Development 130, 969-981

doi:10.1242/dev.00281

Analysis of the mutational effects of the *COP/DET/FUS* loci on genome expression profiles reveals their overlapping yet not identical roles in regulating *Arabidopsis* seedling development

Ligeng Ma[1,2], Hongyu Zhao[3] and Xing Wang Deng[1,2,*]

[1]Peking-Yale Joint Center of Plant Molecular Genetics and Agrobiotechnology, College of Life Sciences, Peking University, Beijing 100871, Peoples Republic of China [2]Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT06520-8104, USA
[3]Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut 06520, USA
*Author for correspondence at address[2] (e-mail: xingwang.deng@yale.edu)

Accepted 29 October 2002

SUMMARY

Microarray gene expression profiling was used to examine the role of pleiotropic *COP/DET/FUS* loci as well as other partially photomorphogenic loci during *Arabidopsis* seedling development and genome expression regulation. Four types of lethal, pleiotropic *cop/det/fus* mutants exhibit qualitatively similar gene expression profiles, yet each has specific differences. Mutations in *COP1* and *DET1* show the most similar genome expression profiles, while the mutations in the COP9 signalosome (*CSN*) and *COP10* exhibit increasingly diverged genome expression profiles in both darkness and light. The genome expression profiles of the viable mutants of *COP1* and *DET1* in darkness mimic those of the physiological light-regulated genome expression profiles, whereas the genome expression profiles of representative lethal mutants belong to another clade and significantly diverge from the normal light control of genome expression. Instead, these lethal pleiotropic mutants show genome expression profiles similar to those from seedlings growth under high light intensity stress. Distinct lethal pleiotropic *cop/det/fus* mutants also result in distinct expression profiles in the small portion of genes examined and exhibit similar relatedness in both light and darkness. The partial *cop/det/fus* mutants affected expression of both light regulated and non-light regulated genes. Our results suggest that pleiotropic *COP/DET/FUS* loci control is largely overlapping but also has separable roles in plant development. The partially photomorphogenic loci regulate a subset of photomorphogenic responses as well as other non-light regulated processes.

Supplemental data available on-line

Key words: *Arabidopsis thaliana*, COP/DET/FUS, Seedling development, Genome expression profile

INTRODUCTION

Arabidopsis seedlings follow two distinct development pathways: skotomorphogenesis in the dark and photomorphogenesis in the light. Dark-grown seedlings develop with long hypocotyls, an apical hook, closed cotyledons and undeveloped chloroplast precursors called etioplasts; whereas light-grown seedlings have short hypocotyls, opened cotyledons and developed chloroplasts (Kendrick and Kronenberg, 1994; Deng and Quail, 1999; Neff et al., 2000).

Genetic screens for *Arabidopsis* mutant seedlings exhibiting a light-grown phenotype when grown in darkness have resulted in the identification of both pleiotropic and partially photomorphogenic mutations (Chory et al., 1989; Deng et al., 1991; Hou et al., 1993; von Arnim and Deng, 1996). The pleiotropic constitutive photomorphogenic (COP) or de-etiolated (DET) mutants were also defined in a FUSCA (FUS) mutant screen (Misera et al., 1994; Wei and Deng, 1999). Together ten pleiotropic *COP/DET/FUS* mutant loci have been defined, including *cop1*, *det1*, *cop8*, *cop9*, *cop10*, *fus5*, *fus6/cop11*, *fus11*, *fus12* and *cop16* (Schwechheimer and Deng, 2000). Recent biochemical characterizations of these 10 loci define four biochemical entities: seven of the loci are required for the COP9 signalosome (CSN) biogenesis and three others, COP1, DET1 and COP10 are not (Wei and Deng, 1999; Serino et al., 1999; Deng et al., 2000). Mutations in these 10 loci result in almost complete photomorphogenic development in darkness, suggesting that their gene products act as light-inactivatable repressors of photomorphogenesis (Wei and Deng, 1999; Osterlund et al., 1999). Recently, it was hypothesized that in darkness all of these four defined functional entities are involved in promoting ubiquitination and proteasome-mediated degradation of photomorphogenesis-promoting transcription factors (Osterlund et al., 2000; Holm et al., 2002). Among them, COP1 and COP10 may constitute E3 and E2 activities and act together with the COP9 signalosome in targeting transcription factors for

ubiquitination and subsequent degradation (Suzuki et al., 2002). Intriguingly, defects in a peroxisome protein were recently reported to suppress weak mutations of both *COP1* and *DET1* (Hu et al., 2002).

It has been generally assumed that the photoreceptors perceive and interpret incident light and transduce the signals to modulate light-responsive nuclear genes, which, in turn, direct appropriate developmental responses during photomorphogenesis (Terzaghi and Cashmore, 1995; Puente et al., 1996; Ma et al., 2001; Tepperman et al., 2001). In our previous reports, we verified that the contrasting developmental patterns are mediated primarily by coordinated changes in genome expression (Ma et al., 2001) and that a large proportion of light-controlled genome expression can be achieved by regulating nuclear COP1 activity (Ma et al., 2002). Thus logical questions to ask are whether all these pleiotropic *COP/DET/FUS* loci are acting together to mediate the light control of genome expression and whether they also have additional and/or distinct roles in regulating plant development and genome expression.

The less pleiotropic or partially photomorphogenic mutants included those with short hypocotyls and/or partially open and developed cotyledons. They included mutations in *COP2*, *COP3* and *COP4* that result in partial photomorphogenic cotyledon development (Hou et al., 1993) and mutations in *DET2* and *DET3* that result in partial photomorphogenic hypocotyls as well as cotyledon development in darkness (Chory et al., 1991; Cabrera y Poch et al., 1993). *DET2* has been shown to encode an enzyme in the biosynthetic pathway of the plant hormone brassinosteroid (Li and Chory, 1996). Interestingly, mutants defective in response to the plant hormone auxin have also been reported to have a partial photomorphogenic phenotype (Dharmasiri and Estelle, 2002). It is not clear exactly how these genes contribute to the light regulation of seedling development and whether they can be considered part of the light signaling pathway. It seems feasible that an analysis of their effect on genome expression may provide some insight into their contribution to photomorphogenesis.

However, we also found that the COP9 signalosome was involved in multifaceted developmental processes besides photomorphogenesis (Peng et al., 2001a; Peng et al., 2001b; Schwechheimer et al., 2001; Schwechheimer et al., 2002). The COP9 signalosome directly interacts with SCF type E3 ubiquitin ligases and regulates their activity in tagging substrates for proteasome-mediated degradation (Schwechheimer et al., 2001; Schwechheimer et al., 2002). Clearly, the COP9 signalosome has a role beyond modulating the hypothesized COP1 (E3) and COP10 (E2) pair in controlling photomorphogenesis, but also in regulating other E3 and E2 pairs and, thus, many additional cellular and developmental processes. An E2 also probably associates with more than one E3 (Hellmann and Estelle, 2002), thus COP10 and COP1 may not necessarily work together exclusively in controlling photomorphogenesis. Therefore a systematic comparison of the genome expression profiles controlled by each of these pleiotropic COP/DET/FUS activities may reveal the extent of overlapping, as well as unique roles, in light regulated and other developmental processes.

In a systematic effort to examine the overlapping and distinct roles of these pleiotropic and partially photomorphogenic genes, we utilized a previously described *Arabidopsis* cDNA microarray with 6126 unique genes (Ma et al., 2001; Ma et al., 2002). A comparison of their genome expression profiles to that of light-regulated genome expression revealed the overlapping as well as distinct roles of the pleiotropic COP/DET/FUS proteins. Furthermore, new insights into the contribution of the partially pleiotropic photomorphogenic loci in overall photomorphogenesis, as well as their specific function, can be derived from this genomic analysis.

MATERIALS AND METHODS

Experimental materials

Unless otherwise indicated, the wild-type *Arabidopsis thaliana* used in this study was in the *Arabidopsis thaliana* Columbia and Wassileskija ecotypes. The *cop1-1*, *cop1-6*, *det1-1*, *cop4* and *tir1-1* mutants used in this study were in the Columbia ecotype (Deng et al., 1991; Deng et al., 1992; Deng and Quail, 1992; McNellis et al., 1994; Chory et al., 1989; Hou et al., 1993; Ruegger et al., 1998). *cop1-5*, *det1-6*, *fus6-1*, *cop9-1*, *cop10-1* and *det2* mutants and the *CRY1* overexpression line were in the Wassileskija ecotype (Wei and Deng, 1992; Wei et al., 1994; McNellis et al., 1994; Chory et al., 1991; Pepper et al., 1994; Lin et al., 1996). *cop1-8* was in the Landsberg *erecta* ecotype (McNellis et al., 1994). Surface sterilization and cold treatment of the seeds have been described previously (Ang and Deng, 1994). The N282 overexpression line was in No-0 ecotype (McNellis et al., 1996), and the wild-type No-0 was used for its control. *Arabidopsis* seedlings were grown on plates of growth medium agar containing 1% sucrose. The seedlings were grown in continuous white light or darkness for 6 days. The white-light intensity used was 150 μmol/m^2/second unless otherwise indicated. The wild-type *Arabidopsis* used in the different light intensity experiments was Columbia ecotype. Low light intensity was obtained by putting various layers of kimwipe tissue papers on top of the plate. High-intensity light was obtained by putting the plate under high-intensity fluorescent light in the greenhouse, with the heat filtered out by a 2 cm deep layer of water between the light and the seedlings. The colored-light growth chamber (Percival Scientific E-30LED2/3) had intensities of 16.2 μmol/m^2/second for blue light (470 nm).

The microarray slide used in this study was described previously (Ma et al., 2001; Ma et al., 2002). Each array contains 9,216 EST clones that represented about 6,126 unique genes. For more information, please see our web sites (http://plantgenomics.biology.yale.edu or http://info.med.yale.edu/wmkeck/dna_arrays.htm).

RNA preparation, fluorescent labeling of probe, slide hybridization, washing and scanning

Total RNA was extracted from the whole seedlings using the Qiagen RNeasy Plant Mini Prep kit. For each treatment, at least two independent biological samples were used for RNA preparations and probe synthesis. 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., 2002). After 3-4 hours of incubation at 42°C, the reaction was stopped by adding 5 μl of 0.5 M EDTA and incubating at 94°C for 3 minutes. The RNA in the mix was hydrolyzed by adding 10 μl of 1 M NaOH and incubating at 65°C for 20 minutes. The 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 450 μl of water and spinning through a Microcon YM-30 filter (Millipore, Bedford, MA) for 9 minutes at 11000 ***g***. The flow-through cDNA solution was spun through the same Microcon filter once more. The purified, labeled

probe was concentrated to a final volume of 5-7 μl. Then, the cDNA probe was further labeled with fluorescent dye by conjugating aa-dUTP and monofunctional Cy-3 or Cy-5 dye (Amersham Pharmacia Biotech, Piscataway, NJ) as follows: 1 μl cDNA solution added to 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 incubated at room temperature in the dark for 60 minutes. 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 minutes. The dye-labeled probe was purified from the unincorporated dye molecules by washing through a Microcon YM-30 filter (Millipore, Bedford, MA) three times, as mentioned above. The probes from the designated sample pairs were combined at the last washing. The purified, labeled probe was concentrated to a final volume of 7 μl.

The protocols for hybridization to the *Arabidopsis* microarray, microarray slide washing and scanning were as described previously (Ma et al., 2001; Ma et al., 2002).

Data analysis

The general approaches were as described in our previous work (Ma et al., 2001; Ma et al., 2002) with minor modifications. Briefly, spot intensities were quantified using Axon GenePix image analysis software (GenePix Pro 3.0). The channel ratio was measured with the GenePix median of ratios method and was then normalized using the 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 or more replicated data sets of each experiment. Different quality control procedures were also conducted before data points were averaged from the replicates. First, all spots that were flagged *Bad* or *Not Found* by GenePix software, were not included in the final data analysis. Second, a very simple outlier searching algorithm was incorporated in GPMERGE; those spots that led 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: (1) signals were higher than the backgrounds for both channels; (2) the signal was twofold higher than the background at least for one channel. For those unique genes that have more than one EST clone, we developed a custom computer program to extract a ratio for the gene as described previously (Ma et al., 2002).

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. (Eisen et al., 1998). Only those genes that had more than twofold changes in expression in at least one of the experimental sets were used in the cluster analysis shown in the figures.

RESULTS

Experimental strategy

To reveal the functional relationship among the selected group of genes, we took advantage of an available EST-based microarray (Ma et al., 2001; Ma et al., 2002) and used the expression profiles of the 6126 unique genes in the microarray as molecular markers to examine the effect of their loss-of-function mutations on genome expression during *Arabidopsis* seedling development. The pleiotropic *COP/DET/FUS* loci belong to four biochemical entities: COP1, DET1, COP10 or the COP9 signalosome (Wei and Deng, 1999; Suzuki et al., 2002). We selected *fus6-1* (for CSN1) and *cop9-1* (for CSN8) mutants to represent the COP9 signalosome defect, and the *cop10-1* mutant to represent *COP10*. For *COP1* and *DET1*, we selected both viable (*cop1-1*, *cop1-6* and *det1-1*) and lethal (*cop1-5*, *cop1-8* and *det1-6*) mutants, respectively. To provide insights into the role of the mutants that display partial photomorphogenesis in darkness, we selected *cop4-1* (Hou et al., 1993), *det2-1* (Chory et al., 1991) and *tir1-1* (Ruegger et al., 1998) mutants for a comparative analysis. Appropriate ecotypes of wild type (Col-O, WS and L*er*) were used as controls.

As in our previous studies (Ma et al., 2001; Ma et al., 2002), at least two independent biological samples for each treatment (6-day old dark or light-grown seedlings, Fig. 1A and 1B) were used for RNA preparations. Each RNA preparation was used to generate probes for hybridization to at least two arrays. In this way we ensured that there were at least four quality data sets for each experimental test. The average of the mean value for each gene expression ratio was used for further analysis and comparison. A commonly used clustering analysis (Eisen et al., 1998) was employed to reveal the relatedness of distinct mutant genome expression, and, thus, to extrapolate the functional relationship of the loci defined by the mutations.

The mutations in pleiotropic *COP/DET/FUS* genes have a similar influence over genome expression as light, but with clear distinctions

We first analyzed the genome expression profile changes caused by the selected mutations in darkness (Fig. 1A) and compared those to the light effect on genome expression in wild-type seedling. All the genes that displayed a differential expression of twofold or more in at least one experimental test were selected to undergo clustering and relatedness analysis. Fig. 1C shows the relatedness of the genome expression changes across these selected mutants and wild types for each experimental test based on the calculation of distance matrices. The light-induced genome expression profiles of the two most frequently used wild-type ecotypes (Col-O and WS) were quite similar to each other (Fig. 1C). The genome expression patterns induced by the pleiotropic *cop/det/fus* mutations were also similar. However, there were clear differences conferred by the distinct pleiotropic loci and even the nature of the mutations (viable versus lethal) from the same locus. Among the representative pleiotropic *cop/det/fus* mutants, genome expression profiles in darkness were most similar between those affected by the *cop1* and *det1* mutations (Fig. 1C). Both the viable (weaker) mutants (*cop1-1* and *det1-1*) and lethal mutants (*cop1-5*, *cop1-8* and *det1-6*) of *COP1* and *DET1* showed very similar genome expression patterns. However, the viable *cop1* and *det1* mutant patterns clustered much closer to each other and exhibited significant differences from their respective lethal mutant cluster. Consistent with our previous studies (Ma et al., 2002), the viable *cop1* and *det1* alleles triggered genome expression profiles most closely resembling those of the white light-grown wild-type seedlings. In contrast, the genome expression profiles caused by the three representative lethal mutations in *COP1* and *DET1* in darkness were more diverged from those of the wild type in white light.

The genome expression profiles of the *csn* mutants (*fus6-1 or cop9-1)* and *cop10-1*, each representing one of the other two functional units, exhibited distinct expression patterns from

either the viable or lethal alleles of the *COP1* and *DET1* clusters. Both *cop9-1* and *fus6-1*, which are lethal mutants in subunits 8 and 1 of the COP9 signalosome, induced similar genome expression patterns in darkness and light (data not shown). Thus, only one of them was used to represent lethal *csn* mutants in most of the data analysis. Among the representative lethal *cop/det/fus* mutants for the four functional units, the lethal *csn* mutant genome expression in darkness was most similar to that of white-light induction in wild-type seedlings (Fig. 1C). However, the *csn1*/*fus6-1* genome expression profile in darkness is slightly more diverged from the white-light control of genome expression than the clade for the viable *cop1* and *det1* mutations. The genome expression profile of the lethal mutation in *COP10* (*cop10-1*) in darkness was the most diverged from that of the white light control (Fig. 1C).

As anticipated, the less pleiotropic constitutive photomorphogenic loci, such as *cop4* and *det2* in darkness, triggered more diverged genome expression profiles than any of the pleiotropic *cop/det/fus* mutations when compared to the expression profile in white light [WT/WL vs. WT/D (WT, wild type; WL, white light; D, dark grown)]. Among the three examined, the mutation in *COP4* resulted in an expression profile that was most similar, followed by det2, and *tir1-1* produced the most divergent profile (Fig. 1C).

Functional relationship among pleiotropic *COP/DET/FUS* in light-grown seedlings

We also carried out a similar genome expression relatedness analysis for the pleiotropic *cop/det/fus* mutants in light and compared them with the light control of genome expression (WT/WL vs. WT/D). As shown in Fig. 1D, it was apparent that all the representative lethal pleiotropic *cop/det/fus* mutations have similar genome expression profiles and fall into one general clade. Within this clade, the specific relationship of the genes representing the four biochemical entities (Fig. 1D) is similar to that revealed by their dark genome expression profiles (Fig. 1C), e.g., *det1-6* and *cop1-8* were the most similar to each other, then the COP9 signalosome representative *cop9-1*, and lastly the *cop10-1* mutant. However, the viable mutants of *cop1* and *det1* showed similar genome expression profiles and were significantly diverged from those of the lethal *cop/det/fus* mutants. In fact, the genome expression profiles triggered by viable *cop1* and *det1* mutations in the light (mutant/WL vs. WT/WL) are closely related to those of white-light regulation (WT/WL vs. WT/D). This implies that in light-grown plants, the viable *cop1* and *det1* mutants may simply enhance the control of genome expression by light.

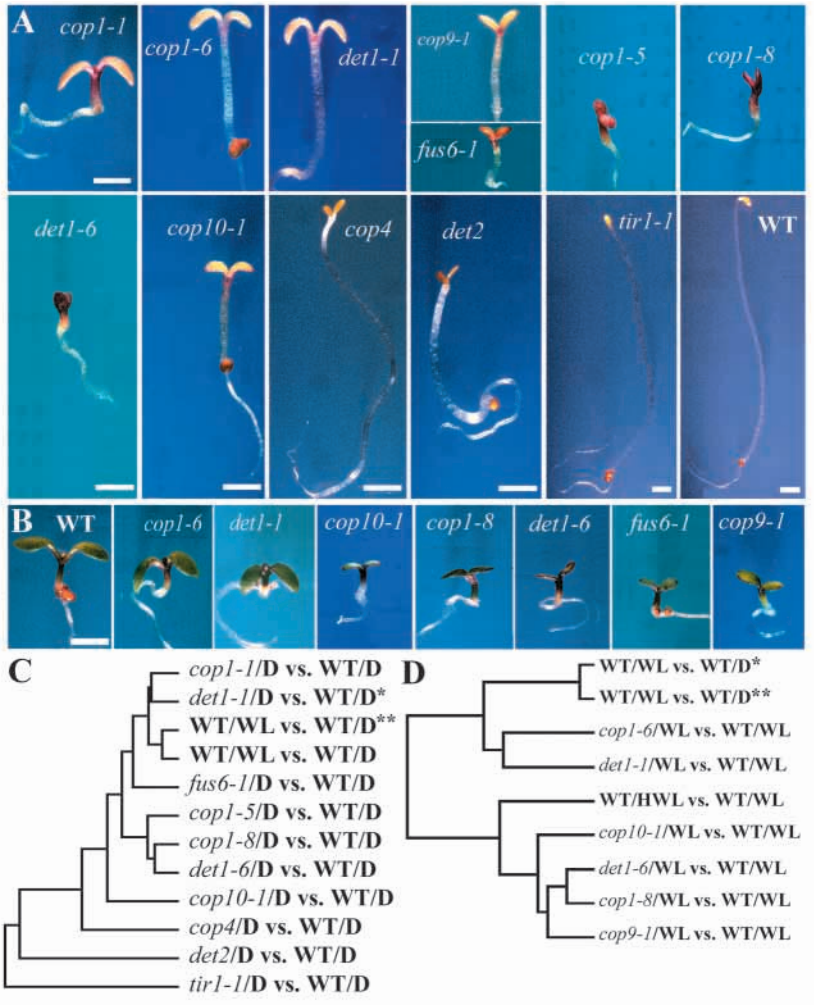

Fig. 1. A summary of the seedling phenotypes of the photomorphogenic mutants and their genome expression profile relatedness. (A) Morphological comparison of dark-grown *Arabidopsis* wild-type (WT), pleiotropic *cop/det/fus* and partial photomorphogenic mutant seedlings. All seedlings were 6-day-old. Scale bars: (in *cop1-1* panel for top row) 1 mm. (B) Morphological comparison of continuous white light-grown wild-type (WT) and pleiotropic *cop/det/fus* mutant seedlings. All seedlings were 6-day-old and photographed at the same magnification. Some of the mutants shown in A and B were used in studies reported in C and D, while some were used in studies reported in subsequent Figures. (C) Hierarchical clustering analysis of overall relatedness for expression ratios from wild-type seedlings grown under normal white light (WL) versus dark-grown (D) siblings, and dark-grown pleiotropic *cop/det/fus* and partial photomorphogenic mutants versus dark-grown wild-type seedlings of the same ecotype. An expression profile from dark-grown *tir1-1* versus dark-grown wild-type seedlings is also included for comparison. Only those genes that exhibited twofold or more differential expression in at least one sample pair of the 13 tested were included. There are 3057 genes included in the cluster analysis (see supplementary data at http://dev.biologists.org/supplemental/ or http://plantgenomics.biology.yale.edu/ for more information). Asterisks denote the ecotype of wild-type *Arabidopsis*: * for Col-0 and ** for WS. (D). Hierarchical clustering analysis of overall relatedness for expression ratios from wild-type seedlings grown under normal white light (WL) versus dark-grown siblings, and high intensity light-grown (HWL) pleiotropic *cop/det/fus* mutants versus normal white light-grown wild-type seedlings of the same ecotype. Only those genes that exhibited twofold or more differential expression at least in one sample pair of the nine tested were included for comparison. There are 2608 genes included in the cluster (see supplementary data at http://dev.biologists.org/supplemental/ or http://plantgenomics.biology.yale.edu/ for more information). Asterisks denote the ecotype of wild-type *Arabidopsis* as in A.

Table 1. Comparison of metabolic pathways regulated by light or the lethal *cop/det/fus* mutations in dark

Strongly activated by light	No obvious difference between light and COP/DET/FUS regulation	Stronger regulation by COP/DET/FUS
Photosynthetic light reactions	Fatty acid biosynthesis	Phenylpropanoid biosynthesis
Photosynthetic carbon assimilation	Fatty acid β oxidation	Protein synthesis in cytoplasm
Starch biosynthesis	Glycoxylate cycle	Water transport across tonoplast
Sucrose biosynthesis	Sulfate assimilation	Water transport across plasma membrane
Photorespiration	Nitrate assimilation	
Chlorophyll synthesis	Ethylene biosynthesis	
	BR biosynthesis	
	Amino acid biosynthesis	
	Glycolysis	
	TCA cycle	
	Protein synthesis in chloroplast	
	Cell wall degradation	
	Starch degradation	
	Cell wall synthesis	

The lethal mutations of pleiotropic *COP/DET/FUS* genes confer distinct effects on different subgroups of light-regulated genes

To reveal what changes in genome expression are responsible for the divergence between the lethal *cop/det/fus* mutations in darkness and white-light control of genome expression (Fig. 1C) and viable *cop1* mutations (Ma et al., 2002), we examined genes belonging to 24 previously defined metabolic pathways that are coordinately up- or down-regulated by light (Ma et al., 2001). As summarized in Table 1, there are clear differential effects of the lethal *cop/det/fus* mutations on the expression of the genes in these pathways. For all pathways directly linked to photosynthesis, including dark and light reactions, starch and sucrose biosynthesis pathways, photorespiration and chlorophyll synthesis, their gene expression in dark-grown lethal *cop/det/fus* mutants are much less activated or even down-regulated, as compared to light activation in wild-type seedlings. However, some pathways, such as phenylpropanoid biosynthesis, cytoplasmic protein synthesis, and water transport across tonoplast and plasma membranes, were more strongly regulated by the lethal *cop/det/fus* mutations in darkness than by white light in wild-type seedlings. For the remaining half of the pathways that are regulated by light, their associated genes seem to be similarly regulated by the lethal *cop/det/fus* mutations in darkness and by light.

The lethal mutations of pleiotropic *COP/DET/FUS* genes mimic a high-intensity light stress effect on light regulated genes

To further examine the effect of the pleiotropic *COP/DET/FUS* group of genes on light regulation of gene expression, we selected 15 genes involved in photosynthetic light reactions and examined their expression levels in various *cop1* or *det1* mutants. Fig. 2A illustrates the expression of 15 genes in the dark-grown viable (weak) mutants of *COP1* (*cop1-6*), *DET1* (*det1-1*), a strong *cop1* mutant (*cop1-1*), lethal mutants of *COP1* (*cop1-8*) and *DET1* (*det1-6)*. All these 15 genes were induced to a certain extent in the dark-grown weak and strong mutants of *COP1* or *DET1* when compared to the dark-grown wild-type seedlings (lanes 2, 3 and 5), but were repressed in both *cop1* and *det1* lethal mutants (lanes 4 and 6).

It has been shown previously that light can exert a quantitative control on photomorphogenesis by proportionally inhibiting nuclear COP1 activity (Osterlund et al., 2000). As the lethal mutant seedlings accumulated high levels of pigments and resembled *Arabidopsis* subjected to high light intensity stress, it is reasonable to hypothesize that the null mutations of *COP1* may mimic the effect of an excess high intensity light environment even when mutants are grown in darkness. To test this hypothesis, we examined whether extremely high light intensity or excess light signaling can cause genome expression profiles in wild-type similar to those of the lethal mutants.

To this end, we employed two experimental approaches. First, we grew wild-type seedlings under normal intensity of white light for 5.5 days, then transferred them to high-intensity white light (HWL; 2500 μmol/m^2/second) for another 12 hours; the resulting genome expression pattern was analyzed. Indeed, the genome expression profile triggered by the high light intensity (WT/HWL vs. WT/D) is closely related to those of the dark-grown lethal mutants, falling between that of the *fus6-1* mutant and those of the lethal mutants in *COP1* or *DET1* (data not shown). The genome expression profile triggered by high intensity white light (2500 μmol/m^2/second) in wild type (WT/HWL vs. WT/WL) is also similar to those caused by the lethal *cop/det/fus* mutations in the light (Fig. 1D). This can be best illustrated by the expression profiles of the 15 selected photosynthetic genes, as their expression levels are all down-regulated by the high-intensity light compared to that of normal light-grown siblings (Fig. 2A, lane 8).

Second, we compared the effect of *CRY1* over-expression (*CRY1 OE*) to that of the lethal *cop/det/fus* mutations on blue-light regulation of genome expression (Ma et al., 2001). In general, the genome expression profile triggered by *CRY1* over-expression is somewhat similar to that of the lethal *cop*/*det/fus* mutations in darkness. In fact, all of the 15 selected photosynthetic genes also exhibited a down-regulation as a result of *CRY1* over-expression when compared to wild-type siblings grown under the same blue-light condition (Fig. 2A, lane 7).

We also analyzed the expression profiles of the 15 selected photosynthetic genes in *cop1* or *det1* mutants grown in normal white light. As shown in Fig. 2B, the light-grown *cop1-6* mutants (the weakest allele) exhibited a slight enhancement of the light activation of all these genes. In the light-grown *cop1-1* and *det1-1* mutants (two other viable mutations), the expression for some genes was slightly enhanced, but most exhibited a slight decrease in light activation. In the lethal

cop1-8 and *det1-6* mutants, the light activation of all 15 genes was completely reversed in these mutants (Fig. 2B, lanes 4 and 6), an effect also caused by the high-intensity light treatment of the normal light-grown wild-type seedlings (Fig. 2B, lane 7).

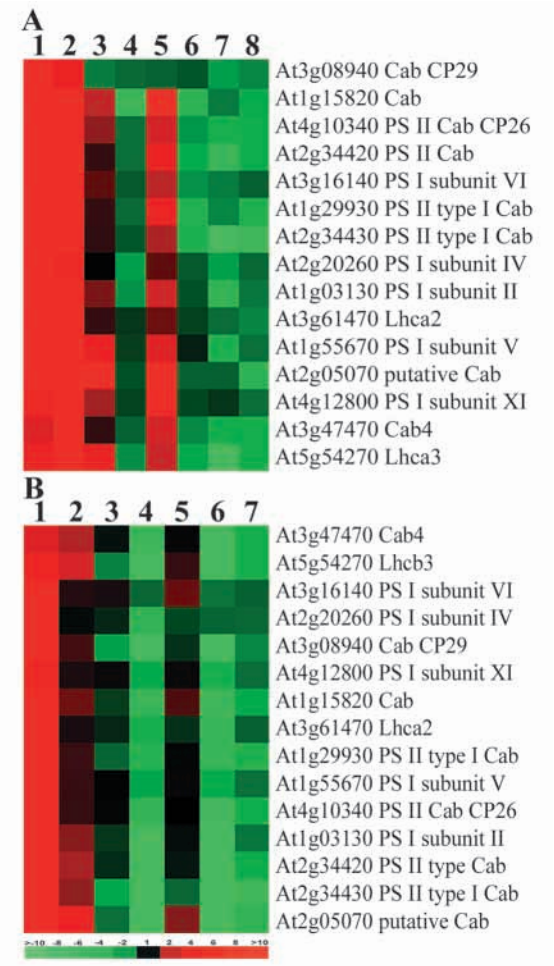

Fig. 2. A comparison of the expression profiles of 15 representative genes encoding photosynthetic light reaction proteins. (A) Hierarchical clustering display of expression ratios from dark-grown pleiotropic *cop/det/fus* mutants seedlings versus dark-grown wild-type seedlings, in comparison with normal and high-light regulation and *CRY1* overexpression affected by blue-light regulation. Lane 1, normal white light and dark-grown wild-type seedlings; lane 2, dark-grown *cop1-6* and wild-type seedlings; lane 3, dark-grown *cop1-1* and wild-type seedlings; lane 4, dark-grown *cop1-8* and wild-type seedlings; lane 5, dark-grown *det1-1* and wild-type seedlings; lane 6, of dark-grown *det1-6* and wild-type seedlings; lane 7, blue light-grown *CRY1 OE* and wild-type seedlings; lane 8, high intensity (2500 μmol/m^2/second) and normal intensity (150 μmol/m^2/second) light-grown wild-type seedlings. (B) Hierarchical clustering display of expression ratios of normal white light-grown pleiotropic *cop/det/fus* mutants seedlings versus white light-grown wild-type seedlings, in comparison with normal white light regulation. Lane 1, white light and dark-grown wild-type seedlings; lane 2, light-grown *cop1-6* and wild-type seedlings; lane 3, light-grown *cop1-1* and wild-type seedlings; lane 4, light-grown *cop1-8* and wild-type seedlings; lane 5, light-grown *det1-1* and wild-type seedlings; lane 6, light-grown *det1-6* and wild-type seedlings; lane 7, high intensity (2500 μmol/m^2/second) and normal intensity (150 μmol/m^2/second) light-grown wild-type seedlings. The color scale for A and B is shown at the bottom of B. Positive numbers represent fold of induction and negative numbers represent fold of repression.

Lethal *cop1* or *det1* mutations induce a new set of genes in both darkness and light

Analysis of the genome expression profiles of light-grown

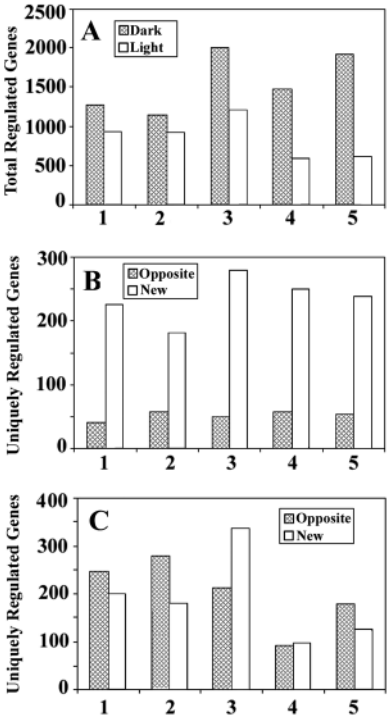

Fig. 3. A comparison of overall genome expression patterns among pleiotropic *cop/det/fus* mutations and high light intensity. (A) The number of differentially expressed genes (≥2-fold regulation) in representative *cop/det/fus* mutations or high-intensity light. Six-day-old dark-grown (hatched bars) and white light-grown (white bars) mutant seedlings were examined. Lane 1, *cop1-8* vs. WT; lane 2, *det1-6* vs. WT; lane 3, *fus6-1* vs. WT; lane 4, *cop10-1* vs. WT; lane 5, high light vs. dark (hatched bar) or normal light (white bar). (B) The number of oppositely regulated and newly regulated genes by the pleiotropic *cop/det/fus* mutations in darkness and high light intensity. Lane 1, *cop1-8*/D vs. WT/D; lane 2, *det1-6*/D vs. WT/D; lane 3, *fus6-1*/D vs. WT/D; lane 4, *cop10-1*/D vs. WT/D; lane 5, WT/HWL vs. WT/D. Only those genes that exhibited twofold or more differential expression in each sample pair and showed oppositely regulation by a *cop/det/fus* mutation and normal light were selected as oppositely regulated genes. Those genes that exhibited twofold or more differential expression in *cop/det/fus* mutants in darkness but did not change (ratio between 0.8 and 1.2) in a normal light sample pair were selected as newly regulated genes. (C) The number of oppositely regulated genes and newly regulated genes by the *cop/det/fus* mutations in normal and high light intensity. Lane 1, *cop1-8*/WL vs. WT/WL; lane 2, *det1-6*/WL vs. WT/WL; lane 3, *fus6-1*/WL vs. WT/WL; lane 4, *cop10-1*/WL vs. WT/WL; lane 5, WT/HWL vs. WT/WL. Only those genes that exhibited twofold or more differential expression in each sample pair and showed opposite regulation between light-grown *cop/det/fus* mutants and light-grown wild type were selected as oppositely regulated genes. Those genes that exhibited twofold or more differential expression in light-grown *cop/det/fus* mutants as compared to wild type but did not change (ratio between 0.8 and 1.2) in normal light versus dark-grown seedlings were selected as newly regulated genes.

lethal *cop/det* mutants revealed a significant fraction of genes whose expressions were oppositely regulated in comparison to that by light. For example, the regulation of more than 250 genes in light-grown lethal *cop1* or *det1* mutants (mutant/WL vs. WT/WL) was found to be opposite that of the same genes from light grown wild-type plants (Fig. 3). Again, the similarity among the pleiotropic *COP/DET/FUS* mutations reflected their relatedness as defined by their genome expression profiles (Fig. 1). The genes that are oppositely regulated in light-grown lethal *cop1* and *det1* mutants are essentially identical. Only a small fraction of genes exhibited opposite regulation by either viable *det1* or *cop1* mutations in light.

In addition to the oppositely regulated genes, there were also genes that were not regulated by light but exhibited significant variation in expression in the lethal *cop/det/fus* mutations. For example, about 200 genes that were not regulated by normal intensity white light in wild type, showed a twofold differential expression in light-grown lethal *cop1* or *det1* mutants (Fig. 3C). Interestingly, about 70% of those genes specifically affected by lethal *cop/det/fus* mutations in normal light are also up- or down-regulated in high-intensity white light in the wild type. This group of genes includes DREB2A (At5g05410), ADP-ribosylation factor (At5g17060) and phospholipase C (At4g38690).

Different light-regulated genes exhibit distinct light intensity dependence in their regulated expression

The clear, distinct effects of the two light intensities on the genome expression profiles described above prompted us to further examine the effects of a large range of light intensities on the genome expression profiles. For this purpose, the wild-type seedlings were grown under 1% (1.5 μmol/m^2/second), 10% (15 μmol/m^2/second) and normal intensity (150 μmol/m^2/second) white light for 6 days. Furthermore, seedlings exposed to high-intensity white light (2500 μmol/m^2/second) for 12 hours after growth in normal intensity white light for 5.5 days were also included in this analysis. The morphology of seedlings are shown in Fig. 4A, and a cluster analysis of their genome expression profiles is shown in Fig. 4B. Among the four increasing light intensities, about 7.7% (471), 15.3% (936), 30.0% (1838) and 31.4% (1922) of genes showed differential expression (≥2-fold) when compared to dark-grown siblings (Fig. 4C). Most of these genes showed the highest variation under normal intensity white light. However, many distinct patterns of light intensity dependence are evident. Twelve representative patterns, with the number of genes following each pattern, are shown in Fig. 5.

Genes involved in photosynthetic light and dark reactions, starch and sucrose synthesis, photorespiration and chlorophyll synthesis pathways were induced even at a very low intensity

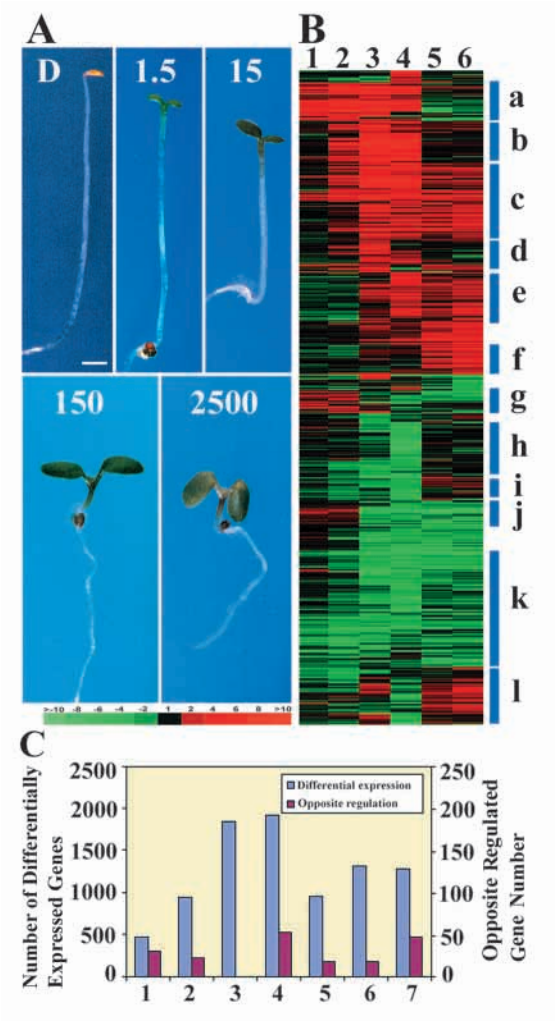

Fig. 4. Comparison of the morphogenic patterns and genome expression profiles of *Arabidopsis* seedlings grown under different light intensity conditions. (A) Phenotype of different intensity white light-grown wild-type seedlings. The seedlings were grown under 1.5 (1.5), 15 (15) or 150 (150) μmol/m^2/second continuous white light or in the dark (D) for 6 days, or under 150 μmol/m^2/second continuous white light for 5.5 days, then transferred to 2500 μmol/m^2/second continuous white light for another 12 hours (2500). Seedlings were photographed at the same magnification. (B) An overview of the hierarchical cluster display of the genome expression profiles for all these different intensity light- and dark-grown wild-type sample pairs is shown in A. The dark-grown *det1-6* or *cop1-8* and wild-type sample pairs were used for comparison. Lane 1, 1.5 μmol/m^{2}second white light- and dark-grown wild-type seedlings; lane 2, 15 μmol/m^{2}second white light- and dark-grown wild-type seedlings; lane 3, 150 μmol/m^2/second white light- and dark-grown wild-type seedlings; lane 4, 2500 μmol/m^2/second white light- and dark-grown wild-type seedlings; lane 5, dark-grown *det1-6* and wild-type seedlings; lane 6, dark-grown *cop1-8* and wild-type seedlings. Only those genes that exhibited twofold or more differential expression in at least one sample pair of the six pairs examined were included for comparison. 2614 genes were included in the cluster analysis (see supplementary data at http://dev.biologists.org/supplemental/ or http://plantgenomics.biology.yale.edu/ for detail). (C) The number of differentially regulated genes by distinct light intensities and different alleles of *COP1*, in comparison with oppositely regulated genes in each case. Lane 1, 1.5 μmol/m^2/second white light- and dark-grown wild-type seedlings; lane 2, 15 μmol/m^2/second white light- and dark-grown wild-type seedlings; lane 3, normal 150 μmol/m^2/second white light- and dark-grown wild-type seedlings; lane 4, 2500 μmol/m^2/second white light- and dark-grown wild-type seedlings; lane 5, dark-grown *cop1-6* and wild-type seedlings; lane 6, dark-grown *cop1-1* and wild-type seedlings; lane 7, dark-grown *cop1-8* and wild-type seedlings. Only those genes that exhibited twofold or more differential expression in each sample pair and showed opposite regulation toward normal light regulation were selected as oppositely regulated genes.

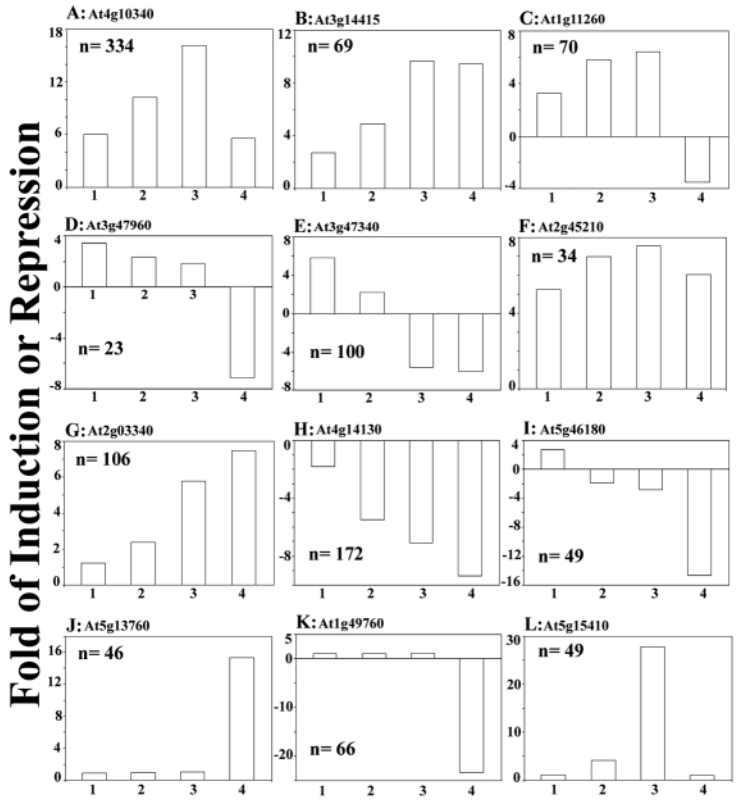

Fig. 5. The 12 distinct response patterns of gene expression to light intensity changes. The profiles of 12 representative genes that showed a different gene expression pattern from wild-type seedlings grown under different intensities of white light. The total number of genes (n) that fit each of the 12 patterns is indicated. The representative genes are: A, At4g10340: chlorophyll a/b binding protein; B, At3g14415: glycolate oxidase; C, At1g11260: glucose transporter; D, At3g47960: peptide transporter PTR1; E, At3g47340: glutamine-dependent asparagine synthetase; F, At2g45210: auxin-regulated protein; G, At2g03340:WRKY DNA-binding protein; H, At4g14130: XET-related protein XTR-7; I, At5g46180: ornithine aminotransferase; J, At5g13760: unknown protein; K, At1g49760: polyA binding protein; L, At5g15410: cyclic nucleotide-gated cation channel. The bars in each graph are: 1, WT/1.5 μmol/m^2/second WL vs. WT/D; 2, WT/15 μmol/m^2/second WL vs. WT/D; 3, WT/150 μmol/m^2/second WL vs. WT/D; 4 WT/2500 μmol/m^2/second WL vs. WT/D.

white light (1.5 μmol/m^2/second). They fall into patterns A, C and F of Fig. 5. Most of these genes reached maximal induction at normal light intensity. Besides genes involved in the metabolic pathways, expression of genes involved in signal transduction, RNA splicing, the auxin-regulated pathway, peptide transport and transcription regulation were also induced in very low intensity white light (1.5 μmol/m^2/second) and some of them were repressed at high light intensity (2500 μmol/m^2/second). Among the 314 genes exhibiting ≥2-fold induction under low light intensity, only 14 of them showed the highest induction at high light intensity (pattern G in Fig. 5). About 24% of low light intensity-induced genes were repressed by high light intensity (patterns C,D,E,I in Fig. 5). The expression of genes encoding phenylpropanoid synthesis and water transport proteins (pattern G or H in Fig. 5) were not affected by very low light intensity (1.5 μmol/m^2/second), but were affected at intermediate light intensities, and reached maximal expression under high light intensity (2500 μmol/m^2/second).

Comparison of the genome expression profiles regulated by different light intensities, together with the expression profiles of two lethal *cop1-8* and *det1-6* mutants (Fig. 4B) revealed different subclusters or expression patterns among different light conditions and mutants. Some of the genes showed similar expression patterns in all 6 tests, such as subclusters c and k. Some genes exhibited opposite expression patterns between low light intensity and lethal *cop1-8* and *det1-6* mutants, such as subclutsers a, e, f, g, i and j. Other genes exhibited different expression patterns between light and the lethal mutations, such as b and h.

A fraction of transcription factor genes are regulated in light intensity-dependent manners

Among the 333 putative transcription factors included in our array, 53 showed differential expression (≥twofold) in at least one light intensity treatment. As shown in Fig. 6A, the expression of the majority of these transcription factors was light intensity dependent. For example, *PAP3* was induced under two low light intensities but was not affected by two higher light intensities. While the expression of two WRKY protein genes was not affected under very low light intensity, they were induced or repressed under higher light intensities. Some transcription factor genes, such as *Pcmyb1*, were similarly regulated by different light intensities, while other transcription factor genes (such as the zinc finger protein At5g58620) were regulated differently in different light intensities.

Interestingly, all these transcription factors also showed differential expression (≥2-fold) in dark-grown *cop1-6* mutant and wild-type seedlings (Ma et al., 2002). The expression pattern triggered by the *cop1-6* mutation in darkness is very similar to that of normal intensity light-grown seedlings, with only two exceptions (At3g59060 and At2g01530, both of which were induced in the dark-grown *cop1-6* mutant but not in normal light). We further checked the expression profiles of these transcription factor genes in different *cop1* mutant alleles (N282, *cop1-6*, *cop1-1* and *cop1-5* to represent very weak, weak, strong and lethal mutations of *COP1*, respectively). As shown by the five representative genes in Fig. 6B, the expression patterns of most of the transcription factor genes behaved in a COP1 activity-dependent manner, and, in general, are consistent with their response to increasing light intensities in wild type. This further substantiates a previous conclusion that increasing the light intensity quantitatively inactivates more COP1 activity, thus promotes stronger photomorphogenesis.

The *COP10* mutation induces high expression of genes involved in cytosolic translation

In comparing the effect of pleiotropic *cop/det/fus* mutations from the four functional groups, we also observed some distinct expression patterns among these groups. As shown in Fig. 3A, the number of distinctly regulated genes in these lethal

mutant seedlings is highly variable, with those of *cop1-8* and *det1-6* being more similar to each other and with *fus6-1* and *cop10-1* diverging. *cop10-1* is, by far, the most diverged in its genome expression profile (Fig. 1C). One striking example is illustrated in Fig. 7, where almost all the genes encoding 60S or 40S ribosomal proteins were much more sensitive to COP10 regulation (lane 5) than any other pleiotropic COP/DET/FUS proteins or to high intensity light. The reason for this is not clear.

The majority of *COP4*-controlled genes are included within light-regulated genes

The *cop4* mutaant belongs to the less pleiotropic or partial photomorphogenic mutants. Seedlings with mutations in the *COP4* locus exhibited open and enlarged cotyledons but long hypocotyls (Hou et al., 1933) (Fig. 1A). Genome expression profile analysis revealed that 496 genes showed twofold or higher differential expression between dark-grown *cop4* and wild-type seedlings, with 246 and 250 genes up- or down-regulated in the mutant, respectively. The genes induced in the *cop4* mutant encode proteins involved in photosynthetic light reactions, carbon assimilation, starch synthesis, sucrose synthesis, photorespiration, chlorophyll synthesis and the TCA cycle. Repressed genes in the *cop4* mutant encoded proteins that are involved in cell wall degradation, water transport across tonoplast and plasma membranes, fatty acid β oxidation, glycoxylate cycle, nitrate assimilation and sulfate assimilation. Most of these COP4-regulated genes are actually light-regulated as well (Ma et al., 2001; Ma et al., 2002). However,

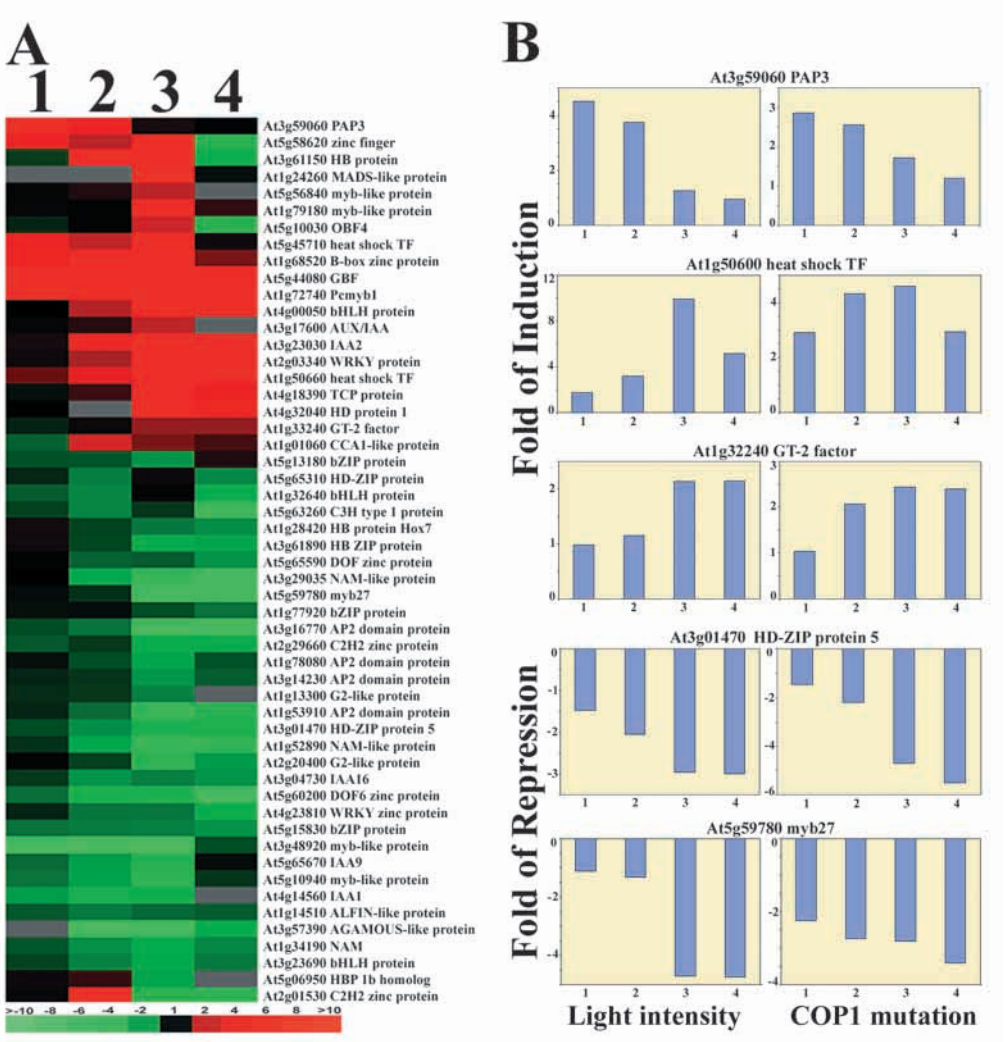

Fig. 6. Clustering analysis of light-regulated transcription factor genes. (A) An overview of the hierarchical cluster display for those transcription factor genes in our microarray regulated by light and COP1. Only those genes that exhibited twofold or more differential change in at least one sample pair of the four light intensity pairs tested were included for comparison. There are 53 genes included in the cluster (see supplementary data at http://dev.biologists.org/supplemental/ or http://plantgenomics.biology.yale.edu/ for detail). The gray color indicates that high quality data was not available. The lanes are as follows: 1, WT/1.5 μmol/m^2/second WL vs. WT/D; 2, WT/15 μmol/m^2/second WL vs. WT/D; 3, WT/150 μmol/m^2/second WL vs. WT/D; 4 WT/2500 μmol/m^2/second WL vs. WT/D. (B) The expression profiles of 5 representative genes from wild-type seedlings grown at four different light intensities and dark-grown *cop1* alleles. The four lanes in the left panels of B are the same as in A, for the four light intensities used. The four lanes in the right panels of B are different *cop1* alleles with increasing severity. Lane 1, dark-grown N282 and wild-type seedlings; lane 2, dark-grown *cop1-6* and wild-type seedlings; lane 3, dark-grown *cop1-1* and wild-type seedlings; lane 4, dark-grown *cop1-5* and wild-type seedlings.

Fig. 7. Hierarchical clustering display of expression profiles of cytosolic ribosomal protein genes from normal light grown and pleiotropic *cop/det/fus* mutations in darkness. Lane 1, expression ratios of white light and dark-grown wild-type seedlings; lane 2, dark-grown *det1-6* and wild-type seedlings; lane 3, dark-grown *cop1-8* and wild-type seedlings; lane 4, dark-grown *cop9-1* and wild-type seedlings; lane 5, dark-grown *cop10-1* and wild-type seedlings. RPL, 60S ribosomal protein genes; RPS, 40S ribosomal protein genes.

the number of differentially expressed genes controlled by COP4 is much fewer than those of light or pleiotropic COP/DET/FUS protein.

To further characterize these COP4-regulated genes, we compared gene expression patterns in *cop4* with the differential gene expression patterns of light-grown wild type and dark-grown *cop/det/fus* mutant seedlings in a cluster analysis (Fig. 8A). The vast majority of the COP4-regulated genes showed qualitatively similar expression in white light or the pleiotropic *cop/det/fus* mutations. In fact, about 72% and 70% of the up-regulated genes that displayed twofold or more differential expression in the *cop4* mutants were included in the white light- or *det1-1*-induced genes, respectively. Likewise, about 73% and 72% of the down-regulated genes displaying ≥2-fold differential expression in the *cop4* mutants were included in the white light or *det1-1* repressed genes, respectively. Less than 5% of COP4-regulated genes show opposite regulation in most pleiotropic *cop/det/fus* mutants, with only the *cop10-1* mutant exhibiting a higher percentage of opposite regulation (Fig. 8C,D). These results support a hypothesis that *COP4* is a locus involved in regulating a subset of photomorphogenic processes (Hou et al., 1993).

A BR synthesis mutation induces a partially overlapping set of light-regulated genes in the dark

We also examined the genome expression profile of another representative partially photomorphogenic locus, *DET2*, which encodes an enzyme involved in brassinosteroid biosynthesis (Li et al., 1996). Dark-grown *det2* mutants had a partially photomorphogenic phenotype with short hypocotyls and small opened cotyledon without an apical hook (Chory et al., 1991) (Fig. 1A). We observed that 253 genes in our array showed twofold or higher differential expression between dark-grown *det2* mutant and wild-type seedlings, while 156 and 97 genes were up- or down-regulated in dark-grown *det2* mutant seedlings, respectively. A comparison of the genome expression profiles between *det2* mutants and light or pleiotropic *cop/det/fus* mutants showed that less than half of the DET2-regulated genes exhibited the same expression pattern with light and pleiotropic COP/DET/FUS regulation. Those genes regulated by both DET2 and light included the genes for the photosynthetic light reaction, cell wall degradation, water transport across the tonoplast, sulfate assimilation and fatty acid β oxidation. Of the approximately 50% of DET2-regulated genes in darkness that were not similarly regulated by light or pleiotropic *cop/det/fus* mutations in the darkness, a small fraction were oppositely regulated by light and/or pleiotropic *cop/det/fus* mutations (Fig. 8E,F). Our result suggested that brassinosteroid also regulates the expression of other unrelated genes in addition to a small subset of light-regulated genes.

DISCUSSION

An evolutionarily conserved group of either pleiotropic or less pleiotropic COP/DET/FUS proteins was initially defined by their ability to repress photomorphogenesis in *Arabidopsis*. Genetic analysis of light control of *Arabidopsis* seedling development revealed that the *COP/DET/FUS* genes play a key role in integrating light signals and modulating developmental pattern formation. In this study, we have systematically investigated COP/DET/FUS-controlled genome expression during *Arabidopsis* seedling development using a cDNA microarray. Our results substantiate the working model build upon molecular genetic studies from the past and provide several new insights regarding the COP/DET/FUS control of gene expression and plant development patterns.

Pleiotropic COP/DET/FUS protein-regulated genome expression exhibits a large overlap with that of light

Phenotypically, all pleiotropic *cop/det/fus* mutants showed an almost complete light-grown response when they were grown in the dark (Wei and Deng, 1999) (Fig. 1A). In this genome expression profiling study, we also found that individual pleiotropic COP/DET/FUS proteins control a largely overlapping set genes; the majority of COP/DET/FUS-regulated genes (more than 80%) overlapped with light-regulated expression at seedling stage as judged by the gene expression profiles. The gene products of the less pleiotropic COP/DET loci also controlled variable fractions of the light-controlled genome expression. So, this genomic study provides an explanation for why mutations in these loci lead to a

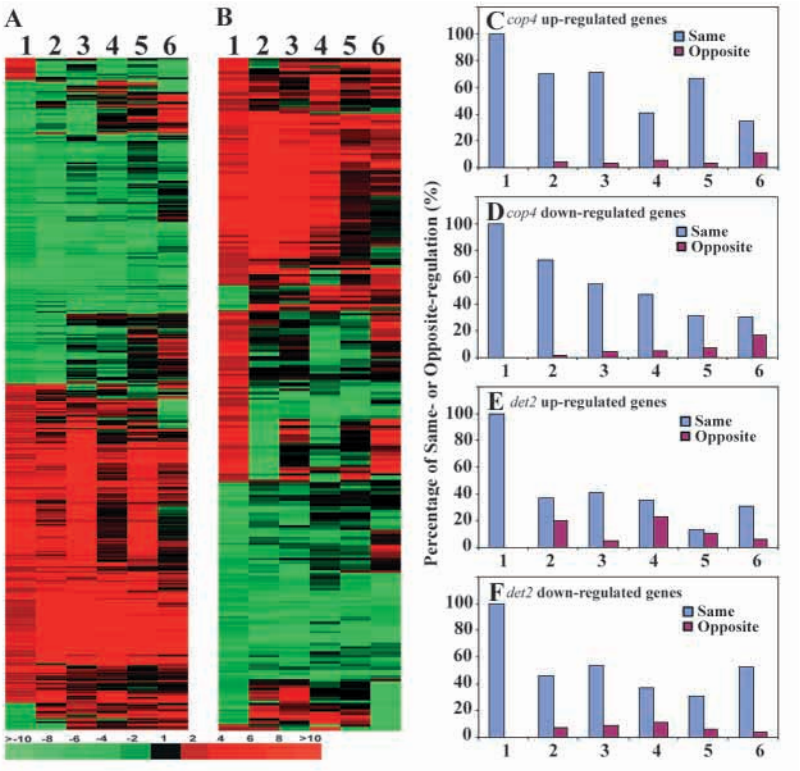

Fig. 8. Comparison of genome expression profiles of COP4 and DET2 with the pleiotropic *cop/det/fus* mutants and light regulation. (A) Hierarchical clustering display of expression profiles for *cop4*, wild type and four representative pleiotropic *cop/det/fus* mutants. The six lanes in the cluster analysis are: 1, *cop4*/D vs. WT/D D; 2, *det1-1*/D vs. WT/D; 3, WT/WL vs. WT/D; 4, *cop1-1*/D vs. WT/D; 5, *cop9-1*/D vs. WT/D; 6, *cop10-1*/D vs. WT/D. Those genes that exhibited twofold or more differential expression in *cop4* mutant and corresponding genes in other pairs were included for comparison. A total of 496 genes were included in the cluster analysis. (B) Hierarchical clustering display of expression profiles for *det2*, wild type and four representative pleiotropic *cop/det/fus* mutants. The six lanes are: 1, *det2*/D vs. WT/D; 2, WT/WL vs. WT/D; 3, *cop9-1*/D vs. WT/D; 4, *det1-1*/D vs. WT/D; 5, *cop1-1*/D vs. WT/D; 6, *cop10-1*/D vs. WT/D. Only those genes that exhibited twofold or more differential expression in the *det2* mutant were included for comparison. A total of 253 genes were included in the cluster. (C,D). The percentage of up- (C) or down-regulated (D) genes (exhibiting twofold or more differential expression) in the *cop4* mutant that are similarly or oppositely regulated by light or each of the 4 representative pleiotropic *cop/det/fus* mutations. The six sample pairs selected (bars 1-6) are the same as in the lanes in A. (E,F) The percentage of up- (E) or down-regulated (F) genes (exhibiting a twofold or more differential expression) in the *det2* mutant that were similarly or oppositely regulated by light or each of the 4 representative pleiotropic *cop/det/fus* mutations. The six bars in the diagram correspond to the lanes in B.

pleiotropic or partial light-grown phenotype when grown in the dark. This is because mutations of an individual pleiotropic COP/DET/FUS locus will de-repress almost the whole set of light-controlled genes, while mutations of a partial COP/DET locus will de-repress a partial set of light-controlled genes. It is not surprising that the former will have an almost completely light-grown phenotype in the dark, while in the later case, the mutations will lead to a partial light-grown phenotype in the dark. This result also supports the notion that contrasting light-controlled developmental patterns are mediated primarily by the change in light-regulated gene expression.

COP1 and DET1 control highly similar genome expression profiles and may function in the close proximity in the pathway

The ten pleiotropic *COP/DET/FUS* loci represent four biochemical entities: the COP9 signalosome, COP1, COP10 and DET1. Recently, it has been proposed that COP1 functions as a putative component of an ubiquitin protein ligase (E3) (Osterlund et al., 2000). *COP10* encodes an ubiquitin-conjugating enzyme (E2) variant (Suzuki et al., 2002) and the COP9 signalosome is structurally similar to the lid of the 19S regulatory particle of the 26S proteasome (Wei and Deng, 1999). While *DET1* encodes a novel nuclear-localized protein (Pepper et al., 1994), its specific biochemical function is not known. These proteins appear to work together to mediate the degradation of photomorphogenesis-promoting transcription factors (Holm et al., 2002).

In this study, we found that among these pleiotropic mutants, *cop1* and *det1* mutant seedlings have the most closely related genome expression profiles both in dark and light growth conditions (Fig. 1C,D). This is true for both the viable mutations or the lethal mutations of *COP1* and *DET1*. This genomic evidence supports the conclusion that COP1 and DET1 work closely together as observed in a previous genetic analysis (Ang et al., 1994). COP1 and DET1 may, therefore, act very closely in repressing photomorphogenesis. Thus signals perceived by photoreceptors act to negatively regulate COP1 and DET1 and relieve their repression of photomorphogenesis.

Light signals can quantitatively regulate the pleiotropic COP1 and DET1 proteins and, thus, the degree of photomorphogenic response

Besides acting as signals to regulate many developmental processes, light also serves as the source of energy for plant photosynthesis. Plants adjust the structure and function of the photosynthetic apparatus in response to changes in their growth environment. The change in the size of the chlorophyll antennae associated with photosystem I and II is very sensitive to light (Niyogi, 1999). Under limiting irradiance conditions, the photosystems acquire large chlorophyll antennae and more extensive thylakoid membrane to absorb more light; while under high irradiance, especially under light stress, chlorophyll antennae sizes are reduced in order to protect the photosynthetic apparatus from damage by repressing LHC gene expression (Escoubas et al., 1995; Maxwell et al., 1995) and/or increasing LHC protein degradation (Lindahl et al., 1995). Also, under light stress, plant cells express some genes encoding specific stress proteins with possible protective functions (Dunaeva and Adamska, 2001).

COP/DET/FUS proteins act as repressors of light-controlled

Arabidopsis seedling development (Wei and Deng, 1999; Osterlund et al., 1999). We have found that light can largely achieve its control of genome expression by negatively regulating COP1 (Ma et al., 2002) and DET1 protein (this work). Therefore, complete loss of COP1 or DET1 function would mimic the action of light stress even in the dark. To provide direct evidence for this, we first checked the expression of representative genes encoding chlorophyll antennae and thylakoid membrane proteins. We found that the expression of these genes was induced by normal intensity light (and even at a very low intensity) and in a dark-grown weak mutant of *cop1* or *det1*, but was repressed in a dark-grown null mutant of *cop1 (cop1-8)* or *det1 (det1-6)* (Fig. 2A). The expression of these genes was also dependent on a COP1 activity in weak, strong and null mutants of *cop1* (Fig. 2A). In addition, all these genes were repressed under high intensity white light (light stress) (Fig. 2A). Second, a predominant portion (80%) of the genes that showed opposite regulation in dark-grown *cop1* or *det1* null mutants compared to normal light regulation also had a similar expression pattern to that resulting from light stress. Some of the light stress-induced marker genes, e.g. late embryogenesis abundant protein (Dunaeva and Adamska, 2001), metallothionein (Dunaeva and Adamska, 2001), HSP70 (Schroda et al., 1999), glutathione reductase (Karpinski et al., 1997) and NAD(P)H dehydrogenases (Endo et al., 1999), were also induced in dark-grown *cop1* or *det1* null mutants. Third, we found that the genes encoding chlorophyll antennae and thylakoid membrane proteins began to be less induced in weak *cop1* mutants, repressed in light-grown strong *cop1* or weak *det1* mutants, and severely repressed in *cop1* or *det1* null mutants (Fig. 2B). Again, more than 80% of the genes that showed opposite regulation in light-grown *cop1* or *det1* null mutants to normal light grown seedlings had a similar expression pattern under light stress. Fourth, comparison of the whole genome expression profiles among pleiotropic *cop/det/fus* mutants and normal and high intensity light induction indicated that the genome expression pattern induced by high light intensity is similar to that of lethal mutants of pleiotropic *cop/det/fus* (Fig. 1 C,D). These genomic results are consistent with the physiological observation that the very short hypocotyls and reduced cotyledon with accumulation of anthocyanin (Fig. 1A) of the *cop/det/fus* mutants are also commonly observed in the plants grown under high intensity light stress. The lethal (null) mutants of *COP/DET/FUS* loci essentially exhibited light stress responses even when grown in the dark.

The *COP/DET/FUS* loci have overlapping yet non-identical roles in regulating *Arabidopsis* seedling development

For those genes that showed opposite regulation between light- and dark-grown *cop1* or *det1* null mutants to regulation by light, most of them do not show opposite regulation in dark-grown *fus6-1*, *cop9-1* or *cop10-1* mutant seedlings. While in light *fus6-1*, *cop9-1* or *cop10-1* mutant seedlings share similar oppositely-regulated genes as the *cop1* and *det1* null mutants or seedlings subjected to light stress, as compared to light regulation. For example, in *fus6-1*, *cop9-1* and *cop10-1* mutants, the genes encoding proteins involved in photosynthetic light reactions were induced in the dark-grown mutant seedlings, whereas they were repressed in dark-grown *cop1-5*, *cop1-8* and *det1-6*. However, the expression of these representative genes was repressed in all light-grown lethal pleiotropic *cop/det/fus* mutant seedlings. All these characteristics that *fus6-1*, *cop9-1* or *cop10*-1 mutants exhibited were similar to some viable *cop1* or *det1* mutants.

In addition, we found that individual pleiotropic COP/DET/FUS proteins also regulated distinct sets of genes. For example, *cop10-1* mutants induced higher expression of cytosolic ribosomal proteins than other pleiotropic *cop/det/fus* mutants (Fig. 6). These results suggest that the pleiotropic COP/DET/FUS proteins, especially the COP9 signalosome and COP10, also function in other developmental processes besides seedling photomorphogenesis.

We thank Drs Elizabeth Strickland and James Sullivan for reading and commenting on this manuscript. We are grateful to the Yale DNA microarray laboratory of the Keck Biological Resource Center for the production of the microarray used in this study (http://info.med.yale.edu/wmkeck/dna_arrays.htm). Our research was supported by a grant from the National Science Foundation of China (#39725002) and grants from National Institutes of Health (GM-47850 to X. W. D. and GM59507 to H. Y. Z.). L. M. is a long-term postdoctoral fellow of the Human Frontier Science Program.

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.

Cabrera y Poch, H. L., Peto, C. A. and Chory, J. (1993). A mutant in the Arabidopsis *DET3* gene uncouples photoregulated leaf development from gene expression and chloroplast biogenesis. *Plant J.* **4**, 671-682.

Chory, J., Nagpal, P. and Peto, C. A. (1991). Phenotypic and genetic analysis of *det2*, a new mutant that affects light-regulated seedling development in Arabidopsis. *Plant Cell* **3**, 445-459.

Chory, J., Peto, C., Feinbaum, R., Pratt, L. and Ausubel, F. (1989). *Arabidopsis thaliana* mutant that develops as a light-grown plant in the absence of light. *Cell* **58**, 991-999.

Deng, X. W. and Quail, P. H. (1992). Genetic and phenotypic characterization of *cop*1 mutants of *Arabidopsis thaliana*. *Plant J.* **2**, 83-95.

Deng, X. W. and Quail, P. H. (1999). Signalling in light-controlled development. *Semin. Cell Dev. Biol.* **10**, 121-129.

Deng, X. W., Caspar, T. and Quail, P. H. (1991). *cop1*: a regulatory locus involved in light-controlled development and gene expression in Arabidopsis. *Genes Dev.* **5**, 1172-1182.

Deng, X. W., Dubiel, W., Wei, N., Hofmann, K., Mundt, K., Colicelli, J., Kato, J., Naumann, M., Segal, D., Seeger, M., Glickman, M., Carr, A. and Chamovitz, D. A. (2000). Unified nomenclature for the COP9 signalosome and its subunits: an essential regulator of development. *Trends Genet.* **16**, 202-203.

Deng, X. W., Matsui, M., Wei, N., Wagner, D., Chu, A. M., Feldmann, K. A. and Quail, P. H. (1992). *COP1*, an Arabidopsis regulatory gene, encodes a protein with both a zinc-binding motif and a G beta homologous domain. *Cell* **71**, 791-801.

Dharmasiri, S. and Estelle, M. (2002). The role of regulated protein degradation in auxin response. *Plant Mol. Biol.* **49**, 401-409.

Dunaeva, M. and Adamska, I. (2001). Identification of genes expressed in response to light stress in leaves of *Arabidopsis thaliana* using RNA differential display. *Eur. J. Biochem.* **268**, 5521-5529.

Endo, T., Shikanai, T., Takabayashi, A., Asada, K. and Sato, F. (1999). The role of chloroplastic NAD(P)H dhydrogenease in photoprotection. *FEBS Lett.* **457**, 5-8.

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.

Escoubas, J. M., Lomas, M., LaRoche, J. and Falkowski, P. G. (1995). Light intensity regulation of *cab* gene transcription is signaled by the redox state of the plastoquinine pool. *Proc. Natl. Acad. Sci. USA* **92**, 10237-10241.

Hellmann, H. and Estelle, M. (2002). Plant development: Regulation by protein degradation. *Science* **297**, 793-797.

Hou, Y., von Arnim, A. G. and Deng, X. W. (1993). A new class of Arabidopsis constitutive photomorphogenic genes involved in regulating cotyledon development. *Plant Cell* **5**, 329-339.

Hu, J., Aguirre, M., Peto, C., Alonso, J., Ecker., J. and Chory, J. (2002). A Role for peroxisomes in photomorphogenesis and development of *Arabidopsis*. *Science* **297**, 405-409.

Holm, M., Ma, L. G., Qu, L. J. and Deng, X. W. (2002). Two interacting bZIP proteins are direct targets of COP1-mediated control of light-dependent gene expression in Arabidopsis. *Genes. Dev.* **16**, 1246-1259.

Karpinski, S., Escobar, C., Karpinska, B., Creissen, G. and Mullineaux, P. M. (1997). Photosynthetic electron transport regulates the expression of cytosolic ascorbate peroxidase genes in Arabidopsis during excess light stress. *Plant Cell* **9**, 627-640.

Kendrick, R. E. and Kronenberg, G. H. M. (1994). *Photomorphogenesis in plants*, 2nd ed. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Li, J, Nagpal, P., Vitart, V., McMorris, T. C. and Chory, J. (1996). A role of brassinosteroids in light-dependent development of Arabidopsis. *Science* **272**, 398-401.

Lindahl, M., Yang, D. H. and Anderson, B. (1995). Regulatory proteolysis of the major light-harvesting chlorophyll *a/b* binding protein of photosystem II by a light-induced membrane-associated enzymic system. *Eur. J. Biochem.* **231**, 503-509.

Lin, C., Ahmad, M. and Cashmore, A. R. (1996). Arabidopsis cryptochrome 1 is a soluble protein mediating blue light-dependent regulation of plant growth and development. *Plant J.* **10**, 893-902.

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, 2383-2412.

Ma, L., Li, J., Qu, L., 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.

Maxwell, D. P., Laudenbach, D. E. and Huner, N. P. A. (1995). Redox regulation of light-harvesting complex II and *cab* mRNA abundance in *Dunaliella salina*. *Plant Physiol.* **109**, 787-795.

McNellis, T. W., Torii, K. U. and Deng, X. W. (1996). Expression of an N-terminal fragment of COP1 confers a dominant-negative effect on light-regulated seedling development in Arabidopsis. Plant Cell **8**, 1491-1503**.**

McNellis, T. W., von Arnim, A. G., Araki, T., Komeda, Y., Misera, S. and Deng, X. W. (1994). Genetic and molecular analysis of an allelic series of *cop1* mutants suggests functional roles for the multiple protein domains. *Plant Cell* **6**, 487-500.

Misera, S., Mueller, A. J., Weiland-Heidecker, U. and Juergens, G. (1994). The *FUSCA* genes of Arabidopsis: negative regulators of light responses. *Mol. Gen. Genet.* **244**, 242-252.

Neff, M. M., Fankhauser, C. and Chory, J. (2000). Light: an indicator of time and place. *Genes Dev.* **14**, 257-271.

Niyogi, K. K. (1999). Photoprotection revisited: genetic and molecular approaches. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **50**, 333-359.

Osterlund, M. T., Ang, L. H. and Deng, X. W. (1999). The role of COP1 in repression of Arabidopsis photomorphogenic development. *Trends Cell Biol.* **9**, 113-118.

Osterlund, M. T., Hardtke, C. S., Wei, N. and Deng, X. W. (2000). Targeted destabilization of HY5 during light-regulated development of Arabidopsis. *Nature* **405**, 462-466.

Peng, Z., Serino, G. and Deng, X. W. (2001a). A role of Arabidopsis COP9 signalosome in multifaceted developmental processes revealed by the characterization of its subunit 3. *Development* **128**, 4277-4288.

Peng, Z., Serino, G. and Deng, X. W. (2001b). Molecular cgaracterization of subunit 6 of the Cop9 signalosome and its role in multifaceted developmental processes in Arabidopsis. *Plant Cell* **13**, 2393-2407.

Pepper, A., Delaney, T., Washburn, T., Poole, D. and Chory, J. (1994). *DET1*, a negative regulator of light-mediated development and gene expression in arabidopsis, encodes a novel nuclear-localized protein. *Cell* **78**, 109-116.

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.

Ruegger, M., Dewey, E., Gray, W. M., Hobbie, L., Turner, J. and Estelle, M. (1998). The TIR1 protein of Arabidopsis function in auxin response and is related to human SKP2 and yeast grr1p. *Genes Dev.* **12**, 198-207.

Schroda, M., Vallon, O., Wollman, F. A. and Beck, C. F. (1999). A chloroplast-targeted heat shock protein 70 (HSP70) contributes to the photoprotection and repair of photosystem II during and after photoinhibition. *Plant Cell* **11**, 1165-1178.

Schwechheimer, C. and Deng, X. W. (2000). The COP/DET/FUS proteins: regulators of eukaryotic growth and development. *Semin. Cell Dev. Biol.* **11**, 495-503.

Schwechheimer, C., Serino, G., Callis, J., Crosby, W., Lyapina, S., Deshaies, R. J., Gray, W. M., Estelle, M. and Deng, X. W. (2001). Interaction of the COP9 signalosome with the E3 ubiquitin ligase SCFTir1 in mediating auxin-response. *Science* **292**, 1379-1382.

Schwechheimer, C., Serino, G. and Deng, X. W. (2002). The COP9 signalosome and AXR1 are required in multiple E3 ubiquitin ligases mediating development processes. *Plant Cell* **14**, 2553-2563.

Serino, G., Tsuge, T., Kwok, S. F., Matsui, M., Wei, N. and Deng, X. W. (1999). Arabidopsis *cop8* and *fus4* mutations define the same gene that encodes subunit 4 of the COP9 signalosome. *Plant Cell* **11**, 1967-1980.

Suzuki, G., Yanagawa, Y., Kwok, S. F., Matsui, M. and Deng, X. W. (2002). Arabidopsis COP10 is a ubiquitin-conjugating enzyme variant that acts together with COP1 and COP9 signaloaome in repressing photomorphogenesis. *Genes Dev.* **16**, 554-559.

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.

von Arnim, A. G. and Deng, X. W. (1996). Light control of seedling development. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 215-243.

Wei, N. and Deng, X. W. (1992). *COP9*: A new genetic locus involved in light-regulated development and gene expression in Arabidopsis. *Plant Cell* **4**, 1507-1518.

Wei, N. and Deng, X. W. (1999). Making sense of the COP9 signalosome: a regulatory protein complex conserved from Arabidopsis to human. *Trends Genet.* **15**, 98-103.

Wei, N., Kwok, S. F., von Arnim, A. G., Lee, A., McNellis, T. W., Piekos, B. and Deng, X. W. (1994). Arabidopsis *COP8*, *COP10*, and *COP11* genes are involved in repression of photomorphogenic development in dark. *Plant Cell* **6**, 629-643.