Plant Physiol. (1996) 110: 731–742

A Complement of Ten Essential and Pleiotropic Arabidopsis *COP/DET/FUS* Genes Is Necessary for Repression of Photomorphogenesis in Darkness[1]

Shing F. Kwok, Barry Piekos, Simon Miséra, and Xing-Wang Deng*

Graduate Program of Genetics, Yale University School of Medicine, New Haven, Connecticut 06520–8005 (S.F.K.); Department of Biology, Yale University, New Haven, Connecticut 06520–8104 (B.P., X.-W.D.); and Institut für Planzengenetik und Kulturpflanzenforschung, Corrensstrasse 3, D–06466, Gatersleben, Germany (S.M.)

Two genetic screens, one for mutations resulting in photomorphogenic development in darkness and the other for mutants with *fusca* phenotype, have thus far identified six pleiotropic Arabidopsis *COP/DET/FUS* genes. Here, we characterized representative mutants that define four additional pleiotropic photomorphogenic loci and a null mutant allele of the previously defined *DET1* locus. Dark-grown seedlings homozygous for these recessive mutations exhibit short hypocotyls and expanded cotyledons and are lethal before reaching reproductive development. Dark-grown mutant seedlings also display characteristic photomorphogenic cellular differentiation and elevated expression of light-inducible genes. In addition, analyses of plastids from dark-grown mutants reveal partial chloroplast differentiation and absence of etioplast development. Root vascular bundle cells of light-grown mutant seedlings develop chloroplasts, suggesting that these *FUS* gene products are important for suppression of chloroplast differentiation in light-grown roots. Double-mutant analyses indicate that these pleiotropic *cop/det/fus* mutations are epistatic to mutations in phytochromes, a blue-light photoreceptor, and a downstream regulatory component, HY5. Therefore, there is a complement of at least 10 essential and pleiotropic Arabidopsis genes that are necessary for repression of photomorphogenic development.

Seedlings of higher plants, such as the dicotyledonous *Arabidopsis thaliana*, are genetically equipped to follow two distinct developmental pathways: skotomorphogenesis in the dark and photomorphogenesis in the light (McNellis and Deng, 1995). Dark-grown seedlings have long hypocotyls, apical hooks, undeveloped (small and unopened) cotyledons that contain etioplasts, and a low level of expression of light-inducible nuclear and chloroplast genes. In contrast, light-grown seedlings have short hypocotyls, no apical hook, opened and enlarged cotyledons with thylakoid stacked chloroplasts, and high levels of expression of light-inducible genes. At least three classes of photoreceptors, phytochromes, blue-light receptors, and UV-light receptors, are involved in sensing red/far-red-, blue-, and UV-light signals from the environment to modulate plant development. Recent identification and characterization of specific mutations in three individual photoreceptors, phytochrome A, phytochrome B, and a blue-light receptor, have suggested that the three photoreceptors have distinct functional roles in sensing high-irradiance far-red-, red-, and blue-light color spectra to control plant development (Bowler and Chua, 1994; Quail, 1994).

To understand the mechanism(s) governing light-regulated plant development after light perception, it is important to identify and isolate genes that play key roles in such processes. One approach has been to isolate mutants that show a light-grown phenotype when grown in complete darkness (Chory, 1993; Deng, 1994). Arabidopsis mutations in 11 loci, 3 *DET* (for de-etiolated) loci and 8 *COP* (for constitutive photomorphogenic) loci, have been reported. Mutations in *DET2*, *DET3*, *COP2*, *COP3*, and *COP4* loci lead to partial light-grown seedling morphology when seedlings are grown in the dark. Thus, these genes likely control a subset of photomorphogenic responses and therefore may function in the branched pathways downstream of the primary development switch. Since mutations in *DET1*, *COP1*, *COP8*, *COP9*, *COP10*, and *COP11* result in the most pleiotropic phenotypes, these loci may encode regulators involved in the primary developmental switch before pathways branch out in controlling individual responses during seedling development. In addition, the recessive nature of the mutations indicates that their genes' products normally function to repress photomorphogenesis in the absence of light, whereas the light perceived by the photoreceptors leads to abrogation of their repressive activities. The fact that overexpression of COP1 leads to suppression of photomorphogenic seedling development under defined light conditions confirmed that at least COP1 can act as a light-inactivable repressor of photomorphogenesis (McNellis et al., 1994b).

In independent screens for mutants with purple cotyledons (high levels of anthocyanin) in young seedlings or mature seeds, more than 200 mutations representing 12 *FUS* loci have been identified (Müller, 1963; Miséra et al., 1994). It is interesting that severe mutations in the pleio-

[1] This work is supported by a grant from the National Science Foundation (MCB-9303029). X.-W.D. is a National Science Foundation Presidential Faculty Fellow and S.F.K. is a recipient of National Institutes of Health and Department of Education predoctoral traineeships.

* Corresponding author; e-mail xdeng@yalevm.ycc.yale.edu; fax 1–203–432–3854.

tropic *DET1*, *COP1*, *COP8*, *COP9*, *COP10*, and *COP11* genes also lead to a similar accumulation of high levels of anthocyanin in the cotyledons of both mature seeds and young seedlings (Wei et al., 1994b). Complementation tests indicated that *DET1*, *COP1*, *COP8*, *COP9*, *COP10*, and *COP11* are identical with *FUS* loci *FUS2*, *FUS1*, *FUS8*, *FUS7*, *FUS9*, and *FUS6*, respectively (Castle and Meinke, 1994; Miséra et al., 1994). Importantly, the *fus* mutant screens identified four new loci, *FUS4*, *FUS5*, *FUS11*, and *FUS12*, in which mutations also lead to dark-grown seedlings that exhibit morphology similar to their light-grown siblings (Miséra et al., 1994). These observations suggest that the four new *FUS* loci may represent additional Arabidopsis pleiotropic *COP/DET/FUS* genes that have not been defined by previous photomorphogenic mutant screens.

In this study, we analyzed in detail representative mutant alleles in the four new photomorphogenic loci, *FUS4*, *FUS5*, *FUS11*, *FUS12*. Furthermore, previous studies have indicated that the weak *det1–1* and the pleiotropic *cop* mutations may have contrasting effects on phytochrome control of seed germination and changes in gene expression pattern during dark adaptation, implying that they may define distinct classes of pleiotropic mutations. To further address this question, we also included a null allele of *FUS2/DET1* for comparison in this work. Our results suggested that severe mutations in all 10 pleiotropic *COP/DET/FUS* loci result in identical or very similar phenotypes. Therefore, Arabidopsis has a complement of 10 essential and pleiotropic genes that are necessary for repression of photomorphogenic development.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Wild type and all *cop/fus* mutants are in the Landsberg *erecta* (Ler) ecotype, except for *fus4*, which is in the Dijon background. For convenience and consistency, all photomorphogenic *FUS* loci have also been given a *COP* designation in our laboratory (Table I). Although two alleles were generally examined, only one allele from each locus was analyzed in great detail and presented. Those alleles are *fus12-U228/cop12–1*, *fus11-U203/cop13–1*, *fus4–414/cop14–1*, *fus5-S234/cop15–1*, and *fus2-U2/det1–8* (Miséra et al., 1994; Pepper et al., 1994). Because all of the mutants are recessive lethal, seeds of heterozygous plants for each mutation were used as seed stocks in all experiments. The growth conditions have been previously described (Wei and Deng, 1992) except seeds were cold treated at 4°C for 7 d (except in germination experiments) before being transferred to growth chambers.

Light Microscopy and EM

The light microscopy and scanning EM were performed as described previously (Hou et al., 1993). Transmission EM was performed as previously described (Wei et al., 1994b).

Table I. *Summary of pleiotropic FUS/COP/DET loci and the total number of alleles identified for each*

FUS loci designations are according to Miséra et al. (1994). The corresponding *COP1*, *COP8*, *COP9*, *COP10*, *COP11*, and *DET1* genes are shown based on complementation tests with known *FUS* genes. For the sake of consistency, we tentatively suggest here the pseudonym designations of *COP12*, *COP13*, *COP14*, and *COP15* for the four new *FUS* loci. The total number of identified alleles corresponding to the *cop/det/fus* mutants are shown in the third column.

FUS Gene	Corresponding COP/DET Gene	Total No. of Identified Alleles	References[a]
FUS1	*COP1*	53	1, 3
FUS2	*DET1*	36	2, 3
FUS3	None[b]	22	3
FUS4[c]	*COP14*	26	3, This study
FUS5[c]	*COP15*	16	3, This study
FUS6	*COP11*	13	3, 4, 5
FUS7	*COP9*	13	3, 4, 5, 6
FUS8	*COP8*	20	3, 4, 5
FUS9	*COP10*	8	3, 4, 5
FUS10	None[b]	7	3
FUS11[c]	*COP13*	5	3, This study
FUS12[c]	*COP12*	3	3, This study

[a] The references are: 1, McNellis et al., 1994a; 2, Pepper et al., 1994; 3, Miséra et al., 1994; 4, Wei et al., 1994b; 5, Castle and Meinke, 1994; 6, Wei and Deng, 1992. [b] Loci do not display a constitutive photomorphogenic phenotype in darkness, although they were identified in the same fusca mutant screens. [c] New FUS loci that are described in this study.

Analysis of Root Chl Fluorescence

Wild-type and mutant seedlings were grown in the light for 7 d. Live seedlings with their roots intact were transferred to a glass slide, gently covered with a coverslip without damaging the roots, and examined directly under a Leitz Diaplan fluorescent microscope (Leica, Heerbrugg, Switzerland) with a fluorescein filter. Chl (and therefore chloroplasts) emits a red fluorescence that can be easily observed through the filter used.

RNA and Protein Gel Blot Analyses

Mutant seedlings used for northern blot analysis were grown in complete darkness or in white light for 8 d at 22°C. For dark adaptation, 8-d-old light-grown seedlings were transferred to complete darkness for an additional 2 d. The dark-grown and dark-adapted seedlings were harvested under a green safelight. Total RNA extraction, electrophoresis and blotting, and hybridization were performed as described previously (Deng et al., 1991).

The protein extraction and immunoblot analysis using affinity-purified polyclonal antibodies against COP1 were performed as previously described (McNellis et al., 1994a; Wei et al., 1994a).

Double-Mutant Construction

The *phyA* mutant allele used for construction of double mutants for analysis was *phyA-1* (Whitelam et al., 1993). The *hy* (for long hypocotyl) mutant alleles used for the

double-mutant construction were *phyB* (*hy3*-Bo64), *hy4* (2.23NO), and *hy5* (Ci88) (Koornneef et al., 1980). All of these mutants are in the Landsberg *erecta* background. Crosses between *phy* or *hy* mutants with *fus4*, *fus5*, *fus11*, and *fus12* mutants were performed as previously described (Wei et al., 1994b).

Seed Germination

After seeds were plated on growth-medium plates and cold treated for 2 d at 4°C in complete darkness, the plates containing wild-type or mutant seeds were then given different light treatments: either no light treatment (complete darkness), a 10-s pulse of saturated far-red light, or a 5-s pulse of saturated red light. After the treatments, plates were incubated at 22°C in complete darkness for 9 d and the number of germinated seedlings was recorded. The plates were then transferred to light for another 9 d, and the number of seedlings germinated was recorded again. The total germinated seedlings, including those after the dark period and subsequent light period, constitute the total germination-competent seeds. The germination rate is the ratio of the germinated seedlings after the dark period to the total germination-competent seeds. Because of a low germination rate of the mutant seeds and high variation among seed batches, this is the only reproducible procedure for estimating germination rate.

RESULTS

Mutations in Ten Arabidopsis Loci Result in Constitutive Photomorphogenic Seedling Development in Darkness

From extensive screens of Arabidopsis *fus* mutants, a total of 12 loci were identified and mutations in 10 of the 12 loci led to dark-grown seedlings with morphology resembling that of their light-grown siblings (Miséra et al., 1994). The mutant alleles identified for the 12 loci through the *fus* and *cop/det* screens are summarized in Table I. The fact that three or more alleles were identified for each independent locus indicated that the screen for mutations with both photomorphogenic and *fus* phenotypes may be near saturation. The 10 photomorphogenic *FUS* loci represent 6 previously identified pleiotropic *COP/DET* loci and 4 novel loci *FUS4*, *FUS5*, *FUS11*, and *FUS12* (Miséra et al., 1994). Therefore, representative alleles from the 4 novel loci were chosen for further characterization. In addition, a likely null allele of the *DET1/FUS2* locus was included in this study. This allele, *fus2-U2/det1–8*, has a premature stop codon mutation at amino acid 15 and likely causes complete loss of function of *FUS2/DET1* (Pepper et al., 1994). Previous studies with a weak mutation suggested that *DET1* may be distinct from the five reported pleiotropic *COP* loci in two specific processes: changes of light-inducible gene expression during dark adaptation and phytochrome control of seed germination (Chory et al., 1989). The inclusion of the *fus2-U2/det1–8* in this work should clarify whether those distinctions are gene specific or allele specific.

Analyses of the gross seedling morphology of representative *fus4*, *fus5*, *fus11*, *fus12*, and *fus2* mutants clearly show that dark-grown mutant seedlings had a morphology similar to their light-grown siblings. Figure 1 shows that 8-d-old dark-grown mutant seedlings had expanded and open cotyledons and short hypocotyls, a phenotype typical of wild-type light-grown seedlings. In both seed and seedling stages of development, these mutants accumulated high levels of anthocyanin pigments in the cotyledons, which gave them a characteristic purple color. The only exception was *fus4* mutants, in which levels of anthocyanin were normal at the seed stage and the cotyledons became purple only after germination. All of the new photomorphogenic *fus* mutants identified were lethal after the seedling stage, similar to the severe pleiotropic *cop/det* mutants.

Because of their near identical morphogenic characteristics to pleiotropic *cop* mutants and also because other *fus* mutants do not display a *cop*-like phenotype in the dark (namely *fus3* and *fus10*, Miséra et al., 1994), we tentatively gave *fus4*, *fus5*, *fus11*, and *fus12* mutants pseudonym designations of *cop14*, *cop15*, *cop13*, and *cop12*, respectively, in our laboratory (Table I). However, we will refer to the mutations as *fus* mutants throughout this study. Overall seedling morphology indicates that the mutant seedlings grow slower than the wild type. Eight-day-old mutant seedlings were at a developmental stage similar to that of 4-d-old wild-type seedlings; thus these two developmental stages were chosen for detailed cellular and molecular characterization.

Dark-Grown Seedlings Homozygous for the New *fus* Mutations Exhibit Photomorphogenic Cell Differentiation in Darkness

To further characterize the phenotypes of the new mutants, the cellular differentiation of cotyledon and hypocotyl cells of dark-grown mutant seedlings was examined. Figure 2 shows the results of scanning electron microscopic examination of cell morphology of whole seedlings, cotyledons, and hypocotyls from light-grown wild-type, *fus12-U228*, *fus11-U203*, *fus4–414*, *fus5-S234*, and *fus2-U2* mutant seedlings. The epidermal cells from wild-type cotyledons frequently displayed open stomatal cells (as indicated by the arrow in Fig. 2B). However, the epidermal cells from the mutant cotyledons were irregular in shape as compared to their wild-type counterparts. In addition, hypocotyl cells of both mutant and wild-type seedlings were relatively short. In fact, in all *fus* mutants, hypocotyls of light-grown seedlings appeared shorter than the wild-type counterparts (in Fig. 2, compare F and C).

Figure 3 shows representative scanning electron micrographs of whole seedlings, cotyledons, and hypocotyls from 4-d-old dark-grown wild-type and 8-d-old dark-grown mutant seedlings. Etiolated wild-type seedlings had small and more regularly shaped epidermal cells and immature stomatal structures without any detectable opening, although indentations were frequently observed between the guard cells (Fig. 3B). The overall seedling morphology of the dark-grown mutants indicated that the cotyledons were expanded and the epidermal cells were enlarged in comparison to etiolated wild-

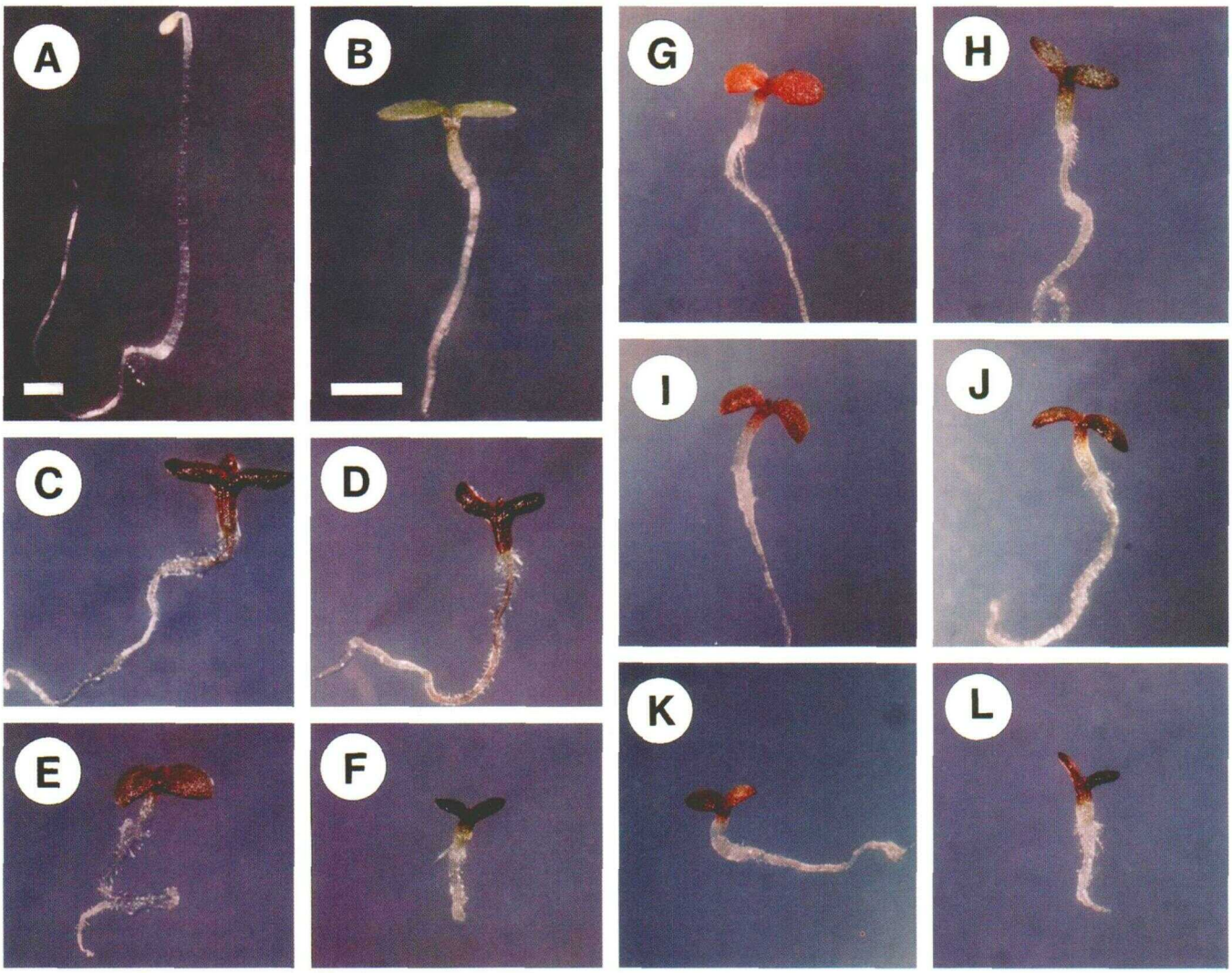

Figure 1. Morphology of 4-d-old wild-type and 8-d-old mutant seedlings. A, Dark-grown wild-type (Landsberg *erecta*) seedling. B, Light-grown wild-type seedling. C, Dark-grown *fus2-U2* seedling. D, Light-grown *fus-U2* seedling. E, Dark-grown *fus12-U228* seedling. F, Light-grown *fus12-U228* seedling. G, Dark-grown *fus11-U203* seedling. H, Light-grown *fus11-U203* seedling. I, Dark-grown *fus4–414* seedling. J, Light-grown *fus4–414* seedling. K, Dark-grown *fus5-S234* seedling. L, Light-grown *fus5-S234* seedling. All seedlings were photographed under the same magnification except for the dark-grown wild-type seedling, which was photographed at 63% of the magnification of the others. Bars represent 1 mm in A and B.

type seedlings. Occasionally, stomatal cells were opened in dark-grown mutant cotyledons (indicated by an arrow in Fig. 3H). In addition, unlike the organized arrangement of wild-type stomatal cells, stomatal cells in some mutants were clustered in groups of two or more (indicated by arrowheads in Fig. 3H). This clustering of stomatal cells and the enlargement of epidermal cells resulted in the rough cotyledon surface seen in mutant seedlings. Dark-grown mutant hypocotyl cells also displayed very little cell elongation when compared to dark-grown wild-type hypocotyl cells and were similar to their light-grown siblings. Furthermore, in some mutants, stomatal precursor cells appeared to be present in the hypocotyl (indicated by an arrow in Fig. 3R). This cellular differentiation pattern has been observed only in light-grown, but not dark-grown, wild-type hypocotyls.

The New *fus* Mutations Alter Plastid Development in Cotyledons of Dark-Grown Seedlings

In higher plants, photosynthetically competent tissues develop chloroplasts from proplastids in the presence of light. However, in darkness, plastids develop into etioplasts that can be transformed into chloroplasts upon exposure to light (Wei et al., 1994b). To investigate whether dark-grown *fus12*, *fus11*, *fus4*, *fus5*, and *fus2* mutants exhibit defects in plastid differentiation, transmission EM was used to examine plastids in cotyledons of these *cop/det/fus* mutants. Figure 4 shows representative plastids from cotyledons of wild-type and mutant seedlings. Wild-type etiolated seedlings had typical etioplasts that contained a large paracrystalline assembly of tubules, termed the prolamellar body (Fig. 4A), whereas wild-type light-grown

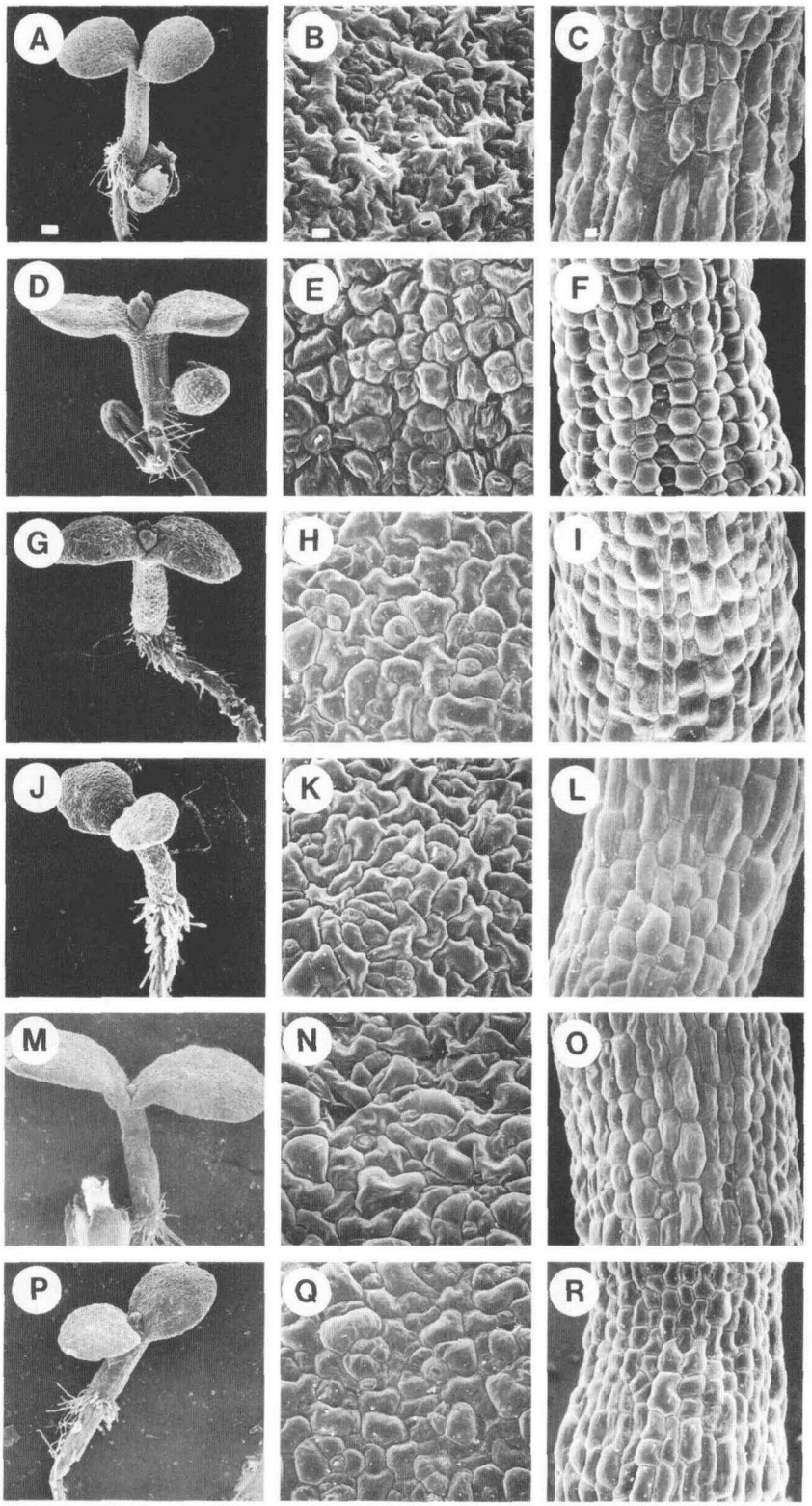

Figure 2. Scanning electron micrographs of whole seedling and epidermal cell surfaces of light-grown wild-type and mutant seedlings. A to C, Wild type; D to F, *fus2-U2*; G to I, *fus12-U228*; J to L, *fus11-U203*; M to O, *fus4–414*; P to R, *fus5-S234*. Left panels show whole seedling morphology; middle panels show cotyledon epidermal cell surface; right panels show hypocotyl cell surface morphology. The arrow in B shows open stomatal cells. Wild-type light-grown seedlings were grown for 4 d, and light-grown mutant seedlings were grown for 8 d. The bar is 100 μm in A, D, G, J, M, and P and 10 μm in B, C, E, F, H, I, K, L, N, O, Q, and R.

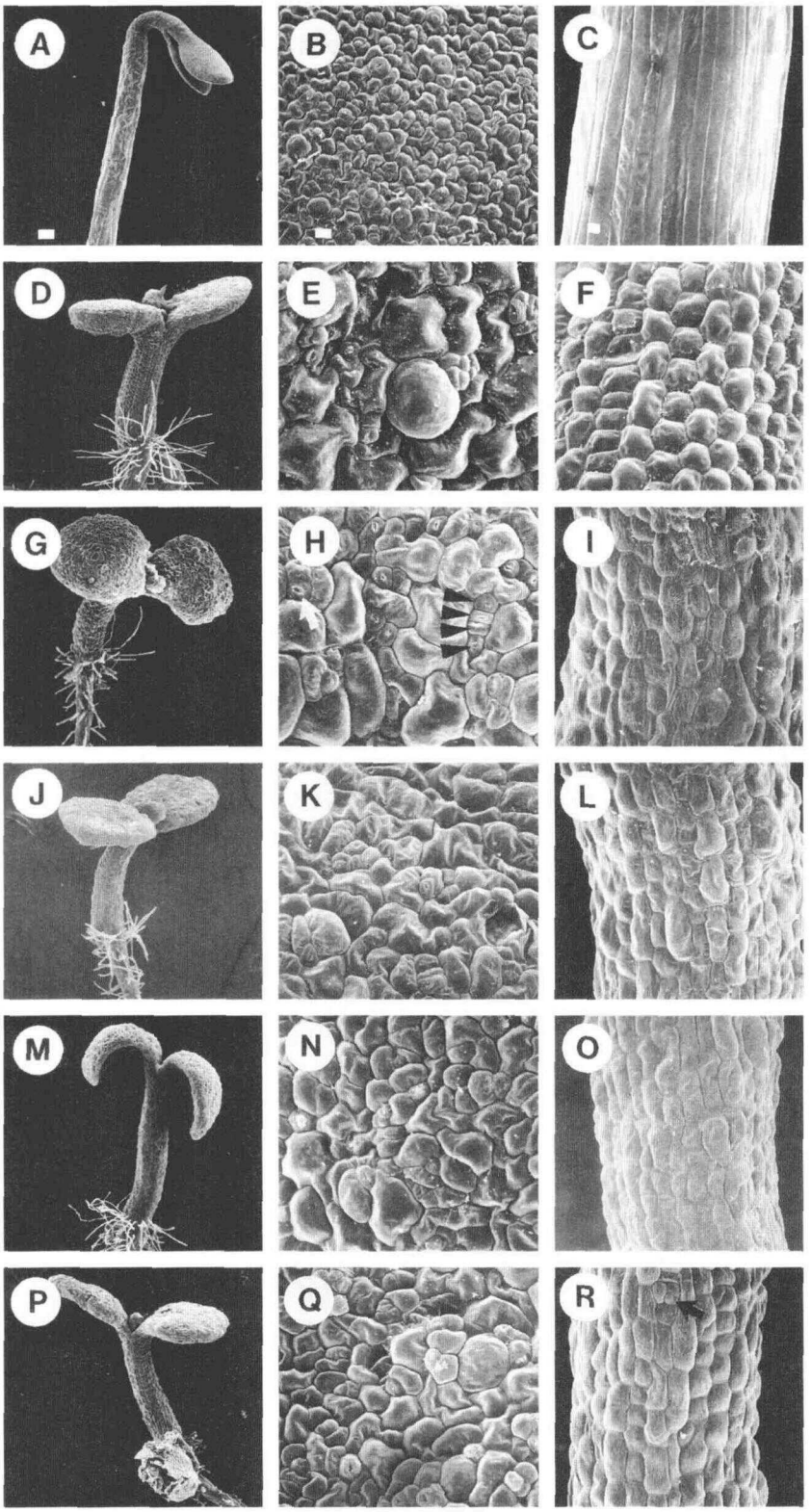

Figure 3. Scanning electron micrographs of whole seedling and epidermal cell surfaces of dark-grown wild-type and mutant seedlings. A to C, Wild type; D to F, *fus2-U2*; G to I, *fus12-U228*; J to L, *fus11-U203*; M to O, *fus4–414*; P to R, *fus5-S234*. Left panels show whole seedling morphology; middle panels show cotyledon epidermal cell surface; right panels show hypocotyl cell surface morphology. The arrow in H shows open stomatal cells. The arrowheads in H indicate clustering of stomatal cells. The arrow in R shows precursors of developing stomatal cells in the hypocotyl. Wild-type dark-grown seedlings were grown for 4 d, and light-grown mutant seedlings were grown for 8 d. The bar is 100 μm in A, D, G, J, M, and P and 10 μm in B, C, E, F, H, I, K, L, N, O, Q, and R.

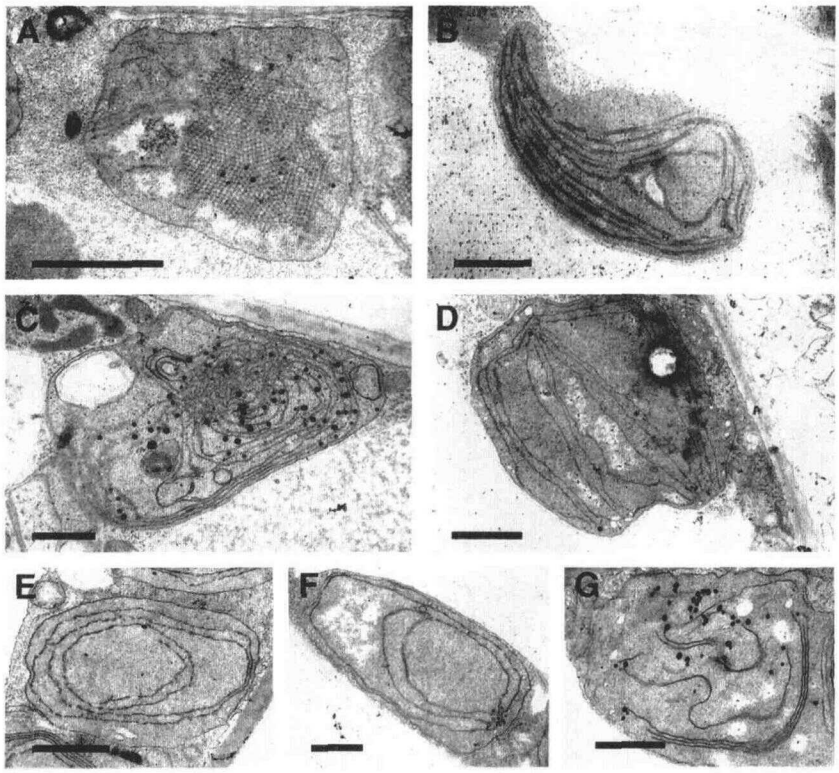

Figure 4. Transmission electron microscopic examination of plastid development in 6-d-old wild-type and 8-d-old mutant seedlings. A, Etioplast from dark-grown wild-type seedling. B, Chloroplast from light-grown wild-type seedling. Also shown are plastids from dark-grown (C) *fus2-U2*, (D) *fus12-U228*, (E) *fus11-U203*, (F) *fus4–414*, and (G) *fus5-S234* seedlings. The scale bars represent 1 μm.

seedlings contained mature chloroplasts with parallel and highly stacked thylakoid membrane structures (Fig. 4B). Examination of cotyledon plastids from all dark-grown mutant seedlings indicated that they contained neither the characteristic prolamellar bodies of etioplasts nor the stacked thylakoid membrane structure of chloroplasts (Fig. 4, C–G). Instead, in these mutants, chloroplast differentiation had been initiated but not completed, although the pathway leading to etioplast development in the dark is evidently impaired in these mutants. In the case of *fus2-U2*, the partially differentiated cotyledon plastids in dark-grown mutant seedlings were very similar to those of the less severe allele, *det1–1* (Chory et al., 1989).

The New *fus* Mutations Result in Chloroplast Differentiation in the Roots of Light-Grown Seedlings

In wild-type Arabidopsis, roots of young light-grown seedlings are nonphotosynthetic and develop amyloplasts instead of chloroplasts. It has been demonstrated by the presence of chloroplasts in the roots of light-grown mutant seedlings that the previously defined six pleiotropic *COP/DET/FUS* loci are required for the suppression of root chloroplast differentiation (Chory and Peto, 1990; Deng and Quail, 1992; Wei and Deng, 1992; Wei et al., 1994b). To investigate whether the new mutations also affect root plastid differentiation, we chose to analyze the presence of Chl, which exists only within chloroplasts, in the light-grown mutant roots. Figure 5 shows characteristic Chl fluorescence in whole, mounted light-grown wild-type and representative mutant roots under fluorescent light microscopy. In wild type, the roots did not display detectable Chl fluorescence and appeared slightly green because of autofluorescence (Fig. 5A). However, all mutants analyzed displayed high levels of Chl fluorescence mostly in the vascular region of the mutant roots. This was indicated by the red granular dots, which represent individual chloroplasts (shown by arrows in Fig. 5, B–F). These results suggest that *FUS12*, *FUS11*, *FUS4*, and *FUS5* are also essential for repressing chloroplast differentiation in the roots of light-grown seedlings.

Dark-Grown and Dark-Adapted Mutant Seedlings Express High Levels of Light-Inducible Genes

The expression of many plant genes is tightly controlled by light. Previous studies have shown that *cop1*, *cop8*, *cop9*, *cop10*, and *cop11* mutants constitutively express light-regulated genes in dark-grown seedlings and dark-adapted light-grown seedlings (Deng et al., 1991; Wei and Deng, 1992; Wei et al., 1994b), whereas *det1–1* mutants constitutively express light-regulated genes in dark-grown seedlings but not in dark-adapted seedlings (Chory et al., 1989). To test whether the new photomorphogenic mutants have similar defects, we examined the expression of four representative light-regulated genes (see legend to Fig. 6) in wild-type and mutant seedlings in response to light treatments. As shown in Figure 6, the wild type displayed high expression of light-inducible genes in light-grown seedlings while exhibiting very low or undetectable levels in both dark-grown seedlings and dark-adapted seedlings (except for the plastid gene *psbA* in the latter case). However, mutations in the *FUS12*, *FUS11*, *FUS4*, *FUS5*, and *FUS2* loci resulted in elevated levels of both nuclear-en-

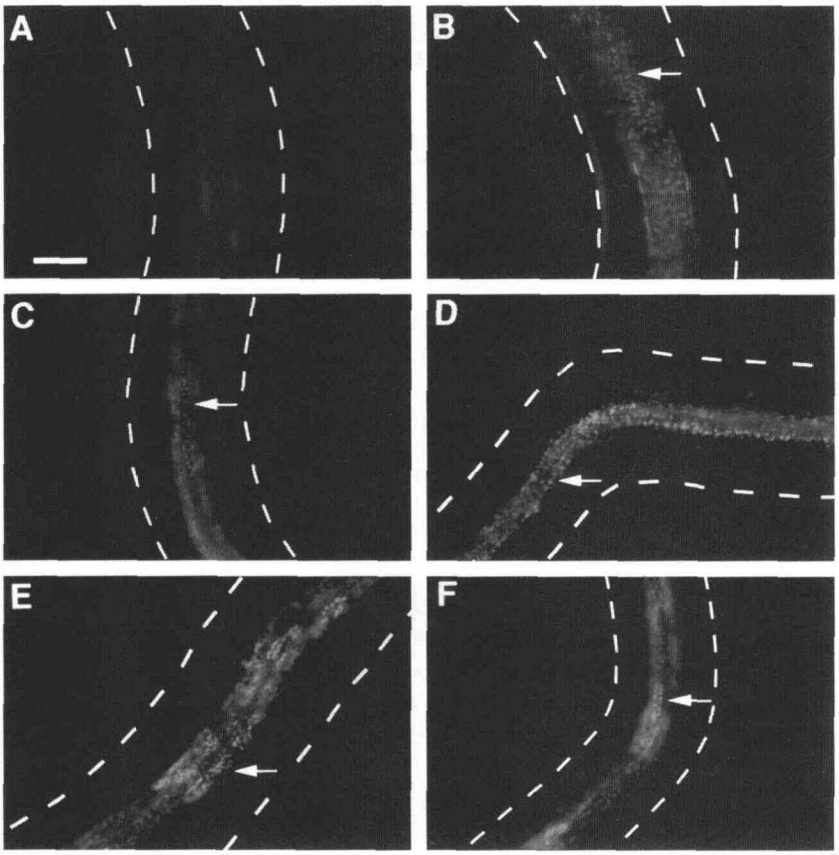

Figure 5. Chl fluorescence in roots of 7-d-old light-grown wild-type and mutant seedlings. Whole-mount wild-type and mutant roots examined through fluorescent microscopy using a fluorescein filter. A, Wild type; B, *fus2-U2*; C, *fus12-U228*; D, *fus11-U203*; E, *fus4–414*; and F, *fus5-S234*. All roots were photographed under the same magnification and the scale bar in A represents 0.1 mm. Dotted lines indicate outline of root structure. The arrowhead points to a representative chloroplast emitting Chl fluorescence.

coded and plastid-encoded mRNAs in dark-grown seedlings. There was also high expression of nuclear-encoded light-inducible genes in dark-adapted mutant seedlings, indicating that lethal mutations in *DET1/FUS2*, *FUS12*, *FUS11*, *FUS4*, and *FUS5* genes severely compromise the ability of plants to undergo this dark-adaptive response. It should be noted that in *fus5* mutants there appeared to be a reduced level of expression of light-induced genes in light-grown versus dark-grown or dark-adapted mutant seedlings. This result is due to an allele-specific, rather than a gene-specific, effect, since other alleles of *fus5* mutants do not display this character (data not shown). Taken to-

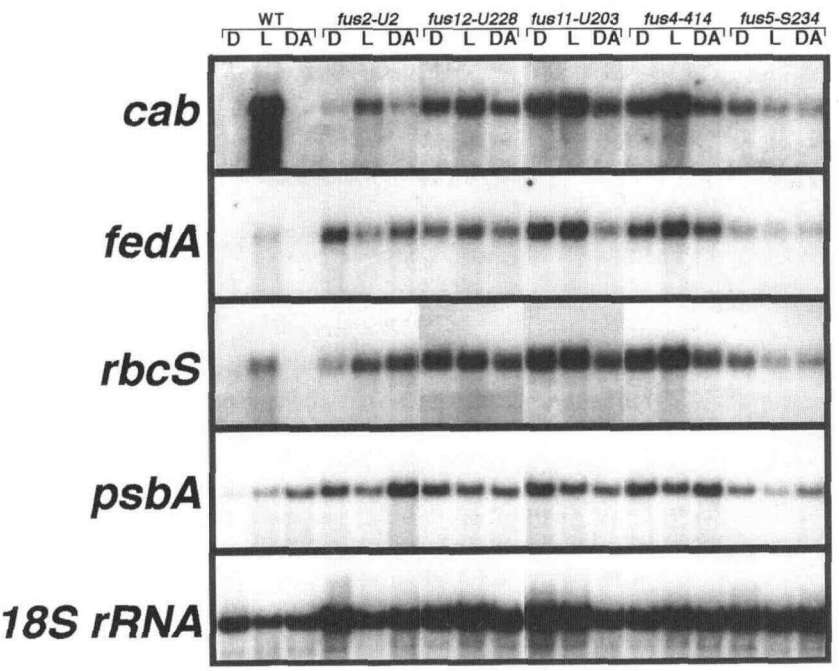

Figure 6. Northern blot analysis of the steady-state mRNA levels of representative light-regulated genes in wild type and mutants. Seedlings of wild type (WT) and *fus2-U2*, *fus12-U228*, *fus11-U203*, *fus4–414*, and *fus5-S234* were grown in the dark (D) or light (L) for 8 d or for 8 d under light followed by 2 d of dark adaptation (DA). Total RNA (2.5 μg in each lane) was used for hybridization. DNA probes for the following genes (Deng et al., 1991) were used for hybridization: *cab*, gene encoding the Chl *a/b*-binding protein of photosynthetic light-harvesting complexes; *fedA*, Fd type A gene; *rbcS*, gene encoding the small subunit of Rubisco; *psbA*, plastid gene encoding the 32-kD protein of PSII; and 18S rRNA, cytoplasmic 18S rRNA.

gether, these results are similar to those seen for mutations in the *COP1, COP8, COP9, COP10,* and *COP11* loci (Deng et al., 1991; Wei and Deng, 1992; Wei et al., 1994b). The absence of the defect in *det1–1* mutants during dark adaptation (Chory et al., 1989) is probably due to the nature of the weak mutation (Pepper et al., 1994), since the result with the null *fus2-U2* mutation indicated that DET1/FUS2 is also essential for dark-adaptive responses of gene expression.

Mutations in *FUS4, FUS5, FUS11, FUS12,* and *FUS2* Do Not Eliminate Phytochrome Control of Seed Germination

In many plant species the active form of phytochrome, Pfr, is required for the promotion of seed germination (Quail, 1994). Prior to any light stimuli, some Pfr is usually stored during seed development and is present in mature seeds. This Pfr can promote seed germination even in the absence of light, and upon absorption of far-red light, nearly all of the Pfr converts to Pr, which is not active for promoting seed germination. The Pr can be photoreversed to Pfr by absorption of red light. Previous studies have shown that one distinction between the *cop1, cop8, cop9, cop10, cop11* mutations and the *det1–1* mutation is their effects on phytochrome control of seed germination. Whereas no pleiotropic *cop* mutant is defective in phytochrome control of seed germination (Ang and Deng, 1994; Wei et al., 1994a, 1994b), the weak *det1–1* mutant does not require active phytochrome to promote seed germination (Chory et al., 1989).

To define whether mutations in *FUS4, FUS5, FUS11,* and *FUS12* and a null mutation of *DET1, fus2-U2,* affect phytochrome control of seed germination, we examined the germination rates of mutant seeds in response to specific red- or far-red-light treatments. The data summarized in Table II indicate that mutations in *DET1/FUS2, FUS12, FUS4,* and *FUS5* still maintain different degrees of phytochrome control of seed germination, as evident by the effects of pulses of red light, which promoted germination, and of far-red light, which inhibited seed germination. Although the overall germination rates of homozygous mutant seeds were very low and were difficult to accurately estimate, our results are qualitatively similar to the effects seen with mutations of *COP1, COP8, COP9, COP10,* and *COP11.* In the case of the *fus11-U203* allele, the far-red inhibition of germination was rather moderate, but reproducible, in three independent experiments (data not shown). It is interesting that *fus2-U2,* a null mutation in *DET1,* did not eliminate the phytochrome control of seed germination, in contrast to the effect of the weak *det1–1* mutation (Chory et al., 1989). This difference may suggest that, although DET1 is not essential for phytochrome control of seed germination, the *det1–1* mutation results in an allele-specific gain-of-function effect and uncoupled phytochrome control of seed germination.

fus4, fus5, fus11, and *fus12* Mutations Are Epistatic to Photoreceptor and *hy5* Mutations and Result in High Levels of COP1 Accumulation

To determine the hierarchical relationship of the new *COP/FUS* loci in the overall light regulatory network, we constructed and analyzed double mutants between the new *fus* mutations and mutations in the phytochrome receptors (*phyA-1* and *phyB),* a blue-light receptor (*hy4*), and *HY5,* a signaling component downstream of both phytochromes and the blue-light receptor. Essentially identical results were obtained from analyses of double mutants involving the four new loci. Figure 7 summarizes characteristics of one representative set involving *fus12-U228.* The results indicate that the double-mutant sets displayed a phenotype similar to that of the parental *fus* mutants, suggesting these new *fus* mutations are epistatic to mutations in *PHYA, PHYB, HY4,* and *HY5.* This result is consistent with a genetic model in which all of the new pleiotropic *COP/DET/FUS* loci act downstream of the photoreceptors and HY5 in the light-signal transduction pathway. This genetic relationship is essentially identical or very similar to that proposed for *DET1, COP1, COP8, COP9, COP10,* and *COP11* (Chory, 1993; Ang and Deng, 1994; Wei et al., 1994a, 1994b).

To define the hierarchical relationship among the 10 pleiotropic *COP/DET/FUS* loci (Deng et al., 1992; Wei et al., 1994b), we examined the effects of the *fus12, fus11, fus4, fus5,* and *fus2* mutations on the levels of COP1 protein. All *cop/det/fus* mutant alleles analyzed accumulated higher levels of COP1 protein in comparison to wild type (data not shown). This result is similar to those with *cop8, cop9, cop10,* and *cop11* mutants (Wei and Deng, 1992; Wei et al., 1994b). Thus, overaccumulation of COP1 protein in mutants of all nine of the other pleiotropic *COP/DET/FUS* loci could suggest a potential feedback regulation on the expression or stability of the COP1 protein.

DISCUSSION

Our results suggest that the 4 new loci and the 6 previously identified pleiotropic *COP/DET/FUS* loci define a full complement of 10 essential and pleiotropic Arabidopsis

Table II. *Germination rates of wild type and fus2-U2, fus4–414, fus5-S234, fus11-U203, and fus12-U228 mutants under different light treatments*

Germination rates were expressed as the percentages of the germinated seedlings/total number of germination-competent seeds, both of which are indicated in the parentheses. Both wild-type and mutant seeds were sown on growth-medium plates and cold treated at 4°C for 2 d. Seeds were then treated with either no light (Dark), a 5-s pulse of red light (Red), or a 10-s pulse of far-red light (Far-red). After light treatments, seeds were kept in darkness at 22°C for 9 d before they were scored for germination. For further details, see "Materials and Methods."

Light Treatment	Wild Type	*fus2-U2*	*fus12-U228*	*fus11-U203*	*fus4–414*	*fus5-S234*
Dark	94 (222/235)	90 (27/30)	63 (41/65)	96 (68/71)	67 (52/78)	70 (23/33)
Red	95 (186/195)	96 (24/25)	75 (46/61)	98 (61/62)	78 (51/65)	80 (28/35)
Far-red	38 (56/148)	32 (8/25)	48 (28/58)	89 (46/54)	57 (34/60)	56 (23/41)

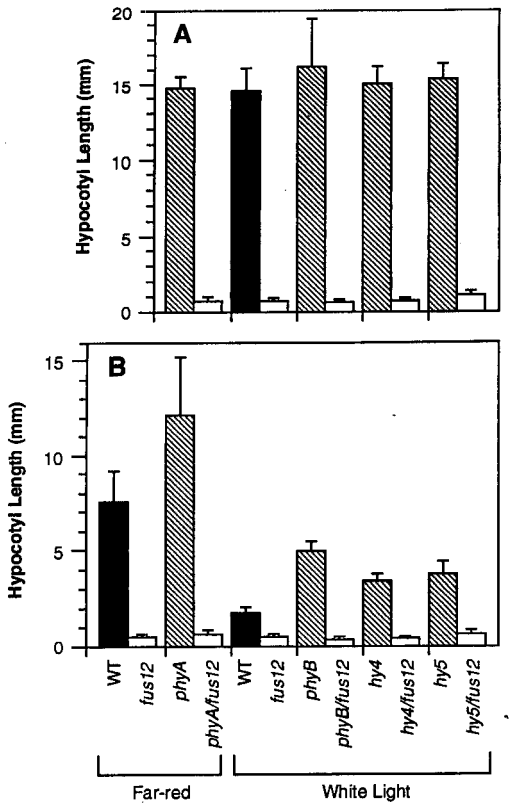

Figure 7. Comparison of hypocotyl lengths of 7-d-old dark (A) and light-grown (B) wild type (WT), *phyA*, *phyB*, *hy4*, *hy5*, *fus12*, *phyA/fus12*, *phyB/fus12*, *hy4/fus12*, and *hy5/fus12* double mutants. The *phyA* mutant phenotype was determined by growing seedlings under continuous far-red light, instead of white light as for *phyB* and all other *hy* mutants. All values represent the averages of more than 25 seedlings for each sample, and error bars represent SD.

genes that are necessary for repression of photomorphogenic development and/or promoting skotomorphogenesis in darkness. The results further support the working hypothesis that photomorphogenic seedling development is the default pathway and a complement of genes is required to suppress this development pathway in the absence of light (Wei et al., 1994b; McNellis and Deng, 1995).

A Complement of Ten Pleiotropic *COP/DET/FUS* Loci Is Involved in Suppressing Photomorphogenic Development in Darkness

The overall morphology, cellular differentiation, and pattern of gene expression of dark-grown *fus12-U228*, *fus11-U203*, *fus4–414*, *fus5-S234*, and *fus2-U2* seedlings, as revealed through this study, are characteristic of light-grown wild-type seedlings and essentially identical or very similar to those of the mutants of the 6 previously identified pleiotropic *COP/DET/FUS* loci. The recessive nature of the pleiotropic mutations suggests that loss of function in any of the 10 pleiotropic Arabidopsis *COP/DET/FUS* loci results in constitutive photomorphogenic development in darkness and fails to promote skotomorphogenesis. Since the ultimate goal of photomorphogenic development is to optimize the potential for photosynthetic growth of the plant, it is not surprising to find that the pleiotropic *COP/DET/FUS* genes play a critical role in controlling plastid differentiation in both green and nongreen tissues. For example, transmission EM analysis indicated that chloroplast differentiation had partially initiated in the cotyledons of dark-grown *cop/det/fus* mutants, whereas normal etioplast differentiation had been abolished. Thus, the gene products of the pleiotropic *COP/DET/FUS* function normally to repress chloroplast development and activate etioplast development in the dark. Furthermore, our analysis of Chl fluorescence in mutant roots suggests that the pleiotropic *COP/DET/FUS* gene products also function to repress chloroplast differentiation in the light in nonphotosynthetic tissues.

The pleiotropic *COP/DET/FUS* loci also play roles beyond the light control of seedling development. First, all severe (possibly null) mutations in the 10 pleiotropic *COP/DET/FUS* loci identified result in lethality after the seedling stage, suggesting that their gene products are essential for normal plant development under light conditions. However, relatively little is known about this aspect of the functions of the pleiotropic *COP/DET/FUS* genes (Castle and Meinke, 1994). Second, substantial evidence suggests that all pleiotropic *COP/DET/FUS* genes are also involved in regulating gene expression during dark adaptation of light-grown plants (Fig. 6; Deng, 1994; Wei et al., 1994b). In the case of *DET1/FUS2*, although a weak mutation failed to show any defect in regulating gene expression during dark adaptation (Chory et al., 1989), the severe (possibly null) mutation (*fus2-U2*) did show clear defects in the dark-adaptation process (Fig. 6).

It is clear that the pleiotropic *COP/DET/FUS* genes are not involved in some light-regulated processes. The best example is phytochrome control of seed germination. Except for the *det1–1* mutation, which specifically affects the first intron of the *DET1* gene and exhibits a weak phenotype (Pepper et al., 1994), no other *cop/det/fus* mutation (including possibly null mutations) blocks phytochrome control of seed germination. Since *fus2-U2/det1–8* mutation is likely a complete loss-of-function mutation and exhibits normal phytochrome control of seed germination, it is reasonable to conclude that none of the 10 pleiotropic *COP/DET/FUS* genes is essential for mediating phytochrome control of seed germination.

The Hierarchical Role of the Pleiotropic *COP/DET/FUS* Loci in the Light-Signaling Network

The results from this study, together with previous work (Deng, 1994), demonstrate that mutations in all 10 pleiotropic *COP/DET/FUS* loci result in dark-grown seedlings that phenocopy light-grown wild-type seedlings in essentially all developmental parameters that have been examined. The pleiotropic nature implies that the *COP/DET/FUS* genes encode products that act early in the pathway before branching out in different regulatory cascades to control individual aspects of seedling development, such as plastid

development, cell differentiation, and gene expression. The recessive nature of the pleiotropic *cop/det/fus* mutations indicates that their respective wild-type gene products act to repress photomorphogenic seedling development in darkness and that light abrogates their suppressive action. This hypothesis is supported by the double-mutant analyses in this report (Fig. 7) as well as those already published (Chory, 1993; Ang and Deng, 1994; Wei et al., 1994a, 1994b). Therefore, a genetic hierarchy of the pleiotropic *COP/DET/FUS* genes can be summarized as shown in Figure 8. In the model, the *COP/DET/FUS* genes act as central processors of the light regulatory network and play the role of a light-regulated developmental master switch(es) for seedling development. In the case of COP1, this working model has been substantiated by overexpression studies (McNellis et al., 1994b).

Potential Functional Relationship of the Pleiotropic *COP/DET/FUS* Loci

The fact that severe (possibly null) mutations in all 10 pleiotropic *COP/DET/FUS* genes result in identical or very similar phenotypes is consistent with at least two alternative hypotheses about their possible relationships in mediating light control of seedling development. First, the products encoded by these loci may function in proximity to each other in the same pathway, or regulate the same target, with the possibility that some of their gene products function as a complex. Consistent with this hypothesis, synthetic lethality and specific epistatic interactions have been observed between weak *det1* and *cop1* mutations (Ang and Deng, 1994; Miséra et al., 1994). Alternatively, those loci may define multiple parallel pathways that independently control the developmental switch between photomorphogenesis and skotomorphogenesis (Quail, 1994). However, a combination of both types of relationship could also occur.

Recently, molecular cloning of four loci, *COP1*, *COP9*, *COP11/FUS6*, and *DET1*, has allowed direct testing of the above models by examining whether any of the gene products indeed acts as a protein complex. In the case of Arabidopsis COP9, it was found to be exclusively in a large (>560 kD) protein complex, which may be subject to light modulation (Wei et al., 1994a). It is interesting that the COP9 complex is not detectable in extracts from *cop8* and *cop11* mutant seedlings. These observations provide evidence that at least some of the pleiotropic *COP/DET/FUS* genes act in the same pathway, possibly as protein complexes.

The molecular cloning and characterization of the pleiotropic *COP/DET/FUS* genes have shed some light on possible cellular and biochemical mechanisms of their suppressive action. Three of the genes, *COP1* (von Arnim and Deng, 1994), *COP9* (N. Wei and X.-W. Deng, unpublished results), and *DET1* (Pepper et al., 1994) are likely nuclear regulators, possibly acting to repress photomorphogenesis in darkness through the regulation of gene expression. Studies using a GUS-COP1 fusion protein as a tool pointed to the tantalizing possibility that COP1 acts in the nucleus to suppress photomorphogenic development and repression of photomorphogenesis by COP1 is relieved by exclusion of COP1 from the nucleus (von Arnim and Deng, 1994). This working model could provide a framework with which to determine the functional roles of other pleiotropic *COP/DET/FUS* genes. For example, some of the other pleiotropic *COP/DET/FUS* gene products may be required for the active import of COP1 from the cytoplasm to the nucleus or the nuclear retention of COP1. Their mutations would perturb the nucleo-cytoplasmic partitioning and yield *cop1*-like phenotypes. Alternatively, some pleiotropic gene products may be essential for COP1 to function in the nucleus to repress photomorphogenic development in darkness. Further investigation to reveal those mechanisms and the nature of the molecular interactions will greatly improve our understanding of light control of plant development.

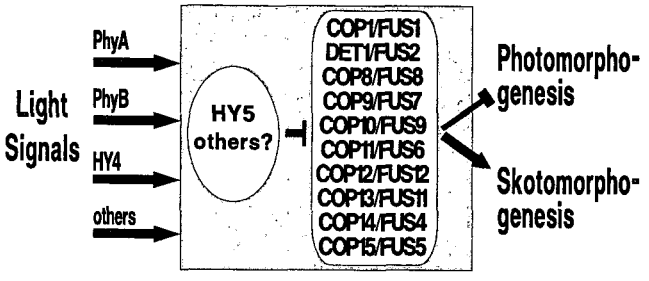

Figure 8. A genetic model for the roles of the pleiotropic *COP/DET/FUS* genes in mediating the light control of Arabidopsis seedling development. Multiple photoreceptors converged to negatively regulate the activity of the pleiotropic *COP/DET/FUS* gene products, and HY5 may represent one of the intermediates involved. The pleiotropic *COP/DET/FUS* gene products act as a master repressor(s) that controls all downstream pathways responsible for seedling development.

ACKNOWLEDGMENTS

We thank Drs. Jeffrey Staub, Ning Wei, and Daniel Chamovitz for their comments concerning this manuscript. We would like to thank the Arabidopsis Resource Center at Ohio State University for kindly providing the Dijon seeds and Dr. Garry Whitelam for providing the *phyA-1* seeds. We would like to dedicate this paper in celebration of Professor Pill Soon Song's 60th birthday.

Received August 29, 1995; accepted November 20, 1995.

LITERATURE CITED

Ang LH, Deng XW (1994) Regulatory hierarchy of photomorphogenic loci: allele-specific and light-dependent interaction between the *HY5* and *COP1* loci. Plant Cell **6:** 613–628

Bowler C, Chua NH (1994) Emerging themes of plant signal transduction. Plant Cell **6:** 1529–1541

Castle LA, Meinke DW (1994) A *FUSCA* gene of *Arabidopsis* encodes a novel protein essential for plant development. Plant Cell **6:** 25–41

Chory J (1993) Out of darkness: mutants reveal pathways controlling light-regulated development in plants. Trends Genet **9:** 167–172

Chory J, Peto CA (1990) Mutations in the *DET1* gene affect cell-type-specific expression of light-regulated genes and chloroplast

development in *Arabidopsis*. Proc Natl Acad Sci USA **87:** 8776–8780

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

Deng XW (1994) Fresh view of light signal transduction in plants. Cell **76:** 423–426

Deng XW, Caspar T, Quail PH (1991) *COP1*: a regulatory locus involved in light-controlled development and gene expression in *Arabidopsis*. Genes Dev **5:** 1172–1182

Deng XW, Matsui M, Wei N, Wagner D, Chu AM, Feldmann KA, Quail PH (1992) *COP1*, an *Arabidopsis* regulatory gene, encodes a protein with both a zinc binding motif and a G_β homologous domain. Cell **71:** 791–801

Deng XW, Quail PH (1992) Genetic and phenotypic characterization of *cop1* mutants of *Arabidopsis thaliana*. Plant Cell **3:** 83–95

Hou Y, von Arnim AG, Deng XW (1993) A new class of *Arabidopsis* constitutive photomorphogenic genes involved in regulating cotyledon development. Plant Cell **5:** 329–339

Koornneef M, Rolff E, Spruit CJP (1980) Genetic control of light-inhibited hypocotyl elongation in *Arabidopsis thaliana* (L.) Heynh. Z Pflanzenphysiol **100:** 147–160

McNellis TW, Deng XW (1995) Light control of seedling morphogenetic pattern. Plant Cell **7:** 1749–1761

McNellis TW, von Arnim AG, Araki T, Komeda Y, Miséra S, Deng XW (1994a) Genetic and molecular analysis of an allelic series of *cop1* mutants suggests functional roles for the multiple protein domains. Plant Cell **6:** 487–500

McNellis TW, von Arnim AG, Deng XW (1994b) Overexpression of *Arabidopsis* COP1 results in partial suppression of light-mediated development: evidence for a light-inactivable repressor of photomorphogenesis. Plant Cell **6:** 487–500

Miséra S, Müller AJ, Weiland-Heidecker U, Jürgens G (1994) The *FUSCA* genes of *Arabidopsis*: negative regulators of light responses. Mol Gen Genet **244:** 242–252

Müller AJ (1963) Embryonentest zum nachweis rezessiver letalfaktoren bei *Arabidopsis thaliana*. Biol Zentralbl **82:** 133–163

Pepper A, Delaney T, Washburn T, Pool D, 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

Quail PH (1994) Photosensory perception and signal transduction in plants. Curr Opin Genet Dev **4:** 652–661

von Arnim AG, Deng XW (1994) Light inactivation of *Arabidopsis* photomorphogenic COP1 involves a cell specific regulation of its nucleo-cytoplasmic partitioning. Cell **79:** 1035–1045

Wei N, Chamovitz DA, Deng XW (1994a) *Arabidopsis* COP9 is a component of a novel signaling complex mediating light control of development. Cell **78:** 117–124

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

Wei N, Kwok SF, von Arnim AG, Lee A, McNellis TW, Piekos B, Deng XW (1994b) *Arabidopsis COP8, COP10,* and *COP11* genes are involved in repression of photomorphogenic development in darkness. Plant Cell **6:** 629–643

Whitelam GC, Johnson E, Peng J, Carol P, Anderson ML, Cowl JS, Harberd NP (1993) Phytochrome A null mutants of *Arabidopsis* display a wild-type phenotype in white light. Plant Cell **5:** 757–768