

A Genome-Wide Analysis of Blue-Light Regulation of Arabidopsis Transcription Factor Gene Expression during Seedling Development^{1[w]}

Yuling Jiao², Hongjuan Yang², Ligeng Ma, Ning Sun, Haiyuan Yu, Tie Liu, Ying Gao, Hongya Gu, Zhangliang Chen, Masamitsu Wada, Mark Gerstein, Hongyu Zhao, Li-Jia Qu*, and Xing Wang Deng*

Peking-Yale Joint Research Center of Plant Molecular Genetics and Agrobiotechnology, College of Life Sciences, Peking University, Beijing 100871, China (Y.J., H.Y., T.L., Y.G., H.G., Z.C., L.-J.Q., X.W.D.); Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, Connecticut 06520-8014 (Y.J., L.M., Y.G., X.W.D.); Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut 06520-8034 (N.S., H.Z.); Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520-8114 (H. Yu, M.G.); and Department of Biological Sciences, Graduate School of Science, Tokyo Metropolitan University, Hachioji, Tokyo 192-0397, Japan (M.W.)

A microarray based on PCR amplicons of 1,864 confirmed and predicted Arabidopsis transcription factor genes was produced and used to profile the global expression pattern in seedlings, specifically their light regulation. We detected expression of 1,371 and 1,241 genes in white-light- and dark-grown 6-d-old seedlings, respectively. Together they account for 84% of the transcription factor genes examined. This array was further used to study the kinetics of transcription factor gene expression change of dark-grown seedlings in response to blue light and the role of specific photoreceptors in this blue-light regulation. The expression of about 20% of those transcription factor genes are responsive to blue-light exposure, with 249 and 115 genes up or down-regulated, respectively. A large portion of blue-light-responsive transcription factor genes exhibited very rapid expression changes in response to blue light, earlier than the bulk of blue-light-regulated genes. This result suggests the involvement of transcription cascades in blue-light control of genome expression. Comparative analysis of the expression profiles of wild type and various photoreceptor mutants demonstrated that during early seedling development cryptochromes are the major photoreceptors for blue-light control of transcription factor gene expression, whereas phytochrome A and phototropins play rather limited roles.

The completed sequence of the Arabidopsis genome by the Arabidopsis Genome Initiative (2000) provides both an opportunity and a challenge to decipher the information hidden behind the vast number of nucleotides. An essential step toward understanding the meaning of the sequences from the Arabidopsis genome is to determine which ones are expressed and how their expression is regulated. The advancement of DNA microarray technology made it possible to monitor the transcription of a large number of genes in the genome in a high-throughput fashion. This approach has recently been used to define the transcriptional activities of all predicted genes in Arabidopsis

chromosome 2 (Kim et al., 2003). It was reported that the expression of a significant fraction of known and predicted genes was confirmed. However, no comprehensive studies have yet been reported that analyze the genome expression profile for an entire functional group of genes in Arabidopsis.

Transcription factor genes constitute one of the most important functional groups of Arabidopsis genes. Because transcription factors perform regulatory roles in the expression of their target genes, the overall expression profiles of transcription factors will in some way reflect the whole-genome transcription activity. Thus it is valuable to learn how transcription factor genes are expressed and regulated at the whole-genome scale. A recent study has examined the expression profiles of 402 Arabidopsis transcription factor genes in response to stress treatments (Chen et al., 2002). Although it investigated just over 20% of the transcription factor genes, valuable insights have been learned. It is obvious, therefore, that an analysis of all transcription factor genes, known and predicted, would provide the most comprehensive and complete picture of the transcription patterns in Arabidopsis.

Being photosynthetic and sessile, plant development is highly regulated by environmental light signals throughout the entire life cycle. It is generally assumed that plants use photoreceptors to receive

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² These authors contributed equally to the paper.

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* Corresponding authors; e-mail xingwang.deng@yale.edu, fax 203-432-3854; e-mail qulj@lsc.pku.edu.cn, fax 86-10-6275-1841.

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light signals and to transduce them to modulate light-responsive gene expression. There are three major types of well-characterized photoreceptors, the red/far-red-light-absorbing phytochromes and blue/UV-A-light-absorbing cryptochromes and phototropins (Neff et al., 2000; Lin, 2002). Recent work using an expressed sequence tag (EST)-based DNA microarray has suggested that nearly one-third of the genome is regulated in white light. In addition, the genome expression patterns largely overlap in 6-d-old seedlings grown under white, far-red, red, and blue light. More than 26 cellular pathways have been found to be commonly regulated by all light signals (Ma et al., 2001). However, the expression of many early-responsive genes to light signals during the dramatic and rapid transitions of seedling development are likely to be missed by examining the effect of light after only 24-h or 6-d irradiation. In an independent microarray study, approximately 10% of about 8,000 genes examined were found to be regulated by phytochrome A (phyA) in far-red light. From a detailed kinetic study, it was evident that a large portion of the genes, which respond to light signals within 1 h, encodes multiple classes of transcription factors (Tepperman et al., 2001). Thus it was concluded that transcriptional cascades are likely to be involved in far-red-light regulation of gene expression.

Despite recent progress, we still do not know whether other photoreceptor systems beyond the phyA-mediated far-red response also employ transcription cascades in mediating light control of genome expression. Furthermore, although phyA and phototropins have been suggested to be involved in blue-light responses, it is not clear what specific role they play in mediating blue-light regulation of gene expression. To address those and other related questions, we developed a new microarray containing currently known and predicted transcription factor genes. This array was used to profile the expression of those genes in 6-d-old *Arabidopsis* seedlings and their regulation by light. A kinetic analysis of blue-light regulation of transcription factor gene expression was carried out to examine the involvement of transcription cascade. In addition, we analyzed the role of phyA and phototropins in blue-light regulation of gene expression. Our results confirmed the transcription activity of vast majority of transcription factor genes and validate most of computationally predicted ones.

RESULTS

Construction of a Whole-Genome Transcription Factor Gene DNA Microarray

Previous reports suggested that there are at least 1,533 transcription factors in the *Arabidopsis* genome (*Arabidopsis* Genome Initiative, 2000; Riechmann et al., 2000; Riechmann, 2002). From a similar analysis based on the reported strategy (Riechmann et al.,

2000), we obtained a slightly larger total gene number (1,864) using the complete genome sequence. The distribution of these genes among the main sub-groups of transcription factor gene families is similar to previous reports, although recent work on the basic helix-loop-helix (bHLH) transcription factor family identified a larger number of genes (147) than we did (108; Toledo-Ortiz et al., 2003). A brief summary of the 1,864 known and predicted transcription factors included in this custom microarray is listed in Table I. A total of 263 highly hypothetical transcription factors are grouped as "Others". A detailed list of all of the selected transcription factor genes with classification and annotation can be found in Supplemental Table S1 (which can be viewed in the online version of this article at <http://www.plantphysiol.org>).

To develop a DNA microarray containing the 1,864 annotated transcription factor genes, primer pairs were designed to amplify about 300- to 500-bp fragments of an exon-rich genomic fragment representing each gene. The PCR-amplified DNA fragments, together with a set of negative and spiking control fragments, were printed in duplicate onto glass slides using a contact microarrayer (see "Materials and Methods" and Supplemental Figure S1). To assure the quality of the DNA microarray, all of the PCR products were quality controlled by agarose gel analysis to assess the DNA fragment purity and abundance (Schenk et al., 2000). Overall, 95.7% of the target sequences were successfully amplified to reasonable abundance and with one major discrete band. Potential cross-hybridizations between highly related transcription factor family members are calculated and listed in Supplemental Table S2.

Most Transcription Factor Genes Are Expressed at the Seedling Stage

To experimentally determine the transcriptional activity of all transcription factors in *Arabidopsis* seedlings, total RNA was isolated from 6-d-old seedlings grown under darkness or white light. Their mRNA was reverse transcribed into cDNA probes and labeled with fluorescent dyes. These labeled probes were hybridized to the array and the signal for each transcription factor gene was quantified (see "Materials and Methods"). The expression patterns of all of the transcription factor genes were derived from 18 experimental repeats using three independent biological samples for the dark-grown seedlings and from six repeats using two independent biological samples for the white-light-grown seedlings (Supplemental Table S3). We followed a recently established benchmark that each expressed gene should have both reproducible and detectable fluorescent signals (Kim et al., 2003; Rinn et al., 2003) to estimate the expression of genes (see "Materials and Methods").

On the basis of our criteria, 1,241 and 1,372 of the 1,864 transcription factor genes represented on the

Table 1. Number of known and predicted *Arabidopsis* transcription factor genes and those with detected gene expression in seedlings

Genes are classified by family on the basis of sequence similarity. All genes are counted only once, regardless of extra signature motif(s), thus this table is non-redundant.

Gene Family	Predicted	Overall Expressed		WL Expressed		Dark Expressed	
			%		%		%
AP2/ERF	127	103	81.1	86	67.7	58	45.7
bHLH	108	90	83.3	78	72.2	60	55.6
MYB	269	220	81.8	194	72.1	160	59.5
C2H2(Zn)	147	108	73.5	98	66.7	48	32.7
NAC	107	78	72.9	54	50.5	69	64.5
HB	95	92	96.8	87	91.6	92	96.8
MADS	93	84	90.3	68	73.1	70	75.3
bZIP	88	77	87.5	66	75	67	76.1
WRKY(Zn)	73	64	87.7	47	64.4	58	79.5
GARP:G2-LIKE	53	50	94.3	44	83	45	84.9
GARP:ARR-B	3	3	100	3	100	3	100
C2C2:DOF	34	33	97.1	29	85.3	23	67.6
C2C2:CO-like	32	28	87.5	25	78.1	23	71.9
C2C2:GATA	7	7	100	7	100	7	100
C2C2:YABBY	5	5	100	4	80	5	100
CCAAT:HAP2	10	8	80	6	60	7	70
CCAAT:HAP3	13	9	69.2	8	61.5	9	69.2
CCAAT:HAP5	13	13	100	13	100	13	100
GRAS	32	30	93.8	30	93.8	23	71.9
Trihelix	8	5	62.5	4	50	5	62.5
HSF	30	21	70	16	53.3	17	56.7
TCP	22	18	81.8	14	63.6	14	63.6
ARF	22	21	95.5	17	77.3	18	81.8
C3H-TYPE1(Zn)	45	35	77.8	33	73.3	13	28.9
C3H-TYPE2(Zn)	26	24	92.3	22	84.6	24	92.3
SBP	16	15	93.8	12	75	15	93.8
Nin-like	14	6	42.9	4	28.6	6	42.9
ABI3/VP1	11	10	90.9	7	63.6	7	63.6
TULP	9	9	100	8	88.9	5	55.6
E2F/DF	7	6	85.7	5	71.4	6	85.7
CPP(Zn)	7	6	85.7	4	57.1	6	85.7
Alfin-like	6	5	83.3	5	83.3	4	66.7
EIL	5	5	100	5	100	4	80
LEAFY	3	3	100	3	100	3	100
Aux/IAA	28	28	100	22	78.6	22	78.6
HMG-box	16	14	87.5	12	75	12	75
ARID	5	5	100	5	100	4	80
JUMONJI	8	8	100	8	100	7	87.5
PcG	4	4	100	4	100	3	75
Others	263	222	84.4	210	79.2	200	76
Total	1,864	1,578	84.7	1,371	73.6	1,241	66.6

array are expressed in 6-d dark- or white-light-grown seedlings, respectively (for detailed gene lists, see Supplemental Table S4). Together, expression of 1,578 (84.7%) transcription factor genes can be detected at the seedling stage. Those expressed genes were distributed among all structural groups (Table I), including 103 APETALA2/Ethylene Response Factor (AP2/ERF) genes (81.1% of this family), 90 bHLH genes (83.3%), 220 MYB superfamily genes (81.8%), 108 C2H2(Zn) genes (73.5%), 78 NAC genes (72.9%), 92 Homeobox (HB) genes (96.8%), 84 MADS genes (90.3%), and 77 bZIP genes (87.5%). For 318 transcription factor genes with representation in a recent EST microarray (Ma et al., 2001, 2002), the expres-

sion for 296 of them are detected in 6-d-old dark- or light-grown seedlings. This result demonstrates that the expression of a majority of the predicted transcription factor genes can be detected at seedling stage.

A Significant Portion of Transcription Factor Genes Are Regulated by Blue Light

We further focused our attention on blue-light regulation of transcription factor gene expression. As shown in Figure 1, white light and blue light perceived by *Arabidopsis* seedling produces largely similar genome expression profiles of transcription factor genes. These results are in agreement with



Figure 1. Hierarchical cluster display of expression ratios of transcription factor expression profiles for dark- and light-grown *Arabidopsis* seedlings. Lane 1, wild-type white-light-grown seedlings versus dark-grown seedlings; lane 2, wild-type blue-light-grown seedlings versus dark-grown seedlings; lane 3, wild-type blue-light-grown seedlings versus *cry1cry2* blue-light-grown seedlings; lane 4, wild-type blue-light-grown seedlings versus *phyA* blue-light-grown seedlings. The color scale is shown at the bottom. Positive numbers represent -fold of induction and negative numbers represent -fold of repression. All seedlings were 6-d-old. All of those genes that exhibited a 1.8-fold or higher differential expression in at least one time point were included.

previous EST-based microarray analysis (Ma et al., 2001). Some selected transcription factors that are differentially regulated by continuous blue light or white light greater than 3-fold are listed in Tables II and III. We note that the majority of these genes have not been studied yet and thus may be of special interest for future investigation.

We further examined blue-light regulation of transcription factor gene expression of 6-d-old seedlings that were grown in darkness and then exposed to blue light for 0.5, 1, 3, 6, 12, 24, 36, and 72 h (Supplemental Table S3). Total RNA samples were extracted from whole seedlings and used to generate probes labeled with Cy-3 and Cy-5 dyes for microarray hybridization and analysis. At least four experimental replicates from two independent biological samples were used for most time points. We used a 1.8-fold or greater change in expression between two samples as the cut-off for regulated versus not-regulated genes (Supplemental Figure S3). Application of this and other criteria (see "Materials and Methods") resulted in identification of 357 differentially expressed transcription factor genes, which is 26% of the total 1,364

transcription factor genes expressed at the seedling stage for at least one time point checked. Among them, 249 were induced and 115 were repressed by blue light. Interestingly, the expression of seven genes showed early induction followed by repression after longer blue-light exposure, or the reverse.

The total number of expressed transcription factor genes remained relatively constant, regardless of the length of exposure to blue light. In contrast, the numbers of genes induced or repressed by blue light were in general elevated with the increasing length of time for blue-light exposure (Fig. 2). About 18% (64) of all of the blue-light-regulated transcription factor genes have an obvious expression level change within 1 h of blue-light irradiation. Among them, 48 transcription factors were up-regulated and 16 were down-regulated.

Most Families of Transcription Factor Genes Have Blue-Light-Regulated Gene Members

Our results showed that many different types of transcription factor genes are regulated by blue light (Table IV). In particular, members from all nine main transcription factor families in *Arabidopsis*, e.g. AP2/ERF, bHLH, MYB superfamily, C2H2, NAC, HB, MADS, bZIP, and WRKY, are found to be regulated by blue light, with either induction or repression. Some of these families have been previously shown to respond to light. For example, some MYB superfamily genes have been implicated in circadian clock and in developmental control (Stracke et al., 2001), and they are likely to be regulated by blue light. The C2C2/GATA family, whose members have been reported to be light related (Takatsuji, 1999), shows blue-light regulation at an early phase for a large fraction of the family. Of 28 members, the Aux/IAA transcription factor family, however, has eight members that are obviously repressed by blue light. A brief summary of these blue-light-regulated gene numbers with each family are shown in Table IV, and the detailed ratio of blue-light regulation for each gene is included in Supplemental Table S5. The clustering of blue-light-regulated transcription factor genes based on expression patterns during blue-light exposure is shown in Figure 3. The expression patterns of several representative known and predicted transcription factor genes are shown in Supplemental Figure S5.

The Role of Cryptochromes in Mediating Blue-Light Regulation of Gene Expression

To determine the role of the cryptochromes (*cry1* and *cry2*) in blue-light regulation of transcription factor gene expression, we examined the effect of the *cry1cry2* double mutations on transcription factor expression profiles in response to blue light. Consistent with previous reports, the *cry1cry2* null mutant dis-

Table II. Summary of white- or blue-light-induced genes with a ratio over 3-fold in 6-d-old continuous light- versus dark-grown seedlings

Arabidopsis Genome Initiative No.	Accession No.	Expression Ratio		Family	Putative Function
		White	Blue		
At5g61470	AB016887	5.908	3.256	C2H2(Zn)	Putative protein
At3g60870	AL162295	5.705	2.632	Others	Putative protein
At1g35490	AC007887	5.392	7.076	bZIP	Hypothetical protein
At1g32750	AC017118	5.326	3.778	HMG-BOX	Hypothetical protein
At5g35810	AB005236	4.868	2.475	Others	Hypothetical protein
At5g65100	AB013395	4.818	3.568	EIL	Putative protein
At1g19040	AC068602	4.628	3.408	NAC	Hypothetical protein
At4g01120	X63895	4.399	2.467	bZIP	G-Box-binding bZIP transcription factor GBF2/AtbZIP54
At4g04880	AL161501	4.257	1.961	bHLH	Putative adenosine deaminase
At5g10120	AL356332	4.218	4.46	EIL	Transcription factor TEIL-like protein
At1g68520	AC011915	4.164	3.22	CO-like	Putative B-box zinc finger protein
At3g49950	AL132978	4.153	3.638	GRAS	Putative protein
At1g63650	AF027732	4.107	4.72	bHLH	bHLH protein (Atmyc-2)
At3g09000	AC009326	4.08	3.364	DOF	Hypothetical protein
At5g46880	AB013394	4.04	3.235	Others	Hypothetical protein
At4g16110	AB016472	4.038	2.722	ARR-B	Hypothetical protein
At3g15510	AB049071	4.029	2.402	NAC	Putative jasmonic acid regulatory protein (AtNAC2)
At5g37020	AF042196	3.987	3.909	ARF	Auxin response factor 8 (ARF8)
At3g57600	AL049660	3.936	3.628	AP2/EREBP	AP2 transcription factor-like protein
At5g66990	N/A	3.839	2.436	NIN-like	Putative protein
At1g25440	AC079281	3.83	2.106	CO-like	Hypothetical protein
At1g63490	N/A	3.828	3.529	ARID	RB-binding protein-like
At3g19580	AY046043	3.755	3.371	C2H2(Zn)	Putative Cys2/His2-type zinc finger protein 2 (AZF2)
At4g23220	AF386946	3.57	3.606	Others	Hypothetical protein
At3g09030	AC009326	3.552	2.179	MYB	Hypothetical protein
At4g36710	AL161589	3.471	3.552	GRAS	SCARECROW-like protein
At3g02830	AF138743	3.43	1.783	C3H-TYPE1	Zinc finger protein 1 (ZFN1)
At1g80840	AC011713	3.425	2.009	WRKY(Zn)	Transcription factor, putative
At1g16060	AY045915	3.397	2.073	AP2/EREBP	Putative transcription factor CKC protein
At1g34670	AC007894	3.397	1.714	MYB	Putative transcription factor (MYB93)
At1g75520	AC006434	3.36	2.561	Others	Putative lateral root primordium protein LRP1
At3g42790	AL138639	3.352	2.627	ALFIN-LIKE	Nucleic acid-binding protein-like
At2g28350	AC006283	3.35	2.275	ARF	Unknown protein
At1g56160	AC069159	3.231	2.432	MYB	Putative transcription factor (MYB72)
At5g44190	AB005239	3.222	2.242	G2-LIKE	golden2-like transcription factor (GLK2)
At4g37940	AL035538	3.22	2.418	MADS	MADS-box protein AGL17
At5g47660	AB025628	3.188	1.993	MYB	Putative protein
At4g20400	N/A	3.027	3.38	JUMONJI	Putative protein
At5g53200	AB025622	2.529	3.506	MYB	Putative protein
At4g29930	AL050352	2.385	3.301	bHLH	Putative protein
At5g61620	AB012239	2.877	3.257	MYB	Transcriptional activator-like protein
At5g44800	AC002342	2.321	3.054	MYB	Unknown protein
At5g46690	AB016882	1.999	3.181	bHLH	Putative protein

played a long hypocotyl and small cotyledons in blue light, compared with wild-type seedlings (Mockler et al., 1999). A kinetic analysis of blue-light-responsive transcription factor genes in *cry1cry2* seedlings was performed (Supplemental Table S3). Eight or more replicate data sets from two independent biological samples were obtained at each time point for further analysis.

Cluster analysis (Eisen et al., 1998) was used to compare the transcription factor gene expression patterns in response to blue light in wild-type and *cry1cry2* double mutant seedlings. To this end, only those transcription factors with a 1.8-fold or greater differential expression for at least one time point in either set were selected for analysis. As shown in

Figure 3, the expression pattern of blue-light-triggered genes in wild-type seedlings depends highly on cryptochromes, especially after a longer time exposure. These results indicate that cryptochromes play a major role in the blue-light regulation of transcription factor gene expression. This finding is consistent with a recent EST-based microarray study of the role of cryptochromes in gene expression under continuous blue light (Ma et al., 2001).

Still, the *cry1cry2* mutants manage to turn on most of the blue-light-responsive genes with extended time, albeit to a significantly reduced extent. On the basis of our cut-off, only 133 transcription factor genes exhibited differential expression between blue-light-grown 6-d-old wild-type and *cry1cry2* double

Table III. Summary of white- or blue-light-repressed genes with a ratio of 0.33-fold or less in 6-d-old continuous light- versus dark-grown seedlings

Arabidopsis Genome Initiative No.	Accession No.	Expression Ratio		Family	Putative Function
		White	Blue		
At1g04250	U49073	0.165	0.17	AUX/IAA	Putative auxin-induced protein IAA17/AXR3-1
At5g59430	N/A	0.168	0.574	MYB	Telomere repeat-binding protein
At1g13260	AB013886	0.178	0.361	AP2/EREBP	RAV1
At3g17050	AB026636	0.181	0.454	C3H-TYPE2	Unknown Gly-rich protein
At1g74840	AC013258	0.194	0.293	MYB	Putative MYB family transcription factor
At1g56010	AF198054	0.2	0.382	NAC	NAC1
At3g21330	AP001305	0.21	0.632	bHLH	Hypothetical protein
At1g04240	U18406	0.212	0.227	AUX/IAA	Putative auxin-induced protein AUX2-11/IAA3
At5g59780	AF062894	0.217	0.584	MYB	Putative transcription factor (MYB59)
At5g51780	AB010074	0.223	0.523	bHLH	Putative protein
At3g16770	AF003096	0.235	0.234	AP2	AP2 domain containing protein RAP2.3
At3g48530	AJ132317	0.245	0.383	Others	AKIN γ
At3g23030	N/A	0.249	0.325	AUX/IAA	Auxin-inducible gene (IAA2)
At3g01470	AC009325	0.258	0.357	HB	Homeobox-Leu zipper protein HAT5
At3g20470	S47414	0.265	0.617	C3H-TYPE2	Gly-rich protein atGRP-5
At3g01330	N/A	0.267	0.345	E2F/DP	Hypothetical protein
At2g02820	N/A	0.27	0.475	MYB	Putative MYB family transcription factor
At4g37240	AL161591	0.279	0.51	MYB	Putative protein
At5g07450	AL163912	0.283	0.458	Others	Hypothetical protein
At3g28910	AF250339	0.285	0.422	MYB	Putative MYB family transcription factor (HSR1)
At1g26960	AC005508	0.287	0.659	HB	Putative DNA-binding protein
At1g69120	Z16421	0.29	0.585	MADS	AP1
At1g75540	AC006434	0.295	0.667	CO-like	Putative zinc finger protein
At4g36930	AF319540	0.298	0.476	bHLH	SPATULA (SPT)
At1g67030	L39649	0.299	0.456	C2H2(Zn)	Zinc finger protein (ZFP6)
At5g64750	AB025637	0.309	0.352	AP2/EREBP	Putative protein
At5g49450	AB023034	0.31	0.439	bZIP	bZIP protein AtbZIP1
At3g55770	X91398	0.311	0.675	Others	Transcription factor L2
At4g34000	AF093546	0.311	0.736	bZIP	Abscisic acid-responsive elements-binding factor
At1g10480	L39648	0.312	0.393	C2H2(Zn)	Zinc finger protein 5 (ZFP5)
At1g49130	AC016041	0.313	0.456	CO-like	Putative B-box zinc finger protein
At1g20280	N/A	0.315	0.465	HB	Hypothetical protein
At4g36900	AJ002598	0.321	0.493	AP2/EREBP	TINY-like protein
At1g61200	AC002294	0.331	0.477	HB	Hypothetical protein
At5g43840	AB026651	0.333	0.311	HSF	Heat shock transcription factor-like protein
At1g72570	AC010926	0.385	0.228	AP2/EREBP	Putative AP2 domain transcription factor

mutant seedlings, whereas 357 transcription factor genes exhibited differential expression between blue-light- and dark-grown wild-type seedlings at the same stage. The majority of the remaining 224 blue-light-regulated genes in wild-type seedlings were also regulated in cryptochrome double mutants following the same trend, but to a lesser extent and below the 1.8-fold cut-off (Fig. 3; Supplemental Table

S5). The weak but clear blue-light response in the *cry1cry2* mutants can be visualized as fainter green or red colors in the mutant part of the cluster display in Figure 3.

Phototropins Primarily Regulate Expression of a Distinct Group of Transcription Factor Genes

Phototropin proteins in Arabidopsis, *phot1* and *phot2*, are flavin-containing plasma membrane photoreceptors mediating blue-light-induced phototropism and other movement responses (Lin, 2002). To examine whether phototropins play an important role in the blue-light regulation of transcription factor gene expression, we did a similar kinetic analysis of blue-light-grown *phot1phot2* double mutant seedlings compared with wild-type seedlings of the same ecotype. Both mutant and wild-type seedlings were exposed to blue light for 0.5, 3, and 24 h before harvest for gene expression analysis (Supplemental Table S3). Figure 4A shows a cluster analysis of transcription factor gene expression profiles in response to blue light for wild type and for the phototropin

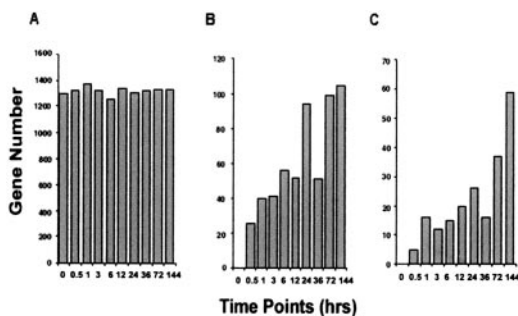


Figure 2. Numbers of transcription factor genes expressed (A), induced (B), and repressed (C) in response to blue-light irradiation in 6-d-old Arabidopsis seedlings.

Table IV. No. of blue-light-regulated known and predicted transcription factor genes categorized by family based on sequence similarity

Genes with mRNA abundance either increased (induced) or decreased (repressed) above 1.8-fold are scored and classified into families. Genes induced or repressed within 1 h of blue-light irradiation were considered as early responded. All genes are counted only once, regardless of extra signature motif(s), thus this table is nonredundant.

Gene Family	Predicted	Induced		Repressed	
		Total	Early Responded	Total	Early Responded
AP2/ERF	127	14	3	17	1
bHLH	108	19	3	4	0
MYB	269	53	12	21	3
C2H2(Zn)	147	23	2	11	1
NAC	107	10	1	5	0
HB	95	10	2	7	4
MADS	93	10	0	1	0
bZIP	88	11	4	6	0
WRKY(Zn)	73	6	1	3	0
GARP:G2-LIKE	53	7	1	1	1
GARP:ARR-B	3	1	0	1	0
C2C2:DOF	34	5	3	0	0
C2C2:CO-like	32	9	6	1	0
C2C2:GATA	7	3	1	1	1
C2C2:YABBY	5	0	0	0	0
CCAAT:HAP2	10	0	0	0	0
CCAAT:HAP3	13	0	0	0	0
CCAAT:HAP5	13	2	0	0	0
GRAS	32	5	0	0	0
Trihelix	8	0	0	1	0
HSF	30	5	3	0	0
TCP	22	1	0	1	0
ARF	22	2	0	1	0
C3H-TYPE1(Zn)	45	3	0	5	0
C3H-TYPE2(Zn)	26	0	0	6	0
SBP	16	4	0	0	0
Nin-like	14	2	0	0	0
ABI3/VP1	11	0	0	0	0
TULP	9	1	1	0	0
E2F/DF	7	1	0	1	1
CPP(Zn)	7	1	0	0	0
Alfin-like	6	1	0	1	0
EIL	5	3	1	0	0
LEAFY	3	0	0	1	0
Aux/IAA	28	1	0	8	2
HMG-box	16	1	0	0	0
ARID	5	1	0	0	0
JUMONJI	8	1	0	1	0
PcG	4	0	0	0	0
Others	263	29	1	8	1
Total	1,864	249	48	115	16

double mutant. There are 104 transcription factor genes showing expression changes equal to or above the 1.8-fold cut-off for at least one of the time points checked. For most of the phototropin-regulated genes, the response to blue light is rapid. Comparison of the transcription factor genes regulated by phototropins (104) with genes regulated by blue light (357) shows only a small group of overlapping genes (16) with similar regulation. Fourteen of these overlapping genes were down-regulated genes, and two were up-regulated. Interestingly, most of the genes

exhibiting differential expression in the phototropin double mutant showed a pattern of regulation that was opposite to that of the cryptochrome double mutant. That is, for those genes that exhibited blue-light induction in a wild-type background, there is a further induction in blue-light-grown phototropin double null mutants instead of a reduction as shown in blue-light-grown cryptochrome mutants. Thus our results suggest that phototropins may antagonize some of the cryptochrome-mediated gene expression in response to blue light.

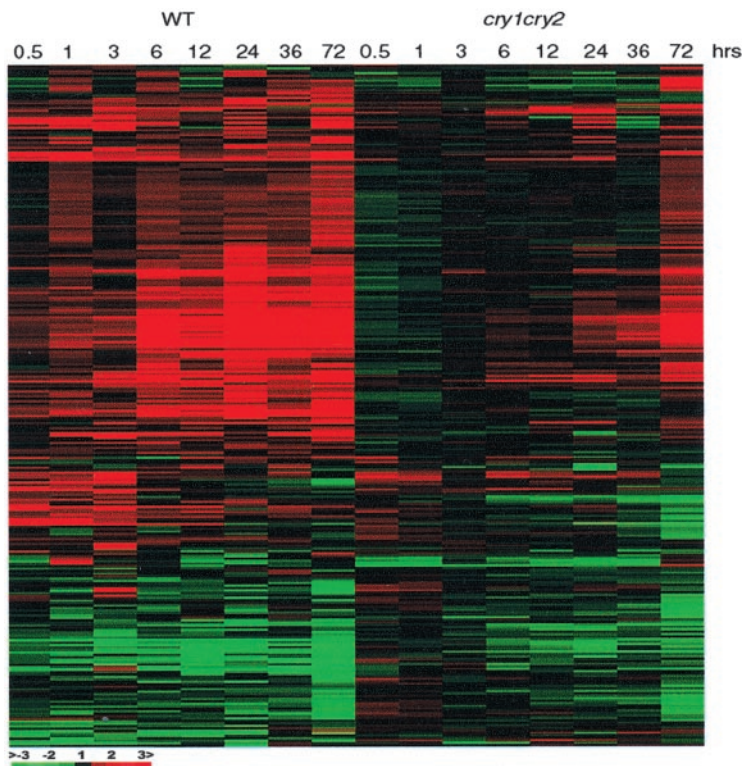


Figure 3. Hierarchical cluster display of transcription factor gene expression changes in response to blue light in wild type (WT/blue versus WT/dark) and in response to cryptochromes in *cry1cry2* mutant seedlings (WT/blue versus *cry1cry2*/blue). Each lane is one time point with the irradiation time length labeled. All genes that exhibited a 1.8-fold or higher differential expression in at least one time point in either wild type or mutant were included.

phyA Plays a Minimal Role in Mediating Blue-Light Regulation of Gene Expression

The classic far-red-light receptor *phyA* has also been reported to act in low intensities of blue light (Somers et al., 1998). We conducted a kinetic experiment similar to those described above to study the role of *phyA* in blue-light regulation of gene expression. Both *phyA* mutant seedlings and wild-type seedlings of the same genetic background were grown in darkness and then transferred to blue light for 0.5, 3, 12, and 24 h before harvest (Supplemental Table S3). A constant blue-light-grown *phyA* mutant was also used to compare with wild-type seedlings. The genes regulated by *phyA* in response to blue light were compared with genes regulated by blue light in wild-type seedlings at the same time points using cluster analysis. As shown in Figures 4B and 1, only a small fraction (30) of transcription factor genes showed significant up-regulation in expression mediated by *phyA* in blue light. The degree in expression changes of those genes as triggered by blue-light exposure seems not to be affected by the duration of light irradiation. It is therefore possible that the expression of this group of genes may be constitutively repressed by *phyA* regardless of light treatment.

DISCUSSION

In this study, we systematically analyzed the genome-scale transcriptional activity of transcription factor genes in *Arabidopsis* seedlings using a

custom-made microarray containing 1,864 known and predicted transcription factor genes. This array allowed us to monitor the expression of almost all genes in an entire functional group in *Arabidopsis*.

The Majority of Transcription Factor Genes Are Expressed in Seedlings

We used whole seedlings, which include different organs and many if not most plant cell types, to maximize the chance of detecting transcription factor expression. We found that 84.7% of known and predicted transcription factor genes were expressed at the seedling stage. We determined that 66.6% of transcription factor genes are transcribed in dark-grown seedlings and 73.6% in white-light-grown seedlings. On the basis of the criteria described above, a total of 84.7% transcription factor genes are expressed in dark- or light-grown seedlings. Our results are in agreement with a variety of other studies on the genome transcription activity in *Arabidopsis* and in other organisms using the microarray approach. For *Arabidopsis* chromosome 2, 84% of predicted genes were expressed in at least one of the selected tissues or physiological conditions examined (Kim et al., 2003). In human, 66% of known and predicted genes were determined to be expressed in any of 69 tissues and disease-specific conditions in chromosome 22q (Shoemaker et al., 2001), 51% in placenta in chromosome 22 (Rinn et al., 2003), and 68% in at least one of 11 human cell lines in chromosomes 21 and 22 (Ka-

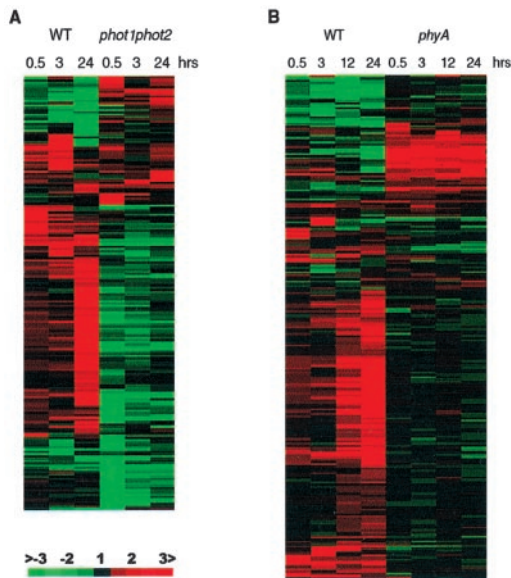


Figure 4. The role of phototropins and phyA in blue-light regulation of gene expression. A, Hierarchical cluster display of expression ratios of transcription factor expression profiles for blue-light-irradiated wild-type seedlings versus dark-grown seedlings (WT) and blue-light-irradiated wild-type seedlings versus blue-light-irradiated *phot1phot2* mutant seedlings (*phot1phot2*); B, blue-light-irradiated wild-type seedlings versus dark-grown seedlings (WT) and blue-light-irradiated wild-type seedlings versus blue-light-irradiated *phyA* mutant seedlings (*phyA*). Each lane is one time point with the irradiation time length labeled. All genes that exhibited a 1.8-fold or higher differential expression in at least one time point were included.

pranov et al., 2002). Our detection rate for Arabidopsis transcription factor gene expression is in line with the overall gene expression percentages reported by others.

We considered both the detection and reproducibility of fluorescent signals in choosing an objective cut-off for gene expression. For the negative controls included in this array, the intensity mean value was 56 ± 104 , based on a total of 180 negative controls from different experiments with the background deduced. The coefficient of variation (CV) of repeats was also used to measure the reproducibility. As shown in Supplemental Figure S2, statistical analysis indicated that signal intensity over an arbitrary unit of 100 would be quite reproducibly detected in our experiments. In this work, we employed a conservative standard of fluorescent intensity unit of 200 as a cut-off for expressed genes. On the basis of an estimate of transcript concentration using yeast non-coding genomic DNA (Ruan et al., 1998), such a cut-off equals a detection level of roughly one to five mRNA copies per cell. Similar protocols and similar signal intensity distributions between our experiments and theirs make this estimation applicable to our results as well. It is worth mentioning that a slight change in the cut-off criteria could cause a considerable change in the number of expressed genes, because the number of genes in each 50 fluo-

rescent intensity unit interval is increasing at the low intensity range (Supplemental Figure S4; Schaffer et al., 2001).

Cross-hybridization is an inherent problem of the DNA fragment-based microarray (Kane et al., 2000). To assess possible cross-hybridization of the closely related family members of transcription factor genes, the PCR-amplified fragments of each gene were blasted against the entire collection of transcription factor genes. Any transcription factor gene pair with 70% or higher identity at the nucleotide level in any given 50-bp window within the PCR-amplified fragments was collected and analyzed. As shown in Supplemental Table S2, a total of 139 pairs of genes could have potential cross-hybridization at the mRNA level.

A Blue-Light-Regulated Transcriptional Cascade

A rapid transcriptional pathway directly targeting light signals to the transcription of primary response genes has been discovered in the phytochrome-signaling network (Quail, 2002). Microarray study showed that 44% of early-responding genes to far-red-light signals are transcription factors (Tepperman et al., 2001). Two transcription factors have been characterized as phytochrome-interacting proteins, PIF3 for both phyA and phytochrome B (phyB; Ni et al., 1998, 1999), and PIF4 for phyB only (Huq and Quail, 2002). PIF3 activates the transcription of several light-responsive genes with G-box sequences in their promoters. Characterized downstream transcription factors, like CCA1 and LHY, further activate the transcription of genes that function in photomorphogenesis and regulate circadian rhythms (Green and Tobin, 2002). It is obvious from this work that far-red- and red-light signals stimulate a direct transcriptional cascade to regulate photomorphogenesis and the circadian clock.

Here, we examined whether blue light is also able to trigger a transcription cascade. Our data suggested that blue light, mainly through the cryptochromes, likely regulates genome expression through a transcriptional cascade. A large portion, 26%, of transcription factors were regulated by blue light, 18% of which responded within 1 h of blue-light irradiation. Many of the transcription factor genes shown to be regulated by blue light are also regulated by far-red light. Those genes include the characterized *HY5*, *CCA1*, *LHY*, and *CONSTANS* genes (Tepperman et al., 2001). This significant overlap between blue-light- and far-red-light-regulated gene expression implies a shared transcriptional regulatory cascade between phyA and cryptochromes. Direct interaction has been reported between phyA and cry1 and between phyB and cry2 at the protein level (Ahmad et al., 1998; Mas et al., 2000). These direct interactions between phytochromes and cryptochromes could be one of the means to achieve regulation of a shared transcription cascade.

Examination of the blue-light-regulated gene expression profile revealed that many transcription factors with similar functions were simultaneously regulated. Many known or putative light response or circadian regulation genes responded to blue light quickly (Fig. 3; supplemental data). Two classic light-regulated transcription factors HY5 and HYH, both members of the bZIP family, were dramatically up-regulated by blue light within 0.5 h. Both HY5 and HYH are able to recognize G-box DNA-binding sites in target gene promoters and regulate deetiolation in light (Ang et al., 1998; Holm et al., 2002). Moreover, HYH is specific to blue light, whereas HY5 responds to all light conditions (Holm et al., 2002). CONSTANS (CO) and COL1, which also show early response to blue light, are characterized members of the light input for circadian regulation and photoperiodic control of flowering-associated CO-like zinc-finger factor subfamily of the C2C2 family (Putterill et al., 1995; Ledger et al., 2001). The MYB superfamily transcription factors CCA1, LHY, and APRR7, which have been shown to be involved in circadian clock regulation (Schaffer et al., 1998; Wang and Tobin, 1998; Sato et al., 2002), displayed rapid transient mRNA level increases followed by a second peak at the end of 24 h after blue-light irradiation (Supplemental Figure S5). The circadian clock itself is known to be regulated by a blue-light signal input (Somers et al., 1998; Harmer et al., 2000). In addition, CCA1 has also been implicated in regulation of light-induced gene expression and is capable of binding to a functionally defined cis-element present in the *CAB* gene promoter region (Wang and Tobin, 1998). It is noteworthy that the MYB superfamily, with many members functioning in light signal transduction (Riechmann, 2002), has the biggest number of transcription factor genes showing an early response to blue light. The GATA and DNA binding with One Finger (DOF) subfamilies of the C2C2 (Zn) family, which have members shown to be light related (Riechmann, 2002), are induced rapidly by blue light. Several transcription factors implicated in drought resistance and abscisic acid (ABA) signal transduction were also early induced by blue light. The AP2/ERF family protein DREB2A, HB protein Athb-12, and bZIP protein ABFs are all important activators in regulating a plant's response to water stress involving ABA-mediated gene expression (Liu et al., 1998; Choi et al., 2000; Lee et al., 2001). After the shift from skotomorphogenic development to photomorphogenic development, more water will be needed for increased respiration and photosynthesis. The activation of water stress-related transcription factor genes coupled with light signals might be a mechanism for seedlings to prepare for the increasing need of water and to avoid low hydrostatic pressure. Nearly 40 other transcription factors without known function also appeared to be involved in rapid blue-light responses and are clearly of interest in future research.

A large percentage of early light-repressed transcription factors are involved in the auxin signal transduction pathway. Auxin is a hormone with a wide variety of effects on plant growth and morphogenesis. One well-known function of auxin is to promote the elongation of stems, but to inhibit root elongation (Kende and Zeevaart, 1997). In *Arabidopsis*, auxin-overproducing mutants have long hypocotyls, epinastic cotyledons, and small leaves (Romano et al., 1995; Zhao et al., 2001). In our experiment, three HB transcription factors HAT2, HAT3, and HAT4, all of which are reported to be induced by auxin (Schindler et al., 1993; Morelli and Ruberti, 2002; Sawa et al., 2002), and IAA3 of the Aux/IAA transcription factor family displayed strong and rapid decreases in mRNA levels under blue light. In addition, six other Aux/IAA family transcription factor genes and NAC1, a transcription factor that is reported to involve in auxin signaling (Xie et al., 2000), are down-regulated after 1 h of blue-light exposure. Together, these results suggest that auxin plays an important role in plant responses to blue light. High levels of auxin response are likely to be needed for skotomorphogenesis to promote hypocotyl cell elongation, to control the expansion of cotyledons, and to inhibit root elongation. Dramatic repression of the auxin-related transcriptional factor genes is coupled to the dramatic and rapid shift of photomorphogenesis in seedling development. Another transcription factor gene detected to be rapidly down-regulated by blue light was Myb30, a MYB superfamily protein that plays a role in the regulation of carbon metabolism (Kleinow et al., 2000). Seedlings will initiate photosynthesis in light, dramatically changing the carbon metabolism based on stored starch during skotomorphogenesis (Muntz et al., 2001; Lemoine, 2000). Our results confirm that seedlings can anticipate carbon metabolism change based on light signals.

A large number of transcription factors controlling diverse developmental processes were blue light activated, but slowly. Well-characterized genes within this group include HB proteins ATHB9 and ATHB14, which are important in the perception of radial positional information in the leaf primordium (McConnell et al., 2001); SCARECROW of the GRAS transcription factor family and ARR11 of the GARP family, both involved in root development (Pysh et al., 1999; Imamura et al., 2003); another HB protein BEH1, which regulates shoot apical meristem development (Smith et al., 2002); squamosa promotor Binding Protein (SBP) family transcription factors SBPL, which function in leaf organogenesis (Cardon et al., 1999); Cystein-rich Polycomb-like Protein (CPP) zinc finger transcription factor TSO1, which is expressed in meristems as well as in leaves and stems (Song et al., 2000); MYB superfamily protein GLABROUS1, which influences trichome development (Herman and Marks, 1989); and MADS box proteins

AGL2 and SPL, expressed at least in flowers (Ma et al., 1991; Yang et al., 1999). Our results agree with observations that seedlings are prepared for further organogenesis under the light, the primary signal for the further developmental steps.

For the majority of transcription factors, however, the function is still missing. Although we have produced expression data for the vast majority of the predicted and known transcription factors, only 60% of them are associated with biochemical function on the basis of sequence similarity (Riechmann, 2002). In fact less than 10% of the transcription factors included in this study have been functionally studied before. It is likely that many important functions of the blue-light-regulated transcription factors and the cellular metabolic and regulatory pathways regulated by them are missing due to the current limited knowledge of transcription factors. However, the expression profiles we have obtained in this study could be an excellent starting point for further investigation.

Cryptochromes Are the Major But Not the Only Blue-Light Photoreceptors for Gene Expression Regulation

Comparison of the clustered transcription factor gene expression profiles of blue-light-regulated as well as cryptochrome-regulated genes revealed a similar pattern of expression for the majority of transcription factor genes (Fig. 3). These whole-genome transcription factor gene expression profiles strongly support previous reports that cryptochromes are the major blue-light photoreceptors mediating blue-light control of genome expression (Ma et al., 2001).

However, there seems some residual level of blue-light regulation even in the cryptochrome double mutant (Fig. 3). Together with the observed partial development of cotyledons, both in shape and color, of the *cry1cry2* double mutant growing in blue light, it is reasonable to suspect that there may be still some level of cryptochrome-like blue-light photoreceptor activity present in the *cry1cry2* mutant. A perusal of the literature suggests that phototropins and phyA might be candidates (Chory, 1997; Briggs and Christie, 2002). Kinetic analysis has also shown that *phot1*, *cry1*, and *phyA*, but not *phyB* affect seedling growth under blue light (Folta and Spalding, 2001a, 2001b). Thus it raised the possibility that these two kinds of photoreceptors also work together with cryptochromes in blue-light control of genome expression. Our efforts to use a blue-light-grown *phot1phot2* double mutant and a *phyA* null mutant to analyze blue-light regulation of gene expression seems not to implicate crucial roles for these photoreceptors in gene expression. Phototropins are mediating some rapid blue-light effects, in agreement with the work of others (Folta and Spalding, 2001a; Baborina et al., 2002), but they are not involved in the majority of the blue-light-regulated gene expression

(Fig. 4A). *PhyA* appears to even has less effect on blue-light control of gene expression in comparison with phototropins (Fig. 4B). It is possible that phototropins and *phyA* perform their function in blue-light response through mechanisms other than regulation of gene expression or that the seedling photomorphogenesis is not the sensitive response to detect the functional role of phototropins and *phyA* in blue-light regulation of gene expression. It is interesting to note that a new cryptochrome family member, cryptochrome 3, has been reported very recently (Brudler et al., 2003; Kleine et al., 2003). Cryptochrome 3 is a photoreceptor located in chloroplasts and mitochondria with DNA-binding affinity. The function of it is not clear yet but provides a another blue-light photoreceptor candidate.

MATERIALS AND METHODS

Microarray Construction

The known and predicted transcription factor genes were selected from the completed Arabidopsis genome sequences in the MIPS Arabidopsis Database (<http://mips.gsf.de/proj/thal/db/index.html>) December 21, 2000 release. Primers with an average length of 20 to 22 bp were designed to produce 300- to 500-bp fragments of exon-rich regions for each selected gene (see Supplemental Table S2 for primer sequences). Each fragment was named using its chromosome locus (e.g. At2g29490). The annotation was based on the same MIPS Arabidopsis Database, with the TIGR Arabidopsis Annotation Database (<http://www.tigr.org/tdb/e2k1/ath1/ath1.shtml>) as supplement. Negative and positive controls were selected from the Arabidopsis Functional Genomics Consortium (AFGC) microarray control set from the Michigan State University DNA Microarray Facility.

As shown in Supplemental Figure S1, the PCR products were amplified from genomic DNA of Arabidopsis Columbia ecotype using the specific primer pairs described above. PCR amplicons were purified by ethanol precipitation. The purified products were resuspended in water, and a sample from each PCR fragment was run on an agarose gel for quality control. More than 95% of the fragments were successfully PCR amplified as single band or multiple bands including the target band, with a DNA concentration above $100 \text{ ng } \mu\text{L}^{-1}$, based on ethidium bromide-staining intensity compared with 1-kb DNA ladder (New England Biolabs, Beverly, MA). For printing, the resuspended PCR fragments were combined 1:1 with dimethyl sulfoxide, and an 8- μL sample of each fragment was transferred to 384-well printing source plates (Whatman, Clifton, NJ). The PCR product was arrayed onto ploy-L-Lys-coated glass slides (Erie, Portsmouth, NH) over a $1.8 \times 1.8\text{-cm}$ area in duplicate using a VersArray ChipWriter Pro System (Bio-Rad, Hercules, CA). Printed slides were allowed to dry at room temperature and were cross-linked at 65 mJ in a Stratalink (Stratagene, La Jolla, CA).

Plant Material and Growth Conditions

The wild-type strain used for white-light and dark gene expression and blue-light/dark gene regulation was Arabidopsis Columbia ecotype. The photoreceptor mutants were *cry1-304cry2-1* in the Columbia ecotype (Mockler et al., 1999), *phot1-101phot2-1* in the Wassilewskija ecotype (Sakai et al., 2001), and *phyA-201* in the Landsberg *erecta* ecotype (Nagatani et al., 1993). Wild-type seeds of the same ecotypes were used to compare with each mutant. Seeds were surface-sterilized and then spread on growth medium agar plates containing 0.3% (w/v) Suc. Seeds were stratified at 4°C for 3 d immediately after plating. Plants were grown in controlled-environment chambers at 22°C. Continuous white light was fluorescent light with an intensity of $150 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Hou et al., 1993), and the continuous blue-light illumination intensity used was $14.2 \mu\text{mol m}^{-2} \text{ s}^{-1}$. For all light shift experiments, seedlings were grown in total darkness before being transferred to blue-light growth chamber for 72, 36, 24, 12, 6, 3, 1, or 0.5 h.

All seedlings were harvested at the 6-d stage, as shown in Supplemental Table S3.

RNA Preparation and Probe Labeling

Whole seedlings were frozen in liquid nitrogen and powdered using a chilled mortar and pestle. Total RNA was isolated using the RNeasy Plant Mini Prep kit (Qiagen, Valencia, CA). At least two independent biological samples for each time point or treatment were prepared for RNA extraction and probe synthesis. The probe-labeling protocols used for this study were modified from those used for EST microarrays (Ma et al., 2002). The total RNA (25 μ g) was labeled by direct incorporation of amino-allyl-modified dUTP (aa-dUTP; Sigma, St. Louis) during reverse transcription. After reverse transcription, template RNA was degraded by RNase treatment. The aa-dUTP labeled cDNAs were purified using a Microcon YM-30 filter (Millipore, Bedford, MA) and resuspended in 0.1 M NaHCO₃. The cDNA probe was further fluorescently labeled by conjugating the monofunctional Cy-3 or Cy-5 dye (Amersham Pharmacia, Piscataway, NJ) to the amino-allyl functional groups. After coupling at room temperature for 45 to 60 min, the labeling reaction was stopped by ethanolamine. The fluorescent dye-labeled probe was separated from unincorporated monofunctional dye using QIAquick PCR Purification kit (Qiagen) and concentrated to a final volume of 3.5 μ L for hybridization using Microcon YM-30 filter.

Slide Hybridization and Scanning

The protocols for microarray hybridization, microarray slide washing, and array scanning were adapted from EST microarray protocols previously described (Ma et al., 2001). Hybridized microarray slides were scanned with a GenePix 4000B scanner (Axon, Foster City, CA), and independent TIFF images for both Cy-3 and Cy-5 channels were normalized first manually and then by GenePix Pro 3.0 Software (Ma et al., 2001, 2002, 2003).

Data Analysis

The GenePix Pro 3.0 output data files for each microarray slide were used to generate the intensities with background deduced and the ratios for each scanned spot. Data from different replicates were further merged to obtain the median using a custom computer program GPMERGE (<http://bioinformatics.med.yale.edu>, for software and manual) and Microsoft Excel. CV, which is a measure of relative dispersion, for all useful replicated spots was calculated for each gene by GPMERGE to check the quality of the spots and slides (GPMERGE manual; Köhler et al., 2003). Average CV of all of the genes for each experiment is listed in Supplemental Table S3.

To objectively determine the transcription factor gene expression level, we used a set of criteria that were applied to each data group individually. Spots with aberrant morphology or internal consistency were removed. With our hybridization and scanning conditions, spots with normalized fluorescence intensity over 200 units after background deduction had measurable signals (Supplemental Figure S4). Spots over this intensity also have a reliable reproducibility (Supplemental Figure S2). Thus we consider genes with an intensity median over 200 units as reliably expressed.

To determine transcription factor genes exhibiting differential expression, we selected genes with expression in at least one channel and an expression ratio of at least 1.8-fold between two channels. We chose the 1.8-fold cut-off instead of the more commonly used 2-fold threshold because transcription factor genes in general show a smaller range of light regulation compared with randomly selected genes using similar biological samples (Supplemental Figure S3; Ma et al., 2001), and a large number of replicates were used in this study. It has also been reported that a cut-off of even 1.4-fold can be used reliably if the data quality is good and there are sufficient replicates (Yue et al., 2001; Zik and Irish, 2003).

The clusters of differentially expressed genes were based on all genes showing more than a 1.8-fold change in expression for at least one time point. Within each group, all of the ratio values were subjected to a log₂-transformation followed by average linkage hierarchical clustering of genes using Cluster and TreeView (Eisen et al., 1998).

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