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A genetic model for light-regulated seedling development in *Arabidopsis*

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Summary

The genetic interactions among mutations that define eight distinct loci involved in light-regulated development in *Arabidopsis thaliana* are described. The mutations in these eight genes define two distinct phenotypic classes with opposite characteristics. Recessive mutations in either one of two genes, *DET*1 or *DET*2, result in dark-grown plants that develop as light-grown wild-type seedlings. Mutants in the second class exhibit a reduced response to light. Recessive mutations in any one of five genes, *HY*1, *HY*2, *HY*3, *HY*5, or *HY*6 cause reduced responses to red-light. Four of these genes, *HY*1, 2, 3, and 6, affect the activity of one or all of the red-light photoreceptors, the phytochromes. The *HY*4 gene product is involved in blue-light perception or action. The experiments described here examine how these eight genes interact to control a particular event, the switch from developmental arrest in the dark (etiolation) to growth in the light (de-etiolation). The phenotypes of doubly mutant strains suggest a hierarchical regulatory network among these genes in the control of the switch from etiolated to de-etiolated growth strategies.

Key words light perception, leaf and chloroplast development, *Arabidopsis*.

Introduction

The mechanisms underlying developmental processses in multicellular eukaryotic organisms are only beginning to be unravelled. In plants, development is controlled by the light environment, and as such, distinct morphologies arise from growing plants under dark or light conditions (Mullet, 1988; Chory, 1991). Dark-grown (etiolated) dicotyledonous seedlings have elongated hypocotyls, small folded cotyledons, and undeveloped chloroplasts (etioplasts) Conversely, light inhibits hypocotyl elongation, and induces leaf expansion and differentiation and chloroplast development (de-etiolation) (Mullet, 1988; Dale, 1988; Gruissem, 1989). The etiolated state is accompanied by little or no expression of several light-regulated genes, including the nuclear genes encoding the light-harvesting chlorophyll a/b binding proteins (*cab*), the small subunit of the RuBP carboxylase/oxygenase (*rbc*S), and chalcone synthase (*chs*) (Silverthorne and Tobin, 1987; Gilmartin et al., 1990). In the light, the expression of these genes is restricted to specific cell types; for instance, *chs* is expressed in the epidermis, and the *rbc*S and *cab* genes are expressed in chloroplast-containing cells (mesophyll) (Chory, 1991). Thus, light and cell-specific factors work in concert to produce specific expression patterns for these genes In addition to light, several phytohormones have been implicated in de-etiolation responses, including cytokinins and gibberellins (Stetler and Laetsch, 1965; Harvey et al., 1974; Flores and Tobin, 1986; Mathis et al., 1989). How light might interact with these hormone signal transduction pathways is not understood.

A complete signal transduction cascade has yet to be elucidated between the steps of photon absorption by a photoreceptor and changes in the expression of light-regulated genes. Several classes of photoreceptors mediate light responses, including protochlorophyllide, blue- and UV-light absorbing receptors, and the red/far-red-light absorbing receptors, the phytochromes (Lagarias, 1985; Colbert, 1988; Senger and Schmidt, 1986) Most efforts to study this process have been directed at the biochemical and molecular characterization of phytochrome. Phytochrome is a soluble pigmented protein that exists in two spectrally distinct, photointerconvertible forms P_r, the red-absorbing form, and P_{fr}, the far-red absorbing form. The spectral qualities of purified P_r and P_{fr} are the results of the combined properties of the 120 x 10^3 M_r apoprotein with its thioether-linked bilitriene chromophore (Lagarias, 1985, Colbert, 1988). Photoconversion of P_r to P_{fr} induces a diverse array of morphogenic responses, whereas reconversion of P_{fr} to P_r cancels the induction of the responses. Thus, P_{fr} is considered the active and P_r the inactive form of the photoreceptor. The molecular mechanism by which P_{fr} induces morphological and gene expression responses in the developing seedling is not known. A further complication is that phytochrome is actually a family of photoreceptors For instance, in the small cruciferous plant, *Arabidopsis thaliana*, there are at least five expressed phytochrome apoprotein genes (Sharrock and Quail, 1989; R. Sharrock, personal communication). The spatial and temporal expression patterns of the different phytochromes and what roles each plays during development are largely unknown.

Blue-light also causes profound changes in the morphology of the developing young seedling (Senger and Schmidt, 1986; Chory, 1991). Though a blue-light receptor has not been chemically defined, recent data suggest the involvement of G-proteins in the signal transduction cascade (Warpeha et al., 1991). Further, the isolation of *Arabidopsis*

mutations that cause defects in just one or a few blue-light responses suggests that the blue-light signal transduction pathways are also likely to be complex (Koornneef et al., 1980; Khurana et al., 1989; Khurana and Poff, 1989; Liscum and Hangarter, 1991).

A combined molecular and classical genetic approach will help dissect what are likely to be very complex photomorphogenetic signal transduction pathways. *Arabidopsis thaliana* is an ideal organism for saturation mutagenesis experiments aimed at identifying genes involved in directing the downstream light-regulated responses. To isolate mutants affected in early seedling development in response to light, we and others have taken advantage of simple screens based on the characteristic morphologies of light- and dark-grown seedlings (Koornneef et al., 1980; Chory et al., 1989a, 1989b, 1991a; Deng et al., 1991; Liscum and Hangarter, 1991). The mutations identified to date fall into two phenotypic classes that define 14 genes. mutations in 10 of these genes produce plants with impaired responses to red (Koornneef et al., 1980; Chory et al., 1989a) or blue light (Koornneef et al., 1980; Liscum and Hangarter, 1991), and mutations in another four genes result in plants that exhibit light-mediated responses more readily than wild-type plants (Chory et al., 1989b, 1991a; Chory, 1991; Deng et al., 1991). This study focuses on eight of these 14 loci

The first phenotypic class of mutants have a partially etiolated morphology when grown in white light. These mutants were designated, *hy*, for long *hy*pocotyl because they do not undergo light-induced inhibition of the rate of hypocotyl elongation (Redei and Horono, 1964). Homozygous recessive *hy* mutations define seven complementation groups, six of which, *hy*1-*hy*6 will be considered here (Koornneef et al., 1980; Chory et al., 1991; J. Chory, unpublished data). Mutations in any one of four genes, *hy*1, *hy*2, *hy*3, or *hy*6 result in phytochrome deficiencies (Koornneef, 1980; Chory et al., 1989a; Parks et al., 1989; Nagatani et al., 1991). *hy*4 mutants, in contrast, show reduced inhibition of hypocotyl elongation in blue light while maintaining normal phytochrome levels and responses (Koornneef et al., 1980). Plants homozygous for *hy*5 alleles are defective in red-light-mediated hypocotyl growth inhibition, though phytochrome activity appears to be normal (Koornneef et al., 1980).

The second phenotypic class of mutants show many characteristics of light-grown plants even when grown in complete darkness. We have designated these mutants *det*, (*de*etiolated), because they are de-etiolated in the dark, instead of having the usual etiolated seedling morphology. Recessive mutations in any one of three *DET* genes result in dark-grown plants that grow as light-grown wild-type seedlings (Chory et al., 1989b, 1991a; J. Chory, unpublished data) More recently, similar mutations in a fourth gene, *COP*1, were identified (Deng et al., 1991).

We have been studying homozygous mutant *det*1 and *det*2 seedlings in the most detail. Dark-grown *det*1 and *det*2 plants have inhibited hypocotyl growth rates, expanded cotyledons, and developed leaves. Additionally, several light-regulated genes, including *cab*, *chs*, and *rbc*S, are expressed at high levels in the dark in these mutants (Chory et al., 1989b, 1991a). Further, light-grown plants appear to require DET1 for spatial repression of light-regulated genes, since *cab*, *chs*, and *rbc*S are ectopically expressed in *det*1 mutants (Chory and Peto, 1990). In contrast, homozygous *det*2 mutations affect photoperiodic responses in light-grown plants, including delayed timing of flowering, reduced dark adaption of *cab* gene expression, and delayed timing of leaf and chloroplast senescence (Chory et al., 1991a). Thus, DET2 may play a negative role in the temporal elaboration of light responses during *Arabidopsis* development.

The experiments described in this paper explore how the activities of the six *HY* and two *DET* genes interact to control the switch from an etiolated to de-etiolated developmental strategy The results from epistasis studies using doubly mutant lines suggest a hierarchical regulatory network among these genes in the control of the downstream light-regulated responses. This analysis should provide the necessary genetic framework for future studies designed to test the molecular basis of these interactions

Materials and methods

Plant material, growth conditions, and genetic methods

General methods for the growth and handling of *Arabidopsis* were described (Somerville and Ogren, 1982) The following mutant strains were used in this work:

Mutant allele	*Reference*
*hy*1 (21.84N)	Koornneef et al 1980, Chory et al 1989a
*hy*1 (d412)	Koornneef et al 1980, Chory et al 1989a
*hy*2 (To76)	Koornneef et al 1980, Chory et al. 1989a
*hy*2-2	Chory et al. 1989a
*hy*3 (Bo64)	Koornneef et al 1980, Nagatani et al. 1991
*hy*4 (2.23N)	Koornneef et al 1980
*hy*5 (Ci88)	Koornneef et al. 1980
*hy*6-1	Chory et al 1989a
*det*1-1	Chory et al. 1989b, Chory and Peto, 1990, Chory, 1991
*det*1-3	this work
*det*2-1	Chory et al. 1991a
*det*2-2	Chory et al. 1991a

All mutant alleles studied are recessive when back-crossed to wild type. The *hy*1-*hy*5 mutations originally identified by Koornneef et al. (1980) are in the Landsberg ecotype carrying the *erecta* mutation. *hy*2-2, *hy*6-1, and the *det* alleles were isolated in the wild-type Columbia background. For double mutant studies involving the *det* mutants and the *hy*1-*hy*5 reference alleles, the *det* mutations were first back-crossed four times into the Landsberg *erecta* background, and the phenotypes of the single and double mutants were compared to a wild-type Landsberg *erecta* line. For double mutants involving the *det* mutants with either the *hy*6-1 or *hy*2-2 alleles, the wild-type was the Columbia line I did not observe significant differences between the ecotype Landsberg and Columbia controls Wild-type alleles are symbolized by upper case italics, the wild-type gene product is in upper case plain text. Mutant alleles are symbolized by lower case italics Doubly mutant strains were created by manual cross-pollination, using homozygous single mutants as parents Most crosses were performed reciprocally, with the exception of crosses that involved *det*2 alleles for which *det*2 was always used as the female parent (see Chory et al., 1991a). The resulting F_1 plants were allowed to self-pollinate and double mutants were scored in the F_2 generation whenever possible. For most crosses, no new phenotype arose in the F_2 plants, so F_2 plants with the phenotype of one or the other single mutant were picked and allowed to self-pollinate. Double mutants were scored in the F_3 generation as segregants in the progeny of such plants In two crosses, namely the *hy*3-*det*1 cross and the *hy*5-*det*1 cross, some ambiguity in scoring

the double mutant was present in the F_3 generation The genotype of the putative double mutant was thus verified by performing crosses to each of the single mutant parents and scoring the F_1 plants

Plants were grown at 20°C under a mixture of fluorescent and incandescent lights at an intensity of 350 $\mu E/m^2/sec$ ("high-white light conditions") Methods for the growth of plants in pots, seed harvesting, and cross-pollination have been described (Somerville and Ogren, 1982, Chory et al , 1989a, 1989b) Single and double mutants and wild type were always grown together under the same light and humidity conditions for the times indicated in the text The treatment of plants with specific wavelengths of light were as follows: red, six 40-W GE cool white bulbs and two incandescent bulbs with red Plexiglas filter, blue: six 40-W GE cool white bulbs with a blue Plexiglas filter. The outputs of the various light sources, measured at seedling level with a Biospherical Instruments Inc QSL-100 light detector were "high" white light. 350 $\mu E/m^2/sec$, "low" white light 60 $\mu E/m^2/sec$; red 55.5 $\mu E/m^2/sec$, and blue· 38 $\mu E/m^2/sec$. Dark-grown seeds were germinated for 24 hours in the light on synthetic medium plus sucrose and then transferred to total darkness for 7-10 days, as previously described (Chory et al., 1989b) A green safelight filter was used during all dark manipulations.

Analytical techniques

Chlorophyll determinations were performed on leaves and stems from wild-type and mutant plants harvested after 12 days growth in high photon fluence rate white light and immediately frozen in liquid nitrogen The frozen tissue was later ground in liquid nitrogen in a mortar and pestle, and the chlorophyll was extracted repeatedly into 80% acetone in the dark until the pellet appeared colorless. Chlorophyll *a* and *b* contents were calculated using MacKinney's specific absorption coefficients (MacKinney, 1941), in which chlorophyll $a = 12.7(A_{663}) - 2.69(A_{645})$ and chlorophyll $b = 22.9(A_{645}) - 4.48(A_{663})$ The total specific chlorophyll content is expressed as micrograms of chlorophyll per seedling

For anthocyanin determinations on dark-grown tissue, 0 1 g of frozen plant tissue was ground in a 1 5 ml microfuge tube with a disposable pestle, and total plant pigments were extracted overnight in 0 3 ml of 1% HCl in methanol. After the addition of 0 2 ml of H_2O, chlorophyll was separated from the anthocyanins by extraction with an equal volume of chloroform. The quantity of anthocyanins was determined by spectrophotometric measurements of the aqueous:methanol phase (A_{530}-A_{657}) and normalized to the total fresh weight of tissue used in each sample (Beggs et al., 1987, Rabino and Mancinelli, 1986). For tissues grown in the light, similar mesurements were made in duplicate (two seedlings/sample) on seedlings grown for 12 days in high photon fluence rate white light.

Hypocotyl elongation was measured with a ruler after growth for 10 days in the various light regimens indicated in Table 5 Measurements for dark-grown seedlings were made after eight days growth. At least 50 seedlings were measured for each sample.

Northern hybridizations

RNA extraction, separation, and gel blot conditions were previously described (Chory et al , 1991a). The DNA probes for nuclear and chloroplast genes used in these studies were published elsewhere (Chory et al , 1989b). To normalize for RNA loading, filters were stripped and rehybridized with an rDNA probe Autoradiograms for different exposure times were scanned with a densitometer. Relative amounts of mRNAs were determined by peak-height measurements, and relative mRNA levels reported are an average of two separate hybridizations

Results

Wild type

Arabidopsis is typical of dicotyledonous seedlings in that dark-grown (etiolated) seedlings are developmentally arrested, having extended hypocotyls, no cotyledon expansion, no leaves or chloroplasts, and no detectable chlorophyll and anthocyanin pigments (Chory et al., 1989b). A set of well-characterized genes, the light-regulated genes, is not expressed or is expressed at a very low level (Chory et al., 1989b). In contrast, when seedlings are exposed to light, there are profound differences in the morphology of the plant, including rapid inhibition of stem elongation, expansion of cotyledons, and development of leaves and chloroplasts (de-etiolation). During de-etiolation in *Arabidopsis*, light-regulated gene expression increases up to 100-fold (Chory et al , 1989b; Chory and Peto, 1990). Upon further exposure to a constant and well-defined light environment, the average *Arabidopsis* wild-type seedling will make approximately 10 vegetative rosette leaves followed by a transition to floral growth at approximately 21 days post-germination (Table 1, and Chory et al., 1991a). The vegetative to floral transition is characterized by rapid growth of the floral bolt accompanied by leaf and chloroplast senescence. During leaf senescence, expression of light-regulated genes is relatively low (a decrease of about 10-fold from early seedling development; Chory, 1991).

Single mutant strains

Phytochrome-deficient hy *mutants,* hy*1,* hy2, hy*3, and* hy*6*

Mutations in three separate loci, *hy*1, *hy*2, or *hy*6 result in seedlings with little to no detectable phytochrome activity in the dark (Koornneef et al., 1980; Chory et al., 1989a; Parks et al., 1989). *hy*1 maps to position 17.0 cM on chromosome 2, *hy*2 maps to chromosome 3, position 0.0 cM, and *hy*6 maps to chromosome 2, position 21 9 cM on the morphological marker map (Koornneef, 1990; Chory et al , 1991a). Though a complete phenotypic analysis of all 19 *hy*1, *hy*2, and *hy*6 alleles has not yet been performed, strong alleles of *hy*1 (*hy*1 21.84N), *hy*2 (*hy*2-2), and *hy*6 (*hy*6-1) have no detectable phytochrome spectral activity (Koornneef et al., 1980; Parks et al., 1989, Chory et al., 1989a), whereas weak alleles of *hy*1 (d412) and *hy*2 (To76) have about 25% of wild-type phytochrome spectroscopic activity (Koornneef et al., 1980; Chory et al., 1989a). It has been proposed that the lesions in the *hy*1, *hy*2, and *hy*6 mutants affect either the synthesis or attachment of the bilitriene chromophore of phytochrome, since these mutants contain both the major light-labile and light-stable phytochrome apoproteins (Parks et al., 1989; Chory et al., 1989a; Nagatani et al., 1991; Parks and Quail, 1991). Since these two major phytochromes, and perhaps all phytochromes, share the same chromophore, these mutants are severely deficient in phytochrome activity In addition to the long hypocotyl phenotype for which they were selected, severe alleles of *hy*1, *hy*2 and *hy*6 are pale yellow, make fewer leaves, have increased apical dominance, and flower prematurely when compared to wild-type plants (See Table 1). Molecular, biochemical, and ultrastructural studies indi-

Table 1. *Summary of phenotypes of various photomorphogenetic mutants*

		Light morphology*				
Genotype	Dark morphology	Color	Hypocotyl length (mm)	Leaf number	Bolt number	Days to flower
wild type	etiolated	green	1 6±0 5	9±2	2-4	21
*det*1-1	de-etiolated	pale green	1 0±0 4	10±2	5-7	21
*det*1-3	de-etiolated	pale green	1 0±0 6	10±2	5-7	21
*det*2-1	de-etiolated	dark green	0 4±0 4	19±3	5-7	30
*det*2-2	de-etiolated	dark green	0 5±0 3	19±2	5-7	30
*hy*1(21 84N)	etiolated	yellow	8 4±1 2	6±1	1-2	14
*hy*1(d412)	etiolated	pale green	7 6±0 8	7±2	1-2	16
*hy*2-2	etiolated	pale green	6 3±0 9	7±1	1-2	14
*hy*2(To76)	etiolated	pale green	6 5±1 0	8±1	1-2	16
*hy*3(Bo64)	etiolated	light green	5 9±0 8	7±2	2-3	19
*hy*4(2 23N)	etiolated	green	4 1±0 5	9±1	2-4	24
*hy*5(Ci88)	etiolated	green	4 6±0 5	9±2	2-4	22
*hy*6-1	etiolated	yellow	7 5±0 9	5±1	1-2	14
*det*1-1 *hy*1(21 84N)	de-etiolated	pale green	1 4±0 3	11±2	5-7	20
*det*1-1 *hy*2-2	de-etiolated	pale green	1 0±0 5	11±2	5-7	21
*det*1-1 *hy*3(Bo64)	de-etiolated	pale green	2 4±0 5	9±1	5-7	21
*det*1-1 *hy*4(2 23N)	de-etiolated	pale green	0 8±0 5	9±2	2-4	25
*det*1-1 *hy*5(Ci88)	partially de-etiolated	green	3 3±0 4	9±2	2-4	20
*det*1-1 *hy*6-1	de-etiolated	pale green	1 2±0 6	9±2	5-7	20
*det*2-1 *hy*1(21 84N)	de-etiolated	yellow	0 5±0 3	16±4	5-7	29
*det*2-1 *hy*2-2	de-etiolated	yellow	0 6±0 4	15±2	5-7	30
*det*2-1 *hy*3(Bo64)	de-etiolated	green	0 5±0 4	16±2	5-7	30
*det*2-1 *hy*4(2 23N)	de-etiolated	green	0 5±0 5	18±2	5-7	29
*det*2-1 *hy*5(Ci88)	partially de-etiolated	green	2 2±0 6	14±3	4-6	29
*det*2-1 *hy*6-1	de-etiolated	yellow	0 7±0 5	18±4	5-7	28
*det*1-3 *det*2-1	de-etiolated	green	0 5±0 4	12±3	up to 20	28

*Data shown are for plants grown in high fluence rate white light on a 16 hour day/8 hour night cycle

cate that these mutants do not complete the leaf and chloroplast developmental program when grown in white light (Chory et al., 1989a).

*hy*3 maps to chromosome 2, position 2.2 cM on the morphological marker map (Koornneef, 1990). Studies in our laboratory and by Somers et al. have recently shown that homozygous recessive *hy*3 alleles have decreased accumulation of the major light-stable phytochrome of *Arabidopsis* to about 5% of wild-type levels (Nagatani et al., 1991; Somers et al., 1991). Our most recent experiments show that the gene that most likely encodes this phytochrome (gene designation *PHY*B, as per Sharrock and Quail, 1989) and *hy*3 mutations are genetically linked (within 0.4 cM on the RFLP map, based on analysis of 220 chromatids) Furthermore, two independent *hy*3 alleles contain mutations in the *PHY*B gene (P. Nagpal, J. Reed, and J. Chory, unpublished data) Thus, it seems likely that *HY*3 encodes the type B phytochrome apoprotein. The *hy*3 mutants have a striking phenotype, including a long hypocotyl, very elongated petioles and leaves, and an elongated flowering bolt (Table 1, Koornneef et al., 1980; Chory et al., 1989a). Plants homozygous for *hy*3 mutations are also slightly defective in greening, accumulating less chlorophyll and having fewer chloroplasts per mesophyll cell than wild-type plants (Table 2, J. C , unpublished data).

Blue-light response mutant, hy*4*

*hy*4 (position 6.2 cM, chromosome 4) mutants show reduced hypocotyl growth inhibition in blue-light while maintaining normal phytochrome levels and red/far-red responses (Koornneef et al., 1980; Koornneef, 1990). *hy*4 (2.23N) may be a null allele since it was generated by fast neutron mutagenesis which has been shown to generate small deletions in

Table 2. *Chlorophyll content and chlorophyll a/b ratio of mutants*

Genotype	Chlorophyll content* (μg Chl/seedling)	Chl a/b (mol/mol)
wild type	15 0	2 6
*det*1-1	12 1	2 7
*det*2-1	18 5	2 7
*hy*1 (21 84N)	5 2	3 6
*hy*2-2	9 1	3 1
*hy*3 (Bo64)	11 5	3 0
*hy*4 (2 23N)	13 7	2 7
*hy*5 (Ci88)	19 0	2 1
*hy*6-1	3 8	6 4
*det*1-1 *hy*1(21 84N)	10 7	3 1
*det*1-1 *hy*2-2	9 9	4 6
*det*1-1 *hy*3(Bo64)	9 9	3 0
*det*1-1 *hy*4(2 23N)	13 5	4 0
*det*1-1 *hy*5(Ci88)	13 9	2 4
*det*1-1 *hy*6-1	11 7	3 6
*det*2-1 *hy*1(21 84N)	5 9	4 0
*det*2-1 *hy*2-2	10 8	5 0
*det*2-1 *hy*3(Bo64)	11 0	3 0
*det*2-1 *hy*4(2 23N)	10 4	2 7
*det*2-1 *hy*5(Ci88)	16.4	2 4
*et*2-1 *hy*6-1	4.1	3 9
*det*1-3 *det*2-1	14 0	2 7

*Measurements were made in duplicate on seedlings grown for 12 days in high photon fluence rate white light.

Arabidopsis DNA. *hy*4 (2.23N) homozygous recessive mutations affect only a subset of blue-light regulated responses, including inhibition of hypocotyl growth (Tables 1, 5) and they cause a 50% decrease in blue-light induced anthocyanin biosynthesis (Table 3). Other known blue-light responses, including stomatal opening, chloroplast development, and phototropism, are normal in these mutants (J. Chory and S. Assmann, unpublished data). The biochemical defects in *hy*4 reduced or loss-of-function alleles are unknown.

Red-light response mutant, hy*5 (Ci88)*

Only two alleles of *hy*5 (chromosome 5, position 1.1 cM) are known, and one (*hy*5 Ci88) was available for this study (Koornneef et al., 1980; Koornneef, 1990). This *hy*5 allele is defective in red-light induced hypocotyl growth inhibition responses, but has normal levels of phytochrome (Koornneef et al., 1980). In addition, *hy*5 (Ci88) mutants have a higher specific chlorophyll content, and a lower chlorophyll *a*/*b* ratio than wild-type *Arabidopsis* grown under the same incident light conditions (Table 2). Though the molecular lesion in *hy*5 mutants is not known, a *hy*5 mutation in combination with either a *hy*1, *hy*2, or *hy*3 mutation, has an additive effect on inhibition of hypocotyl elongation (Koornneef et al., 1980). This indicates the possibility that *HY*5 encodes a component on a unique red-light transduction pathway.

det*1*

*det*1 maps at position 13.4 cM on the fourth chromosome in the *Arabidopsis* RFLP map of Chang et al., (1988); (T. Delaney and J. Chory, unpublished data). Six alleles of *det*1 exist. All six are completely recessive when backcrossed to the wild-type Columbia line (H. Cabrera and J. Chory, unpublished data). Detailed descriptions of the light- and dark-grown phenotypes of *det*1-1 have been published (Chory et al., 1989b, 1991) and are summarized here. When grown in the dark, *det*1-1 alleles have the gross morphology of light-grown plants, including hypocotyl growth rate inhibition, anthocyanin production, and the development of chloroplasts and rosette leaves. Phytochrome spectral activity and regulation appear to be similar to wild-type etiolated seedlings though several light-regulated genes, including *cab*, *chs*, and *rbc*S, are expressed in the dark in these mutants

Table 3. *Anthocyanin content of light and dark-grown mutants*

	Normalized anthocyanin content	
Genotype	Light-grown*	Dark-grown†
wild type	1.0	1.0
*det*1-1	5.7	13.0
*det*2-1	1.4	3.3
*hy*1 (21.84N)	0.85	1.0
*hy*2-2	0.85	1.0
*hy*3 (Bo64)	0.82	0.8
*hy*4 (2.23N)	0.48	0.5
*hy*5 (Ci88)	0.72	1.4
*hy*6-1	0.67	0.6
*det*1-1 *hy*1(21.84N)	4.4	10.7
*det*1-1 *hy*2-2	3.2	11.3
*det*1-1 *hy*3(Bo64)	3.3	11.5
*det*1-1 *hy*4(2.23N)	39.4	12.3
*det*1-1 *hy*5(Ci88)	1.5	4.3
*det*1-1 *hy*6-1	5.3	12.6
*det*2-1 *hy*1(21.84N)	1.6	3.2
*det*2-1 *hy*2-2	1.7	3.0
*det*2-1 *hy*3(Bo64)	2.1	3.4
*det*2-1 *hy*4(2.23N)	2.0	2.9
*det*2-1 *hy*5(Ci88)	1.8	1.3
*det*2-1 *hy*6-1	1.5	3.0
*det*1-3 *det*2-1	7.5	15.0

*Measurements were made in duplicate (two seedlings/sample) on seedlings grown for 12 days in high photon fluence rate white light.
†Measurements were made on 0.1 g wet weight dark-grown tissue. Values are normalized so that wild-type=1.

(Chory et al., 1989b). Further, light-grown plants require DET1 for tissue-specific repression of light-regulated genes, since *cab*, *chs*, and *rbc*S are ectopically expressed in *det*1-1 alleles (Chory and Peto, 1990). The dark- and light-grown seedling phenotypes of *det*1-3 homozygotes are similar to those of *det*1-1 (Table 1). Homozygous *det*1-2 alleles are slightly less severe in that fewer rosette leaves develop in the dark than for either *det*1-1 or *det*1-3 mutants (J.C., unpublished data).

det2

*det*2 maps to position 32.9 cM on chromosome 2 (Chory et al., 1991a) of the morphological map of Koornneef (1990).

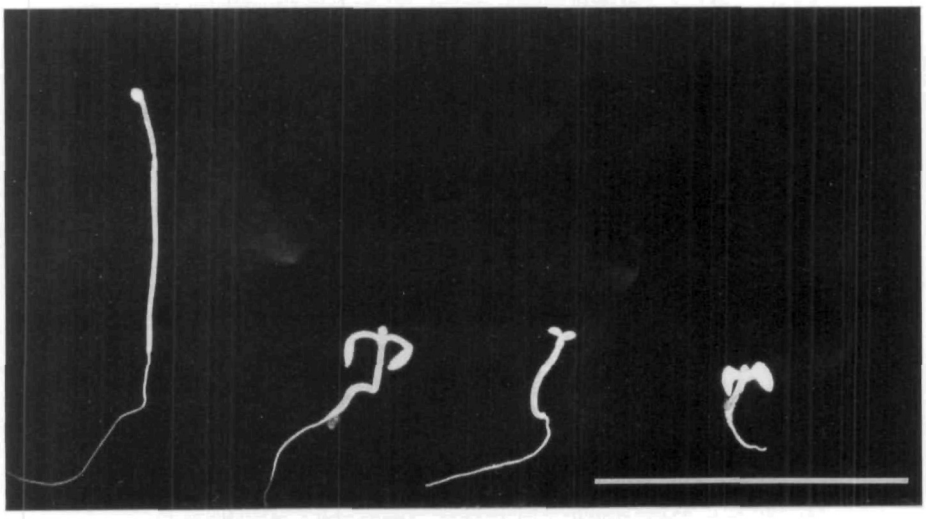

Fig. 1. Phenotypes of seven-day-old dark-grown wild-type and mutant seedlings. From left to right are: wild-type (etiolated), *det*1-3, *det*2-1, and *det*1-3 *det*2-1. Bar = 1 cm.

There are five *det2* alleles and each is completely recessive when backcrossed to wild type Since all five alleles have the same phenotype (Chory et al., 1991a), only *det2*-1 is described here. Like *det*1 mutants, dark-grown homozygous *det2*-1 mutants have normal levels of phytochrome, increased expression of light-regulated genes, and show cotyledon expansion and an inhibition of the rate of hypocotyl elongation in the absence of light (Chory et al., 1991a; L Pratt and J. Chory, unpublished data). Unlike *det*1 mutants, dark-grown *det2* seedlings develop only primary leaf buds, but not rosette leaves. Though there is cotyledon development and some leaf differentiation in dark-grown *det2* mutants, chloroplasts do not differentiate. Light-grown *det2* mutants appear to have defects in photoperiodic timing, characterized by a prolonged vegetative phase, a delay in leaf and chloroplast senescence (accompanied by high levels of *cab* and *rbc*S gene expression after flowering), and a failure to repress the accumulation of light-regulated RNAs during dark periods (Chory et al., 1991a).

Table 4. *Germination frequencies of mutants grown in different light qualities*

Genotype	Germination frequency (% of white light)			
	White light	Red light	Blue light	Dark
Wild type	100	102	97	67
*det*1-1	100	120	101	120
det2-1	100	99	98	82
*hy*1 (21 84N)	100	91	102	51
*hy*2-2	100	100	110	24
*hy*3 (Bo64)	100	104	103	52
*hy*4 (2 23N)	100	110	100	67
*hy*5 (Ci88)	100	102	98	70
*hy*6-1	100	110	56	34
*det*1-1 *hy*1(21 84N)	100	105	103	104
*det*1-1 *hy*2-2	100	110	106	100
*det*1-1 *hy*3(Bo64)	100	100	90	101
*det*1-1 *hy*4(2 23N)	100	101	87	96
*det*1-1 *hy*5(Ci88)	100	109	97	80
*det*1-1 *hy*6-1	100	110	98	103
det2-1 *hy*1(21 84N)	100	96	87	80
det2-1 *hy*2-2	100	101	72	80
det2-1 *hy*3(Bo64)	100	102	90	75
det2-1 *hy*4(2 23N)	100	87	66	88
det2-1 *hy*5(Ci88)	100	74	89	70
det2-1 *hy*6-1	100	97	79	75
*det*1-3 *det2*-1	100	99	101	102

Double mutants

The phenotypic and genetic analysis of *det* and *hy* single mutant lines suggests that DET1 and DET2 proteins are required to couple the red- and blue-light signals to the downstream light-regulated developmental and gene expression responses in *Arabidopsis* In a variety of genetic systems, epistasis tests have helped define functional relationships between mutationally defined genes (e.g., Ferguson et al., 1987; Ambros, 1989; Bowman et al., 1991; Chant and Herskowitz, 1991). I reasoned that by comparing the phenotypes of doubly mutant lines to single mutants, I could provide a genetic framework for the interactions between the various photomorphogenetic genes. This genetic formalism, which does not presuppose any particular molecular mechanism for the interactions, will be useful for future molecular analysis of these interactions.

To investigate the functional relationships among the activities of *hy*1-*hy*6, *det*1 and *det2*, doubly mutant strains were constructed carrying mutations in one *hy* gene and one *det* gene or in two *det* genes. The effects of the double mutant

Table 5. *Degree of hypocotyl elongation in single and double mutants grown in different light qualities*

Genotype	Hypocotyl length (mm)				
	High white light	Low white light	Red light	Blue light	Dark
Wild type	1 6±0 5	4 0±0 7	7 5±1 6	5 6±0 7	19 0±2 7
*det*1-1	1 0±0 4	2 4±0 6	2 0±0 4	1 7±0 6	4.4±0 9
det2-1	0 4±0 4	0.6±0 3	1 1±0 6	0 6±0 4	3.2±0 9
*hy*1 (21 84N)	8 4±1 2	14 5±0 6	19 0±1 5	13 3±1 6	18 6±1 0
*hy*2-2	6 3±0 9	12 8±1 2	19 0±1 8	12 8±1 2	18 3±1 5
*hy*3 (Bo64)	5 9±0 8	12 0±1 5	13 5±2 1	11 4±1 3	22 2±3 0
*hy*4 (2 23N)	4 1±0 5	8 8±1 3	6 8±0 8	13 7±0 9	16 7±2 9
*hy*5 (Ci88)	4 6±0 5	10 5±0 7	13 3±1 9	12 2±2 4	17 9±1 8
*hy*6-1	7 5±0 9	14 3±0 9	15 7±1 7	8 9±1 0	18 3±1 2
*det*1-1 *hy*1(21 84N)	1 4±0 3	2 9±0 8	2 3±1 3	2 1±0 4	4.6±1 0
*det*1-1 *hy*2-2	1 0±0 5	2 4±1 1	3 6±1 1	2 5±0 5	4.5±0 7
*det*1-1 *hy*3(Bo64)	2 4±0 5	5 9±0 5	4 5±1 1	3 1±0.6	8 2±2 1
*det*1-1 *hy*4(2 23N)	0 8±0 5	1 9±0 5	1 1±0 3	1 5±0.5	5 0±1 2
*det*1-1 *hy*5(Ci88)	3 6±0 4	7 6±1 2	6 8±1 1	6 6±0.6	14 9±1 8
*det*1-1 *hy*6-1	1 2±0 6	2 5±0 7	2 2±0 6	1 9±0.7	4 0±0 8
det2-1 *hy*1(21 84N)	0 5±0 3	1 0±0 5	1 0±0 5	1 0±0.6	3 4±1 0
det2-1 *hy*2-2	0 6±0 4	2 1±0 4	3 7±0 7	1 6±0.5	3 3±0 6
det2-1 *hy*3(Bo64)	0 5±0 4	0 7±0 5	1 3±0 4	0 6±0.5	3 0±0 9
det2-1 *hy*4(2 23N)	0 5±0 5	0 8±0 7	0 6±0 4	1 0±0	3 6±0 8
det2-1 *hy*5(Ci88)	2 2±0 6	2 4±0 6	2 9±0 2	2 3±0.6	8 4±1 2
det2-1 *hy*6-1	0 7±0 5	0 8±0 4	1 2±0 6	0 5±0.4	3 2±1 0
*det*1-3 *det2*-1	0 5±0 4	0 5±0 5	0 2±0 5	0 5±0 5	2 0±1 0

Seedlings were grown for 10 days in the various light conditions or for 8 days in complete darkness
The fluence rates for the various light growth conditions are described in Materials and methods

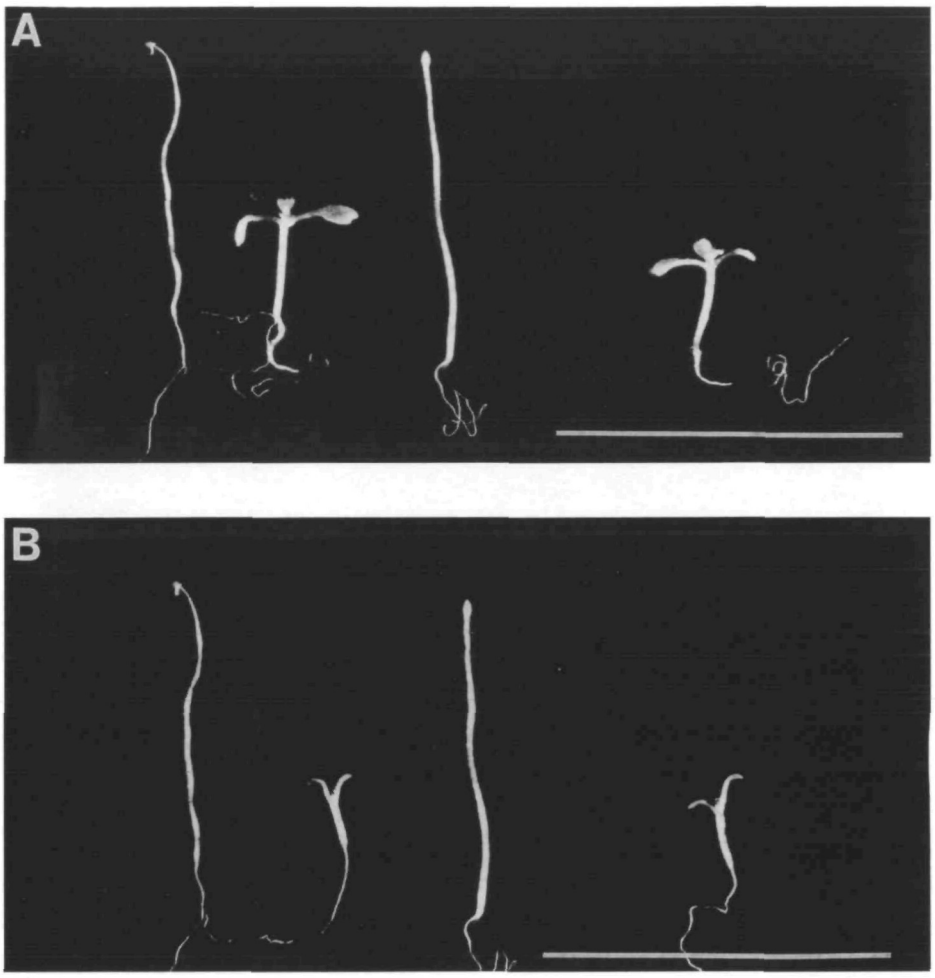

Fig. 2. Phenotypes of seven-day-old dark-grown wild-type and *hy*1, *det*1, *det*2, and *hy*1 *det*1, and *hy*1 *det*2 mutant seedlings (A) Pictured from left to right are wild type (etiolated), *det*1-1, *hy*1 (21.84N), and *det*1-1 *hy*1 (21 84N) The phenotypes of the *hy*2-2 *det*1-1 double mutant or the *hy*6-1 *det*1-1 double mutant were identical to the *hy*1 *det*1 double mutant and are not shown. (B) Pictured from left to right are wild type, *det*2-1, *hy*1 (21.84N), and *det*2-1 *hy*1 (21.84N) The phenotypes of the *hy*2-2 *det*2-1 double mutant or the *hy*6-1 *det*2-1 double mutant were identical to the *hy*1 *det*2 double mutant and are not shown. Bar = 1 cm

combinations on the morphology and gene expression responses in light- and dark-grown plants were examined. In particular, the responses studied included: hypocotyl growth, chlorophyll and anthocyanin accumulation, floral induction, and mRNA accumulation of light-induced genes in dark-grown seedlings. The data for each individual analysis, e.g., chlorophyll accumulation, are presented in a separate table that compares all the single and double mutants with the wild type. For these experiments, I used the most severe allele(s) available for each gene, with the exception of *hy*5 for which I had only one allele, Ci88. I do not know if any of the alleles used were null; however, all the alleles used were fully recessive, with the exception of *hy*3 alleles which are semi-dominant. For a given experiment, when epistasis of one mutation to a second was observed, I assumed an order of gene action could be inferred. However, in a couple of cases, the double mutant phenotype was a superimposition of the phenotypes associated with each of the single mutant parents. Though the additive results suggest that the genes involved affect pathways that proceed independently of each other, I could not rule out the possibility that the single mutants were simply leaky mutations in genes required for regulating the downstream developmental responses.

det*1* det*2* *double mutants*

We had previously shown that plants homozygous for *det*1-1 and *det*2-1 mutations have a simple additive phenotype of the single mutant parents (Chory et al., 1991a). Similar results were observed with *det*1-1 *det*2-2 double mutants (Chory et al , 1991a). In this study, a *det*1-3 *det*2-1 double mutant was constructed. The double mutant had an additive phenotype in the dark (Fig. 1) and in the light (Tables 1-5). The morphological characteristics scored were: hypocotyl length and leaf development in the dark (Table 5) and pigment synthesis (Tables 2, 3), hypocotyl length (Table 5), germination frequencies (Table 4), leaf and bolt number (Table 1), and flowering time in the light (Table 1). Therefore, all *det*1 *det*2 double combinations examined to date have a phenotype that is a superimposition of the phenotype associated with the single mutants. This suggests that *det*1 and *det*2 affect independent pathways.

Phytochrome-deficient hy*1,* hy2, *or* hy6 *mutants and* det*1 or* det*2 double mutants*

Plants homozygous for *hy*1 (21.84N) and *det*1-1 exhibit the dark-grown and light-grown phenotypes of *det*1-1, including the elevated expression levels of light-regulated nuclear (e.g , *rbc*S) and chloroplast (e.g., *psb*A) mRNAs in dark-grown seedlings (Figs 2, 3, 4, Tables 1-6). By the same criteria, *det*1 is also epistatic to *hy*2-2 and *hy*6-1 (Tables 1-6). A puzzling result was obtained when plants homozygous for the less severe *hy*1 allele (d412) and *det*1 were constructed.

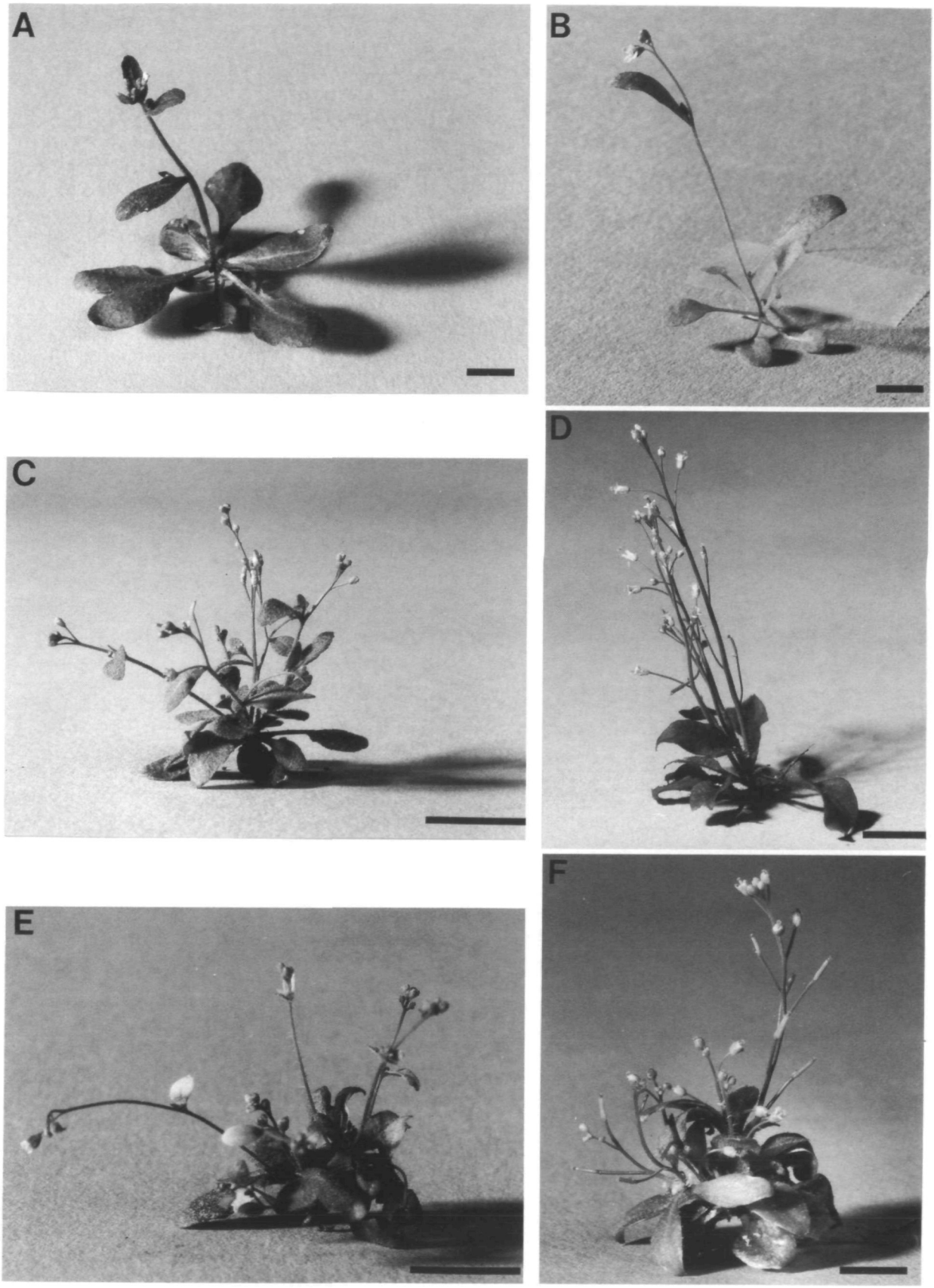

Fig. 3. Phenotypes of light-grown wild-type, and *hy*1, *det*1, *det*2, *hy*1 *det*1, and *hy*1 *det*2 mutant seedlings. (A) 26-day-old ecotype Landsberg *erecta*., (B) 18-day-old *hy*1 (21.84N); (C) 30-day-old *det*1-1, (D) 36-day-old *det*2-1, (E) 30-day-old *hy*1 (21 84N) *det*1-1 double mutant; (F) 33-day-old *hy*1 (21.84N) *det*2-1 double mutant. Bar = 1 cm.

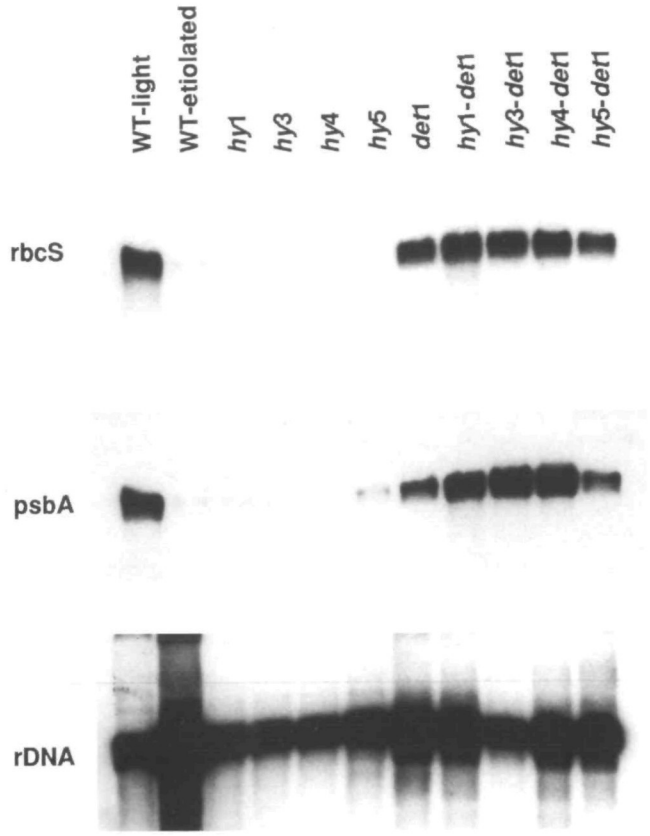

Fig. 4. Accumulation of mRNAs for light-regulated nuclear (*rbc*S) and chloroplast (*psb*A) genes in 7-day-old dark-grown wild type, single, and double mutant combinations involving *det*1-1 Top: accumulation of *rbc*S, a representative nuclear-encoded mRNA, Middle. accumulation of *psb*A, which encodes one of the subunits of photosystem II and is used here as a representative chloroplast-encoded mRNA; and Bottom accumulation of rRNA for normalization of RNA load Very little mRNA accumulated in etiolated wild type (lane 2) or for any of the etiolated *hy* single mutants See Table 6 for quantitation of the amount of RNA accumulated 3 μg of total RNA was loaded per lane.

This double mutant essentially looked like *det*1, except that the double mutant hypocotyl was slightly longer than a *det*1 hypocotyl (3.9 mm versus 2.4 mm in the *det*1 single mutant; data not shown). We originally assessed the severity of the *hy*1 (d412) allele by the amount of phytochrome activity measured in dark-grown seedlings, a measurement that mostly quantifies the activity of the type A phytochrome. However, it is likely that the type B phytochrome is responsible for hypocotyl growth inhibition responses, which cannot be assessed spectrally in green plants Therefore, the less severe *hy*1 alleles may actually be more severe with respect to the activity of the type B phytochrome.

Plants homozygous for *hy*1 (21.84N) and *det*2-1 exhibit the dark-grown and light-grown phenotypes of *det*2-1, with one exception: the *hy*1 *det*2 double mutant had the pale-green phenotype of *hy*1 mutants (Figs 2, 3, 5, and Tables 1-6). Similar results were observed when *det*2-1 was put in combination with *hy*2-2 or *hy*6-1 (Tables 1-6). *hy*1(d412) *det*2-1 strains showed the same phenotype as the above, but the

Table 6. *Expression of light-regulated genes in dark-grown single and double mutants*

	Gene expression (% of wild-type levels in light)*		
Genotype	*rbc*S	*cab*	*psb*A
Wild type	5	5	5
*det*1-1	80	30	100
*det*2-1	20	10	25
*hy*1 (21 84N)	5	5	5
*hy*2-2	5	5	5
*hy*3 (Bo64)	5	5	5
*hy*4 (2 23N)	5	5	5
*hy*5 (Ci88)	5	5	5
*hy*6-1	5	5	5
*det*1-1 *hy*1(21 84N)	90	25	100
*det*1-1 *hy*2-2	80	30	100
*det*1-1 *hy*3(Bo64)	100	30	100
*det*1-1 *hy*4(2 23N)	90	30	100
*det*1-1 *hy*5(Ci88)	40	10	50
*det*1-1 *hy*6-1	90	35	100
*det*2-1 *hy*1(21 84N)	20	10	30
*det*2-1 *hy*2-2	20	10	30
*det*2-1 *hy*3(Bo64)	20	10	30
*det*2-1 *hy*4(2 23N)	20	10	30
*det*2-1 *hy*5(Ci88)	20	10	30
*det*2-1 *hy*6-1	20	10	30
*det*1-3 *det*2-1	100	50	100

*Gene expression values were quantitated as described in Materials and methods and are rounded up to the nearest 5% value

hypocotyl was slightly longer than the hypocotyl from *det*2 single mutant lines (2 1 mm versus 0.7 mm), consistent with the *hy*1 (d412) *det*1-1 results just described. Therefore, *det*2 is partially, but incompletely epistatic to the phytochrome-deficient *hy* mutants.

hy*3 (Bo64) and* det*1-1 or* det*2-1 double mutants*

The double mutant combination of *hy*3 (Bo64) and *det*1-1 results in plants that look largely like the *det*1-1 single mutant except that the hypocotyl elongates to a greater degree in dark- or light-grown seedlings, and the floral bolt is slightly longer in the double mutant (Figs 4, 6, 7 and Tables 1-6). For most responses, except stem elongation, *det*1-1 is epistatic to *hy*3 (Bo64). Likewise, the double mutant, *hy*3 (Bo64) *det*2-1, has the phenotype of *det*2-1 by every criterion, except that the hypocotyl elongates to a greater degree in the double mutant (Figs 5, 6, 8, and Tables 1-6). *det*2-1 is therefore also epistatic to *hy*3.

hy*4 (2 23N) and* det*1-1 or* det*2-1 double mutants*

Plants homozygous for *hy*4 (2.23N) and *det*1-1 have the dark-grown de-etiolated phenotype of *det*1-1 (Figs 4, 9). When grown in high white-light conditions, the *hy*4 *det*1 double mutant has the phenotype of *det*1-1 seedlings, with one exception. *hy*4 *det*1 double mutant plants are extremely purple during the early stages of seedling growth (Figs 10, 11). This is due to a 40-fold increase in anthocyanin accumulation in the double mutants (Table 3). To examine further the molecular basis for aberrant accumulation in the double mutants, I looked at accumulation of *chs* mRNA in the single and double mutants (Fig. 11). The condensation of 3 acetate units with 4-coumaroyl-CoA to naringenin chalcone by chal-

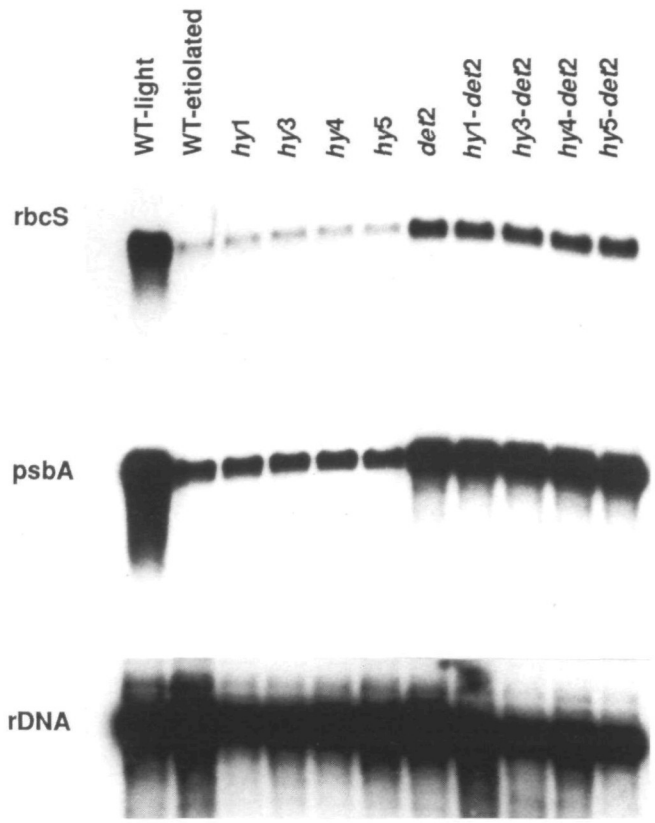

Fig. 5. Accumulation of mRNAs for light-regulated genes in 7-day-old dark-grown wild type, single and double mutant combinations involving *det2*-1. Top accumulation of *rbc*S, a representative nuclear-encoded mRNA, Middle: accumulation of *psb*A, a representative chloroplast-encoded mRNA; and Bottom. accumulation of rRNA for normalization of the RNA load in each sample. The mRNA levels for etiolated wild-type and *hy* single mutants appear higher than in Fig. 4 due to the longer exposure times required to see the mRNA levels in *det2* and *det2* double mutants See Table 6 for quantitation of the amount of RNA accumulated. 3 μg of total RNA was loaded per lane

cone synthase (*chs*) is the first committed step in the anthocyanin biosynthetic pathway (Heller and Hahlbrock, 1980). *chs* is a single copy gene whose expression is regulated by blue light in *Arabidopsis* (Feinbaum et al., 1991). Dark-grown *det*1 single mutants accumulate anthocyanins in the dark and light-grown *det*1 mutants have 5-fold higher levels of anthocyanins than wild type (Chory et al., 1989b, Table 3). *hy*4 single mutants, in contrast, have reduced anthocyanin accumulation (about 2-fold less than wild type) in light-grown seedlings (Table 3). *chs* mRNA accumulates approximately 0.5× in the *hy*4 single mutant and 5× in the *det*1 single mutant compared with wild-type seedlings, as might have been predicted by the anthocyanin results (Fig. 11). However, the *hy*4 *det*1 double mutant had only about a 5-fold increase in *chs* mRNA accumulation over wild type and approximately equal amounts to the *det*1 single mutant parent (Fig. 11). I conclude from these studies that *det*1 is epistatic to *hy*4; however, HY4 may play an additional role in anthocyanin biosynthesis.

*hy*4 (2.23N) *det*2-1 double mutants have the dark- and light-grown phenotypes of *det*2-1 single mutant plants, including anthocyanin production (Figs. 5, 8, 9, and Tables 1-6). *det*2-1 is epistatic to *hy*4 (2.23N).

hy5 (Ci88) and det1-1 or det2-1 double mutants

Plants homozygous for *hy*5 (Ci88) and *det*1-1 mutations are only partially de-etiolated when grown in the dark, making fewer leaves than *det*1-1 single mutants. In addition, dark-grown *hy*5 *det*1-1 double mutants have a very elongated hypocotyl, which is similar in length to the *hy*5 single mutant parent (Fig 12 and Table 5) There was only about half the accumulation of *cab*, *rbc*S, and *psb*A mRNAs in dark-grown *det*1 *hy*5 double mutants as in *det*1 single mutants (Fig. 4, Table 6). However, these mRNA levels were several-fold higher than those of *hy*5 single mutants or wild type (Fig. 4, Table 6) Light-grown *hy*5 *det*1 double mutant plants had a superimposition of the phenotypes of *hy*5 and *det*1 single mutants with respect to hypocotyl elongation (Table 5), leaf size (Table 1 and Fig. 13), bolt length (Fig 13), germination frequencies (Table 4), and accumulation of anthocyanins and chlorophylls (Tables 2 and 3)

Dark-grown *hy*5 (Ci88) *det*2-1 double homozygotes have a hypocotyl of intermediate length between *hy*5 and *det*2 single mutants (Fig. 12, Table 5). Also, anthocyanins accumulate to an intermediate level between *det*2 and *hy*5. In the light, the *hy*5 *det*2-1 double mutants have an additive phenotype with respect to bolt number (Table 1, Fig. 8) and flowering time (Table 1). Significantly, *hy*5 *det*2 double mutants made a large number of seeds when self-fertilized, unlike the *det*2 single mutant, which is almost completely male-sterile. Thus, the light and dark phenotypes of both the *hy*5 *det*1 and *hy*5 *det*2 double mutant combinations were additive.

Discussion

Phenotypic analysis of double mutant combinations between the *hy* and *det* mutants suggests a simple branched pathway (Fig 14). The nearly additive interactions observed between *det*1-1 and *hy*5 (Ci88), *det*2-1 and *hy*5 (Ci88), and *det*1 and *det*2 alleles suggest that these 3 gene products act on distinct signal transduction pathways to affect the downstream light-regulated responses (Fig. 14A). Plants homozygous for *hy*5 (Ci88) are defective in red-light controlled hypocotyl growth inhibition responses; however, physiological studies with *hy*5 and double mutant combinations of *hy*5 with the phytochrome-deficient *hy*1 or *hy*2 mutants imply that HY5 defines a red-light action pathway that is separate from phytochrome (Koornneef et al., 1980).

An alternative model to explain the double mutant phenotypes is presented in Fig. 14B. In this model, HY5 is a transduction element on a phytochrome action pathway that is influenced by DET1. This model takes into account that I have no available means to assess if the mutant alleles used were null. For instance, the *hy*5 (Ci88) allele may be leaky, a result that would be consistent with the nearly additive interactions that were also observed between *hy*5 (Ci88) and *hy*1 (21.84N) or *hy*5 (Ci88) and *hy*2 (To76) (Koornneef et al., 1980). The *det*1 *hy*5 double homozygote appeared very similar to a wild-type plant when grown in the light, having a

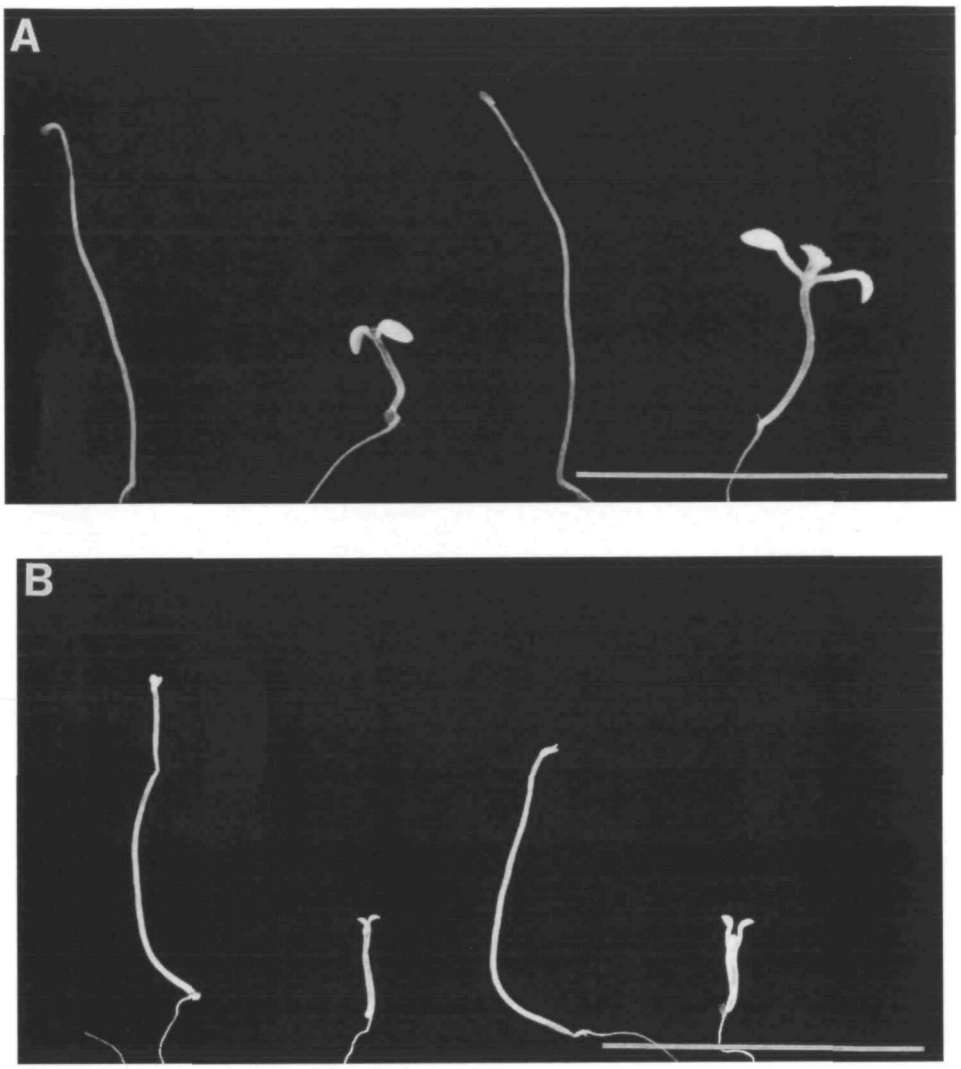

Fig. 6. Phenotypes of seven-day-old dark-grown wild-type and *hy*3, *det*1, *det*2, *hy*3 *det*1, and *hy*3 *det*2 mutant seedlings. (A) Pictured from left to right are: etiolated wild type, *det*1-1, *hy*3 (Bo64), and *det*1-1 *hy*3 (Bo64) (B) Pictured from left to right are etiolated wild type, *det*2-1, *hy*3 (Bo64), and *det*2-1 *hy*3 (Bo64). Bar = 1 cm.

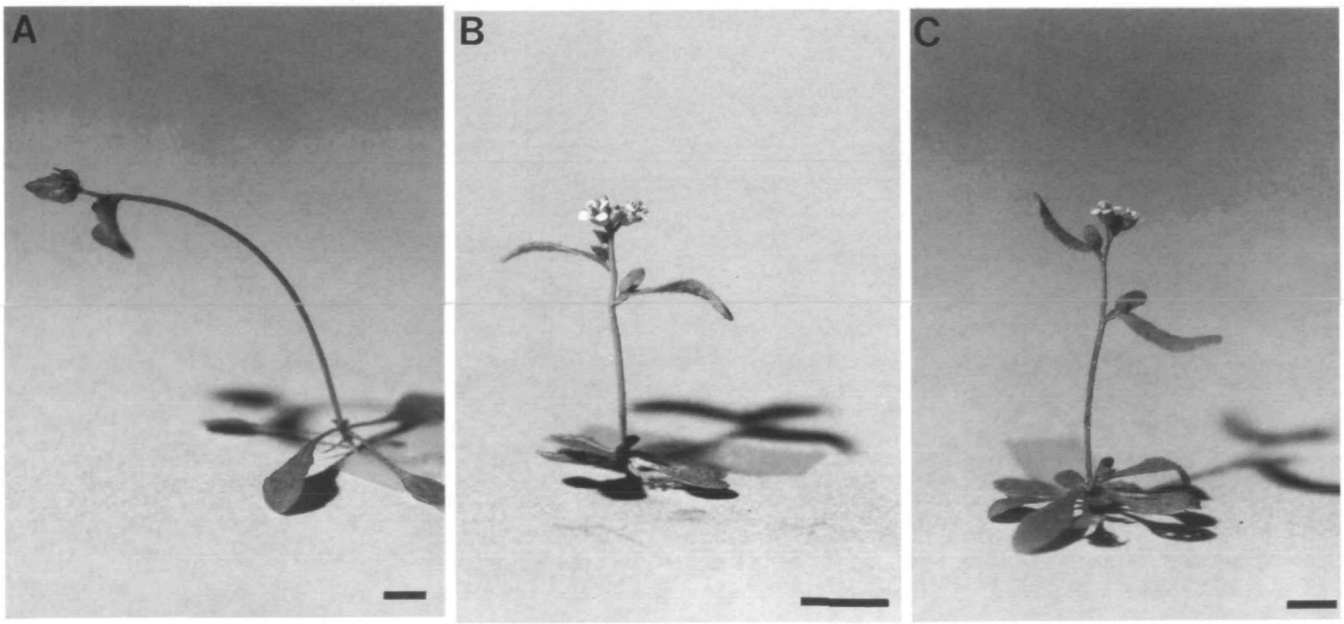

Fig. 7. Phenotypes of light-grown *hy*3 (Bo64), *det*1-1, and *hy*3 (Bo64) *det*1-1 double mutant. (A) 24-day-old *hy*3 (Bo64); (B) 26-day-old *det*1-1; and (C) 26-day-old *hy*3 (Bo64) *det*1-1 double mutant Bar = 1 cm

Fig. 8. Phenotypes of light-grown *det*2-1 and *det*2-1 hy double mutant combinations (A) 38-day-old *det*2-1; (B) 38-day-old *det*2-1 *hy*5 (Ci88) double mutant, (C) 36-day-old *det*2-1 *hy*3 (Bo64) double mutant, and (D) 36-day-old *det*2-1 *hy*4 (2 23N). Bar = 1 cm

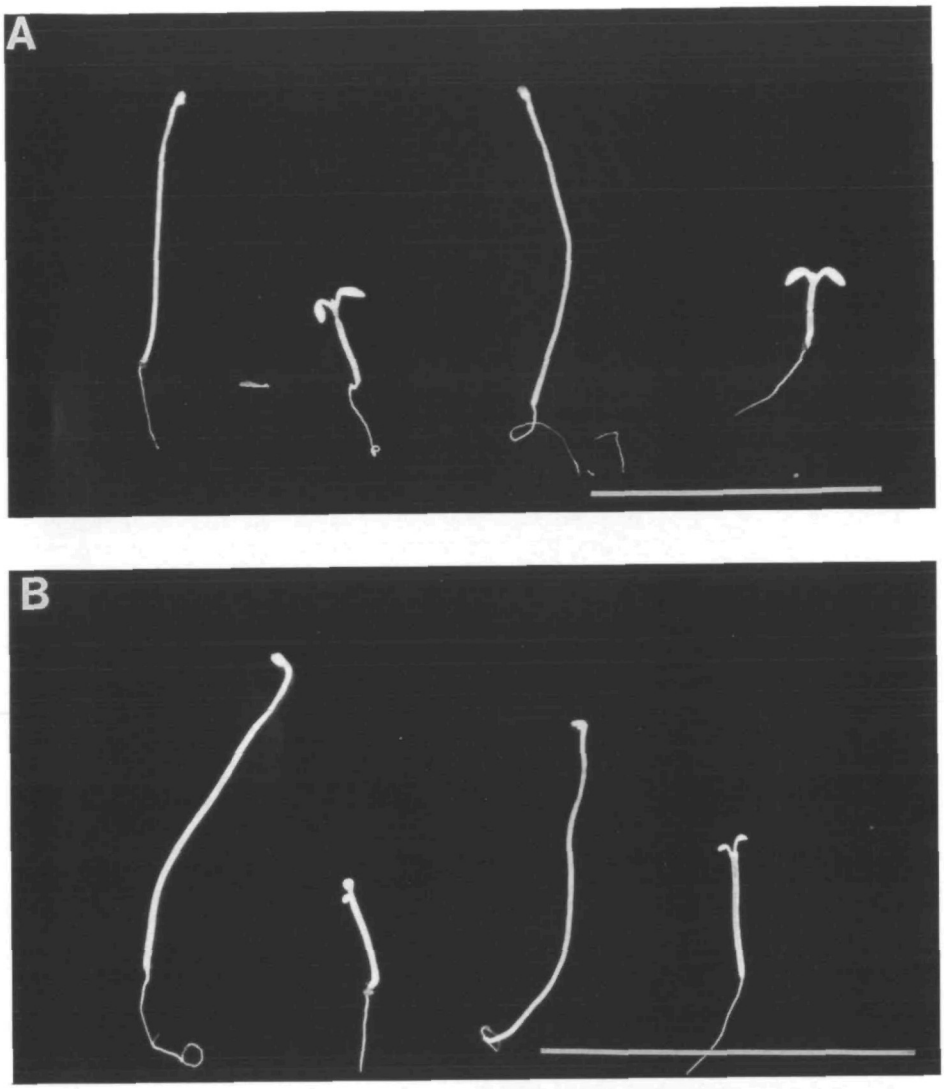

Fig. 9. Phenotypes of seven-day-old dark-grown wild type and *det*1, *det*2, *hy*4, *det*1 *hy*4, and *det*2 *hy*4 (A) Pictured from left to right are wild type, *det*1-1, *hy*4 (2 23N), and *hy*4 (2 23N) *det*1-1 (B) Pictured from left to right are. wild type, *det*2-1, *hy*4 (2.23N), and *hy*4 (2 23N) *det*2-1. Bar = 1 cm

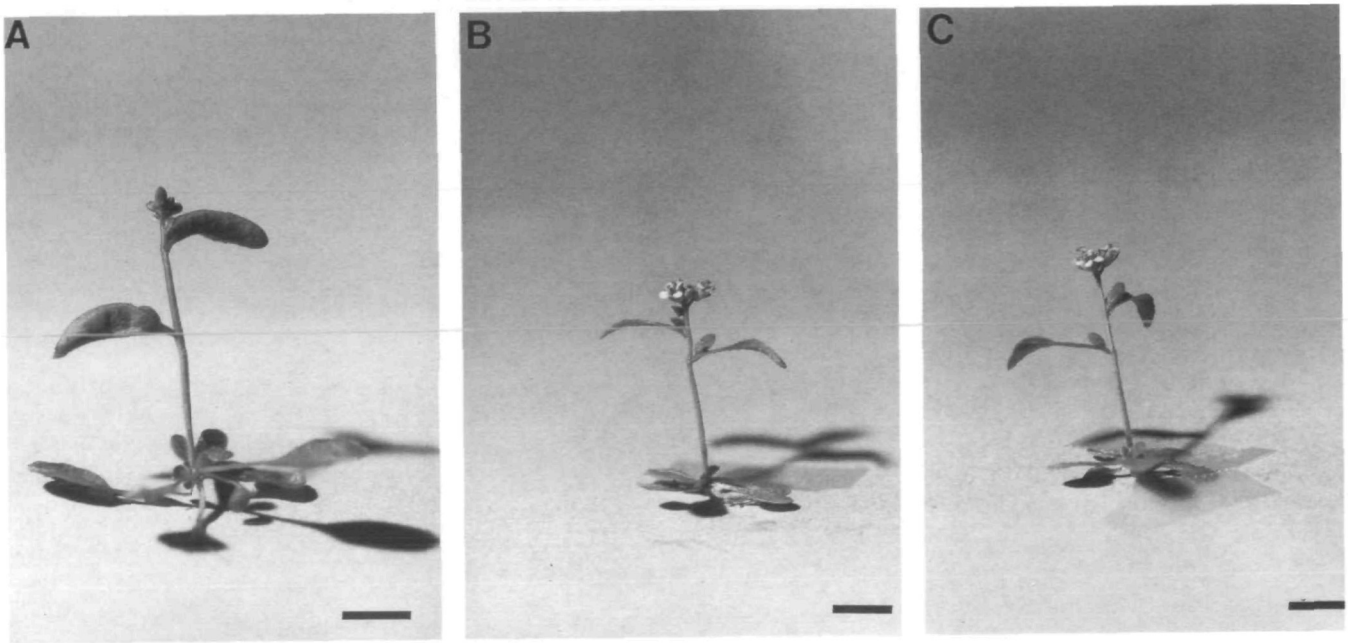

Fig. 10. Phenotypes of light-grown *hy*4 (2.23N), *det*1-1, and *hy*4 (2 23N) *det*1-1 double mutant (A) 28-day-old *hy*4 (2.23N), (B) 28-day-old *det*1-1; and (C) 28-day-old *hy*4 *det*1-1 double mutant. Bar = 1 cm.

much more dramatic phenotype than light-grown *det2 hy5* double mutants. This could be due to the fact that *det1-1* is itself leaky, which is consistent with our ability to isolate dominant extragenic suppressors of *det1-1* (A. Pepper and J. Chory, unpublished data). Still, the phenotype of *det1 hy5* double mutants is interesting because it points to the opposite roles that DET1 and HY5 appear to play during early development in response to light. To discriminate between the two models, we are currently isolating new mutant alleles of all the genes in question.

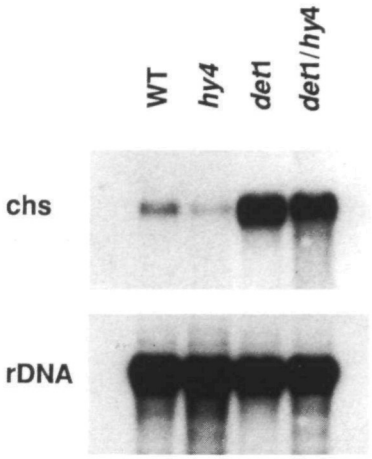

Fig. 11. Accumulation of *chs* mRNA in 12-day-old light-grown *det1 hy4* double mutant compared with single mutants and wild type. 5 μg of total RNA was loaded per lane.

In the absence of leaf and chloroplast development, a small, but measurable, induction of *cab*, *chs*, and *rbc*S gene expression can be seen after pulses of red or blue light are applied to etiolated seedlings (e.g., Karlin-Neumann et al., 1988; Feinbaum et al , 1991). This is indicated by a dotted line in Fig. 14A. The intermediates that regulate gene expression by this probably simple signal transduction pathway are unknown, and may possibly be a distinct set of molecules from those defined by *hy5*, *det1* or *det2* mutations. We have recently identified a class of *trans*-acting mutations in *Arabidopsis* for which *cab* gene expression is increased in the dark in the absence of de-etiolation (L. Altschmied and J. Chory, unpublished data). These mutations may define such signal transduction components.

det1 and *det2* are epistatic to all strong alleles of *hy1*, *hy2*, and *hy6*. Since *det1* and *det2* mutants contain wild-type levels of phytochrome activity, I propose an order of gene action that places *hy1*, *hy2*, *hy3*, and *hy6* before *det1* or *det2* on a

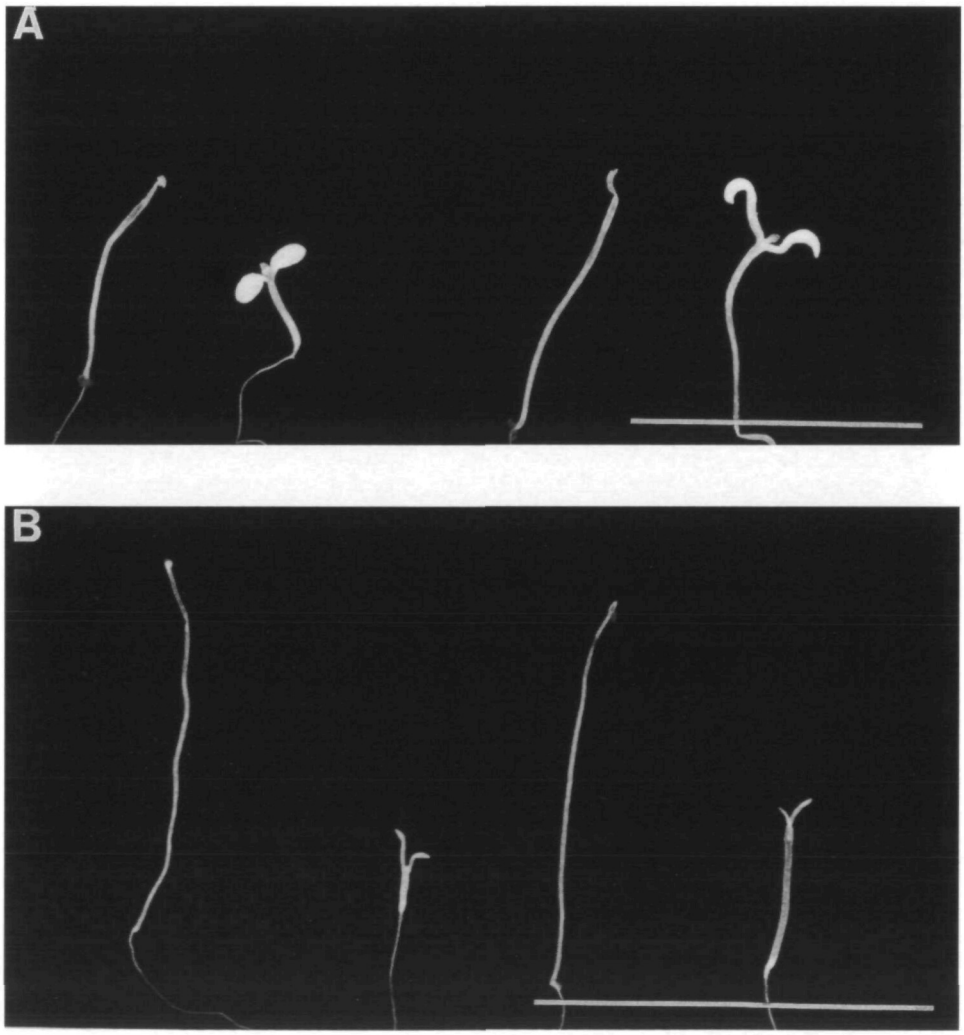

Fig. 12. Phenotypes of 7-day-old dark-grown wild type, *det1-1*, *det2-1*, *hy5* (Ci88), *det1-1 hy5* double mutant, and *det2-1 hy5* double mutant (A) Pictured from left to right are. etiolated wild type, *det1-1*, *hy5* (Ci88), and *hy5* (Ci88) *det1-1* (B) Pictured from left to right are: wild type, *det2-1*, *hy5* (Ci88), and *hy5* (Ci88) *det2-1*. Bar = 1 cm.

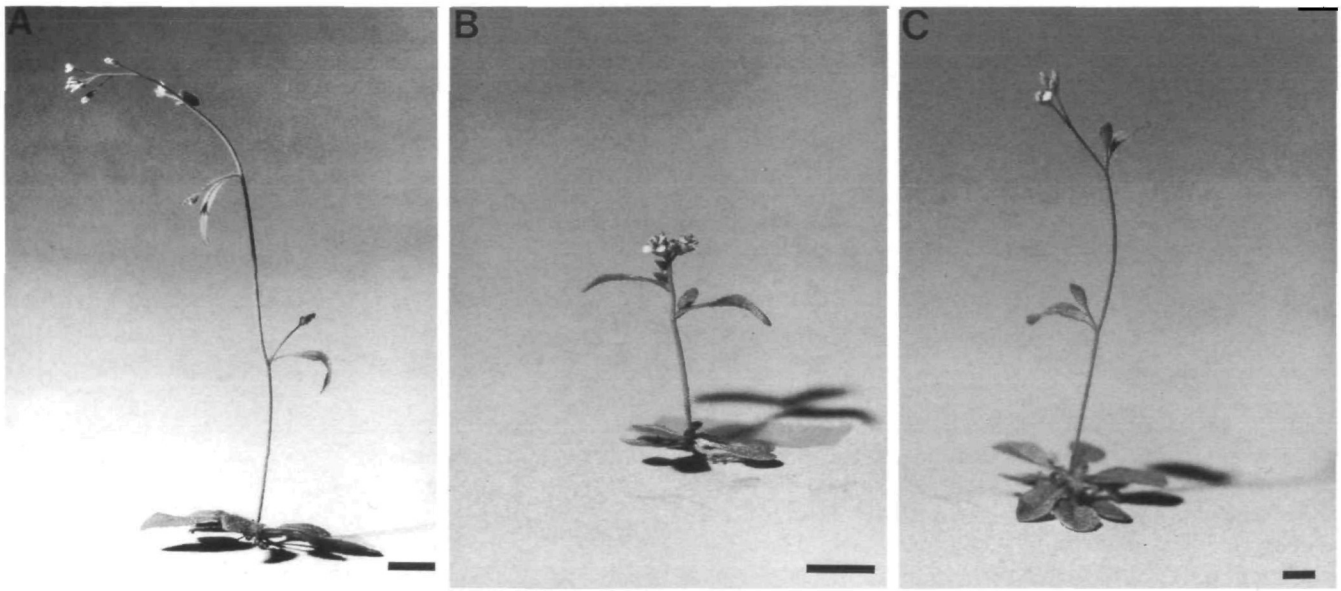

Fig. 13. Phenotypes of light-grown *hy*5 (Ci88), *det*1-1, and *hy*5 (Ci88) *det*1-1 double mutant (A) 28-day-old *hy*5 (Ci88); (B) 28-day-old *det*1-1, and (C) 28-day-old *hy*5 (Ci88) *det*1-1 double mutant. Bar = 1 cm.

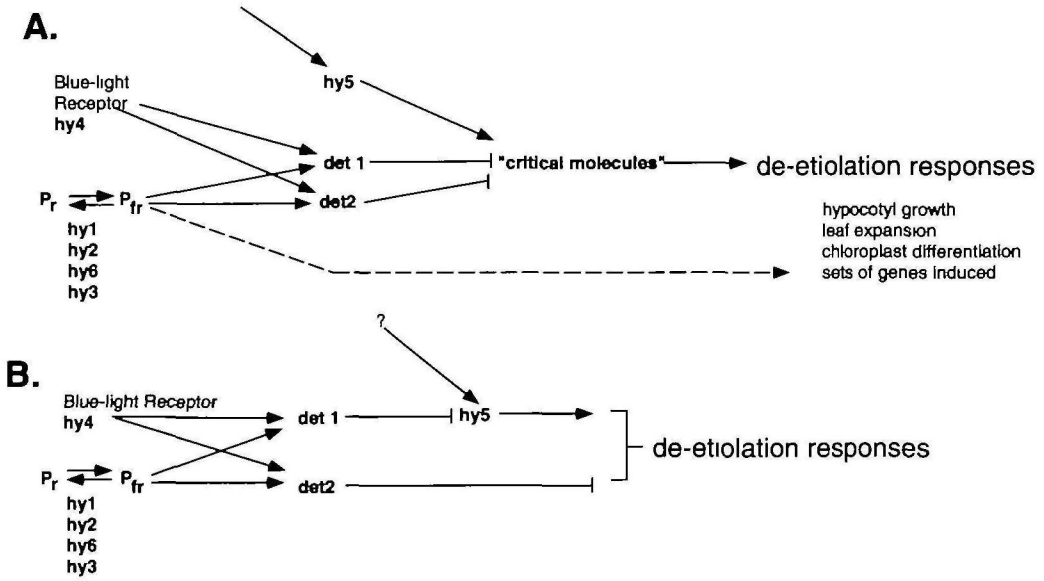

Fig. 14. Models for the functional relationships among the *DET* and *HY* genes. The phenotypes of doubly mutant lines suggest a hierarchical regulatory network among genes controlling the downstream light-regulated responses (inhibition of hypocotyl elongation, and promotion of leaf expansion, chloroplast differentiation, and gene expression) The models are formal, and make no prediction as to the precise molecular nature of the proposed interactions among genes or gene products (A) *DET*1 and *DET*2 are negative regulators of de-etiolation responses. Formation of Pfr results in a decrease in activity of DET1 or DET2 leading to derepression of the downstream light-regulated morphological and gene expression responses. *DET*1 and *DET*2 activities are influenced similarly by blue-light signals (*hy*4). *hy*5 *det*1 or *hy*5 *det*2 double mutant plants show aspects of both single mutant parents suggesting that the genes affect pathways that proceed independently of each other. The activities of *DET*1, *DET*2, and *HY*5 may be direct, or by unidentified intermediate regulators, e.g , cytokinins (as implied here). The dotted arrow indicates a separate signal transduction pathway that exists in etiolated seedlings and affects the expression of certain phytochrome-regulated genes (pathway inferred from physiological studies with red- and far-red-light treatments). (B) An alternative model to explain the double mutant phenotypes. Because the null phenotype for the various mutations is not known, I propose the second model to explain the additive interactions between *hy*5 and *det*1 and *hy*5 and the phytochrome-deficient hy mutants This model would account for the data if *hy*5 or any of the other alleles were leaky. In this model, HY5 is a transduction element on a phytochrome action pathway whose activity is influenced by DET1.

phytochrome signal transduction pathway. In this model, formation of the active form of phytochrome, P_{fr}, results in a decrease in activity of either DET1 or DET2 which in turn leads to derepression of the downstream light-regulated morphological and gene expression responses. DET1 appears to play a role in the negative control of all downstream light-regulated responses during growth in the dark (Chory et al., 1989b). Further, DET1 exerts its negative effects spatially on adult light-grown plants since in *det*1-mutants there is ectopic expression of *chs*, *rbc*S, and *cab*, and chloroplasts develop ectopically in *det*1-roots (Chory and Peto, 1990). *det*2 is not fully epistatic to the strong *hy*1, *hy*2, and *hy*6 alleles, in that *det*2 *hy* mutant combinations are pale yellow-green in color as are the single *hy*1, *hy*2, or *hy*6 parents. This result suggests that DET2 acts on a branch of a pathway downstream from phytochrome that does not affect chlorophyll biosynthesis per se. Further, the prolonged juvenile phase and delayed senescence phenotypes of *det*2 single mutants implies a temporal negative regulatory role for DET2 during vegetative growth in *Arabidopsis* (Chory et al., 1991a). In wild-type plants, it is predicted that the activity of DET2 is high prior to light-induced morphogenesis, low during the early stages of chloroplast differentiation and build-up, and relatively high during the later stages of vegetative growth (senescence) when the light developmental program is turned down (Chory, 1991)

*hy*3 mutations specifically affect accumulation of the type B phytochrome (Nagatani et al , 1991, Somers et al., 1991). *hy*3 mutants have elongated hypocotyls, petioles, and floral bolts, suggesting that B phytochrome deficiencies alone may be responsible for the long hypocotyl phenotype seen in the *hy*1, *hy*2, and *hy*6 mutants. DET1 and DET2 appear to be on a branched pathway downstream from formation of P_{fr}(B), however, since we do not have mutants deficient in any one of the other phytochromes, it is not yet clear whether DET1 and DET2 act in a pathway downstream from the other phytochromes as well. Since the temporal and spatial expression patterns for any of the phytochromes or DET1 or DET2 is not known, it is impossible to predict if the individual DETs will act in concert with a particular phytochrome during development.

*det*1 and *det*2 are also epistatic to *hy*4, suggesting that blue-light signals are involved in decreasing DET1 and DET2 activities (Fig. 14). This is consistent with data from studies of *det*1 and *det*2 single mutants which showed that blue-light regulated gene expression and anthocyanin accumulation were derepressed in the dark (Table 3, and Chory et al., 1989b, 1991a). One puzzling observation with regard to anthocyanin accumulation, though, is that *det*1-1 *hy*4 (2.23N) double homozygotes had eight-fold higher levels of anthocyanins than *det*1 single mutants and 40-fold higher levels than wild type (Table 3). *chs* mRNA and anthocyanin accumulation is 5-fold higher in the *det*1 mutant than in wild-type seedlings; however, in the *det*1 *hy*4 double homozygote, *chs* mRNA is 5-fold higher than in wild type (similar to *det*1) (Fig. 11), while anthocyanins are 40-fold increased (Table 3) A possible explanation for these observations is that DET1 is the primary negative regulator of the anthocyanin biosynthetic gene, *chs*, when DET1 repression of *chs* is released (*det*1 background), some other mRNA or gene product in the anthocyanin biosynthetic pathway becomes limiting. Reduced activity, or loss-of-function of, HY4 alleviates this second block. Thus, *HY*4 might play a negative regulatory role in anthocyanin biosynthesis that is only revealed in the *det*1 background. Alternatively, since anthocyanins also accumulate during stress conditions in *Arabidopsis*, a *hy*4 *det*1 double mutant may have high anthocyanin levels due to metabolic stress

DET1, DET2, and HY5 may act directly to affect the downstream de-etiolation responses, or they may act through unidentified intermediate regulators, such as hormones, as implied in Fig. 14A. What might this critical regulator be? One suggestion from the literature, (Stetler and Laetsch, 1965; Flores and Tobin, 1986), and from our studies of *det*1 and *det*2 mutants is that cytokinin is a critical growth regulator for de-etiolation responses (Chory et al., 1991b). The similarity of red-light and cytokinin effects was first noted in 1956 (Miller, 1956). Since then, cytokinins have been shown to promote chloroplast development, shoot development and expression of genes for chloroplast-destined proteins in tissue culture cells (e.g , Teyssendier de la Serve et al., 1985; Harvey et al., 1974; Flores and Tobin, 1986). Increased cytokinin levels also correlate with the delayed senescence phenotype of tobacco plants transformed with a gene that increases cytokinin production (Smart et al., 1991) Work in my lab has shown that many of the phenotypes of *det*1 and *det*2 mutants can be mimicked by the addition of the cytokinin, 2-isopentenyl adenine, to the growth medium of wild-type plants (Chory et al., 1991b). Like *det*1 mutants, dark-grown wild-type seedlings grown in the presence of cytokinins have short hypocotyls, expanded cotyledons and leaves, contain chloroplasts, and have high levels of expression of genes that are normally light-regulated. This intriguing result implies that an increase in available cytokinins in dark-grown seedlings is sufficient to override a light requirement for leaf and chloroplast development and gene expression in *Arabidopsis*. DET1, DET2, and HY5 may be involved in regulating the activity or availabilty of cytokinins in *Arabidopsis* (as indicated in Fig 14A). Alternatively, phytochrome, blue-light, and cytokinins may independently modulate the pool size of common intermediates (DET1, DET2, HY5) that directly regulate gene expression.

Clearly, the model proposed here does not define all the elements in the light signal transduction pathways that affect early seedling development. As mentioned above, there are a minimum of five phytochromes defined by DNA sequence homology in *Arabidopsis* (Sharrock and Quail, 1989, R. Sharrock, personal communication) Though some information is available for the expression pattern of phytochrome A apoprotein in etiolated seedlings, there is little available data on the cell-type specific expression of the different phytochrome genes or proteins in green plants (Komeda et al., 1991). Further, no information is available for the temporal or spatial regulation of any of the five *Arabidopsis* phytochrome genes or the *DET*1 and *DET*2 genes (which are not yet cloned). New mutants that lack a particular phytochrome would help to further define the red-light controlled signal transduction pathways. The blue-light signal transduction pathways are even less well defined However, a new class of photomorphogenetic mutant with long hypocotyls only in blue-light has recently been defined (Liscum and Hangarter, 1991) and mutants with impaired blue-light phototropism

have also been described (Khurana et al., 1989; Khurana and Poff, 1989). These two classes of mutants may shed some light on the complexity of blue-light signal transduction pathway(s) in *Arabidopsis*. Lastly, mutations in *DET3*, *HY7*, (J. Chory, unpublished), *COP1*, (Deng et al., 1991) and the other photomorphogenetic loci need to be integrated into this pathway.

In summary, these studies define just a few steps in what is likely to be a complex regulatory network that involves a large number of gene products that affect early dicotyledonous seedling development in response to red and blue light signals. Clearly, further genetic studies are required to identify mutations that affect only a subset of the downstream light-regulated responses and additional epistasis experiments on extant mutant alleles need to be performed. Lastly, the phenotypic and genetic properties of the various photomorphogenetic genes and the formal nature of their functional interactions may reflect any of a variety of cellular and molecular mechanisms. Resolution of such issues requires that the genes defined by the various mutations be cloned and characterized. Such studies may determine whether the products of these genes interact directly or whether they participate in processes occurring in distinct cellular compartments or at discrete times during development.

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