993). *phyA-1*, *fhy1-1* are in the Ler ecotype background; *phyA-211*, *fhy3-1*, *fin219*, *rep1-1* are in the COL ecotype background; *far1-2* and *fhy3-4* are in the No-0 ecotype background; *spa1-3* is in the RLD ecotype background (Table 1). The *far1-2FHY3* and *fhy3-1FAR1* transgenic lines are generated in the *far1-2* and *fhy3-1* background, and overexpress FHY3 and FAR1, respectively. The FHY3CT transgenic line is generated in the COL ecotype and overexpresses a carboxyl-terminal fragment of FHY3 (amino acids 473–839, Wang and Deng, 2002a). Surface sterilization and cold treatment of the seeds were described previously (Ang and Deng, 1994). *Arabidopsis* seedlings were grown in growth medium agar plates containing 0.3% sucrose. The seedlings were grown in continuous white light for 1 day to induce germination, then transferred to far red light (approximately $160 \mu\text{mol m}^{-2} \text{sec}^{-1}$) or darkness for 5 days.

The microarray slide used in this study was described previously. There were 9216 EST clones in the array, represented about 6126 unique genes (see Ma *et al.*, 2001, 2002). For more information, please check <http://plantgenomics.biology.yale.edu> or http://info.med.yale.edu/wmkeck/dna_arrays.htm.

RNA preparation and fluorescent labeling of probe

Total RNA was extracted from the whole seedlings using the Qiagen RNeasy Plant Mini prep kit. An amount of 50 μg total RNA was first labeled with aminoallyl-dUTP (aa-dUTP, Sigma, St. Louis, Missouri) by direct incorporation of aa-dUTP during reverse transcription as described previously (Ma *et al.*, 2001), but instead of Cy-3 or Cy-5 dUTP. After 3-h incubation at 42°C, the reaction was stopped by adding 5 μl of 0.5 M EDTA and incubating at 94°C for 3 min, and RNA was hydrolyzed by adding 10 μl of 1 M NaOH and incubating at 65°C for 20 min. This reaction was neutralized by adding 6 μl of 1 M HCl and 2 μl of 1 M HCl-Tris (pH 7.5). The aa-dUTP labeled cDNA was purified from the unincorporated aa-dUTP molecules by adding 400 μl of water, and spinning through a Microcon YM-30 filter (Millipore, Bedford,

MA) for 7 min at $11000 \times g$ and is washed again. The purified, labeled probe was concentrated to a final volume of 7 μl . Then, cDNA probe was further labeled with fluorescent dye by conjugating aa-dUTP and monofunctional Cy-3 or Cy-5 (Amersham Pharmacia Biotech, Piscataway, NJ) as follows: 1 volume cDNA solution added 0.1 volume 1 M sodium bicarbonate (Sigma, St. Louis, Missouri) and 1 μl Cy3 or Cy-5 dye (solved in DMSO). The mixture was mixed with a pipette tip, and incubated at room temperature in the dark for 60–90 min. After incubation, the labeling reaction was stopped by adding 1 μl 2 M ethanolamine (Sigma, St. Louis, Missouri), and further incubated at room temperature for 5 min. The dye-labeled probe was purified from the unincorporated dye molecules by washing through a Microcon YM-30 filter (Millipore, Bedford, MA) as mentioned above for three times, and combined the two sample pairs at the last washing. The purified, labeled probe was concentrated to a final volume of 7 μl .

Hybridization, washing and scanning

The protocol for hybridization to the *Arabidopsis* microarray, microarray slide washing, and scanning were as described previously (Ma *et al.*, 2001). The separate TIFF images for Cy-3 and Cy-5 channels were obtained by scanning with Axon GenePix 4000B scanner (Foster City, CA) at 10-nm resolution. Laser and photomultiplier tube (PMT) voltages were adjusted manually to minimize background and the number of spots that have saturated signal values. The normalization of the two channels with respect to signal intensity was also obtained by adjusting the PMT and laser power settings. We chose the PMT voltages to let the signal ratio of the majority of control genes to be as close to 1.0 as possible.

Data analysis and quality control

Microarray experiments are inherently associated with biological and technical variations. Biological variations are usually defined as that observed in multiple independent RNA preparations, and technical variations are observed between duplicate array experiments from each one of the RNA preparations. To minimize these variations in our experiments we took following precautions. First, for each biological sample, we isolated at least two independent RNA samples (for some wild-type controls, three independent samples were prepared) from separate experiments, which reduces the biological variations. Second, we use each RNA sample to probe two microarrays, which reduces technical variations. Thus, we generated at least four data sets for each experimental pair. Third, all four data sets were pooled together and analyzed.

Data analysis was conducted essentially as described in our previous work (Ma *et al.*, 2001, 2002) with minor modifications. Briefly, spot intensities were quantified using Axon GenePix image analysis software (GenePix Pro 4.0). The channel ratio was measured with GenePix median of ratio method, and was then normalized using the corresponding GenePix default normalization factor. In order to merge the replicated GenePix output data files in a reasonable way, we developed a computer program called GPMERGE (<http://bioinformatics.med.yale.edu/software.html>). With this program, we pooled the four data sets of each experiment together. Different quality control procedures were conducted before data points were averaged from the four data sets. First, all spots, which were flagged *Bad* or *Not Found* by GenePix, were not taken into account in the final data analysis. Second, a very simple outlier searching algorithm was incorporated in GPMERGE, those spots which lead to a large difference

between the ratio mean and the ratio median were defined as outliers and eliminated from the analysis. Third, only those spots that met both of the following two conditions were considered in further data analysis: (i) signals were higher than the backgrounds for both channels; (ii) the signal was two-fold higher than the background at least for one channel.

Different kinds of expression pattern identification and pattern matching were conducted within or across these experiment groups. Within each group a hierarchical clustering analysis was performed as described by Eisen *et al.* (1998). Only those genes that had more than 1.5- or 2-fold changes in expression (as specified in the text and Figure legend) in at least one of the experiment sets were used in the cluster analysis shown in the figures.

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References

- Ang, L.H. and Deng, X.W. (1994) Regulatory hierarchy of photomorphogenic loci: allele-specific and light-dependent interaction between the HY5 and COP1 loci. *Plant Cell*, **6**, 613–628.
- Arabidopsis genome initiative (AGI). (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature*, **408**, 796–815.
- Ballesteros, M., Bolle, C., Lois, L.M., Moore, J.M., Vielle-Calzada, J.-P., Grossniklaus, U. and Chua, N.-H. (2001) LAF1, a MYB transcription activator for phytochrome A signaling. *Genes Dev.* **15**, 2613–2625.
- Barnes, S.A., Nishizawa, N.K., Quaggio, R.B., Whitelam, G.C. and Chua, N.-H. (1996b) Far-red light blocks greening of *Arabidopsis* seedlings via a phytochrome A-mediated change in plastid development. *Plant Cell*, **8**, 601–615.
- Barnes, S.A., Quaggio, R.B., Whitelam, G.C. and Chua, N.-H. (1996a) *fhyl* defines a branch point in phytochrome A signal transduction pathways for gene expression. *Plant J.* **10**, 1155–1161.
- Bolle, C., Koncz, C. and Chua, N.-H. (2000) PAT1, a new member of the GRAS family, is involved in phytochrome A signal transduction. *Genes Dev.* **14**, 1269–1278.
- Borevitz, J.O., Maloof, J.N. *et al.* (2002) Quantitative trait loci controlling light and hormone response in two accessions of *Arabidopsis thaliana*. *Genetics*, **160**, 683–696.
- Chattopadhyay, S., Ang, L.H., Puente, P., Deng, X.W. and Wei, N. (1998) *Arabidopsis* bZIP protein HY5 directly interacts with light-responsive promoters in mediating light control of gene expression. *Plant Cell*, **10**, 673–683.
- Deng, X.W. and Quail, P.H. (1999) Signalling in light-controlled development. *Semi. Cell Dev. Biol.* **10**, 121–129.
- Desnos, T., Puente, P., Whitelam, G.C. and Harberd, N.P. (2001) FHY1: a phytochrome A-specific signal transducer. *Genes Dev.* **15**, 2980–2990.
- Dieterle, M., Zhou, Y.-C., Schäfer, E., Funk, M. and Kretsch, T. (2001) EID1, an F-box protein involved in phytochrome A-specific light signaling. *Genes Dev.* **15**, 939–944.
- Eichenberg, K., Hennig, L., Martin, A. and Schafer, E. (2000) Variation in dynamics of phytochrome A in *Arabidopsis* ecotypes and mutants. *Plant Cell Environ.* **23**, 311–319.
- Eisen, M.B., Spellman, P.T., Brown, P.O. and Botstein, D. (1998) Cluster analysis and display of genome-wide expression patterns. *Proc. Natl Acad. Sci. USA*, **95**, 14863–14868.
- Enard, W. *et al.* (2002) Intra- and interspecific variation in primate gene expression patterns. *Science*, **296**, 340–343.
- Fairchild, C.D., Schumaker, M.A. and Quail, P.H. (2000) *HFR1* encodes an atypical bHLH protein that acts in phytochrome A signal transduction. *Genes Dev.* **14**, 2377–2391.
- Fankhauser, C. and Chory, J. (1997) Light control of plant development. *Annu. Rev. Cell Dev. Biol.* **13**, 203–229.
- Harmer, S.L., Hogenesch, J.B., Straume, M., Chang, H.-S., Han, B., Wang, X., Kreps, J.A. and Kay, S.A. (2000) Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock. *Science*, **290**, 2110–2113.
- Hoecker, U., Tepperman, J.M. and Quail, P.H. (1999) SPA1, a WD-repeat protein specific to phytochrome A signal transduction. *Science*, **284**, 496–499.
- Hoecker, U., Xu, Y. and Quail, P.H. (1998) *SPA1*: a new genetic locus involved in phytochrome A-specific signal transduction. *Plant Cell*, **10**, 19–33.
- Hsieh, H.-L., Okamoto, H., Wang, M., Ang, L.-H., Matsui, M., Goodman, H. and Deng, X.W. (2000) *FIN219*, an auxin-regulated gene, defines a link between phytochrome A and the downstream regulator COP1 in light control of *Arabidopsis* development. *Genes Dev.* **14**, 1958–1970.
- Hudson, M.E. (2000) The genetics of phytochrome signalling in *Arabidopsis*. *Semi. Cell Dev. Biol.* **11**, 475–483.
- Hudson, M., Ringli, C., Boylan, M.T. and Quail, P.H. (1999) The *FAR1* locus encodes a novel nuclear protein specific to phytochrome A signaling. *Genes Dev.* **13**, 2017–2027.
- Kuno, N. and Furuya, M. (2000) Phytochrome regulation of nuclear gene expression in plants. *Semi. Cell Dev. Biol.* **11**, 485–493.
- Kuno, N., Muramatsu, T., Hamazato, F. and Furuya, M. (2000) Identification by large-scale screening of phytochrome-regulated genes in etiolated seedlings of *Arabidopsis* using a fluorescent differential display technique. *Plant Physiol.* **122**, 15–24.
- Ma, L., Gao, Y., Qu, L., Chen, Z., Li, J., Zhao, H. and Deng, X.W. (2002) Genomic evidence for COP1 as a repressor of light-regulated gene expression and development in *Arabidopsis*. *Plant Cell*, **14**, 2383–2398.
- Ma, L., Li, J., Qu, L., Hager, J., Chen, Z., Zhao, H. and Deng, X.W. (2001) Light control of *Arabidopsis* development entails coordinated regulation of genome expression and cellular pathways. *Plant Cell*, **13**, 2589–2607.
- Maloof, J.N., Borevitz, J.O., Dabi, T., Lutes, J., Nehring, R.B. *et al.* (2001) Natural variation in light sensitivity of *Arabidopsis*. *Nat. Genet.* **29**, 441–446.
- Maloof, J.N., Borevitz, J.O., Weigel, D., Chory, J. *et al.* (2000) Natural variation in phytochrome signaling. *Semi. Cell Dev. Biol.* **11**, 525–530.
- Martínez-García, J.F., Huq, E. and Quail, P.H. (2000) Direct targeting of light signals to a promoter element-bound transcription factor. *Science*, **288**, 859–863.

- Møller, S.G., Kunkel, T. and Chua, N.-H.** (2001) A plastidic ABC protein involved in intercompartmental communication of light signaling. *Genes Dev.* **15**, 90–103.
- Nagatani, A., Reed, J.W. and Chory, J.** (1993) Isolation and initial characterization of *Arabidopsis* mutants that are deficient in phytochrome A. *Plant Physiol.* **102**, 269–277.
- Nagy, F., Kircher, S. and Schäfer, E.** (2000) Nucleo-cytoplasmic partitioning of the plant photoreceptors phytochromes. *Semi. Cell Dev. Biol.* **11**, 505–510.
- Nagy, F. and Schäfer, E.** (2002) Phytochromes control photomorphogenesis by differentially regulated, interacting signaling pathways in higher plants. *Annu. Rev. Plant Biol.* **53**, 329–355.
- Neff, M.M., Fanhauser, C. and Chory, J.** (2000) Light: An indicator of time and place. *Genes Dev.* **14**, 257–271.
- Perez-Amador, M.A., Lidder, P., Johnson, M.A., Landgraf, J., Wisman, E. and Green, P.J.** (2001) New molecular phenotypes in the *dst* mutants of *Arabidopsis* revealed by DNA microarray analysis. *Plant Cell*, **13**, 2703–2717.
- Puente, P., Wei, N. and Deng, X.W.** (1996) Combinatorial interplay of promoter elements constitutes minimal determinants for light and developmental control of gene expression in *Arabidopsis*. *EMBO J.* **15**, 3732–3743.
- Quail, P.H.** (2002a) Phytochrome photogransensory signalling networks. *Nat. Rev. Mol. Cell Biol.* **3**, 85–93.
- Quail, P.H.** (2002b) Photosensory perception and signalling in plant cells: new paradigms? *Curr. Opin. Cell Biol.* **14**, 180–188.
- Quail, P.H., Boylan, M.T., Parks, B.M., Short, T.W., Xu, Y. and Wagner, D.** (1995) Phytochromes: photosensory perception and signal transduction. *Science*, **268**, 675–680.
- Reed, J.W., Nagatani, A., Elich, T.D., Fagan, M. and Chory, J.** (1994) Phytochrome A and phytochrome B have overlapping but distinct functions in *Arabidopsis* development. *Plant Physiol.* **104**, 1139–1149.
- Schaffer, R., Landgraf, J., Accerbi, M., Simon, V., Larson, M. and Wisman, E.** (2001) Microarray analysis of diurnal and circadian-regulated genes in *Arabidopsis*. *Plant Cell*, **13**, 113–123.
- Soh, M.S., Kim, Y.M., Han, S.J. and Song, P.S.** (2000) REP1, a basic helix-loop-helix protein, is required for a branch pathway of phytochrome A signaling in *Arabidopsis*. *Plant Cell*, **12**, 2061–2074.
- Tepperman, J.M., Zhu, T., Chang, H.S., Wang, X. and Quail, P.H.** (2001) Multiple transcription-factor genes are early targets of phytochrome A signaling. *Proc. Natl Acad. Sci. USA*, **98**, 9437–9442.
- Terzaghi, W.B. and Cashmore, A.R.** (1995) Light-regulated transcription. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **46**, 445–474.
- Wang, H. and Deng, X.W.** (2002a) *Arabidopsis* FHY3 defines a key phytochrome A signaling component directly interacting with its homologous partner FAR1. *EMBO J.* **21**, 1339–1349.
- Wang, H. and Deng, X.W.** (2002b) Phytochrome signaling mechanism. In *The Arabidopsis Book* (Meyerowitz, E. and Somerville, C., eds). American Society of Plant Biologists). Available at <http://www.aspb.org/publications/arabidopsis/>.
- Whitelam, G.C., Johnson, E., Peng, J., Carol, P., Anderson, M.L., Cowl, J.S. and Harberd, N.P.** (1993) Phytochrome A null mutants of *Arabidopsis* display a wild-type phenotype in white light. *Plant Cell*, **5**, 757–768.
- Yanovsky, M.J., Casal, J.J. and Luppi, J.P.** (1997) The *VLF* loci, polymorphic between ecotypes *landsberg erecta* and *Columbia*, dissect two branches of phytochrome A signal transduction that correspond to very-low-fluence and high-irradiance responses. *Plant J.* **12**, 659–667.
- Yanovsky, M.J., Whitelam, G.C. and Casal, J.J.** (2000) *fhy3-1* retains inductive responses of phytochrome A. *Plant Physiol.* **123**, 235–242.
- Yue, H., Eastman, P.S., Wang, B.B., Minor, J., Doctolero, M.H., Nuttall, R.L., Stack, R., Becker, J.W., Montgomery, J.R., Vainer, M. and Johnston, R.** (2001) An evaluation of the performance of cDNA microarrays for detecting changes in global mRNA expression. *Nucl Acids Res.* **29**, E41.