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From seed germination to flowering, light controls plant development via the pigment phytochrome

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ABSTRACT **Plant growth and development are regulated by interactions between the environment and endogenous developmental programs. Of the various environmental factors controlling plant development, light plays an especially important role, in photosynthesis, in seasonal and diurnal time sensing, and as a cue for altering developmental pattern. Recently, several laboratories have devised a variety of genetic screens using *Arabidopsis thaliana* to dissect the signal transduction pathways of the various photoreceptor systems. Genetic analysis demonstrates that light responses are not simply endpoints of linear signal transduction pathways but are the result of the integration of information from a variety of photoreceptors through a complex network of interacting signaling components. These signaling components include the red/far-red light receptors, phytochromes, at least one blue light receptor, and negative regulatory genes (*DET*, *COP*, and *FUS*) that act downstream from the photoreceptors in the nucleus. In addition, a steroid hormone, brassinolide, also plays a role in light-regulated development and gene expression in *Arabidopsis*. These molecular and genetic data are allowing us to construct models of the mechanisms by which light controls development and gene expression in *Arabidopsis*. In the future, this knowledge can be used as a framework for understanding how all land plants respond to changes in their environment.**

Plant development is flexible and subject to modulation by environmental cues such as light, water, and gravity. Because plants are photosynthetic, they are exquisitely sensitive to light in their environment, carefully monitoring light intensity, quality, and duration to control such developmental decisions as when to germinate or flower. Light has particularly dramatic effects on the morphogenesis of seedlings (1–3). As such, distinct morphologies arise from growing plants under dark or light conditions (Fig. 1*A*). Dark-grown (etiolated) dicotyledonous seedlings have elongated hypocotyls, small folded cotyledons, and undeveloped chloroplasts. In contrast, light inhibits hypocotyl elongation and induces leaf expansion, differentiation, and chloroplast development. The etiolated state is accompanied by little or no expression of several light-regulated nuclear genes involved in photosynthetic function or pigment synthesis. During the transition from dark- to light-grown morphology (de-etiolation), light signals are integrated with intrinsic developmental programs to specify correct spatial and temporal regulation of gene expression, organelle development, and cellular differentiation. These developmental programs may include the action of several different phytohormones (4–6). How light might interact with these hormone signal transduction pathways is not understood.

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The light-dependent development of plants is a complex process involving the combined action of several photoreceptors. These include red/far-red photoreceptors, called phytochromes (7), encoded by five different genes in *Arabidopsis* (*PHYA–PHYE*); one or more blue/UV-A receptors, called cryptochromes (8); and UV-B receptors of unknown photochemistry (9). As will be detailed below, control of light responses is complex because these multiple photoreceptors have partially overlapping functions. Given the number of photoreceptors and the diverse array of developmental events regulated by light, it seems likely that light responses result from integration of information from a variety of signals through a complex network of interacting signaling components. This review summarizes some of the results from molecular genetic analysis using the model plant *Arabidopsis* that support this view. The emphasis is placed on the signal transduction pathways emanating from phytochromes A and B (PHYA and PHYB); the blue-light signaling pathway defined by the *HY4* photoreceptor is mentioned in context of its possible redundant interactions with phytochrome-regulated responses.

Phytochromes: Structure, Function, and Signal Transduction Pathways

Phytochrome is a soluble pigmented protein (purified from plants as a homodimer of two 120-kDa polypeptides) that can exist in two spectrally distinct, photointerconvertible forms: Pr, a red-absorbing form, and Pfr, a far-red-absorbing form (7). For most responses, photoconversion of Pr to Pfr induces a diverse array of morphogenetic responses, whereas reconversion of Pfr to Pr cancels the induction of the responses. Thus, with the exception of seed germination under certain light conditions (10, 11), Pfr is considered to be the active form and Pr the inactive form of the photoreceptor. The unique spectral properties of purified Pr (λmax = 660 nm) and Pfr (λmax = 730 nm) result from the combined properties of apoprotein with its thioether-linked linear tetrapyrrole chromophore.

Despite the work of many, the molecular mechanism by which Pfr induces the downstream developmental responses is not known. A reasonable hypothesis for phytochrome action is that conformational changes associated with Pr and Pfr photoconversion result in differential interactions with downstream component(s) of signal transduction chain(s) linking phytochrome to physiological responses. The construction of transgenic plants expressing domains of phytochrome and the analysis of missense mutations in the *Arabidopsis* phytochrome A and B genes have defined several regions of the apoprotein that are important for its function, including regions for

Abbreviation: BR, brassinosteroid.

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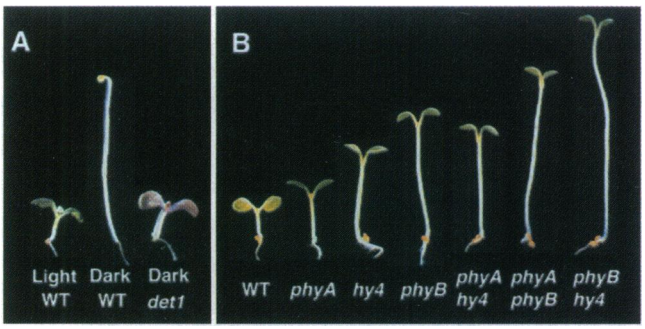

FIG. 1. Phenotypes of light- and dark-grown wild-type *Arabidopsis* seedlings and phototransduction pathway mutants. (*A*) The seedling morphology of light- and dark-grown wild type and dark-grown *det1*. Dark-grown *det1* mutants have a strikingly similar phenotype to light-grown wild-type seedlings. (*B*) The elongated hypocotyl phenotypes of light-grown *phyA*, *hy4*, and *phyB* mutants and double mutant combinations.

chromophore attachment (N terminus), for signaling (several domains), and for dimerization (C terminus). These domains of phytochrome have been reviewed recently (7) and will not be discussed further here. Very recently, however, analysis of transgenic *Arabidopsis* plants containing reciprocal N- and C-terminal domain swaps of rice PHYB with oat PHYA have indicated that these two phytochromes may interact with the same reaction partner (12). The identity of this partner is not known.

Relatively little is known about the downstream signaling components in the phototransduction pathways. Biochemical and microinjection experiments indicate a role for a heterotrimeric GTP-binding protein in phytochrome and blue light signal transduction pathways, and for cGMP, calcium, and calmodulin in phytochrome-regulated gene expression (13). In addition, numerous reports have suggested an involvement of phosphorylation in phytochrome responses. These include reports of red light-regulated protein phosphorylation, phosphorylation-regulated binding of factors to promoters of light-regulated genes, and light-regulated induction of genes encoding protein kinases (14–16). Ongoing biochemical and genetic studies should help to confirm these results.

Molecular and genetic analysis of light-insensitive mutants (identified based on their inability to restrict hypocotyl growth in response to light of different wavelengths) has allowed the identification of loci involved in phytochrome chromophore biosynthesis, in a blue-light photoreceptor, *HY4*, and in the two major phytochrome apoprotein genes, *PHYA* and *PHYB* (3). These mutants have been extremely useful in determining the function of the three major photoreceptors controlling seedling responses to red (*PHYB*), far-red (*PHYA*), and blue/UV-A (*HY4*) light. Each of these three photoreceptors plays a role in multiple processes, including cell expansion and gene expression. They do so by unique and redundant mechanisms.

Null mutations in *PHYB* result in plants with elongated cells in hypocotyl, petioles, inflorescence stems, and root hairs, primarily in response to red light. PHYB also affects chlorophyll accumulation, chloroplast development, and flowering time, and contributes to the red light-induced expression of downstream light-regulated genes. Thus, PHYB controls *Arabidopsis* development at numerous stages and in multiple tissues, and a major role for this phytochrome is in sensing whether the plant is being shaded by other vegetation. If this is so, PHYB initiates an altered program of growth designed to increase overall plant height.

In contrast, PHYA appears to have a more specialized role in *Arabidopsis* development, primarily in germination and seedling responses to far-red light. However, an important role for PHYA later in development is in sensing photoperiod, so that flowering is initiated at the proper time (3, 11). PHYA is not simply a far-red light sensor, however. For instance, when *phyA phyB* doubly null mutants are made, it is clear that PHYA plays a significant role in de-etiolation in red light and, together with PHYB, regulates expression of light-regulated promoters in response to a pulse of red light (11).

The blue and red light signal transduction pathways are at least partially redundant, since doubly null mutant combinations show more elongated hypocotyls in broad spectrum white light (17, 18). As shown in Fig. 1*B*, PHYA, PHYB, and the blue light receptor HY4 each contribute significantly to the hypocotyl growth inhibition response. In the future, we need to answer the question of whether PHYA, PHYB, and HY4 affect the downstream light-regulated processes via shared or parallel signal transduction pathways acting in the same cells. Because these photoreceptors seem to play overlapping roles in plant development, one might postulate that the photoreceptors can activate a shared signal transduction pathway, perhaps by interactions with a common component. However, the finding that each of these photoreceptors also regulates a specific subset of responses suggests that there may also be components that interact uniquely with PHYA, PHYB, or HY4.

Do Negative Regulators Integrate the Information from Multiple Photoreceptors?

Mutations that affect the entire morphogenetic program of young seedlings in the dark have been isolated in several laboratories (2, 3, 19). Recessive mutations in any one of 16 de-etiolated (*det*), constitutively photomorphogenic (*cop*), constitutive photomorphogenesis and dwarfism (*cpd*), embryo defective (*emb*), and fusca (*fus*) genes cause seedlings to exhibit varying degrees of developmental characteristics of light-grown plants, even when the mutants are grown in complete darkness, including changes in gene expression, morphology, and plastid state (Fig. 1*A*; 3). Phenotypes of double mutant plants carrying a mutation of the *det/cop/fus* class and blue light or phytochrome receptor mutations suggest that the *DET/COP/FUS* genes lie downstream of known photoreceptors (20–22). The 10 most pleiotropic mutations result in seedling lethality, suggesting these gene products play an essential role in both light and dark development of *Arabidopsis*. Although all 10 of these loci have been identified in different screens in several laboratories, historical considerations suggest that the various loci can most simply be designated with the following gene names: *DET1*, *COP1*, *COP9*, *FUS4*, *FUS5*, *FUS6*, *FUS8*, *FUS9*, *FUS11*, and *FUS12*. It has been suggested that these gene products act in a common signal transduction pathway, perhaps in a large multiprotein complex. Weak mutations in two of these 10 genes, *cop1* and *det1*, have been identified (23, 24); these weak alleles provide compelling evidence for a role of these two genes in photoregulated development because partial loss-of-function mutations in either the *det1* or *cop1* gene result in dark-grown plants that most exactly phenocopy light-grown wild-type plants. The simplest model that explains the existence of *det*, *fus*, and *cop* mutants is that their gene products are negative regulators that couple light or other signals to the downstream light-regulated program in developing seedlings. The existence of these regulators implies that de-etiolation is neither a simple nor direct series of positive regulatory events leading from light perception to gene induction and other light-dependent processes. Rather, dark-grown cells appear to be poised in a repressed state, ready to respond once light is perceived.

Several of these loci have been cloned, including *COP1* (25), *COP9* (26), *FUS6* (27), *DET1* (23), *DET2* (28), and *CPD* (29). *DET2* and *CPD* are involved in steroid hormone biosynthesis and will be discussed in detail below. In contrast, *COP1*, *COP9*, and *DET1* encode novel nuclear proteins that may be negative regulators of gene expression. *FUS6* also encodes a novel

hydrophilic protein, although its subcellular localization has not been reported. COP9 is found in a large molecular mass (≈550 kDa) complex, whose formation depends on the activity of at least two other genes, *FUS6* and *FUS8*. The deduced COP1 protein contains a Zn-binding ring finger, a coiled-coil motif, and a series of WD-40 repeats. A β-glucoronidase–COP1 fusion protein is nuclear-localized in dark-grown *Arabidopsis* hypocotyls, but the fusion protein may be depleted from the nucleus when plants are transferred to the light (30). This suggests that COP1 exerts its effects as a repressor by its regulated translocation to the nucleus. This is an attractive model that suggests a mechanism by which light can regulate gene expression; however, there are some questions raised by the data. The first is that these results do not explain the observations that *cop1* mutations are lethal in light-grown *Arabidopsis* seedlings. This implies that COP1 must play a role in light-grown seedlings as well as dark-grown seedlings, presumably by functioning in the nucleus. A second confusing result is that the kinetics of COP1 depletion from the nucleus are too slow to be consistent with what is known about phytochrome-regulated gene expression. Additional studies should help clarify these observations.

The deduced DET1 protein sequence has no revealing homologies, although it is hydrophilic and has substantial predicted α-helical content. Consistent with its presumed role in gene regulation, a DET1–β-glucoronidase fusion protein localizes to the nucleus; this nuclear localization does not appear to be light-regulated (23). Moreover, *Arabidopsis* appears to be very sensitive to the levels of DET1. Mutants that are heterozygous for weak or intermediate alleles of *det1* show increased expression of photoregulated genes in the dark, in the absence of morphological changes to the etiolated seedling. In contrast, dark-grown homozygous *det1* mutants with partial DET1 activity develop as light-grown plants. *det1* severe and null mutants share this phenotype, but also display severe defects in temporal and spatial regulation of gene expression. These genetic results suggest that *Arabidopsis* plants respond exquisitely to the levels of active DET1 protein. For instance, the semidominant effects on gene expression observed in *det1-4* heterozygous plants suggests that light-regulated promoters, in the context of the correct cell type for light-induced expression, are very sensitive to the level of DET1 activity. In contrast, seedling morphology is altered only in homozygous mutant backgrounds, suggesting that developmental pattern is mediated by promoters that are less sensitive to the level of DET1 activity. Light-regulated promoters that are not in the correct cell type for light-induced expression are only affected in severe *det1* mutants and may be even less sensitive to the level of DET1. Thus, DET1 appears to be required for correct spatial control of gene expression; in addition, other factors, perhaps ones that interact directly with DET1, must be required to specify the proper temporal and spatial pattern of photoregulated gene expression.

Consistent with this hypothesis, DET1 does not appear to bind DNA. Using partially purified recombinant DET1 protein or *in vitro*-transcribed and -translated DET1, we have so far been unable to detect DNA binding activity on single-stranded or double-stranded DNA cellulose columns (A.P., R.K.C., and J.C., unpublished data). Proteins that repress transcription, but do not directly bind DNA, have been described in yeast and mammals (31–34). These proteins act via protein–protein interactions. For SIN3, SSN6, and Id, it has been proposed that these interactions are mediated by amphipathic helices. DET1 is predicted to contain >25% amphipathic helices and a mutation in *det1-4* changes Gly-58 to Arg in a predicted amphipathic helix. This mutation may thus weaken or prevent an important protein contact. Therefore, it appears that DET1 represses transcription by interactions with other proteins. Three possible models for how DET1 might repress transcription are shown in Fig. 2. Similar models might be invoked for

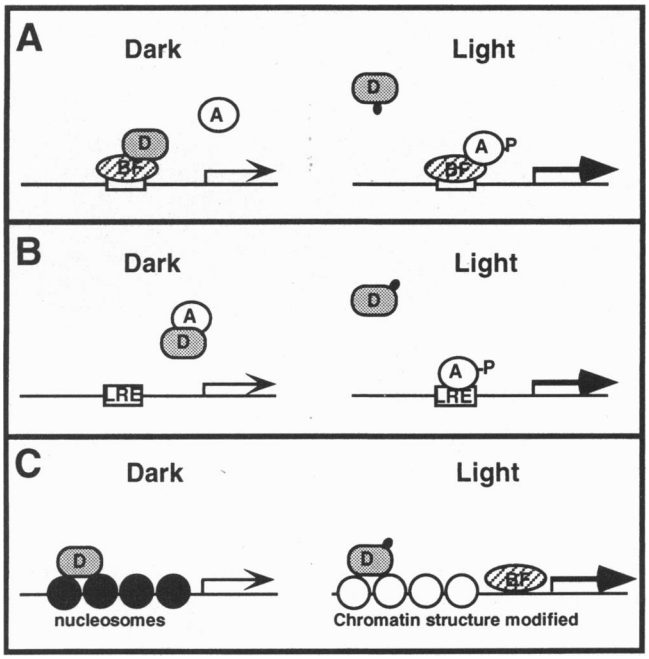

FIG. 2. Models for how DET1 might act to repress a light-regulated promoter. (*A*) DET1 is an active transcriptional repressor that binds the basal transcription machinery, blocking transcription in the dark. (*B*) DET1 represses gene expression in the dark by titrating out transcriptional activators, thereby making them unavailable to bind light-regulatory elements in the upstream region of light-regulated promoters. (*C*) DET1 might be part of a repressive complex that acts to create a repressive chromatin state. D, DET1; BF, basal transcription complex; A, activator; and LRE, light-regulatory element.

COP1 as well.

The simplest model for *DET1* and *COP1* action based on genetic and molecular analyses and studies of the phenotypes of weak and null alleles is that DET1 and COP1 are general repressors of transcription, negatively controlling the expression of photoregulated and other developmental genes. Examples of gene repression in eukaryotes have only recently been described, and the number of examples is increasing dramatically (31–36). Repression seems to be mediated by several mechanisms that inhibit the initiation of transcription. "Anti-activation" can result from competition for DNA-binding sites between transcriptional activators and repressors, or through repressor–activator interactions that block activator functions. Similarly, interactions between repressors and basal transcription proteins can inhibit complex formation directly. This model is attractive in terms of COP1 which contains WD-40 repeats which are also found in $TAF_{II}80$, a component of the basal transcription machinery in *Drosophila* (37). A third model for repression involves the packaging of promoters into nucleosomes such that binding of both activator and basal transcription proteins are blocked (Fig. 2*C*). There are two modes of repression that have been described that involve chromatin remodeling: reversible repression by the global regulator complex, SSN6–TUP1, and permanent silencing (e.g., at heterochromatin). These models are not necessarily mutually exclusive. For instance, experimental evidence supports models for TUP1–SSN6 action by interactions with the basal transcriptional machinery and also by altering chromatin structure (33, 38). These models for repression make sense in terms of what is known about eukaryotic gene expression. Eukaryotic genes typically respond to several, and often many, transcriptional activator proteins. To repress transcription of such genes selectively by a mechanism that directly compromises activator function, a dedicated repressor would, in principle, be needed for each activator. Because DET1 and

COP1 repress the activity of many different genes that are under positive control of a diverse set of activator proteins, models for DET1 and COP1 as global repressors are attractive.

The general repressor model raises the question: how directly are DET1, COP1, COP9, and the FUSCAs involved in signal transduction from the photoreceptors? As mentioned above, the analysis of null alleles at these loci result in seedling lethality and suggests the involvement of these genes in a number of different developmental pathways. Indeed, phenotypic characterization of a subset of the *fusca* mutants suggests a limited role in light-regulated gene expression and chloroplast development. Thus, it has been proposed that *FUS6* is involved in a signal transduction network that acts independently of light (27). Another possibility is that other signal transduction pathways that impact upon light-controlled gene expression become constitutively activated in *fus* mutants. It is known, for example, that carbohydrates, hormones, and signals from the plastid can also control the expression of light-regulated nuclear genes (3). Nonetheless, several independent lines of evidence derived from genetic and molecular studies with *DET1* and *COP1* suggest that these two loci are important regulators of light-regulated gene expression and development. First, *DET1* appears to be a dosage-sensitive regulator of light-regulated genes. Second, overexpression of COP1 from a highly and constitutively expressed promoter results in plants with a partially light-insensitive phenotype (39). Third, the short circadian period of *CAB* gene expression in weak and null *det1* alleles and a weak *cop1* allele suggests that these two gene products act on a light input pathway that sets the circadian oscillator (40). Lastly, extragenic suppressors of *det1* weak and intermediate alleles have light-insensitive phenotypes (A.P. and J.C., unpublished data). Together, these results argue that DET1 and COP1 are global repressors that play a specific role in photomorphogenesis.

A Working Model for Light-Regulated Development of Plants

The genetic and molecular studies suggest a model for light-regulated seedling development of *Arabidopsis* (Fig. 3). In this model, the action of multiple photoreceptors is integrated through global repressors (*DET1*, *COP*, and *FUS*), which then act through specific regulators [e.g., *DET3* (41), *DOC1* (42), and *HY5* (17)] to repress gene expression and morphogenesis in dark-grown seedlings. When this repression is relieved, cell

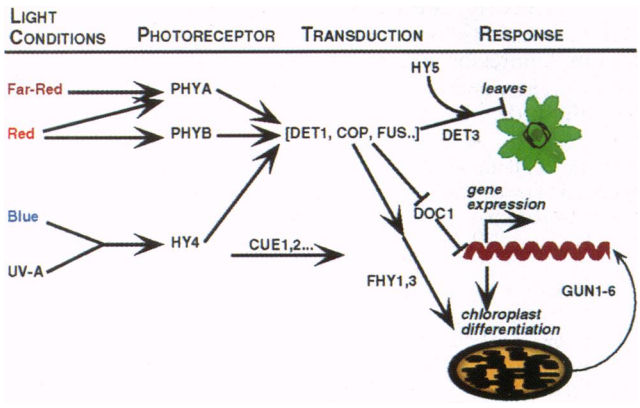

FIG. 3. Model of phototransduction pathways in *Arabidopsis* based on genetic studies. The phenotypes of double mutant lines suggest a hierarchical regulatory network among genes controlling the downstream light-regulated responses (inhibition of hypocotyl elongation and promotion of leaf expansion, chloroplast differentiation, and gene expression). The possible functions of the depicted genes are described in the text. The model is formal and makes no prediction as to the precise molecular nature of the proposed interactions among genes or gene products.

type-specific positive regulators [e.g., *CUE1* (43)] can act to induce gene expression and development. In addition, two loci, *FHY1* and *FHY3* (44), which are positive regulators of far-red light-regulated responses specifically, appear to act downstream of PHYA. Finally, the expression of light-regulated nuclear genes is also controlled by a retrograde pathway that involves signaling from the chloroplast to the nucleus (defined by six genes, *GUN1–GUN6*; ref. 45). Although this model is simplistic and does not address the actual mechanisms involved, it suggests a framework with which to address the mode of action and the interactions of the various gene products.

Hormones and Light Signal Transduction

Plant hormones can induce germination, bolting, flowering, gene expression, and other responses identical to those initiated by phytochrome. The overlapping role of light and plant hormones in development raises the interesting question of whether light and hormones act independently to affect development or whether plant hormones are involved in the sequence of events initiated by physiologically active photoreceptors. Considerable evidence is amassing that phytochrome and hormone metabolism or signal transduction are intimately entwined. For instance, in several plants, alterations in gibberellin metabolism or response can cause phenotypes that resemble the elongation and flowering phenotypes of *phyB* mutants (46, 47). Moreover, induction of flowering by long days in rosette plants has been correlated with increased gibberellin levels (5), and gibberellin-deficient *Arabidopsis* mutants fail to flower in short days (48). Conversely, an *Arabidopsis phyB* null mutant (49) and presumed *phyB* mutants of sorghum, pea, cucumber, and *Brassica* have altered gibberellin metabolism or an increased responsiveness to applied gibberellins (46, 47, 50, 51). Transgenic tobacco and tomato plants overexpressing phytochrome A have a dwarfed phenotype (52, 53). The tobacco lines contain lower levels of several gibberellins than the wild type implying that phytochrome A can inhibit gibberellin biosynthesis (54).

Phytochrome may also act through auxins to control stem elongation. Several studies suggest that phytochrome may regulate stem elongation rates by depleting auxin within the epidermis, which, in turn, could constrain the growth of the entire stem. Thus, the redistribution of auxins might be an additional important determinant in phytochrome-mediated growth suppression (55, 56).

Alterations in hormone metabolism or responsiveness can also alter the morphology of dark-grown seedlings (reviewed in ref. 3). Several auxin response mutants that are insensitive to high levels of applied auxins have short hypocotyls and unfolded cotyledons in the dark, reminiscent of *det*, *cop,* and *fus* mutants. The *Arabidopsis amp* mutant (for altered meristem program) has a short hypocotyl, produces leaves in the dark, and was shown to overproduce cytokinins by 6-fold over the wild type. In addition, applied cytokinin mimics the phenotypes of *Arabidopsis det* mutants, causing a de-etiolated morphology, development of chloroplasts, and expression of light-regulated genes in dark-grown wild-type *Arabidopsis* seedlings. Cytokinin levels are normal in several *det* mutants; however, these mutants show an increased responsiveness to cytokinins in cell culture or in a detached leaf senescence assay. Finally, the constitutive ethylene response mutant (*ctr*), as well as a number of ethylene overproducing mutants (*eto*), also have significant inhibition of hypocotyl elongation in the dark.

Very recently, the study of a subset of *Arabidopsis* de-etiolated mutants with a dwarf stature in the light has led to renewed interest in a class of plant steroids, called brassinosteroids (BRs; ref. 63), and implicated a role for brassinolide (the most active BR) in light-dependent development of plants (28, 29, 58). In the dark, these mutants are short, have thick

hypocotyls, accumulate anthocyanins, have open, expanded cotyledons, and develop primary leaf buds (29, 59). Furthermore, these morphological changes are accompanied by a 10- to 20-fold derepression of several light-responsive genes. In the light, the mutants are smaller and darker green than wild type, show reduced cell size in several tissues, have reduced apical dominance and male fertility, and have altered photoperiodic responses (59). The mutants defined by these traits include: *det2*, *cpd*, and several *dwf* (dwarf) and *cbb* (cabbage) lines. It now appears that *dwf1* is allelic to *dim* and *cbb1*, and that *cpd* is allelic to *cbb3*. *DET2* and *CPD* have been cloned and shown to encode a steroid 5α-reductase (28) and a cytochrome P450 monooxygenase (similar to steroid hydroxylases; ref. 29) respectively, suggesting a possible role for these genes in steroid biosynthesis. *DIM/DWF1/CBB1* has also been cloned (57). Although it was originally proposed that *DIM* might be a regulatory gene because it contained possible nuclear localization signals, more recently it was suggested that *DIM* encodes an oxidase based on a conserved motif (60). Moreover, the phenotypes of *det2*, *cpd/cbb3*, and *cbb1/dwf1/dim* can be suppressed by applied brassinolide, but not by other plant hormones, suggesting that these mutants do indeed function in the biosynthetic pathway of brassinolide (Fig. 4 *B* and *C*). Feeding experiments and biochemical quantitation suggest that *DET2* functions in the first committed step in the proposed biosynthetic pathway (refs. 28 and 61; Fig. 4*A*). The level of campestanol in *det2* null alleles is $\approx$10% of wild type (S. Fujioka, A. Sakurai, J.L., and J.C., unpublished results). This suggests that there is a second steroid 5α-reductase in *Arabidopsis* or an alternative pathway for the production of campestanol. *CPD* appears to act later in the pathway, in the conversion of cathasterone to teasterone (29). *DIM* may function after *CPD* in the formation of typhasterol (29). Taken together, these studies provide a function for brassinolide in *Arabidopsis* in repressing light- and stress-regulated gene expression and in promoting cell expansion, leaf senescence, and flowering. Thus, brassinolide is involved—either directly or indirectly—in light-regulated processes in *Arabidopsis*. In particular, one can now predict a role for brassinolide in the differential growth responses (inhibition of hypocotyl elongation and expansion of cotyledons and leaves) that result in response to light. One prediction is that light negatively regulates BR synthesis or responsiveness in the hypocotyl, while simultaneously promoting BR synthesis or responsiveness in leaf cells. These hypotheses can now be tested by quantifying BRs after different light treatments. Additionally, two BR-insensitive mutants of *Arabidopsis* (*bri* and *cbb2*) have been described (58, 62) and a previously described de-etiolated mutant, *det3*, also appears to be insensitive to exogenously added brassinolide (refs. 29 and 41; and J.L. and J.C., unpublished results). Cloning of these genes may lead to molecular information on how cells perceive and respond to BRs.

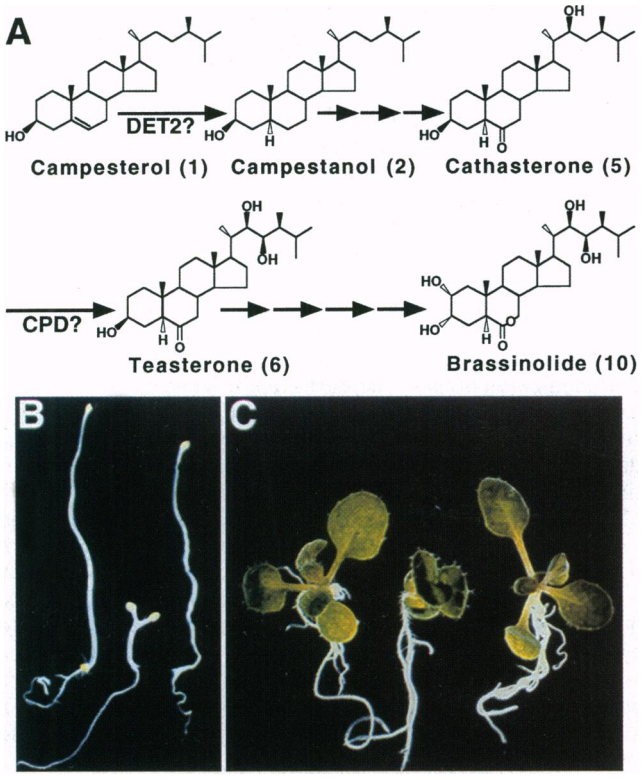

FIG. 4. (*A*) Proposed brassinolide biosynthetic pathway, depicting the biosynthetic steps at which DET2 and CPD act. (*B* and *C*) Rescue of *det2* phenotypes by brassinolide. Dark-grown 10-day-old seedlings (*B*) and light-grown 12-day-old (*C*) seedlings. From left to right in each panel are wild-type, *det2*, and brassinolide-treated *det2* plants. *B* and *C* are reprinted with permission from ref. 28. Copyright (1996) AAAS.

It has been proposed that *DET1* and *DET2* act downstream from multiple photoreceptors based on epistasis analysis with null photoreceptor mutations. Since *det1* and *det2* appear to have wild-type phytochrome spectral activity, these data are consistent with the order of gene action proposed from the genetic studies. Moreover, the genetic studies suggest that *DET1* and *DET2* act on separate pathways. Szekeres *et al.* (29) recently questioned the validity of placing *DET1* and *DET2* on independent pathways. Instead they suggested that *det1*, as well as *axr2* and a number of *cop/fus* mutants, are involved in BR synthesis or perception because they observed an increase in hypocotyl elongation in these dark-grown plants following addition of 10^{-6} M brassinolide. However, our results comparing the response of dark-grown *det1* and *axr2* with light-grown wild-type seedlings to increasing doses of brassinolide clearly show that *det1* and *axr2* are not brassinolide mutants, but merely respond to applied brassinolide in a manner analogous to the wild type (ref. 28; J.L. and J.C., unpublished results). Thus, a subset of hypocotyl elongation mutants may be affected in BR responses, and there are likely to be other developmental pathways that are regulated by light. *DET1* may define one such pathway.

Conclusions and Perspectives

The importance of light in plant development cannot be overestimated. However, much still needs to be learned about the intermediate steps between light reception, hormone action, and physiological responses. In the future, a combination of suppressor screens, the determination of function of cloned genes, and the isolation of interacting proteins should help fill in the sizable gaps in our knowledge.

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