Extragenic Suppressors of the Arabidopsis *det1* Mutant Identify Elements of Flowering-Time and Light-Response Regulatory Pathways

Alan E. Pepper* and Joanne Chory

*Plant Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, California 92037 and *Department of Biology and Crop Biotechnology Center, Texas A & M University, College Station, Texas 77843*

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ABSTRACT

Light regulation of seedling morphogenesis is mediated by photoreceptors that perceive red, far-red, blue and UV light. Photomorphogenetic mutants of Arabidopsis have identified several of the primary photoreceptors, as well as a set of negative regulators of seedling photomorphogenesis, including *DET1*, that appear to act downstream of the photoreceptors. To study the regulatory context in which *DET1* acts to repress photomorphogenesis, we used a simple morphological screen to isolate extragenic mutations in six loci, designated *ted* (for reversal of the *det* phenotype), that partially or fully suppress the seedling morphological phenotype of *det1-1*. Genetic analyses indicate that mutations in the *ted4* and *ted5* loci identify new alleles of the previously described photomorphogenetic loci *hy1* and *hy5*, respectively. Molecular analyses indicate that the *ted* mutations partially suppress the dark-grown gene expression phenotype of *det1-1*, and that the mechanism of suppression does not involve direct remediation of the splicing defect caused by the *det1-1* mutation. The *ted* mutations also partially suppress the light-grown morphological phenotype of mature *det1-1* plants, and *ted1* and *ted2* suppress a daylength insensitivity phenotype of *det1*. *TED1*, *TED2* and *TED3* are newly described genes, whose function appears closely associated with that of *DET1*. In addition, alleles of *ted1* are associated with a moderate late-flowering phenotype, suggesting that *TED1* plays a role in the pathways that regulate both seedling morphogenesis and the initiation of flowering.

THROUGHOUT their life history, plants display remarkable developmental plasticity in response to prevailing conditions of light quality and quantity. These responses include the regulation of seed germination, growth phototropisms and the timing of flowering. In part because of their small size, young Arabidopsis seedlings are particularly well-suited as subjects for rigorous genetic analyses of developmental responses to light (reviewed by CHORY 1993). Seeds that germinate in darkness adopt a light-foraging strategy, termed etiolated growth, characterized by elongation of the seedling stem (the hypocotyl) and arrested development of leaves and chloroplasts. Upon exposure to red, far-red, or blue-UV light, seedlings undergo de-etiolation, a process in which seedlings slow their rate of hypocotyl elongation and induce leaf and chloroplast development. Arabidopsis mutants deficient in de-etiolation responses have identified several photoreceptors that mediate de-etiolation. For example, long *hypocotyl* mutant *hy1* is deficient in the biosynthesis of the linear tetrapyrrole chromophore of the red/far red photoreceptor phytochrome (KOORNNEEF *et al.* 1980). Mutants *phyB* and *phyA* are defective in the apoprotein components of the phytochomes PHYB and PHYA that mediate de-etiolation responses to red and far-red light, respectively (NAGATANI *et al.* 1993; PARKS and QUAIL 1993; REED *et al.* 1993; WHITELAM *et al.* 1993). Similarly, the blue-light insensitive mutant *cry1* (*hy4*) is defective in a blue light photoreceptor with amino acid sequence homology to an *Escherichia coli* blue-light-dependent photolyase (AHMAD and CASHMORE 1993). The *hy5* mutant is deficient in red, far-red, and blue light responses and may act downstream from the photoreceptors (CHORY 1992). *HY5* encodes a basic leucine zipper transcription factor (T. OYAMA and K. OKADA, personal communication).

Arabidopsis mutants have also been isolated that resemble light-grown plants when grown in complete darkness. These include the *deetiolated* mutants *det1* (CHORY *et al.* 1989), *det2* (CHORY *et al.* 1991) and *det3* (CABRERA Y POCH *et al.* 1993), and the *constitutive photomorphogenesis* mutants *cop1* (DENG *et al.* 1991), *cop9* (WEI and DENG 1992), *cop8*, *cop10*, and *cop11/fus6* (CASTLE and MEINKE 1994; WEI *et al.* 1994). In the dark, *det1* seedlings have a short hypocotyl, open and expanded cotyledons and readily visible leaf primordia. Eventually, *det1* plants will make a full set of leaves and flower in complete darkness (PEPPER *et al.* 1994). At the subcellular level, dark-grown *det1* seedlings display partial development of chloroplasts and high levels of expression of genes normally induced by light, such as *cab*, *rbcS* and *chs* (CHORY *et al.* 1989). Based on the recessive nature of *det1* we hypothesized that *DET1* encodes a negative regulator of seedling deetiolation responses.

Corresponding author: Alan E. Pepper, Department of Biology, Texas A & M University, College Station, TX 77843-3258.
E-mail: apepper@bio.tamu.edu.

The *DET1* gene was isolated by a genetic map-based cloning strategy and was found to encode a novel 62.2-kDa protein (PEPPER *et al.* 1994). The *DET1* polypeptide does not appear to bind DNA (A. PEPPER, K. COOK and J. CHORY, unpublished observations) but does contain sequences with a strong similarity to consensus SV40 bipartite-type and yeast *MATα* nuclear localization signals. A DET1/β-glucuronidase fusion polypeptide is predominantly localized to the plant cell nucleus, suggesting that DET1 itself may function as a nuclear protein, perhaps as a non-DNA binding repressor of transcription that acts via protein/protein contacts (PEPPER *et al.* 1994). Transcription of *DET1* is not regulated by light, suggesting a possible role as a signal transduction element, rather than as a downstream effector molecule acting in a transcriptional cascade.

The role of DET1, however, is not limited to light regulation of seedling morphogenesis. Plants homozygous for all known *det1* and *cop* alleles display mutant phenotypes as mature plants when grown in the light or the dark. Light-grown *det1-1* plants are small, have reduced apical dominance and reduced fecundity. Plants homozygous for null alleles of *det1*, such as *det1-6* (PEPPER *et al.* 1994), have greatly reduced size and produce extremely high levels of anthocyanin in the light and in the dark. The phenotype of the *det1* null mutant is, in fact, similar to strong alleles of *cop1*, *cop9*, and other members of the *fusca* class of seedling mutants (MISERA *et al.* 1994), such as *fus6/cop11* (CASTLE and MEINKE 1994). CASTLE and MEINKE (1994) determined that the *fus6* mutation affects a number of physiological and developmental pathways including responses to exogenous sugars and phytohormones. Effects of *fus6* on light responses have also been observed (WEI *et al.* 1994). Finally, the dark-grown seedling phenotypes of weak and moderately strong alleles of *DET1* and *COP1* can be partially phenocopied by the addition of high levels of exogenous cytokinin in the growth media (CHORY *et al.* 1994). These observations, considered together, suggest that *DET1*, and other members of the *DET/COP/FUS* class of genes, participate in a number of (potentially networked) regulatory pathways, including the light-signaling pathways that mediate deetiolation (for a review and commentary see REED and CHORY 1994).

Two related and nonexclusive hypotheses for the interaction of *DET1* with light-signaling pathways can be proposed. In both models, DET1 is postulated to be a semi-specific negative regulatory molecule, perhaps a transcriptional repressor. One hypothesis stipulates that in response to appropriate light signals, deetiolation is initiated by posttranscriptional down-regulation of DET1 activity. This could involve any one of several mechanisms such as protein modification of DET1, or the presentation of alternative protein contacts (thus titrating DET1 from contacts associated with repression). In an alternative scenario, DET1 plays a less direct role. Light or other environmental and developmental signals activate an as yet undefined transcriptional regulator that is to some degree insensitive to repression by DET1. When this regulator is activated, DET1 activity is *de facto* down-regulated. In the *det1* mutant, one would postulate that the positive regulatory molecule(s) initiates deetiolation in the absence of the appropriate signal(s).

A critical element in our study of the pathways by which light stimulates deetiolation is the elucidation of how DET1-mediated repression of deetiolation is overcome upon exposure to light. To study the regulatory environment in which DET1 is acting, we set out to identify extragenic suppressors of *det1*, which would restore repression of deetiolation responses in the dark. Based on analysis of mutant phenotypes in the *det1* allelic series, *det1-1* is partially functional (PEPPER *et al.* 1994). Thus, in formal genetic terms, suppressors of *det1-1* could be mutant alleles of genes that normally act upstream of *DET1*, downstream of *DET1*, or in pathways that bypass *DET1* activity. In this work, we describe the isolation and characterization of suppressors of *det1-1* using a simple screen based on the gross morphology of dark grown seedlings.

MATERIALS AND METHODS

Plant strains and growth conditions: Plant media and growth conditions were as described previously (PEPPER *et al.* 1994) unless otherwise noted. Previously described *Arabidopsis thaliana* (L.) Heynh. strains used in this work are summarized in Table 1. Genetic nomenclature follows the accepted guidelines for *A. thaliana* (MEINKE 1995).

Mutageneses and screens for suppressors of *det1-1*: A *det1-1* homozygous line was bulk-propagated in isolation in a growth chamber. These seeds were mutagenized by imbibition in 0.3% EMS (Sigma) for 16 hr, followed by extensive washing with dH_2O. Two sets of mutagenized seeds were utilized. In the first, 30,000 M_1 plants were propagated in greenhouse conditions, from which M_2 seeds (140,000 total) were harvested from pools of 600 M_1 plants. In the second, 15,000 M_1 plants were propagated, from which M_2 seeds (150,000 total) were harvested from pools of 20 M_1 plants. Approximately 2000–3000 seeds from each large pool and 100–200 seeds from each small pool were sterilized and grown in the dark on agar plates for 7 days (CHORY *et al.* 1989), then screened for plants with a long hypocotyl and/or small, unexpanded cotyledons.

Putative suppressor mutants were transferred to light for recovery, then backcrossed to *det1-1* before further genetic and phenotypic analyses. We used a PCR-based diagnostic for the *det1-1* allele (described below) to eliminate candidate suppressors that were no longer homozygous for the *det1-1* mutation (possibly arising from pollen contamination, seed contamination, or exact reversion of the *det1-1* mutation).

Genetic analysis: Standard methods of Arabidopsis genetic analysis were employed (SOMERVILLE and OGREN 1981) unless otherwise noted. Dominance or recessiveness of putative suppressors was determined by analysis of dark-grown F_1 and F_2 phenotypes in crosses to *det1-1* homozygous lines. To discriminate intragenic from extragenic suppressors, two independent tests for linkage of suppressor mutations to the *det1* locus were employed. In the first approach, putative suppressors

TABLE 1

Previously described *A. thaliana* strains

Strain/genotype	Ecotype	References
DET1 (wild type)	Col-0	REDEI (1992)
det1-1	Col-0	CHORY *et al.* (1989); PEPPER *et al.* (1994)
det1-4	Col-0	PEPPER *et al.* (1994)
det1-6	La-*er*	MISÉRA *et al.* (1994); PEPPER *et al.* (1994)
La-*det1-1*	La-*er*/Col-0	PEPPER *et al.* (1994)
hy1-1	La-*er*	KOORNNEEF *et al.* (1980)
hy2-1	La-*er*	KOORNNEEF *et al.* (1980)
hy5-1	La-*er*	KOORNNEEF *et al.* (1980)
PhyA-201	La-*er*	REED *et al.* (1994)
PhyB-1	La-*er*	KOORNNEEF *et al.* (1980)

were crossed to wild-type Col-0, and resulting F_2 populations were examined for the segregation of deetiolated seedlings; the absence of deetiolated seedlings in a large F_2 population derived from this cross was taken as evidence of complete linkage of the suppressor mutation to the *det1-1* locus. In a second approach, suppressors were tested for linkage (using methods described below) to the simple sequence length polymorphism (SSLP) marker nga8 (BELL and ECKER 1994), located ~3 cM from DET1.

Recessive mutations were assigned to complementation groups through the analysis of F_1 and F_2 phenotypes. Dominant and semi-dominant extragenic suppressors were assigned to allelic groups based upon two independent lines of evidence: (1) examination of the F_2 progeny of repulsion-phase crosses between suppressor lines (the absence of deetiolated seedlings in the F_2 progeny was taken as evidence of allelism) and (2) genetic mapping of the suppressor loci (described below).

Mapping methods: At present, DNA-based mapping methods in Arabidopsis require that the locus of interest be segregating in a mapping population derived from an interecotypic cross (*e.g.*, La-*er* × Col-0). Yet, the suppressor phenotype can only be scored in plants that are homozygous for the *det1-1* mutation (derived from the Col-0 ecotype). To circumvent this problem, we introgressed the *det1-1* mutation into the Landsberg *erecta* (La-*er*) ecotypic background through eight successive outcrosses to La-*er*, creating La-*det1-1* (PEPPER *et al.* 1994). Fingerprinting analysis using a set of 12 SSLPs and seven cleaved amplified polymorphic sequence (CAPS) markers (KONIECZNY and AUSUBEL 1993) indicated that, with the exception of a *DET1* CAPS marker, the La-*det1-1* line had homozygous La-*er* genotypes at markers throughout the genome, including *DET1*-flanking markers nga8 and AG (chromosome *IV*, 35 and 72.6 cM, respectively) (LISTER and DEAN 1993; http://cbil.humgen.upenn.edu/atgc/genetic-mapping/ListerFeb95.html). Thus, La-*det1-1* retained a segment of Col-0 chromosome containing *det1-1* that is <38 cM in size. To create an F_2 mapping population, suppressors were crossed to La-*det1-1*. After phenotyping, F_2 seedlings were transferred to fresh agar plates and grown in the light for 2 weeks before harvest for DNA. Dominant/semi-dominant suppressors were mapped by genotyping plants with a nonsuppressed (deetiolated) phenotype in the F_2. Preliminary mapping was performed using a set of 12 SSLP markers and six CAPS markers located at intervals throughout the genome. All linkage data reported is based on raw recombination frequency.

Genomic DNA isolations: Fresh individual seedlings were ground for 5 sec in 1.5-ml microcentrifuge tubes using Teflon pestles (VWR). Extraction buffer (200 mM Tris pH 7.5, 250 mM NaCl, 25 mM EDTA pH 8, 0.5% SDS) was added, and tissues were further ground. After a brief centrifugation to remove solids, nucleic acids were precipitated from the extract with isopropyl alcohol. The precipitate was resuspended in 50 mM Tris pH 7.5, 10 mM EDTA pH 8 and centrifuged to remove undissolved solids. Nucleic acids were then again precipitated from the extract with isopropyl alcohol and resuspended in 0.1 ml of 10 mM Tris pH 7.5, 1 mM EDTA. Two microliters of the nucleic acid solution prepared by this method was used as substrate for PCR.

Phenotypic analysis of suppressor mutants: All phenotypic analysis of suppressor mutants was performed in the *det1-1* homozygous genetic background unless otherwise stated. Analysis of splicing of intron 1 of the *DET1* transcript in suppressors of *det1-1* was performed by reverse transcriptase-PCR (RT-PCR) methods. Total RNA was isolated (NAPOLI *et al.* 1990) and digested with 15 units of DNAase I (FPLC-pure RNAase-free, Pharmacia) for 2 hr at 37° to eliminate contaminating genomic DNA. RNA samples were then extracted with phenol/chloroform, and 10-μg aliquots were reverse transcribed for 2 hr at 42° using random hexamer primers and 200 units of M-MLV RT (Gibco-BRL). Reverse transcription products were diluted 1:20 in dH_2O, and 5-μl aliquots of diluted first-strand cDNA were used as template for 50 μl PCR reactions. *DET1*-specific oligos 13-1G (CTTCGAACGTCAGATTCGAACTCCTC) and 7-1H (CAAATCTCCCTGAAGATAA) were used to amplify transcribed regions extending from exon 1 to exon 3. To estimate the efficiency of splicing of intron 1, a quantitative RT-PCR method was employed. For this analysis, 20 cycles of PCR were performed. The presence of intron 1 and intron 2 in RT-PCR products was assayed by digestion with *Bst*BI, which cleaves the PCR product asymetrically (inside exon2), giving rise to digestion products of characteristic sizes: (1) 678 bp, corresponding to transcripts with unspliced intron 2, or contaminating genomic DNA; (2) 604 bp, corresponding to transcripts with properly spliced exon 2; (3) 345 bp, corresponding to transcripts unspliced at intron 1; and (4) 254 bp, corresponding to transcripts in which intron 1 is properly spliced. To complete the quantitative analyses, digestion products were Southern blotted, probed with a full length *DET1* genomic DNA, and quantitated by Phosphorimager and ImageQuant software (Molecular Dynamics).

Northern blots and probes were as described previously (PEPPER *et al.* 1994). Northern analysis was performed using duplicate RNA samples, quantified by PhosphorImage analysis, and normalized to signals from an rDNA probe. Apical dominance in mature light-grown plants was quantitated by counting the number of inflorescence axes at the time of appearance of the first mature, yellow silique (stage 6.5 in the Arabidopsis developmental key) (http://genome-www.stanford.edu/Arabidopsis/comguide/chap_1_plants/5_developmental_key.html). Developmental time-to-flow-

ering was measured by counting the number of rosette and cauline leaves on the primary inflorescence axis. At the transition to reproductive growth, the shoot apical meristem stops producing leaves and starts producing flowers. Leaf number is thus a measurement of "developmental time" to flowering that minimizes experimental noise due to uneven germination and microenvironmental differences. Our rationale for counting both rosette and cauline leaves, rather than rosette leaves only, is based on the recent electron micrographic characterization of floral induction by HEMPEL and FELDMAN (1995). This analysis indicated that the meristematic transition to reproductive growth occurs before any elongation of the primary inflorescence axis (the flowering stem), and that at the time of floral induction, the cauline leaf primordia were already formed were initially indistinguishable from rosette leaf primordia. The total number of leaves produced (rosette and cauline) is therefore the best available measure of developmental time to flowering. Long-day (16 hr) and short-day (10 hr) grown plants were given 150 $\mu\epsilon$-m^{-2}-sec^{-1} and 240 $\mu\epsilon$-m^{-2}-sec^{-1}, respectively, of white light supplied by an equal mix of Phillips Cool-White and Gro-lux wide-spectrum bulbs.

PCR-based diagnostic assays for *DET1* alleles: Plants carrying various *det1* alleles (*det1-1, det1-4, det1-6*) (PEPPER *et al.* 1994) were identified by PCR-based diagnostics. The presence of the *det1-1* lesion was determined by a CAPS assay. The *det1-1* mutation destroys a *Bsm*FI recognition site in the *DET1* gene. A genomic DNA fragment containing the site of the *det1-1* lesion was amplified using primers 13-1G (CTTCGAACGTCAGATTCGAACTCCTC) and 13-1revB (CATTGAAGGTAAAGAGATAAGC) then tested for cleavage by *Bsm*FI. To test for the *det1-4* and *det1-6* lesions, a PCR-primer introduced restriction site analysis method (SORSCHER and HUANG 1989) was employed. Primer det1-4 (ACTGTTTCCGCAAATTC**C**CAGAAG) contains a mismatch to genomic DNA (indicated in bold) which, when incorportated in a PCR product, creates an artificial restriction enzyme recognition site for *Bsl*I (**C**CNNNNNNNG̲G) that spans the 3′ end of the oligo and includes the nucleotide that is mutated from G to A in *det1-4* (underlined). Primers det1-4 and 7-1revB (ACCAGGAACAGCGTCATTAG) were used to amplify a 321-bp PCR product. Amplification products not carrying the *det1-4* mutation are cleaved by *Bsl*I into 298- and 23-bp fragments. Similarly, oligo det1-6 (CTTTTGCTTATCTCTTTACCT**C**CAATG) (mismatch indicated in bold) was used in combination with 13-1revA (ATGAGGAAGAGTCCGTCTTC) to amplify a 143-bp PCR product. Amplification products not carrying the *det1-6* mutation are cleaved by *Bsl*I into 115- and 28-bp fragments. Restriction products were analyzed using 2.5% MetaPhor agarose gels (FMC Corp.).

RESULTS

Screens for suppressors of *det1-1*: From an estimated total of 45,000 M_1 plants and 290,000 M_2 seeds screened, 40 putative suppressors were isolated. More than half of the collection (20/40) had at least one wild-type *DET1* (*Bsm*FI cleavable) allele when tested with the *det1-1* PCR-based diagnostic assay. (Interestingly, all plants that displayed complete phenotypic suppression of *det1-1* fell into this category.) The majority of the DET1-containing lines (14/20) were heterozygous for *Bsm*FI cleavage. The high level of heterozygosity for *det1-1* seen in these lines suggests that they arose from pollen contamination onto stigmata of M_1 *det1-1* plants or EMS-induced reversion of the *det1-1* lesion rather than from seed contamination.

The remaining 20 plants, homozygous for *det1-1*, were considered candidate extragenic or intragenic suppressors of *det1-1* and analyzed further. In some cases, putative suppressors identified in M_2 screens failed to deetiolate when transferred to light. When these occurred in small (20 M_1) pools, they were recovered from siblings. In screens of small pools, putative suppressors from the same pool were assumed to be siblings, and only one representative was recovered for further analysis.

Genetic characterization of suppressors of *det1-1*: Analysis of F_1 and F_2 phenotypes of backcrosses to *det1-1* indicated that 10 of the suppressor mutations were recessive and 10 were dominant or semi-dominant. Four of the dominant/semi-dominant mutations showed tight linkage to nga8 (no recombinants with nga8 were observed in analyses of 24 or more F_2 seedlings). In crosses of these four lines to wild-type Col-0, fully deetiolated seedlings were not observed in the F_2 (at least 400 seedlings examined in each cross). Thus, two independent lines of evidence indicated that in these four mutants suppression was closely linked to the *det1-1* locus. Further analysis of these linked suppressors, which may be intragenic, will be described elsewhere.

Extragenic suppressor mutants were designated *ted* (for reversal of the *det* phenotype). Dominant and semi-dominant mutations were assigned to three classes, *ted1, ted2* and *ted3*, on the basis of allelism in repulsion phase crosses and preliminary mapping data. Four semi-dominant alleles were assigned to the *ted1* group (designated *ted1-1SD*, etc.), while *ted2* and *ted3* contained one dominant allele each (designated *ted2-1D* and *ted3-1D*, respectively). Recessive mutations were assigned to three complementation groups, *ted4, ted5* and *ted6*. The *ted4* group included five alleles and the *ted5* group included four alleles. The *ted6* complementation group contained only one allele, which suppressed *det1-1* very weakly, and will not be described further. The dark-grown seedling phenotypes of *ted1-1SD, ted2-1D, ted3-1D, ted4-1* and *ted5-1* in the *det1-1* genetic background are shown in Figure 1. In each case, the suppressor line shows a significantly elongated hypocotyl and reduced development of the cotyledons and leaf primordia relative to *det1-1*.

The preliminary genetic analysis of multiply backcrossed representative *ted* alleles is summarized in Table 2. In further backcrosses to *det1-1* (Table 2A), the suppression phenotype appears to be monogenic in each case. Outcrosses to wild-type *DET1* demonstrate that each locus is unlikely to be linked to *det1-1*, except for *ted1*, which showed linkage of ~19 cM, based on an analysis of 1606 F_2 seedlings. Twelve of the 27 deetiolated F_2 seedlings from this cross were grown to maturity and F_3 seeds were isolated. All progeny from these deetiolated F_2 plants had a deetiolated phenotype, indicat-

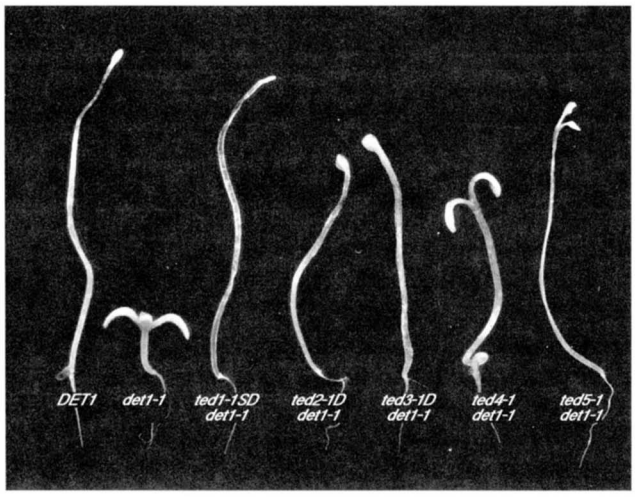

FIGURE 1.—Morphological phenotypes of 7-day-old dark-grown wild type (*DET1*), the *det1-1* mutant and *ted det1-1* seedlings.

ing that *ted1* is not an intragenic suppressor with incomplete penetrance.

In repulsion phase crosses between representative dominant and semi-dominant mutants (Table 2B) deetiolated seedlings were segregating in all crosses indicating that these representative mutations are not allelic to one another. However, the ratio of suppressed/deetiolated seedlings in the *ted1-1SD* × *ted2-1SD* (8.38:1) was less than the 15:1 expected for two unlinked dominant markers, and the ratio for *ted2-1D* × *ted3-1D* (31.3:1) was greater than that expected for two unlinked dominant markers (only the former was rejected by chi-squared test, $P < 0.025$). One explanation for the *ted1-1SD* × *ted2-1SD* results is an interaction between *ted1-1SD* and *ted2-1D*, perhaps effecting germination efficiency.

To routinely assess the degree of suppression of *det1-1*, hypocotyl length of dark grown seedlings was used as an easily measurable quantitative trait (Table 3). Hypocotyls of dark-grown F_1 seedlings from *ted det1-1* × *det1-1* crosses were measured and compared to homozygous parental controls. Both *ted2-1D* and *ted3-1D* were essentially dominant for hypocotyl phenotype, although the heterozygous individuals displayed a higher frequency of open cotyledons than in their respective homozygous *ted det1-1* parents (not shown). *ted1-1* was semi-dominant for both hypocotyl length and frequency of open cotyledons, while *ted4-1* and *ted 5-1* were fully recessive for both hypocotyl and cotyledon phenotypes.

Genetic mapping of *ted* loci: Map positions of the *ted* loci were estimated using small F_2 populations (120–180 individuals total, from which 28–48 individuals were genotyped at 12 SSLP and six CAPS loci). As discussed above, *ted1-1SD* displayed moderate linkage (19 cM) to *det1* in crosses to wild-type *DET1*. Based on the analysis of 34 F_2 seedlings, *ted1* showed linkage to nga8 at a distance of ~25 cM. Analysis of this same set of plants also indicated linkage (~25 cM) to AG. These independent lines of evidence, considered together, indicate linkage of *ted1* to chromosome *IV* at a position between *DET1* and *AG*.

Genotyping of 42 F_2 seedlings indicated that *ted2* is

TABLE 2

Genetic analysis of *ted* suppressor lines

Cross	sup	det	Ratio	n.h.	*P*
A. F_2 analysis of *ted* backcrosses to *det1-1* and crosses to *DET1*					
ted1-1SD det1-1 × *det1-1*	198	63	3.14:1	3:1	> 0.5
ted2-1D det1-1 × *det1-1*	96	36	2.67:1	3:1	> 0.5
ted3-1D det1-1 × *det1-1*	105	34	3.09:1	3:1	> 0.9
ted4-1 det1-1 × *det1-1*	16	51	1:3.18	1:3	> 0.5
ted5-1 det1-1 × *det1-1*	21	66	1:3.14	1:3	> 0.5
ted6-1 det1-1 × *det1-1*	35	87	1:2.49	1:3	> 0.1
DET1 × *ted1-1SD det1-1*	1579	27	58.5:1	15:1	< 0.005 r
DET1 × *ted2-1D det1-1*	233	17	13.7:1	15:1	> 0.5
DET1 × *ted3-1 det1-1*	168	10	16.8:1	15:1	> 0.5
DET1 × *ted4-1 det1-1*	153	37	4.14:1	13:3	> 0.5
DET1 × *ted5-1 det1-1*	46	9	5.11:1	13:3	> 0.5
DET1 × *ted6-1 det1-1*	369	89	4.15:1	13:3	> 0.5
B. F_2 analysis of repulsion phase crosses between dominant/semidominant *ted* mutants					
ted1-1SD det1-1 × *ted2-1D det1-1*	151	18	8.38:1	15:1	<0.025 r
ted1-1SD det1-1 × *ted3-1D det1-1*	116	9	12.8:1	15:1	> 0.5
ted2-1D det1-1 × *ted3-1D det1-1*	125	4	31.3:1	15:1	> 0.1

In these experiments, suppressed (sup) is used to describe a wide range of phenotypes, from weak suppression of *det1-1* (as in *ted4-1 det1-1*) to fully etiolated (as in the wild-type *DET1*). Deetiolated (det) is used to describe phenotypes similar to *det1-1*. Segregation data was evaluated with chi-squared analysis using the null hypothesis (n.h.) indicated. Chi-squared probabilities (*P*) are indicated. Rejection of the null hypothesis is indicated (r).

TABLE 3

Hypocotyl length of 6-day-old dark-grown parental and F_1 seedlings

Parental line	Hypocotyl length	
	Parent	F_1 (× *det1-1*)
det1-1	2.9 ± 1.1 (21)	ND
DET1	13.6 ± 1.2 (30)	12.7 ± 1.3 (15)
ted1-1SD det1-1	13.4 ± 1.0 (22)	8.5 ± 0.8 (9)
ted2-1D det1-1	12.2 ± 1.0 (22)	11.6 ± 1.3 (8)
ted3-1 det1-1	12.0 ± 1.0 (22)	13.9 ± 1.1 (10)
ted4-1 det1-1	7.4 ± 1.7 (22)	3.4 ± 1.2 (7)
ted5-1 det1-1	12.1 ± 1.7 (22)	3.4 ± 0.9 (7)

Number tested is indicated in parentheses. Error shown is standard deviation.

ND, experiment not done.

located on chromosome *I* ~5 cM from nga248 (*I*, 57.1 cM), between nga248 and GAPB (*I*, 86.2 cM). On the basis of genotyping 28 F_2 seedlings, *ted3* mapped to chromosome *I*, ~10 cM from nga 280 (*I*, 114.6 cM), between nga 280 and nga111 (*I*, 149.2 cM). Further evidence indicating that *ted2* and *ted3* are independent loci was obtained in repulsion phase crosses of *ted2-1* × *ted3-1*. In the F_2 of this cross, deetiolated seedlings were observed at a frequency of 0.035 (Table 2B), suggesting two independent, but perhaps linked suppressors. Unlike the homozygous parents, F_1 plants from the *ted2-1* × *ted3-1* cross have an essentially wild-type (*DET1*) phenotype, both as dark grown seedlings and as mature light-grown plants. This additivity of phenotype in the F_1 of two dominant mutants suggests that the mechanisms of suppression by the two mutations are different. These results, considered together, suggest that *ted2-1D* and *ted3-1D* are independent mutations located on chromosome *I* that suppress *det1-1* by different mechanisms. Finally, *ted4* showed linkage (12 cM) to nga168 (*II*, 78.5 cM) on the basis of 45 F_2 seedlings genotyped and *ted5* was linked (20 cM) to nga225 (*V*, 11 cM) as determined by the analysis of 42 F_2 seedlings.

The *ted* mutations do not restore correct splicing of the *det1-1* transcript: The *det1-1* mutation is a G to A transition located just inside intron 1, five nucleotides from the 5′ splice junction (Pepper *et al.* 1994). Previous Northern analysis indicated that the mature *DET1* transcript is ~100 nucleotides larger in the *det1-1* mutant than in the wild-type Col-0. This apparent mobility shift closely matches what would be expected for transcripts that have not spliced out the 85 nucleotides of intron 1. In addition, an intron 1-specific probe hybridizes to the abnormally long transcript observed in the *det1-1* mutant. Translation of mRNAs containing intron 1 terminates prematurely at two consecutive stop codons (TAG, TGA) located within intron 1, producing a truncated polypeptide consisting of 24 wild-type residues and 26 residues encoded by the intron. We hypothesized that the apparent partial *DET1* activity in the *det1-1* mutant might be due to a low level of correct splicing of intron 1, giving rise to residual wild-type DET1 polypeptide. Based on this model, several possible mechanisms of suppression can be imagined that involved modification of the transcription or splicing machinery to produce more correctly spliced *DET1* transcript from the *det1-1* template. Suppression by translational mechanisms (*e.g.*, nonsense suppression) was considered to be exceedingly unlikely given the adjacent TAG (amber) and TGA (opal) stop codons.

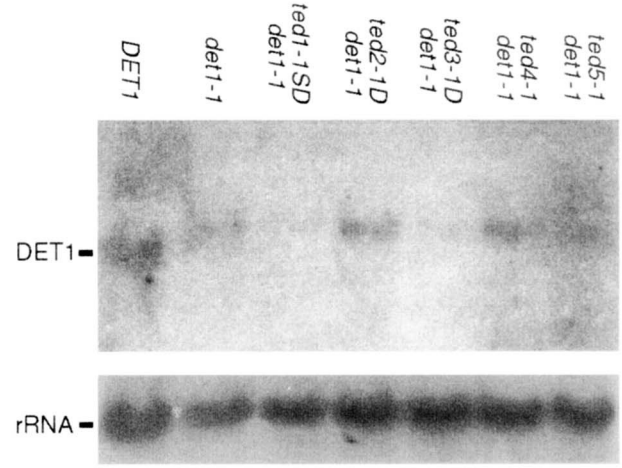

FIGURE 2.—Northern analysis of the *DET1* transcript in wild type (*DET1*), the *det1-1* mutant and in *ted det1-1* lines. RNAs were isolated from 21-day old, light-grown plants. Fifteen micrograms of total RNA (measured by $O.D._{260}$) were loaded onto each lane. The *DET1* transcript was analyzed by probing with 4-kb genomic DNA fragment containing the entire *DET1* gene (Pepper *et al.* 1994). *DET1* blot was exposed for 290 hr onto BIOMAX MS film (Kodak). Ribosomal RNA probe (Chory *et al.* 1989) was exposed for 45 min onto RX film (Fuji).

Figure 2 shows a Northern analysis of *DET1* transcripts in *DET1*, *det1-1*, and in the *ted* mutants. As shown, the overall levels of the *DET1* transcript were not appreciably higher in any of the *ted* mutants than in *det1-1*, indicating that suppression is not achieved by an overall increase in *det1-1* transcription or mRNA stability (that would compensate for reduced efficiency of splicing of intron 1). In addition, no novel transcripts were observed, indicating that suppression was not the result of a gross alteration in the transcribed sequences or the splicing pattern of the *det1-1* transcript. To investigate possible subtle effects on mRNA structure, total RNA from *det1-1* and from *ted* mutants in the *det1-1* background was isolated, reverse transcribed, and a DNA fragment extending from exon 1 to exon 3 was amplified by PCR. The resulting RT-PCR products were analyzed by restriction digestion with the frequently cutting 4-bp recognition site restriction enzymes *Alu*I, *Mbo*II, *Mnl*I, *Sau*3A. No discernible differences between *det1-1* and any of the suppressor lines were observed (data not shown).

To investigate possible quantitative changes in splic-

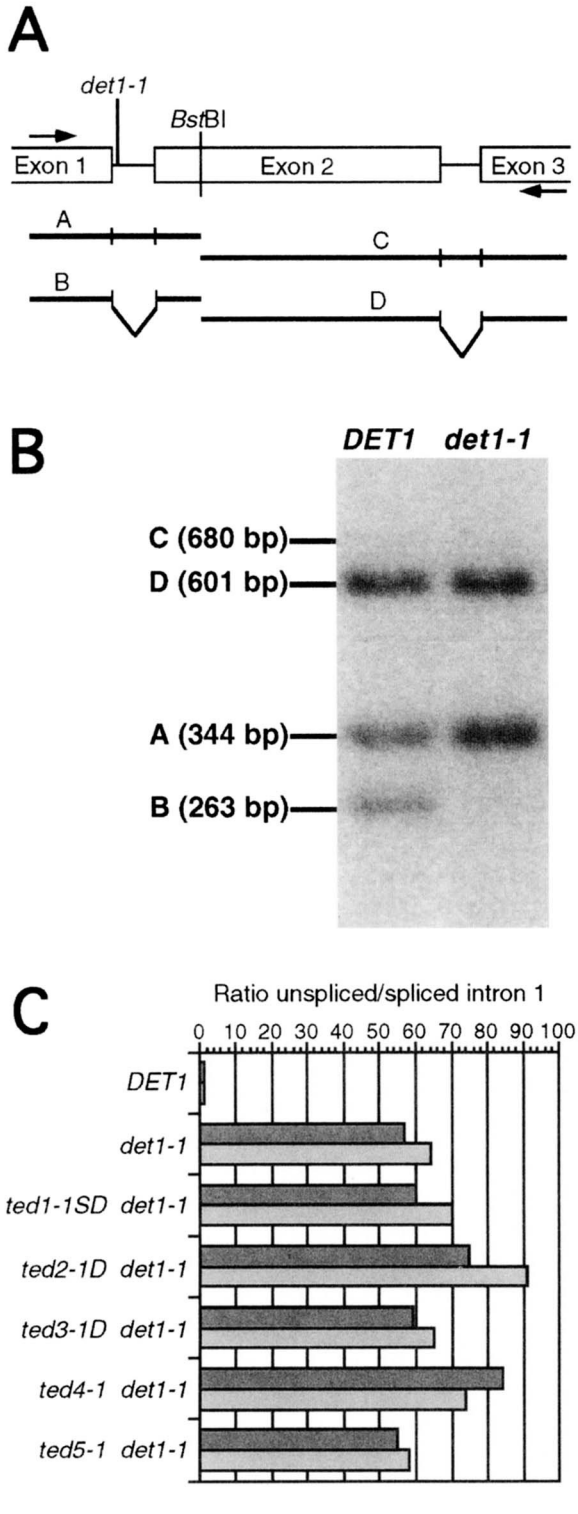

FIGURE 3.—(A) Method for analysis of intron 1 splicing in *DET1* transcripts. Partial intron/exon structure of the *DET1* gene is shown (PEPPER *et al.* 1994). The location of the *det1-1* mutation inside intron 1 is shown. Opposing arrows indicate primers used for RT-PCR. The site of asymmetric cleavage of RT-PCR products by *Bst*BI is indicated. Fragments A (344 bp) and C (680 bp) are the cleavage products derived from unspliced RNAs or contaminating genomic DNA. Fragments B (263 bp) and D (601 bp) are the cleavage products derived from spliced RNAs. (B) Phosphorimage of RT-PCR products from wild-type (*DET1*) and *det1-1* plants. (C) Quantitated Phosphorimage analysis of wild type (*DET1*), the *det1-1* and *ted det1-1* seedlings. Duplicate RT-PCR reactions were performed on a single RNA sample from each genotype. Individual bands (*A*, *B* and *C*) were quantitated, along with appropriate background samples for each band, and approximate ratios were calculated. The ratio of unspliced to spliced intron 1 was estimated by the following formula:

$$\frac{\text{Unspliced}}{\text{Spliced}} = \frac{[(A - b_A)/334] - [(C - b_C)/680]}{(B - b_B)/263}$$

where b_A, b_C, b_B and are background samples for bands *A*, *B* and *C*, respectively. Background samples had the same area as the quantitated band and were taken from the same lane.

ing of intron 1 that might give rise to suppression, a quantitative RT-PCR assay was employed. Figure 3A outlines the assay for estimating the relative efficiency of splicing of intron 1. This assay was designed to minimize potential artifacts arising from hnRNA and contaminating genomic DNA. Primers located in exon 1 and exon 3 are used to amplify a DNA fragment spanning intron 1 and intron 2. These amplification products were digested with *Bst*BI to produce asymmetric fragments. Products containing intron 2 (arising from hnRNA, genomic DNA and mature mRNAs in which intron 2 has not been spliced) are detected as a 680-bp digestion product (fragment C). Efficiency of splicing of intron 1 was estimated by comparing the ratio of phosphorimage signal from 344-bp fragment A to that of the 263-bp fragment B. In Figure 3B, RNAs from 21-day old light-grown *DET1* and *det1-1* were reversed transcribed and the resulting RT-PCR products analyzed by Southern analysis. In the wild-type, splicing of intron 1 was not complete <50% of the mRNAs were spliced. In contrast, *det1-1* displayed an overwhelming preponderance of unspliced mRNA. Quantitative analysis of several long phosphorimage exposures, subtracting appropriate background samples, reproducibly demonstrated that *det1-1* retains a low but detectable level of correctly spliced message (1–2% of the total). Thus, the partial *DET1* activity in *det1-1* (that is indicated by the phenotypes of the *det1* allelic series) is probably due to greatly reduced, but still extant levels of wild-type DET1 polypeptide. Further analysis of quantitated RT-PCR blots (Figure 3C) indicated that the ratio of unspliced to spliced transcript in the *ted* mutants was not significantly less than in *det1-1*. Thus, quantitative changes in efficiency of splicing of intron 1 were not observed as a mechanism of suppression. Taken together, the results of these investigations of the *det1-1* transcript in the *ted* mutants indicate that suppression was not occurring through any discernible alterations in *det1-1* mRNA structure, transcription rate, mRNA stability or splicing efficiency.

The *ted* mutations suppress dark-grown gene expression phenotypes of *det1-1*: Since the *ted* mutants were isolated solely on the basis of the morphology of dark-grown seedlings, it was of interest to determine whether

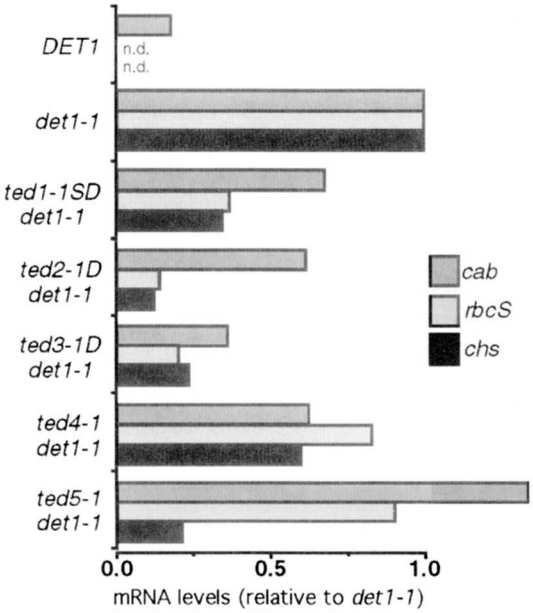

FIGURE 4.—Northern analysis of several photoregulated transcripts of 7-day-old dark-grown wild-type (*DET1*), *det1-1*, and *ted det1-1* seedlings. Two micrograms of total RNA from duplicate RNA preparations were analyzed with *cab, rbcS* and *chs* probes (CHORY *et al.* 1989). RNAs were quantitated by phosphorimage analysis with subtraction of appropriate background values. Signal from each probe was normalized to signals from a ribosomal RNA probe. An average of the two normalized values from each genotype is compared to the *det1-1* mutant. Within each pair of normalized values, the greater value was in all cases <20% higher than the lesser value, and usually <10%. n.d., not detected significantly above background.

they suppress other aspects of the *det1-1* phenotype. Figure 4 shows quantitated Northern analysis of the photoregulated transcripts *cab, chs* and *rbcS* in seven-day old dark-grown *DET1* (wild-type Col-0), *det1-1,* and *ted* seedlings. All suppressor lines except *ted4-1* show a significant diminution in dark-grown seedling transcript levels in at least a subset of these genes, indicating that suppression of the *det1-1* defect is affecting molecular, as well as morphological aspects of the *det1* phenotype. It is interesting to note that *ted5-1,* in contrast to the others, restores repression of *chs,* but *rbcS* and *cab* are unaffected. This observation indicated that *ted5-1* is only suppressing a subset of the *det1-1* molecular phenotypes, and may therefore be acting in one of several (branched) pathways downstream of *DET1.*

***ted* mutations suppress the light-grown phenotypes of *det1-1*:** When grown in the light, *det1-1* had reduced size, reduced apical dominance and reduced fertility. As shown in Figure 5, each of the *ted* mutations at least partially suppressed the size phenotype of *det1-1* (in the case of *ted4-1,* the effect was limited to the elongation of the inflorescences). Apical dominance, measured by the number of inflorescence axes at maturity in long-day grown plants, was restored by *ted1, ted2* and *ted5,* but not by *ted3* or *ted4* (Table 4).

***ted1-1SD* and *ted2-1D* suppress a daylength insensitivity phenotype of *det1-1*:** Arabidopsis is a quantitative long-day plant, flowering much later in short days (10 hr) than in long days (16 hr). Flowering responses to daylength in the wild-type, *det1-1* and *ted det1-1* lines were measured by counting the number of leaves on the primary shoot axis (Table 4, see MATERIALS AND METHODS). Wild-type plants initiate nearly three times as many leaves before flowering in short days than they do in long days. In contrast, *det1-1* was completely insensitive to day length, flowering after initiating ~10 leaves in both long- and short-days.

As shown in Table 4, *ted1-1* and *ted2-1* partially restored sensitivity to day length (SD/LD ratios of 1.9 and 1.7, respectively), while the other *ted* mutations had little or no effect. Measurements of short-day and long-day flowering in 60 F_2 progeny from *ted1-1SD* × *det1-1* and *ted2-1D* × *det1-1* crosses indicated that suppression of *det1-1* (as evidenced by size and apical dominance in mature light-grown plants) cosegregated with restoration of daylength sensitivity. This observation strongly suggests that restoration of daylength sensitivity in each case was due either to the *ted* mutation or a closely linked mutation.

The *ted1-1SD* mutation is associated with a late flowering phenotype: *ted1-1SD det1-1* displayed a moderate late-flowering phenotype, relative to both *det1-1* and *DET1,* in both long days and short days. In all F_2 individuals from several successive *ted1-1SD* × *det1-1* backcrosses, late flowering (in long days) was associated with suppression of the *det1-1* phenotype, suggesting that late flowering is due to *ted1-1SD* or a closely linked mutation. Additionally, three of the four primary mutants assigned to the *ted1* allelic class (on the basis of linkage data and allelism tests) had a discernible late-flowering phenotype. These lines of evidence, considered together, provide compelling support for the hypothesis that the late flowering phenotype is a result of the *ted1* mutation.

The location of the *ted1* locus, between *DET1* and *AG,* is near that of the previously described late flowering locus *fca* (KOORNNEEF *et al.* 1991). However, all known alleles of *fca* are recessive, while all *ted1* alleles appear to be dominant/semi-dominant for the flowering time phenotype (data not shown). In addition, *ted1* did not respond to vernalization treatment (imbibed seeds were treated for 45 days at 4°) whereas this same treatment fully suppressed the phenotype of *fca-1.* A cross of *fca-1* to *det1-1* gave rise to rare late-flowering *det1* progeny in the F_2 generation. These putative *fca-1 det1* double mutants showed slight suppression of the mature light-grown phenotype of *det1* (these plants were slightly larger, and had greater apical dominance than *det1-1*) but, in contrast to *ted1* lines, had the archetypal *det1-1* phenotype as dark grown seedlings. Considered together, these results suggest that *ted1* is either a new moderately late flowering locus, uncovered in the

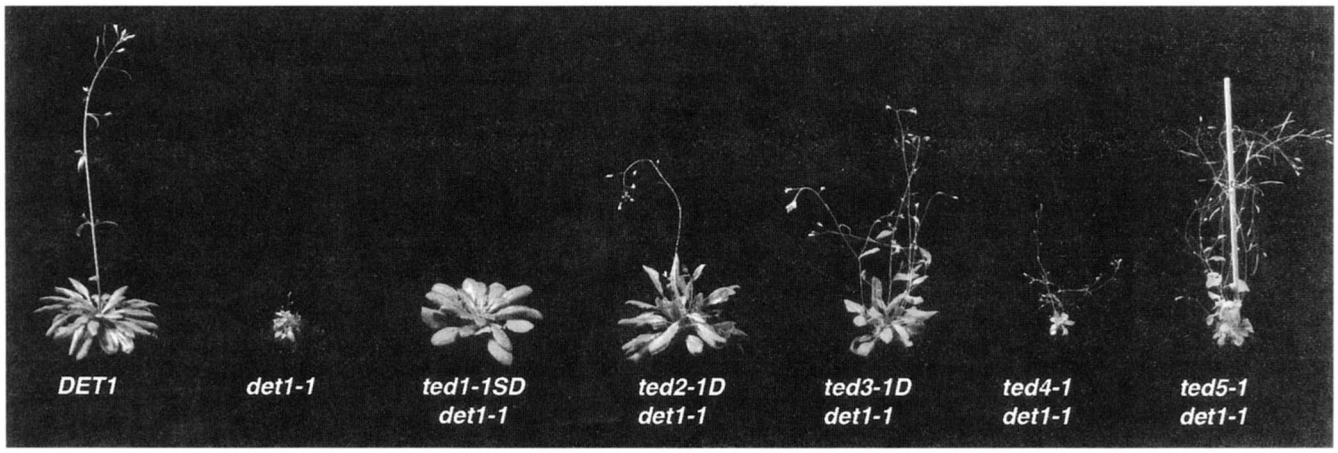

FIGURE 5.—Mature wild-type (*DET1*), *det1-1* and *ted det1-1* plants. Plants were grown in short days (10 hr).

det1 genetic background, or is a new and distinct class of alleles of *fca-1*.

The *ted* loci do not suppress *det2*: To determine whether the *ted* mutations are general suppressors of all mutants in the *det/cop* class, they were introduced into the *det2-1* genetic background. *det2-1* was selected because it is likely to be defective in a pathway that is independent of *det1* (CHORY 1992; LI *et al.* 1996; see DISCUSSION) and because the light- and dark-grown phenotypes of *det2-1* are distinct from those of *det1-1* and from the *det2-1 det1-1* double mutant (CHORY 1992). To detect suppression of *det2-1*, we crossed each *ted det1-1* line with *det2-1* and directly quantitated the number of nonsuppressed *det2-1* seedlings in the F_2. Analysis of crosses of *ted1-1SD*, *ted2-1* and *ted3-1D* to *det2-1* failed to detect significant ($P > 0.05$) likelihood of suppression (Table 5). Crosses of recessive alleles (*ted4-1* and *ted5-1*) were more difficult to evaluate statistically, but the only clear evidence of suppression was obtained from *ted5-1*, which weakly suppressed *det2-1* mutants (as evidenced by longer hypocotyls in dark-grown seedlings detected in the F_2 at a frequency of 0.08). This frequency was consistent with the presence of an unlinked, recessive weak suppressor of *det2-1* coming from the *ted5-1 det1-1* parental background ($P > 0.5$). Thus, with the possible exception of *ted5-1*, the *ted* loci are not universal suppressors of *det/cop* loci.

Analysis of suppression of *det1-4* and *det1-6*: *ted det1-1* lines were directly crossed to *det1-4* and *det1-6*. F_2 seedlings (>50) from each cross were grown in the light and in the dark, and scored on the basis of degree of phenotypic suppression relative to control parental lines (*e.g.*, *det1-1*, *ted1-1SD det1-1*, and *det1-4*). After phenotyping, seedlings were transferred to fresh agar plates and grown in the light for 2 weeks. Genomic DNAs were harvested and the *det1* allelic composition of each plant was determined by PCR-based diagnostic tests for *det1-1* in combination with either *det1-4*, or *det1-6*. The degree of suppression of *det1-4* or *det1-6* was assessed by analysis of segregation of suppressed phenotypes that were homozygous for either *det1-4* and *det1-6*, respectively (Table 5). *det1-4* is a moderately weak allele that has a missense (glycine to arginine) mutation in a predicted amphipathic alpha-helical region (PEPPER *et al.* 1994). The *DET1* mRNA level is normal in *det1-4*, implying that normal levels of a defective DET1 molecule

TABLE 4

Mature light-grown phenotypes of *DET1*, *det1-1* and *ted det1-1* lines

	Inflorescence number[a]	Leaf number (SD)[b]	Leaf number (LD)[c]	SD/LD
DET1	3.1 ± 0.9	25.7 ± 3.0	8.9 ± 1.0	2.9
det1-1	9.8 ± 3.5	9.8 ± 0.8	9.9 ± 1.6	1.0
ted1-1SD det1-1	2.6 ± 1.7	32.0 ± 5.5	16.5 ± 1.3	1.9
ted2-1D det1-1	3.5 ± 1.1	16.6 ± 2.4	9.9 ± 1.4	1.7
ted3-1D det1-1	6.7 ± 1.9	9.5 ± 1.3	7.6 ± 1.5	1.3
ted4-1 det1-1	7.8 ± 2.3	6.6 ± 0.7	5.6 ± 0.8	1.2
ted5-1 det1-1	3.8 ± 1.2	8.7 ± 2.0	8.0 ± 1.2	1.1

[a] Inflorescence axes in mature long-day grown plants (at time of first yellow silique, stage 6.5 in the Arabidopsis developmental key; see MATERIALS AND METHODS). $N = 20$ plants of each genotype measured.

[b] Total leaf number (rosette and cauline leaves on primary shoot axis) grown under 10 hr day-length (see MATERIALS AND METHODS). $N = 20$ plants of each genotype measured.

[c] Total leaf number (rosette and cauline leaves on primary shoot axis) grown under 16 hr day-length (see MATERIALS AND METHODS). $N = 20$ plants of each genotype measured.

TABLE 5

Interactions of *ted* loci with *det1-4* and *det1-6*

ted locus	*det2-1*	*det1-4*	*det1-6*
ted1-1SD	−*	++	±
ted2-1D	−*	++	±
ted3-1D	−*	±	−
ted4-1	−**	++	(−)
ted5-1	±**	++	±

Suppressor lines were crossed to *det2-1*, *det1-4* (a missense allele of *det1*) and *det1-6* (a null allele of *det1*). Suppression was scored in light- and dark-grown seedlings. Analysis of suppression of *det2-1* are based on chi-squared analysis of F_2 segregation. * indicates that suppression was ruled out ($P < 0.05$). ** indicates that the hypothesis shown had a higher probability than alternative hypotheses. Crosses to *det1-4* and *det1-6* were analysed by PCR-based genotyping of suppressed segregants. −, no evidence of suppression based on morphological phenotype; (−), very weak suppression of anthocyanin levels; ±, less suppressed than the corresponding *ted* mutation in the *det1-1* background; +, similar to the corresponding *ted* mutation in the *det1-1* background; ++, more suppressed than the corresponding *ted* mutation in the *det1-1* background.

might be produced. In the homozygous *det1-4* background all of the *ted* loci showed strong suppression activity, except for *ted3-1D*, which showed only slight suppression in *det1-4* seedlings. None of the *ted* mutations was completely specific for the *det1-1* allele, supporting the previously discussed molecular evidence that suppression is not achieved by mechanisms related to the *det1-1* lesion (affecting splicing or transcript abundance). *ted1-1SD*, *ted2-1* and *ted5-1* showed significant suppression of the homozygous *det1-6* (null) mutation. This result implies that these genes normally act in morphogenetic pathways that are either downstream of *DET1* or that bypass the repression activity of *DET1*. *ted3-1D* showed no evidence for suppression of *det1-6*, indicating that this suppressor acts by a mechanism that requires at least some DET1 activity (as seen in *det1-1* and *det1-4*). Finally, *ted4-1* showed only very slight suppression in the visible levels of anthocyanin in dark- and light-grown *det1-6* seedlings.

Phenotypes of the *ted* mutants in the *DET1* genetic background: In the F_2 progeny of *ted4-1* and *ted5-1* crosses to *DET1*, seedlings with extreme long hypocotyl phenotypes (relative to the *DET1* parent) were observed. The segregation patterns were consistent with the Mendelian segregation of recessive long hypocotyl mutations that were unlinked to the *det1* locus, but to which *det1-1* was epistatic (frequencies of long hypocotyl individuals of 0.16 and 0.21 in the *ted4-1* and *ted5-1* crosses, respectively). To further investigate the long hypocotyl segregants, they were each subjected to complementation tests with a suite of known recessive long hypocotyl mutants (*hy1-1*, *hy2-1*, *hy5-1*, *phyA-201*, *phyB-1*, *cry1-1*, *hy5-1*). An analysis of hypocotyl phenotype in the F_1 and F_2 generations of these crosses indicated that the mutation giving rise to the long hypocotyl phenotype in *ted4-1* × Col-0 is allelic to *hy1-1*, and the mutation giving rise to the long hypocotyl phenotype in *ted5-1* × Col-0 is allelic to *hy5-1*. In each case, all F_1 progeny had a long hypocotyl phenotype and all F_2 progeny (N = 147 for the *ted4-1* cross and N = 280 for the *ted5-1* cross) also had a long hypocotyl phenotype. Five independently isolated *ted4* alleles and four independently isolated *ted5* alleles were then tested directly against *hy1-1* and *hy5-1*, respectively, in F_1 complementation tests (scoring for the extreme long hypocotyl phenotype). In each case, *ted4* alleles failed to complement *hy1*, and *ted5* alleles failed to complement *hy5*. Preliminary mapping data for *ted4-1* and *ted5-1* (described above) placed *ted4-1* and *ted5-1* near the previously mapped locations of *hy1* and *hy5*, respectively. These results, taken together, furnish conclusive evidence that the suppression of *det1-1* observed in *ted4* and *ted5* is due to mutations in the previously described loci *hy1* and *hy5*, respectively.

In the cross of *ted1-1SD det1-1* × *DET1-1*, moderately long hypocotyl segregants were observed at a frequency of 0.032 (192 F_2 plants phenotyped). Three long hypocotyl segregants from this cross were recovered and were found to be homozygous for the wild-type *DET1* allele. All three were moderately early flowering in long days. One of these lines was found to complement the long hypocotyl mutations present in all of the long hypocotyl tester lines (described above). Among the F_2 progeny of the *ted1-1SD det1-1* × *DET1-1*, a range of flowering-time phenotypes were observed, from moderately early to moderately late flowering. Further molecular and genetic analysis of these segregants, to determine their genotypes at the *det1* and *ted1* loci, will be required to further explore this segregation pattern. In a cross of *ted2-1SD det1-1* × *DET1-1*, plants with delayed leaf development were observed segregating in the F_2 generation. Again, additional molecular and genetic analysis of these segregants will be required to determine the significance of this observation.

DISCUSSION

In a number of genetic systems, including yeast, Caenorhabditis and Drosophila, extragenic suppressors have proven to be an effective tool for elucidating complex developmental pathways. We have used a simple morphological screen to isolate mutations in six genes (designated *ted*) that suppress the *det1-1* mutation. We have obtained conclusive evidence that two of these genes are allelic to the previously described photomorphogenetic mutants *hy1* and *hy5*. The *ted* mutations suppress not only the seedling morphological phenotype of *det1-1*, but also partially suppress the dark-grown gene expression phenotype and the mature light-grown morphological phenotype. *ted1-1SD* and *ted2-1* suppress a daylength insensitivity phenotype of *det1-1* as well. Thus,

the suppressors affect many or all aspects of the *det1* defect, suggesting that the mechanism of suppression in each case is intimately related to DET1 function.

With the possible exception of *ted5-1* (*hy5*), the *ted* mutations fail to suppress *det2-1*. There is compelling evidence that *DET1* and *DET2* act in separate regulatory pathways. The *det1-1/det2-1* double mutant has an additive phenotype (CHORY *et al.* 1991). Given that *det2-1* is probably a null mutation (LI *et al.* 1996), this additivity indicates that *DET2* and *DET1* act in separate genetic pathways. We have recently shown that the *DET2* gene encodes a steroid 5α-reductase involved in the biosynthesis of the brassinosteroid phytohormone brassinolide, which appears to be required for elongation of seedling hypocotyls during etiolated growth. In contrast, the sequence of *DET1* does not suggest an enzymatic activity nor are *det1* mutants rescued by brassinolide application (PEPPER *et al.* 1994; LI *et al.* 1996). It is therefore unlikely that *DET1* catalyzes a step in this biosynthetic pathway. The observation that none of the *ted* mutations suppress *det2-1* provides further evidence that the suppressors are not acting by a general mechanism.

A phenotypic analysis of mutants in the *det1* allelic series indicates that the *det1-1* mutation results in only partial loss of *DET1* activity. The presence of a low level of correctly spliced *DET1* message in the *det1-1* mutant implies that partial activity is due to residual production of small amounts of wild-type DET1 polypeptide. In theory, suppressors of *det1-1* might therefore include the following: (1) intragenic or extragenic mutations that enhance correct splicing of intron 1 of the *DET1* message, or otherwise ameliorate the splicing defect (*e.g.*, by increasing overall transcription rate), (2) intragenic mutations or "upstream" extragenic mutations that increase the activity of residual *DET1* molecules, thus restoring repression of deetiolation, (3) "downstream" extragenic mutations that block deetiolation even though *DET1* activity is reduced by mutation and (4) mutations that restore repression of deetiolation by a pathway that bypasses the *DET1* gene product.

Although the available evidence suggests that the *ted* mutations act in pathways that are closely related to *DET1* function, we found no indication that they suppress *det1* by a mechanism that is specific to the *det1-1* allele. All of the *ted* loci showed suppression of the missense allele *det1-4*. (In the case of *ted3-1D*, the suppression of *det1-4* was less than that observed in *det1-1*). One class of potential extragenic suppressors of *det1-1* would include mutations in elements of the splicing machinery, such as the U2 snRNA, that might enhance correct splicing. Similarly, a transcription factor mutation that leads to the overexpression of *det1-1*-derived transcript would compensate for reduced efficiency of splicing of intron 1. However, our Northern analysis, restriction mapping of RT-PCR products, and quantitative RT-PCR analysis of the *det1-1* transcript in the *ted* genetic backgrounds failed to demonstrate that any of the extragenic suppressors act by simple transcriptional or posttranscriptional (*e.g.*, splicing, stability) mechanisms. Alternatively, the *ted* mutations might act either by stabilizing the small amount of DET1 polypeptide that is presumed to be present in *det1-1*, or by somehow compensating for reduced levels of DET1 polypeptide present in the mutant. Unfortunately, we do not yet have anti-DET1 antibodies of a high enough quality to explore these possibilities by biochemical methods. However, genetic experiments indicate that *ted1-1SD*, *ted2-1D* and *ted5-1* suppress a null allele of *det1* that does not produce any detectable *DET1* mRNA. Thus, these suppressors do not act by stabilizing extant DET1 polypeptide. Interestingly, *ted3-1D* fails to suppress *det1-6*, implying that *ted3-1* requires *DET1* polypeptide to be present for suppression. *ted3-1* suppresses *det1-4* weakly (compared to suppression of *det1-1*), therefore the mechanism of suppression is partially allele-specific. One hypothesis that explains these results is that *ted3-1* stabilizes the DET1 polypeptide, and therefore has a significant effect on *det1-1* (which presumably produces vastly reduced levels of normal polypeptide), but less of an effect on *det1-4*, which presumably produces normal levels of a defective polypeptide (PEPPER *et al.* 1994). Given that the *ted* mutations are acting in what are likely to be *DET1*-related pathways, but not simply by remediating the *det1-1* molecular defect, they are likely to be of significant interest in studies of the regulatory context of DET1 action.

The *ted1-1SD*, *ted2-1D* and *ted3-1D* mutations show significant suppression of the *det1* morphological phenotypes as dark-grown seedlings and as light-grown mature plants. In addition, these mutants partially suppress the dark gene expression phenotype of *det1-1*. Unlike *ted3-1D*, however, *ted1-1SD* and *ted2-1D* both suppress the null allele of *det1*. Therefore, *TED1* and *TED2* might normally act downstream of *DET1*, perhaps as targets for DET1 activity. Alternatively, the *ted* mutations might bypass the need for *DET1* activity. For example, *ted2-1D* might be envisioned as a hypermorphic allele of a negative regulator that is under the control of, or independent of DET1. Alternatively, *ted2-1D* might be a dominant-negative allele of a gene product that acts in a complex to promote deetiolation in the *det1* mutant. It is interesting to note that only one dominant allele was obtained at the *ted2* and *ted3* loci, while multiple alleles were obtained at the *ted1*, *ted4* (*hy1*) and *ted5* (*hy5*) loci. Although mutation frequencies vary widely among different loci, it is possible that the *ted2-1D* and *ted3-1D* are rare gain-of-function alleles and *ted1* alleles are loss-of-function. The strongest *ted1* allele, *ted1-1SD* is clearly semi-dominant. It is possible that the *ted1* mutations give rise to a semi-dominant phenotype through haplo-insufficiency. This hypothesis leaves open the possibility that *ted1* acts as a positive regulator of deetiolation that is a target for *DET1* activity. In this model

residual *TED1* activity present in the *det1* mutant leads to deetiolation, while haplo-insufficiency for *TED1* restores repression of deetiolation. Intriguingly, we observed F_2 progeny from a *ted1-1SD* × *det1-1* cross that displayed a moderate long hypocotyl phenotype, similar to weak alleles of *phyB* (REED *et al.* 1993). The significance of this finding remains to be determined.

Suppression of the *det1* null by *hy5* (*ted5*), when considered together with the fact that *ted5* suppresses a subset of the *det1* phenotypes (particularly at the dark gene expression level), suggests that HY5 acts downstream from DET1, perhaps as a target for DET1 activity that regulates hypocotyl elongation and *chs* expression. The *HY5* gene has recently been cloned and found to encode a protein with similarity to b-zip transcription factors (K. OKADA, personal communication). In preliminary experiments, HY5 and DET1 appear to interact directly in GST immunoprecipitation assays (M. CHATTERJEE and J. CHORY, unpublished data). The phenotype of *hy5* suggests that HY5 is a positive regulator of seedling deetiolation that specifically effects hypocotyl elongation. These results, considered together, suggest that DET1 represses deetiolation by direct physical interaction with HY5 and other positive regulators of deetiolation.

Mutations at the *hy1* (*ted4*) locus were found to weakly suppress *det1-1*. Despite the weak phenotype we considered mutations at this locus significant because (1) several alleles were isolated and (2) the isolation of these alleles demonstrates the identification of photoperception mutants by this screen. Interestingly, *hy1* appears to weakly suppress *det1-1* in the dark, where phytochomes are presumably inactive. It is possible, however, that the phytochrome deficiency has an effect on developmental trajectory during embryogenesis and seed maturation, or during an 8-hr light pretreatment (to stimulate germination) given before placing seedlings in the dark (CHORY *et al.* 1989). Alternatively, the phytochrome holoprotein might mediate a residual "current" of signal transduction activity in the dark. In this scenario, the "dark current" would be required for full expression of the *det1* mutant phenotype. *hy1* does not significantly suppress the null allele *det1-6*. This finding is consistent with our model, based on epistasis analysis, which places *det1* downstream from the photoreceptors including phytochrome (CHORY 1993).

In this work, we report that the *det1-1* mutant does not display repression of flowering when grown in short days. This result has two implications: (1) flowering is actively repressed under short-day conditions and (2) *DET1* is a component of this active repression. *ted1-1SD* and *ted2-1D* partially restore sensitivity to daylength in *det1*. These results are consistent with the activities of these mutations in suppressing a variety of *det1* phenotypes. In addition, *ted1-1SD* is apparently associated with a further late-flowering phenotype in long- and short days. It seems likely that *TED1*, much like *PHYB*, *DET1* and several other loci (WEIGEL 1995; AUKERMAN and AMASINO 1996), may play a role in the networked pathways that regulate both deetiolation and the timing of flowering.

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LITERATURE CITED

AHMAD, M., and A. R. CASHMORE, 1993 *HY4* gene of *A. thaliana* encodes a protein with characteristics of a blue-light photoreceptor. Nature **366:** 162–166.

AUKERMAN, M. J., and R. M. AMASINO, 1996 Molecular genetic analysis of flowering time in *Arabidopsis*. Sem. Cell Dev. Biol. **7:** 427–433.

BELL, C. J., and J. R. ECKER, 1994 Assignment of 30 microsatellite loci to the linkage map of Arabidopsis. Genomics **19:** 137–144.

CABRERA Y POCH, H. L., C. A. PETO and J. CHORY, 1993 A mutation in the *Arabidopsis DET3* gene uncouples photoregulated leaf development from gene expression and chloroplast biogenesis. Plant J. **4:** 671–682.

CASTLE, L., and D. MEINKE, 1994 A *FUSCA* gene of *Arabidopsis* encodes a novel protein essential for plant development. Plant Cell **6:** 25–41.

CHORY, J., 1992 A genetic model for light-regulated seedling development in *Arabidopsis*. Development **115:** 337–354.

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

CHORY, J., C. PETO, R. FEINBAUM, L. PRATT and F. AUSUBEL, 1989 *Arabidopsis thaliana* mutant that develops as a light-grown plant in the absence of light. Cell **58:** 991–999.

CHORY, J., P. NAGPAL and C. A. PETO, 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., D. REINECKE, S. SIM, T. WASHBURN and M. BRENNER, 1994 A role for cytokinins in de-etiolation in *Arabidopsis*. Plant Physiol. **104:** 339–347.

DENG, X.-W., T. CASPAR and P.H. QUAIL, 1991 *COP1:* a regulatory locus involved in light-controlled development and gene expression in *Arabidopsis*. Genes Dev. **5:** 1172–1182.

HEMPEL, F. D., and L. J. FELDMAN, 1995 Specification of chimeric flowering shoots in wild-type Arabidopsis. Plant J. **8:** 725–731.

KONIECZNY, A., and F. M. AUSUBEL, 1993 A procedure for mapping Arabidopsis mutations using co-dominant ecotype specific PCR-markers. Plant J. **4:** 403–410.

KOORNNEEF, M., E. ROLFF and C. J. P. SPRUIT, 1980 Genetic control of light-inhibited hypocotyl elongation in *Arabidopsis thaliana* (L.) Heynh. Z. Pflanzenphysiol. **100:** 147–160.

KOORNNEEF, M., C. J. HANHART and J. H. VAN DER VEEN, 1991 A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. Mol. Gen. Genet. **229:** 57–66.

LI, J., P. NAGPAL, V. VITART, T. MCMORRIS and J. CHORY, 1996 A role for brassinosteroids in light-dependent development of *Arabidopsis*. Science **272:** 398–401.

LISTER, C., and C. DEAN, 1993 Recombinant inbred lines for mapping RFLP and phenotypic markers in *Arabidopsis thaliana*. Plant J. **4:** 745–750.

MEINKE, D., 1995 Genetic nomenclature guide. *Arabidopsis thaliana*. Trends Genet. **11:** 22–23.

MISERA, S., A. J. MULLER, U. WEILAND-HEIDECKER and G. JURGENS, 1994 The *FUSCA* genes of *Arabidopsis:* negative regulators of light responses. Mol. Gen. Genet. **244:** 242–252.

NAGATANI, A., J. W. REED and J. CHORY, 1993 Isolation and initial characterization of *Arabidopsis* mutants that are deficient in phytochrome A. Plant Physiol. **102:** 269–277.

NAPOLI, C., C. LEMIEUX and R. JORGENSEN, 1990 Introduction of a

chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. Plant Cell **2:** 279–289.

PARKS, B. M., and P. H. QUAIL, 1993 *hy8,* a new class of *Arabidopsis* long hypocotyl mutants deficient in functional phytochrome A. Plant Cell **5:** 39–48.

PEPPER, A., T. DELANEY, T. WASHBURN, D. POOLE and J. CHORY, 1994 *DET1,* a negative regulator of light-mediated development and gene expression in *Arabidopsis,* encodes a novel nuclear-localized protein. Cell **78:** 109–116.

REDEI, G. P., 1992 A heuristic glance at the past of *Arabidopsis* genetics, pp. 1–15 in *Methods in Arabidopsis Research,* edited by C. KONCZ, N. H. CHUA and J. SCHELL. World Scientific, Singapore.

REED, J. W., and J. CHORY, 1994 Mutational analyses of light-controlled seedling development in Arabidopsis. Sem. Cell Biol. **5:** 327–334.

REED, J. W., P. NAGPAL, D. S. POOLE, M. FURUYA and J. CHORY, 1993 Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout *Arabidopsis* development. Plant Cell **5:** 147–157.

SOMERVILLE, C. R., and W. L. OGREN, 1981 Isolation of photorespiration mutants in *Arabidopsis thaliana,* pp. 129–139 in *Methods in Chloroplast Molecular Biology,* edited by M. EDELMAN, R. B. HALLICK and N. H. CHUA. Elsevier Biomedical Press, New York.

SORSCHER E. J., and Z. HUANG, 1989 Diagnosis of genetic disease by primer-specified restriction map modification, with application to cystic fibrosis and retinitis pigmentosa. Lancet **337:** 1115–1118.

WEI, N., and X.-W. DENG, 1992 *COP9:* a new genetic locus involved in light-regulated development and gene expression in *Arabidopsis.* Plant Cell **4:** 1507–1518.

WEI, N., S. F. KWOK, A. G. VON ARNIM, A. LEE, T. MCNELLIS *et al.,* 1994 Arabidopsis *COP8, COP10,* and *COP11* genes are involved in repression of photomorphogenic development in darkness. Plant Cell **6:** 629–643.

WEIGEL, D., 1994 The genetics of flower development: from floral induction to ovule morphogenesis, pp. 19–39 in *Annual Review of Genetics,* edited by A. CAMBELL. Annual Reviews Inc., Palo Alto, CA.

WHITELAM, G. C., E. JOHNSON, J. PENG, P. CAROL, M. L. ANDERSON *et al.,* 1993 Phytochrome A null mutants of *Arabidopsis* display a wild-type phenotype in white light. Plant Cell **5:** 757–768.

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