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Signal-transduction pathways controlling light-regulated development in *Arabidopsis*

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SUMMARY

All metazoan cells are able to make decisions about cell division or cellular differentiation based, in part, on environmental cues. Accordingly, cells express receptor systems that allow them to detect the presence of hormones, growth factors and other signals that manipulate the regulatory processes of the cell. In plants, an unusual signal – light – is required for the induction and regulation of many developmental processes. Past physiological and molecular studies have revealed the variety and complexity of plant responses to light but until recently very little was known about the mechanisms of those responses. Two major breakthroughs have allowed the identification of some photoreceptor signalling intermediates: the identification of photoreceptor and signal transduction mutants in *Arabidopsis*, and the development of single-cell microinjection assays in which outcomes of photoreceptor signalling can be visualized. Here, we review recent genetic advances which support the notion that light responses are not simply endpoints of linear signal transduction pathways, but are the result of the integration of a variety of input signals through a complex network of interacting signalling components.

1. INTRODUCTION

All cells possess the capacity to receive and process information from their surroundings. Because they are fixed in space, plants – more than most organisms – need to be especially plastic and flexible in response to external stimuli. Numerous environmental factors influence plant development, including temperature, light, touch, water and gravity. Of these factors, light has an especially important role, affecting almost every stage of the plant life cycle, from germination through floral induction (Mullet 1988; Chory 1991). Light has particularly dramatic effects on the morphogenesis of seedlings, which become etiolated in the absence of light, displaying elongated hypocotyls, small folded cotyledons and undeveloped chloroplasts called etioplasts (Mullet 1988; Gruissem 1989; Chory 1991). Conversely, light inhibits hypocotyl elongation and induces leaf expansion and differentiation, chloroplast development (a process called de-etiolation), as well as the expression of several 'light'-regulated nuclear genes, including the genes encoding the light-harvesting chlorophyll a/b binding proteins (*cab*), the small subunit of the RuBP carboxylase/oxygenase (*rbcS*), and chalcone synthase (*chs*) (Gilmartin *et al.* 1990; Li *et al.* 1993). Because these genes are activated in cell-type specific patterns in light-grown plants, cell-specific factors and light must work in concert to regulate their expression. In addition to light, several plant growth regulators, including cytokinins and gibberellins, have been implicated in de-etiolation responses. How light might interact with these hormone signal-transduction pathways is not understood (Stetler & Laetsch 1965; Harvey *et al.* 1974; Flores & Tobin 1986; Mathes *et al.* 1989; Bartholomew *et al.* 1991; Chory *et al.* 1994). Finally, light modulates the circadian clock to control endogenous rhythms that help the plant to measure daylength (Millar *et al.* 1995). Thus, given the complexity of the input signals and the diverse array of developmental events regulated by light, it seems likely that light responses result from integration of a variety of signals through a complex network of interacting signalling components (see figure 1).

2. LIGHT PERCEPTION

Several classes of photoreceptors mediate light responses, including protochlorophyllide, blue- and UV-light-absorbing receptors, and phytochrome, which is the most intensively studied photoreceptor (Gallagher *et al.* 1988; Chory 1991; Quail 1991; Ahmad & Cashmore 1993; Terry *et al.* 1993). This soluble chromoprotein exists as a dimer of two 120 kDa polypeptides, each with a covalently attached linear tetrapyrrole chromophore which is responsible for visible light absorption. The phytochrome polypeptide

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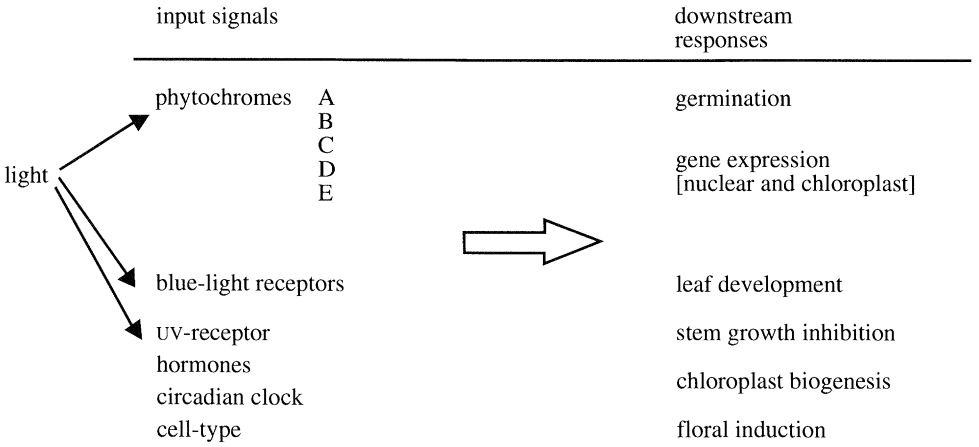

Figure 1. Diagram indicating the diversity of input signals and downstream responses regulated by light. Light affects plant development through the integrated action of multiple photoreceptor systems acting in the context of other input signals, for example cytokinins, ethylene, abscisic acid and gibberellins. Downstream photoregulated responses are also complex, occuring in the same cell as the stimulus (gene expression and chloroplast development), or at the level of entire organs (stem growth inhibition, germination, leaf development), or even the entire organism (floral induction).

folds into two major domains separated by a protease-susceptible hinge region: an approximately 70 kDa N-terminal chromophore-bearing domain and an approximately 55 kDa C-terminal domain. Native phytochrome exists as a dimer in solution. The dimerization site resides on the C-terminal domain and thus the molecule is visualized as an apparent tripartite structure by electron microscopy (Jones & Edgerton 1994). The pigment-protein can undergo a reversible photo-induced conversion between two spectrally and biochemically distinct forms, a red-absorbing form, Pr (λmax = 660 nm), and a far-red-absorbing form, Pfr (λmax = 730 nm). The mode of action of phytochrome is best characterized for dark-grown seedlings that are irradiated with low fluences of red light, which converts Pr to Pfr and concomitantly causes the induction of several responses, including the transcriptional activation of nuclear genes. As these responses can be canceled by a pulse of far-red light, which photoconverts Pfr back to Pr, it is generally believed that Pfr is the active form of phytochrome.

Photoconversion of Pr to Pfr is associated with an isomerization of the tetrapyrrole, which apparently causes a conformational change of the apoprotein (reviewed in Terry *et al.* 1993). An obvious and long held hypothesis is that these conformational changes result in differential interactions with subsequent component(s) of the signal transduction chain(s) linking phytochrome to physiological responses. What these subsequent component(s) are remains unknown. Recent microinjection experiments have suggested that photoconversion of phytochrome leads to the activation of heterotrimeric G-protein(s), which in turn activates three subsequent pathways: one involving cGMP; one involving Ca^{2+}/calmodulin; and one requiring both cGMP and Ca^{2+}/calmodulin (Neuhaus *et al.* 1993; Bowler *et al.* 1994*a*). There is considerable crosstalk between the various branched pathways (Bowler *et al.* 1994*b*). Because G-protein activation is the earliest event in the proposed pathway, heterotrimeric G-proteins are presently the best candidates for a phytochrome-interacting component. It is important to note, however, that phytochrome is localized in the cytoplasm and that G-proteins, which are membrane-associated, are normally coupled to transmembrane receptors. This suggests the requirement for additional (perhaps novel) components to couple phytochrome photoconversion to G-protein regulated pathways. These experiments are clearly an important contribution to our understanding of phytochrome signalling, however, the approach itself is limited by the fact that it relies on the known activity of pharmacological compounds defined in animal systems. Thus any novel mechanisms of phytochrome signalling might not be revealed from these studies.

Another complicating problem in phytochrome research is the presence *in vivo* of at least two pools of phytochromes, which can be distinguished by physiological, spectrophotometric, and immunochemical studies (Quail 1991; Terry *et al.* 1993). One pool, the light-labile type I, which has been purified and extensively characterized, predominates in etiolated tissues, whereas type II phytochrome is light-stable and present at approximately the same, relatively low levels in light-grown and etiolated tissues. The molecular basis for this heterogeneity of phytochromes has been revealed through the cloning of the structural genes, which turned out to constitute a family that comprises at least five different genes, named *PHYA* through *PHYE*, in *Arabidopsis* (Sharrock & Quail 1989; Clack *et al.* 1994). Preliminary characterization of the protein products of the first three demonstrated that *PHYA* encodes a light-labile phytochrome whereas *PHYB* and *PHYC* encode light-stable phytochromes. The finding of multiple phytochrome genes immediately suggested that individual phytochromes might have specific physiological roles, which was recently confirmed by genetic studies.

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3. PHOTORECEPTOR MUTANTS

Mutations in photoreceptor genes, as well as in downstream components of the light-signal transduction pathway, have been isolated in screens for mutants defective in the light-inhibited hypocotyl elongation of young *Arabidopsis* seedlings (Chory 1993). Screens under different light conditions have identified mutations in the gene for phytochrome A (*phyA*) (Dehesh *et al.* 1993; Nagatani *et al.* 1993; Whitelam *et al.* 1993), in the gene for phytochrome B (*phyB*, previously called *hy3*) (Koornneef *et al.* 1980; Reed *et al.* 1993), in loci affecting chromophore synthesis or availability (*hy1*, *hy2*, *hy6*) (Koornneef *et al.* 1980; Chory *et al.* 1989*a*; Parks *et al.* 1989; Parks & Quail 1991), and in a gene encoding a putative blue light receptor (*hy4*) (Koornneef *et al.* 1980; Ahmad & Cashmore 1993). In addition, mutations in putative signal transduction components, acting downstream of phytochrome A (*fhy1*, *fhy2*) have been isolated (Whitelam *et al.* 1993). The *hy5* mutation was identified in white light screens, but appears to affect responses to light of red, blue or UV frequencies, and therefore affects either a step downstream of the different photoreceptors, or a separate input pathway controlled by some other type of signal (Koornneef *et al.* 1980; Chory 1992).

Isolation and characterization of these mutants – by ourselves and others – has helped to unravel some of the complexity of light responses. First, the mutants have confirmed that there are indeed photoreceptors specific to blue and UV light, which are distinct from the phytochromes. Second, characterization of the different phytochrome mutants has allowed for the dissection of red/far-red responses. For example, the *phyA* mutants show their most drastic physiological defects – poor germination, elongated hypocotyl and failure to expand cotyledons – when grown under far-red light as opposed to white or red light (Reed *et al.* 1994). In contrast, the *phyB* mutants germinate poorly in the dark or in response to red light, have elongated hypocotyls in red or white light, and are generally elongated in several different tissues as adults (Reed *et al.* 1993, 1994; Shinomura *et al.* 1994;). In far-red light, they grow essentially as the wild type.

The simplest conclusion from these experiments is that PHYA and PHYB control similar responses, but that PHYA senses far-red light, whereas PHYB senses red light. However, our analysis of the phenotypes of a *phyA phyB* double mutant suggests that the functions of these two phytochromes are not entirely separate because the *phyA phyB* double mutant has a more severe deficiency in de-etiolation under red light than does the *phyB* single mutant (the *phyA* single mutant appears normal under red light and elongated in far-red light) (Reed *et al.* 1994). The deficiencies observed included: a reduction in *cab* mRNA accumulation by a pulse of red light in the double mutant (but not in either single mutant), a reduction in the potentiation of chlorophyll accumulation by pulses of red light, and poorly developed cotyledons and severely elongated hypocotyls in continuous red light (Reed *et al.* 1994). These results indicate that PHYA and PHYB act together to promote de-etiolation in response to red light. Thus for these responses, the two phytochromes appear to be partly redundant, and absorption of light by either phytochrome may activate a common signal transduction pathway. The relative importance of PHYA and PHYB in activating that pathway will depend on the ambient light conditions.

The phytochrome mutants have also helped uncover a role for the Pr form of phytochrome in the control of germination and shoot gravitropism. Liscum & Hangarter (1993) have shown that wild-type seedlings grow less upright in red light than in the dark. The increased gravitropism in the dark is dependent on phyB activity, indicating that the Pr form of PHYB must promote correct gravitropism. This conclusion is supported by the finding that *hy2* mutant seedlings, in which PHYB is presumably 'locked' in the Pr form because of a chromophore deficiency, grow upright in both red light and the dark. That the Pr form of PHYB has physiological activity, is further supported by our observation that *phyB* mutant seeds germinate in far-red light better than wild-type seeds do, indicating that the Pr form of PHYB must inhibit germination under these conditions, whereas the Pfr form of PHYB promotes germination, as described above (Reed *et al.* 1994; Shinomura *et al.* 1994). Lastly, both *phyA* and *phyB* mutations affect flowering time. In contrast to wild-type, flowering of *phyA* mutants is not accelerated by a light break in the middle of the night, indicating that PHYA promotes flowering (Reed *et al.* 1994). Conversely, *phyB* mutants flower early in both long and short day cycles, suggesting that PHYB normally plays a role in inhibiting flowering in *Arabidopsis* (Goto *et al.* 1991; Reed *et al.* 1993).

Taken together, these results indicate that both the Pr and Pfr forms of PHYB have activities, and that these activities may act in opposing directions. This conclusion is consistent with the notion that PHYB signals developmental responses after sensing the ratio of incident red and far-red light. PHYB then, and perhaps other light-stable phytochromes, may transmit information about its Pr/Pfr steady state down the signal transduction chain, and the opposing activities of the Pr and Pfr forms make this transmission more sensitive. In contrast, there is to date no evidence for activity of the Pr form of the light-labile phytochrome A. Rather than acting to sense the ratio of red and far-red light, PHYA appears to function mainly as a switch, recognizing the onset of light before de-etiolation (and perhaps at dawn, to regulate flowering), and doing so most effectively when the proportion of far-red light is very high.

4. SIGNAL-TRANSDUCTION PATHWAY MUTANTS

Originally, mutations that affect the entire morphogenetic programme of young seedlings in the dark were isolated by ourselves; this work was followed by subsequent similar screens in other laboratories. Recessive mutations in any one of a number of *det* (de-etiolated) (Chory *et al.* 1989*b*; 1991; Chory & Peto

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1990; Chory 1992; Cabrera y Poch *et al.* 1993), *cop* (constitutively photomorphogenic) (Deng *et al.* 1991; Wei & Deng 1992; Hou *et al.* 1993; Wei *et al.* 1994*b*) or *fus* (fusca) (Castle & Meinke 1994; Miséra *et al.* 1994) genes cause seedlings to exhibit many phenotypic characteristics of light-grown plants even when grown in complete darkness, including changes in gene expression, morphology, and plastid state. Not surprisingly, phenotypes of double mutant plants carrying a mutation of the *det/cop/fus* class and one of the long hypocotyl mutations indicate that the *DET/COP/FUS* genes lie downstream of known photoreceptors (Chory 1992; Ang & Deng 1994; Miséra *et al.* 1994). The simplest model that explains the existence of *det* and *cop* type mutants is that their gene products are negative regulators which couple light signals to the downstream light-regulated programme 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 (Chory *et al.* 1989*b*).

Four loci from the *DET/COP/FUS* class have been cloned, including *COP1* (Deng *et al.* 1992), *DET1* (Pepper *et al.* 1994), *COP9* (Wei *et al.* 1994*a*), and *FUS6* (Castle & Meinke 1994). The deduced COP1 protein sequence contains a Zn-binding motif, a coiled-coil motif, and a series of WD-40 repeats found in β subunits of trimeric G-proteins and in a number of transcription factors. The C-terminal portion of COP1 has homology to $TAF_{II}80$ (Dynlach *et al.* 1993), a subunit of the complex of proteins associated with TFIID in *Drosophila*, suggesting that COP1 might have a role in general control of transcription. However, the lack of homology at the N-terminus indicates that the two proteins have different functions. A recent study reported that when COP1 is fused to a reporter gene, the fusion protein remains cytoplasmic in the light, while it becomes localized to the nucleus in root cells or dark-grown hypocotyl cells. The authors suggest that COP1 acts as a repressor in the nucleus of dark-grown plants, and it is translocated from the nucleus or degraded in light-grown plants, thus relieving the negative activity of COP1 on light-regulated processes (von Arnim & Deng 1994). However, these results are confusing in that they do not explain the observations that *cop1* mutations are lethal in light-grown *Arabidopsis* seedlings. Thus COP1 must play a role in light-grown seedlings as well as dark-grown seedlings, presumably by functioning in the nucleus. Additional studies to elucidate the role of COP1 in light-grown plants will clarify these apparent contradictory results. 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, DET1 appears to localize to the nucleus (Pepper *et al.* 1994). Analysis of a number of *det1* mutants suggests that DET1 plays a role in both light and dark-grown *Arabidopsis* plants. In darkness, DET1 acts to repress the de-etiolation programme, while in the light, DET1 acts as a spatial repressor of light-regulated gene expression and chloroplast development (Chory & Peto 1990; Pepper *et al.* 1994). Null alleles are seedling lethal, suggesting that there is a derepression of expression of other (still unknown) developmental genes. The prediction is that DET1 is a global transcriptional repressor, affecting the expression of many genes, of which the light-regulated genes are a subset that respond sensitively to the levels of active DET1 (Pepper *et al.* 1994). *FUS6* and *COP9* also encode novel proteins. Though no information is available on the subcellular localization of the gene products, COP9 appears to be part of a large molecular mass complex whose formation requires FUS6 (Wei *et al.* 1994*a*). To date, we do not understand how any of these gene products control morphogenesis, nor do they explain the mechanism by which the photoreceptors act.

To obtain a complete picture of the complexities of the phototransduction pathways, it will be necessary to define loci which when mutated result in defects only in a subset of light-regulated responses. To this end, we have identified a number of new loci that are affected in particular light responses without having the pleiotropic effects of the *det1/cop1* class. These include *det3* (Cabrera y Poch *et al.* 1993) as well as a number of mutants identified in screens for altered expression of genes that are induced by light (Li *et al.* 1994). The *det3* mutant has a short hypocotyl and makes leaves in the dark, but does not express light-regulated genes or show chloroplast differentiation. Conversely, other mutants have normal dark morphology, but altered gene expression. The *doc* (dark overexpression of *cab*) mutants express *cab* genes at three- to eightfold higher levels than the wild type, while maintaining normal etiolated morphology, suggesting that they lack a function downstream of the more global regulators identified by mutants of the *det* class (Li *et al.* 1994). Consistent with this hypothesis, a *det1–1 doc1* double mutant expresses *cab* genes at about the same level in the dark as the *det1* single mutant. In contrast, a *det1–1 doc2* double mutant expresses *cab* at a higher level in the dark than either single mutant, suggesting that – if *doc2–1* is a null mutation – *DOC2* and *DET1* may fall in separate pathways.

Only three positively acting signal transduction candidates have been identified in the mutational analyses performed to date: *hy5*, *fhy1*, and *fhy3*. To understand each positively acting component of the signal transduction network from the photoreceptors, we devised a negative selection for mutants that do not express a *cab* promoter at high levels in the light. This screen has identified mutations in at least eleven distinct loci, including the photoreceptor-encoding loci, *PHYB* and *HY1* (E. Lopez, H. m. Li & J. Chory, unpublished data). These results suggest that this screen should uncover all the positively acting loci that affect *cab* gene expression, including those that act downstream from phytochrome. Mutations in one of these genes, *CUE1* (for cab-underexpressed), have been most intensively studied. *cue1* mutations cause a reduction of *cab* and *rbcS* expression levels in the light by about 90%, with no effects on dark-expression levels of these genes (Li *et al.* 1995). In contrast, the light-regulated expression of *chs*, an epidermal cell-specific expressed gene, is not affected. Moreover, the *cab* and *rbcS*

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mRNAs are not induced by either red or blue light pulses, suggesting that, as with the *det* and *doc* mutants, *CUE1* may act downstream in the phototransduction pathways after the action of multiple photoreceptors has already been integrated (Li *et al.* 1995). Together these results suggest that CUE1 is a mesophyll-cell-specific postively acting component of the light signalling pathways in *Arabidopsis*.

5. OTHER INPUT SIGNALS REGULATING SEEDLING DEVELOPMENT

A complication in the analysis of phytochrome signal transduction exists in the plethora of literature documenting that light and hormones cause similar effects in developing plants. For instance, control of germination has been shown to involve gibberellins, cytokinin, and abscisic acid in various species (Moore 1979; Jacobsen & Chandler 1987). Gibberellins, auxins and ethylene are each involved in the control of cell elongation and morphological responses required for seedling emergence from the soil (Evans 1985; Potts *et al.* 1985; Abeles *et al.* 1992; Kieber *et al.* 1993). Cytokinins promote cotyledon expansion, leaf development and chloroplast differentiation (Miller 1956; Stetler & Laetsch 1965; Huff & Ross 1975). During later vegetative growth, cytokinins and ethylene control the onset of leaf senescence (Leopold & Kawase 1964; Gepstein & Thimann 1981). Finally the ratio of cytokinins to auxins is a primary determinant in the control of apical dominance (Moore 1979). The overlapping roles of light and plant hormones in development raises the interesting question of whether light and hormones act independently to affect developmental responses or whether plant hormones are involved in the sequence of events initiated by physiologically active photoreceptors. The answer to this question remains elusive, but may emerge from genetic studies.

Hormone mutants have indicated that hormone metabolism alters development of dark-grown seedlings. For example, the *amp* (altered meristem programme) mutant produces six times as much cytokinin as the wild type, has a short hypocotyl and makes leaf-like structures in the dark (Chaudhury *et al.* 1993). Several auxin resistant mutants likewise have distinct phenotypes in the dark (Lincoln *et al.* 1990). These results suggest that lowering the effective auxin: cytokinin ratio can induce de-etiolation in dark-grown plants, and that light might act through changes in auxin or cytokinin metabolism. In fact, treatment of dark-grown wild-type *Arabidopsis* seedlings with cytokinin causes a de-etiolated morphology and expression of genes normally induced by light (Chory *et al.* 1994). Cytokinin levels are normal in *det1* and *det2* seedlings, but these mutants have an altered responsivity to exogenously added cytokinins (Chory *et al.* 1994). Ethylene also plays a role in the morphology of dark-grown seedlings. For example the *ctr* mutant which displays a constitutive ethylene signalling response has a short hypocotyl in the dark, presumably because in the dark ethylene signals supersede light control of hypocotyl elongation (Kieber *et al.* 1993). Lastly, abscisic acid can cause decreases in the accumulation of photoregulated mRNAs (Bartholomew *et al.* 1991).

6. CONCLUSIONS AND PERSPECTIVES

The future goals of phytochrome research are to identify and characterize each component of the signalling pathway(s) and to understand the mechanisms by which light controls gene expression, organ differentiation and floral induction. To date, mutational analyses have indicated that greater than 40 loci are involved in light signalling in *Arabidopsis*, but this is likely to be an underestimation because most of the genetic screens have not been done to saturation. Immediate goals include cloning the loci involved, analysing the function of the cloned gene products, and identifying relevant protein-protein interactions. In the long term, understanding the relation of the phototransduction pathways to other endogenous developmental programmes, such as those initiated by the growth regulators, will aid in generating a broader picture of signal transduction in plants.

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