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Introduction

As photoautotrophs, plants are exquisitely sensitive to their light environment. Light affects many developmental and physiological responses throughout plants' life histories, including germination (Seed Dormancy and Germination doi/10.1199/tab.0050), flowering (Flowering doi/10.1199/tab.0055), and direction of growth (Phototropism, Mechanisms and Outcomes doi/10.1199/tab.0042). In *Arabidopsis*, there are four major classes of photoreceptors: the phytochromes acting predominantly in red/far-red wavelengths (Phytochrome Signaling Mechanisms doi/10.1199/tab.0074), the cryptochromes responding in blue and UVA (reviewed in Lin, 2000), the phototropins responding in blue (Phototropism, Mechanisms and Outcomes doi/10.1199/tab.0042), and UVB photoreceptors yet to be extensively characterized. The phytochromes are encoded by five related genes, called *PHYA-E*. The cryptochromes are encoded by *CRY1* and *CRY2*. *PHOT1* and *PHOT2* (formerly known as *NPH1* and *NPL1*, respectively) are the two characterized phototropins.

The focus of this chapter will be on the crucial period of time between seed germination and the development of the first true leaves. During this time, the seedling must determine the appropriate mode of action to best achieve photosynthetic and eventual reproductive success. If light is limiting, the seedling will exhibit etiolated growth —a developmentally arrested growth mode characterized by an elongated hypocotyl topped by tightly-closed, underdeveloped cotyledons and a limited root system. In contrast, *Arabidopsis* seedlings grown in continuous bright white light have thick, short hypocotyls, broad, open cotyledons, and an elaborated root system (Fig. 1A). These seedlings also show accelerated production of true leaves, and are relatively quick to flower. There is a dizzying array of inputs determining where along this growth spectrum a given plant will be found, including the quality, quantity, duration, and intensity of light, as well as genetic factors. It is perhaps not surprising that such a complex web of regulation controls photomorphogenesis, because in this brief window of time, a plant matures from an endosperm-dependent embryo to a self-sufficient photoautotroph. Correct assessment of the environment is quite literally a matter of life and death. Moreover, perfect coordination of growth response across the entire plant is essential to avoid disruption of the plant body. The following sections will focus on our current understanding of interactions between input pathways and describe possible mechanisms for integration of internal and external environmental signals into a discrete growth response.

THE LIGHT ENVIRONMENT

The initial period of *Arabidopsis* seedling growth can be divided into three major stages (Fig. 1B; reviewed in Casal et al., 1998; Maloof et al., 2000; Neff et al., 2000). The first is germination. Factors including availability of water and temperature, in addition to light, play a large role in determining the timing of seedling emergence (Seed Dormancy and Germination doi/10.1199/tab.0050). The next phase of growth involves assessing photosynthetic opportunity. If the seedling is buried, thereby receiving very little light, hypocotyl elongation and repression of cotyledon development are achieved via the very low irradiance response (VLFR), primarily under the control of PHYA. Once the

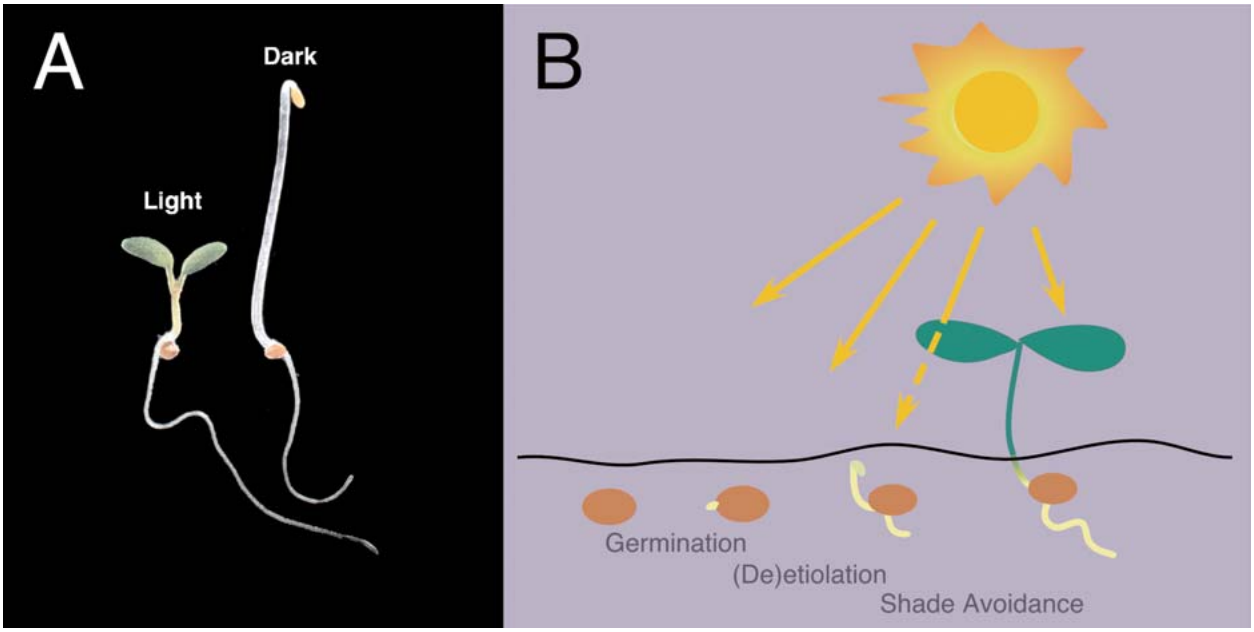

Figure 1. A. Arabidopsis seedlings grown in light (deetiolated) or dark (etiolated). B. Different aspects of light detection are experienced by seedlings in their initial phase of growth.

seedling emerges into the light, PHYA is rapidly degraded, and the effects of PHYB and the cryptochromes begin to dominate. In these conditions, cotyledons unfold and expand, and the photosynthetic apparatus differentiates. At this time, the third phase of light sensing becomes apparent. Neighbor detection, or shade avoidance, involves assessment of both the red/far-red ratio reaching the seedling and the direction of illumination. Overhanging vegetation selectively removes red and blue light, while allowing far-red light through. A shift of the red/far-red ratio from 1.2 to 0.8 can radically alter the growth habit of the seedling (Casal et al., 1998). High red/far-red ratios severely inhibit hypocotyl elongation, and conversely, low red/far-red ratios promote elongation. In low red/far-red conditions, both the PHYB-mediated low fluence response (LFR) and the PHYA-mediated high irradiance response (HIR) are acting in opposition to regulate the growth of the plant. PHOT1 detects asymmetric light on the hypocotyl caused by near neighbors or other obstacles and likely alters the distribution of auxin in the hypocotyl, resulting in phototropic bending (Christie et al., 1998; Friml et al., 2002).

The adaptive value of a shade avoidance response is somewhat cryptic in a rosette plant like *Arabidopsis*. However, studies using dense canopy shade overgrowing *Arabidopsis* seedlings clearly demonstrate a vital role for PHYA-mediated HIR (Yanovsky et al., 1995). *phyA* mutant seedlings in heavy shade will elongate until they die, never making true leaves. This finding suggests that the HIR may act as a 'back-up' deetiolation system. In effect, HIR forces the plant into the photomorphogenetic program in suboptimal environments when additional hypocotyl growth promoted by shade is insufficient for seedling emergence into bright light. As *Arabidopsis* seeds are quite small, seedlings must balance the need for beneficial light environments with limited food stores. Studies to determine fitness benefits and costs associated with various modes of hypocotyl elongation responses in various *Arabidopsis* accessions are currently underway (Dorn et al., 2000).

INTERACTIONS BETWEEN THE PHOTORECEPTORS DETERMINE GROWTH RESPONSE

Of course most plants grow in a light environment composed of a mixture of light qualities and quantities, simul-

taneously activating several photoreceptors. The signals from these receptors appear to be integrated by a wealth of shared downstream components and by direct interactions between at least a subset of the photoreceptors themselves. Phototropic bending of the hypocotyl is one well-characterized example of cross-talk between photoreceptors. While the major effects of this asymmetric growth occurs through phototropin-mediated blue light perception, it has long been known that there is a red/far-red reversible modulation of this response (Stowe-Evans et al., 2001). Recent studies have shown that this red light enhancement of phototropic curvature is mediated by PHYA, in a thus far unique example of a PHYA low fluence, far-red-reversible response (Stowe-Evans et al., 2001). Cryptochromes interact genetically with multiple phytochromes (Neff et al., 2000). PHYB and CRY2 have been shown to tightly co-localize in vivo (Mas et al., 2000), and PHYA can phosphorylate CRY1 and CRY2 in vitro (Ahmad et al., 1998).

Recent microarray studies performed in far-red light suggest a rapid, massive change in gene expression in response to light treatment, with a particularly large effect on transcription factors (Tepperman et al., 2001). Such a complex response is likely observed in all light conditions and may be mediated by different classes of photoreceptor-interacting proteins. As is described in detail in Phytochrome Signaling Mechanisms doi/10.1199/tab.0074 (and reviewed in Schwechheimer and Deng, 2001), much of the light response is channeled through the DET/COP/FUS repressors of deetiolation. COP1 shuttles between the nucleus and cytosol in a light-dependent manner and represses deetiolation at least in part by targeting the nuclear-localized, light-response-promoting transcription factor HY5 for degradation in the dark. This degradation is likely mediated by the action of the COP9 complex or COP9 signalosome (hereafter called CSN). COP1, HY5, and the CSN act downstream of PHYA, PHYB, CRY1, and CRY2. Mutations in the gene encoding SUB1, a Ca^{+2} binding protein, cause hypersensitivity to both blue and far-red light (Guo et al., 2001). SUB1 likely acts as a point of integration between cryptochromes and phytochromes by negatively regulating light-induced accumulation of HY5. Somewhat surprisingly, COP1 has recently been shown to directly interact with CRY1, and possibly also with CRY2 and PHYB (Wang et al., 2001; Yang et al., 2001). DET1, a nuclear protein with likely functions in chromatin remodeling, is also downstream of all of the major photoreceptors (D. Schroeder and J.C., unpublished results and Reed and Chory, 1994).

In addition to the positive coordination of the light response signals, there is also evidence of negative interactions, particularly between PHYA and PHYB. Recent studies have genetically separated the VLFR and HIR responses, attributed predominately to PHYA function (Hennig et al., 2001; Luccioni et al., 2002). Several lines of evidence suggest that VLFR acts antagonistically with the PHYB pathway, while HIR interactions with PHYB are synergistic. This makes some degree of sense, as the naturally occurring situations likely to correspond to these two fluence conditions promote largely etiolated growth in the case of VLFR and deetiolated growth in the case of HIR. On the other hand, overexpression of PHYB decreases the inhibition of hypocotyl growth in far-red light, implying that PHYB is interfering with PHYA function (Hennig et al., 2001). This complex molecular interaction may partially explain the continuous spectrum of growth habits observed by providing a highly sensitized detection of subtle changes in absolute amount of light, as well as degrees of shading. Indeed, careful analysis of the earliest stages of light perception support a model where many photoreceptors contribute to the light response within defined windows of time and light environment (Parks et al., 2001).

OTHER FACTORS IMPINGE ON THE LIGHT RESPONSE

Photoreceptor response is also mediated by the circadian clock (The Arabidopsis Circadian Clock doi/10.1199/tab.0044). In *Arabidopsis*, circadian rhythmicity in hypocotyl growth has been well-documented (Dowson-Day and Millar, 1999; http://www.bio.warwick.ac.uk/millar/video.html). Hypocotyls elongate primarily in the dark, with the slowest growth occurring at subjective dawn, and the fastest growth at subjective dusk (Dowson-Day and Millar, 1999). This rhythmicity is apparent immediately upon germination and is coincident with the cycle of cotyledon raising and lowering. Mutants in clock components have altered cycles of hypocotyl growth resulting in hypocotyl length phenotypes (Dowson-Day and Millar, 1999). In *toc1*, for example, a shortened circadian period is observed throughout the plant and results in a reduction in the overall length of the hypocotyl. Recent evidence suggests that this may result in part from clock-gating of photoreceptor function (Reed et al., 2000). For instance, nuclear localization of PHYB appears to follow a circadian fluctuation even after plants are shifted to complete dark or continuous light (Nagy, 2001). Interestingly, although single mutants in *phyA*, *phyB*, *cry1*, and *cry2* can alter circadian period in specific light environments, a quadruple mutant maintains normal rhythmicity in white light, despite a nearly completely etiolated phenotype (Somers, 1999; Yanovsky et al., 2000). This finding suggests that many photoreceptors

may contribute to a robust clock-setting mechanism.

Chloroplast development and photomorphogenesis are unsurprisingly tightly linked (Chloroplast Development doi/10.1199/tab.0068). Regulation of the light response by carbohydrates has been extensively studied. Exposure of the aerial part of seedlings to exogenous sucrose leads to a variety of aberrant light responses in dark-grown plants (Roldan et al., 1999). Microarray studies also suggest that several key regulatory elements within the sucrose/starch pathway exhibit circadian fluctuations (Harmer et al., 2000), and, as might be expected, a large number of genes are regulated by the presence or absence of sucrose (M.Chen and J.C., unpublished results). In addition, the presence of metabolizable carbohydrates in the media strongly enhances PHYB's inhibitory effects on PHYA (Short, 1999). In many ways, the regulatory effects of sugar may be analogous to the 'gating' regulation of the light response by the circadian clock. Levels of sucrose within the plant cell may modulate the ability of the photoreceptors to respond to the light cue. It is worth noting, however, that the lab conditions under which many of these studies were performed may never be observed in nature. As the presence of exogenous sucrose is rare in natural settings and the wavelengths of light required to drive photosynthesis are quite similar to the range absorbed by the photoreceptors, it is likely that in most cases endogenous sucrose levels are largely correlated with the intensity of the photoreceptor-mediated light response.

HORMONES AS TRANSDUCERS OF THE LIGHT SIGNAL

Genetic and physiological studies have provided insight into the complexity of the photomorphogenesis decision-making process. While a relatively clear picture exists defining which photoreceptors act in different light regimes, the picture is decidedly more muddy as the signal moves downstream towards the eventual cell mechanics of expansion, division, and differentiation. Hormones of virtually every persuasion have been implicated in this growth, with cytokinin promoting photomorphogenesis, and auxin, brassinosteroids (BRs), and gibberellins (GAs) acting in opposition. Abscisic acid (ABA) acts in opposition to GAs and BRs in some contexts, yet the ABA response also appears necessary to maintain etiolated growth. Analysis of ethylene response mutants suggests that ethylene can act either to promote or inhibit photomorphogenetic growth in a tissue and environment-dependent manner.

The following sections cover each hormone in turn, highlighting components identified in studies of one hormone and subsequently shown to act in at least two distinct signaling pathways during seedling photomorphogenesis. Figure 2 attempts to illustrate the complexity of this cross-talk with each input pathway having its own color, described in the section headings below. The light response is shown in red. The large number of connections depicted in the figure will be built up one by one, starting with auxin, where perhaps the most elaborated case for cross-talk between light and hormones can be seen. In fact, in this area of the 'signaling map', some components cannot be unambiguously assigned to either light or auxin pathways and so are depicted as belonging to both (i.e., BIG). In other cases where the exact nature of the signaling component is not known, it is depicted associated with the pathway where it was originally identified. The overview of each hormone is by necessity highly abbreviated and readers are encouraged to refer to Auxin (doi/10.1199/tab.0057), ABA (doi/10.1199/tab.0058), Cytokinins (doi/10.1199/tab.0063), Ethylene (doi/10.1199/tab.0071), and Brassinosteroids (doi/10.1199/tab.0009) for a more complete review of the current understanding of each of these fields.

Auxin (dark blue)

Several lines of evidence indicate that auxin plays a major role in promoting hypocotyl elongation and acts as a primary target for the photoreceptors' signal to inhibit this growth (Tian and Reed, 2001). Auxin response appears to be regulated at four distinct levels: biosynthesis, metabolism, transport and response (Auxin doi/10.1199/tab.0057). Light has been shown to affect both auxin transport and response. Polar auxin transport (PAT) is studied primarily through the use of inhibitors. These inhibitors reveal that PAT is not required for hypocotyl elongation in the dark and that PAT is light quality dependent (Jensen et al., 1998). Mutants in various photoreceptors show reduced response to PAT inhibitors (Jensen et al., 1998). Interestingly, shade conditions produce plants with decreased vascular and root development, in addition to elongated hypocotyls. It has been postulated that this altered growth could result from decreased auxin flux (Morelli and Ruberti, 2000). Normally, auxin is produced primarily in the apical tip of the growing shoot and transported towards the roots in the differenti-

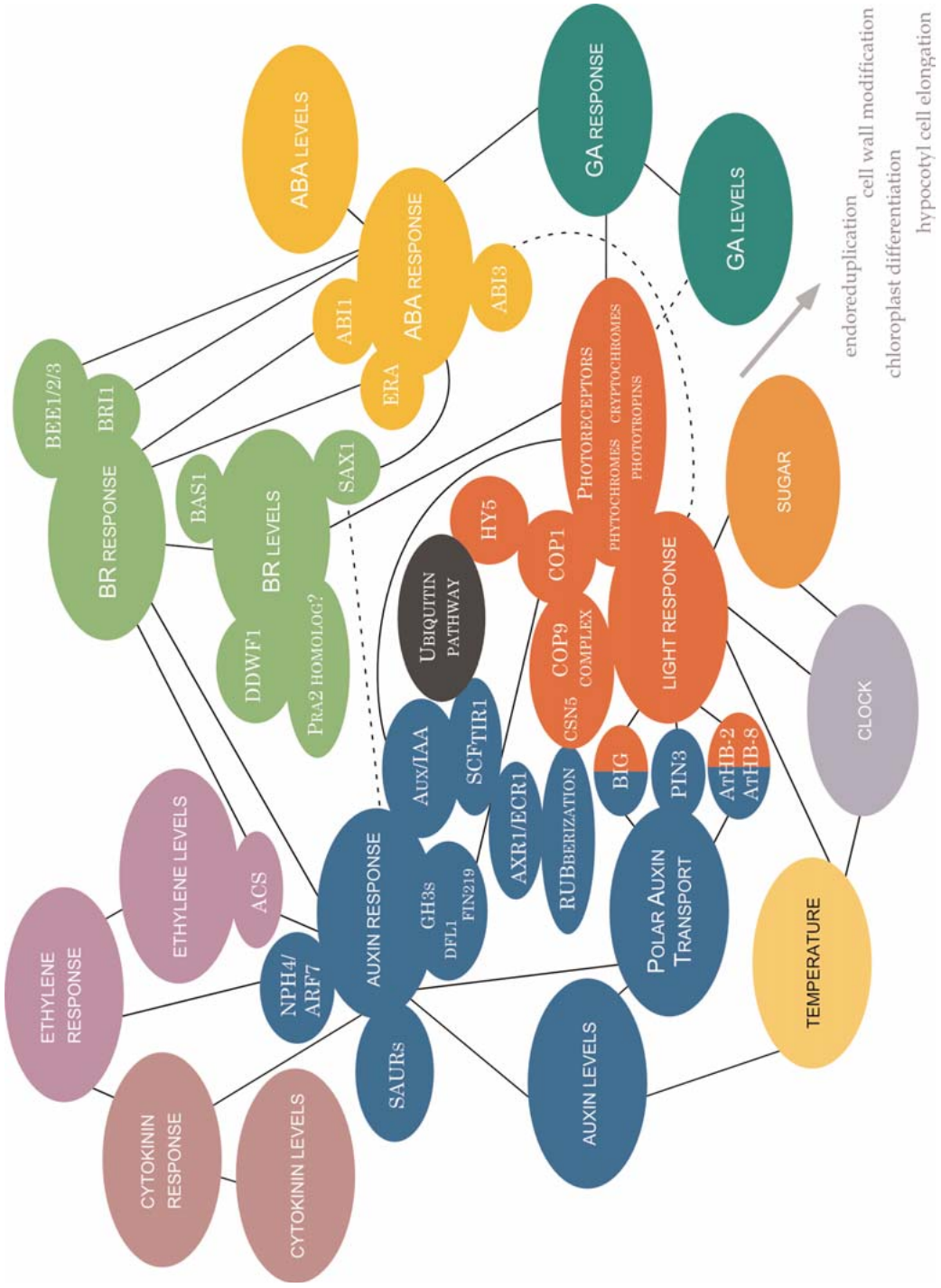

Figure 2. A simplified model of cross-talk during seedling photomorphogenesis. Factors mentioned in the text and known to connect at least two response pathways are shown. Large ovals represent major input pathways. Associated factors are shown as smaller ovals attached to these pathways. Cross-talk between pathways is represented by lines connecting pathways or connecting individual proteins where these have been identified. Dashed lines represent relationships that are not well-characterized in the literature to date.

ating vasculature. If this polar basipetal transport was redirected laterally towards epidermal cells of the hypocotyl, those cells would elongate, while the cells downstream of the auxin flow, namely the basal vasculature and roots would show reduced growth. As mentioned earlier, an asymmetric redistribution of auxin transport, or altered auxin responsiveness, mediated by PHOT1 may be the mechanism of seedling phototropism. Analyses of the *nph4* mutant and PIN3 auxin transporter strongly suggest that PHYA's effect on the phototropic response is via auxin (Friml et al., 2002; Stowe-Evans et al., 1998). It is still unclear whether PHYA acts by altering cellular levels of auxin or modifying responsiveness to the hormone. Other evidence for the relationship between PAT and light response comes from studies of a homeodomain-ZIP protein called ATHB-2 whose expression is rapidly induced by decreases in red/far-red ratios (Steindler et al., 1999). ATHB-2 has been postulated to suppress PHYB-mediated shade avoidance behavior by altering PAT. It is thought that ATHB-2 may be acting in opposition to another HD-ZIP, called ATHB-8, which is rapidly induced in response to auxin and is required for proper differentiation of vasculature. In another recent study, *doc1* mutants which inappropriately express light-induced genes in the dark, were shown to be allelic to the polar auxin transport mutant, *tir3*, and can be largely rescued by overproducing auxin (Gil et al., 2001). This result, in addition to the recent finding that polar auxin transport inhibitors cause general membrane disruption (Geldner et al., 2001), suggest that auxin response is among the most sensitive ways for plant cells to monitor the environment.

The auxin response is coordinated through an astonishingly large number of genes which are upregulated within minutes of auxin application (Guilfoyle et al., 1998). These include the *GH3*, *SAUR*, and *Aux/IAA* families, all of which are proposed to act as modulators of transcription. Regulated turn-over of either mRNA (for the *SAUR*s) or protein (for the *Aux/IAA*s) is also postulated to play an important role in regulation. Mutations in several *Aux/IAA* family members have now been identified and shown to stabilize the proteins and cause reduced responsiveness to exogenous auxin (Tian and Reed, 2001). Many of these mutants also show deetiolation in the dark, further supporting a role for auxin in opposition to the light signal. One member of this family, *SHY2/IAA3*, was initially identified for its ability to suppress the phenotype of *hy2* mutants, deficient in phytochrome chromophore biosynthesis, as well as suppressing *phyB* mutants (Tian and Reed, 1999). Phytochrome A has been shown to phosphorylate some Aux/IAA proteins, and it is thought that phosphorylation may be required for efficient targeting of proteins for ubiquitin-mediated degradation (Colon-Carmona et al., 2000). A screen to identify suppressors of *cop1*, which exhibits a constitutive light response, found *fin219* which exhibits a far-red specific long hypocotyl phenotype (Hsieh et al., 2000). This phenotype was reported to result from an epigenetic modification causing decreased expression of the auxin-inducible gene, *GH3-11*. *dfl1-D*, caused by overexpression of a different *GH3* gene, shows a light-specific dwarf phenotype and is resistant to exogenous auxin (Nakazawa et al., 2001). The temperature-sensitive allele of *CSN5*, a subunit of the COP9 signalosome, shows decreased auxin responsiveness, and has been implicated in regulating RUBberization of the cullin subunit of the SCF ubiquitin ligase, a known modulator of auxin response (Schwechheimer et al., 2001). It is interesting to note that mutations in *HY5*, a target of COP1/CSN-mediated degradation in light, cause several phenotypes reminiscent of decreased auxin sensitivity (Oyama et al., 1997).

Cytokinin (plum)

Cytokinin, auxin's traditional antagonist, has also been shown to have a role in photomorphogenesis. Exogenous application of cytokinins promotes deetiolation even in the absence of light (Chory et al., 1994). Dark-grown plants exposed to exogenous cytokinins exhibit expanded cotyledons, develop leaves, have short hypocotyls, activate light-regulated promoters, and contain partially developed chloroplasts. In the light, treated plants also closely resemble *det1* mutants. They are short, pale green, with reduced fertility and apical dominance. One cytokinin insensitive mutant, *cin4*, was found to be allelic to *cop10*, providing further evidence for cytokinin involvement in light perception (Vogel et al., 1998). However, there is no detectable regulation of cytokinin levels in wild-type or *det1* plants exposed to differing light regimes, suggesting neither absolute amounts of cytokinin nor its biosynthesis account for deetiolation under normal conditions (Chory et al., 1994). It is tempting to speculate that antagonism with auxin or brassinosteroids may explain part of cytokinin's effects. The paucity of cytokinin mutants has made assessing this question difficult. Recent identification of several putative cytokinin signal transduction components may clarify the natural role of cytokinin in regulating the deetiolation response (Hwang and Sheen, 2001). Loss of function mutants in the recently cloned receptor *CRE1* do not show an obvious light response phenotype, but *CRE1* expression is limited to the vasculature (Inoue et al., 2001; Yamada et al., 2001). Identification of related proteins expressed in other tissue types and the identification of loss of function mutants in downstream response genes are essential for elucidating the connection between cytokinin and light.

Gibberellins (dark green)

Gibberellins (GAs) are another hormone class with a clear role in many growth processes, including deetiolation (Sun, 2000). GA-deficient mutants are dwarfs, and conversely exogenous application of GA can elongate hypocotyls. *phyB* mutants have been shown to have increased responsiveness to GA (Chory and Li, 1997; Reed et al., 1996). In addition, analysis of double mutants between the GA-deficient *ga1* and *phyB* show dramatically increased responsiveness to exogenous GA (Reed et al., 1996). As the effect is of a larger magnitude than what can be obtained by adding GA to either single mutant or wild-type, it is likely that *PHYB* acts to negatively regulate GA responsiveness in the hypocotyl, rather than regulating GA biosynthesis. This alteration of sensitivity to GA could be directly on GA response components or it may be achieved by *PHYB* exerting influence on other, interacting hormone pathways. Though studies in other plants have suggested light-regulation of GA biosynthesis, the results in *Arabidopsis* have been less clear. Accumulation of GA-20 oxidase mRNA has been linked to light, and this accumulation may promote flowering, in association with a *PHYB*-controlled photoperiod response (Xu et al., 1995).

In flowering, GA appears to regulate an endogenous developmental clock which can be overridden to some degree by changes in light condition, temperature, or nutrient levels (Blazquez and Weigel, 2000). It may be that GA's role in hypocotyl elongation is similar, acting as the default growth-promoting factor in the absence of the same group of potentially conflicting signals. These signals (light, temperature, nutrient levels) are perhaps mediated by other hormones, such as auxin and brassinosteroids. GA-induced hypocotyl elongation has been shown to be independent of the auxin response and has been demonstrated to act additively with brassinosteroids in other systems (Collett et al., 2000; Mandava, 1988).

Brassinosteroids (lime)

Brassinosteroids (BRs) were among the first plant hormones linked to the process of deetiolation. Mutations causing decreased BR levels or decreased BR response, as well as treatment with BR biosynthesis inhibitors, cause dark-grown plants to deetiolate. *DET2* and *CPD* were the first genes in the biosynthesis pathway cloned, and have been shown to encode a steroid 5a-reductase and a C23-steroid hydroxylase, respectively (Li et al., 1996; Szekeres et al., 1996). In the dark, *det2* and *cpd* mutant plants have reduced hypocotyl length, opened cotyledons, and even produce rudimentary leaves. In addition, the expression of several light-responsive genes is derepressed in *det2* and *cpd* mutants, suggesting that this is truly a 'misreading' of the light conditions, rather than a consequence of growth inhibition. In light growth, brassinosteroid-deficient plants are quite severely affected in several growth processes. In most cases, rosettes are small, dark-green, and very compact. Leaves are rounded and petioles are severely reduced in length. In *det2* plants, circadian rhythm, as measured by CAB mRNA expression, is shortened, and flowering time as well as senescence, is delayed (Li et al., 1996; Millar et al., 1995). These phenotypes can largely be rescued by exogenous application of brassinolide, the most biologically active BR, and not by the addition of other hormones, including GA and auxin. Exogenous brassinolide has little effect on hypocotyl elongation in mutants defective in biosynthesis or response to other hormones (i.e., *ga5*, *eto1*), with the notable exception of *axr2* which shows a 2-3 fold increase in hypocotyl elongation, as well as significantly increased cotyledon expansion (Szekeres et al., 1996). *axr2* mutants exhibit a variety of auxin response defects and contain a gain-of-function mutation in an *Aux/IAA* family member (Nagpal et al., 2000). Interestingly, a mutation in *SAX1*, a gene involved in brassinosteroid biosynthesis, was identified in a screen for auxin hypersensitivity (Ephritikhine et al., 1999a; Ephritikhine et al., 1999b). In addition to a 2-3 fold increase in sensitivity to auxin, *sax1* mutants also exhibit greatly increased sensitivity to abscisic acid and resistance to exogenous gibberellins and ethylene, providing a compelling example of the interconnectedness of hormone signaling pathways.

Response of wild-type plants to exogenous brassinolide is dependent on both quality and quantity of illumination used (J.N. and J.C., unpublished results). BAS1, a steroid 26-hydroxylase involved in the regulated inactivation of BRs, provides one possible mechanistic link between brassinosteroid biosynthesis and light (Neff et al., 1999). *bas1-D*, a gain-of-function mutant, was isolated in an activation tagging screen for suppressors of an intermediate *phyB* mutant allele. Increased expression of *BAS1* results in severely reduced production of brassinolide and is able to fully suppress both intermediate and null alleles of *phyB* in red light; however, overexpression does not rescue *phyA* null mutants in far-red light and only partially suppresses *cry1* null mutants in blue light. Moreover, antisense lines of *bas1* are hyperresponsive to brassinolide, and show a decreased response to white, blue, and far-red light, but no change in their red light response. Together these data suggest that regulation of brassinolide levels via BAS1-mediated inactivation may represent one pathway whereby light inhibits hypocotyl elongation. Brassinosteroids

have also been implicated in repressing PHYA-mediated VLFR (Luccioni et al., 2002).

Recent work by Kang et al. also suggests a role for phytochrome in regulating brassinolide levels (Kang et al., 2001). Pra2, a dark-inducible, phytochrome-repressed small G protein from pea was used as bait in a yeast two-hybrid screen to pull out a cytochrome P450 hydroxylase, which they named DDWF1. Overexpression and antisense constructs of the pea *DDWF1* expressed in transgenic *Arabidopsis* plants results in brassinosteroid overproduction and brassinosteroid-deficient phenotypes, respectively. In addition, the short hypocotyl phenotype in antisense plants can be rescued by addition of brassinolide or castasterone, a brassinolide precursor downstream of the biosynthetic step proposed to be regulated by DDWF1. It is proposed that light-controlled regulation of this DDWF1 biosynthetic step also occurs in *Arabidopsis* via a Pra2 homolog, though this has not been shown to date.

Mutations in the proposed BR receptor, the serine/threonine kinase BRI1, also result in deetiolation phenotypes in the dark as well as decreased hypocotyl elongation in the light and overall dwarfism (Clouse et al., 1996; Li and Chory, 1997). Recent identification of a triple mutant in BR early response genes, *BEE1 BEE2* and *BEE3*, also support a role for BR response in hypocotyl elongation in both light and dark (Friedrichsen et al, submitted). Interestingly, analysis of the *BEE* genes reveals a direct interaction of the antagonistic abscisic acid pathway in regulating these early signaling intermediates and subsequent elongation responses in hypocotyl and root.

Abscisic Acid (mustard)

Abscisic acid (ABA) has traditionally been characterized by its antagonism with GA, particularly in seed germination. Recent studies have shown that ABA also acts in opposition to BRs in both seed germination and hypocotyl growth, as mentioned above (Steber and McCourt, 2001). While not much is known about ABA's role in light response, one gene involved in ABA response, *ABSCISIC ACID INSENSITIVE3*, has been shown to play a role in etiolated growth (Rohde et al., 2000). ABI3 is a transcription factor with high similarity to the *VIVIPAROUS1* gene of maize and is known to regulate a variety of seed-specific genes in response to ABA. Seeds of *abi3* mutants, in addition to being hyperresponsive to germination, also show abnormal differentiation of chloroplasts. *ABI3* has a role in determining plastid identity as well as maintaining repression of leaf development in the dark. Moreover, analysis of *det1 abi3* mutants clearly shows that *DET1* is required for full expression of *ABI3* in seeds, during etiolated growth, as well as in flowering time, suggesting that ABA has a role in the light response in all of these conditions.

Ethylene (purple)

Ethylene has been shown to regulate cell expansion in a light- and tissue-dependent manner. In the dark, ethylene inhibits cell elongation, while in the light ethylene promotes the opening of the apical hook, a process involving cell expansion, as well as promoting elongation of the hypocotyl (Raz and Ecker, 1999; Smalle et al., 1997). Constitutive ethylene response mutants show decreased size of cells throughout the plant, while ethylene insensitive mutants show aberrant cell expansion in the hypocotyl and/or cell division in roots. There is a growing body of evidence connecting ethylene response with several other hormones, best-characterized in the close relationship between ethylene and auxin. Several observations suggest that the effect of ethylene on hook formation may be via effects on auxin transport or perception (Stepanova and Ecker, 2000). A mutation in *NPH4*/ *ARF7*, an auxin signal transduction component, results in loss of auxin-mediated phototropic bending in the hypocotyl, as previously mentioned. Strikingly, this mutant phenotype may be largely rescued by addition of exogenous ethylene, suggesting that ethylene may act directly to modify auxin responsiveness (Stowe-Evans et al., 1998; Stowe-Evans et al., 2001). Several auxin-resistant mutants, including *axr1* and *aux1*, are also partially insensitive to ethylene (Stepanova and Ecker, 2000). Both brassinolide and auxin upregulate ACC synthase genes, likely resulting in increased endogenous ethylene (S. Mora-Garcia, Y. Yin, Y. Zhao, and J.C., unpublished results). Cytokinin has also been reported to negatively regulate the ethylene pathway (Vogel et al., 1998). In roots, it has been shown that the growth inhibition response to high doses of ABA requires functional ethylene signaling components (Ghassemian et al., 2000).

HOW DOES THE HYPOCOTYL GROW?

Ultimately, the effects of light and hormones on the morphology of *Arabidopsis* seedlings are enacted at the cellular level. *Arabidopsis* hypocotyl cells are formed in the embryo and undergo little to no additional divisions during seedling growth (Gendreau et al., 1997). As a result, all

growth occurs through cell expansion, which in the case of dark-grown tissues may mean a greater than 100-fold increase in cell length over one week's time. As in all plant cells, hypocotyl cells are bounded by the highly organized structure of the cell wall, composed primarily of two major polysaccharide networks (The Cell Wall doi/10.1199/tab.0019). Cellulose and xyloglucan fibers provide tensile strength while a gel-like pectin layer resists compression. All cell expansion requires loosening of this structure, with enzymes like hydrolases and xyloglucan endo-transglycosylases, synthesizing new wall components and integrating them into the wall, and expanding the cytoplasm to fill the new space. Some physiological and genetic evidence suggests that this last process is carried out by increasing the uptake of water into the vacuole thus swelling the cellular volume and providing turgor pressure. Mutants in *DET3*, a vacuolar ATPase assembly subunit, show decreased hypocotyl length in the dark, perhaps due to a defect in this aspect of cellular growth (Schumacher et al., 1999). Auxins and brassinosteroids have been linked to transcriptional upregulation of several cell wall loosening and expanding agents, including xyloglucan endo-transglycosylases, hydrolases, and expansins, as well as aquaporins (Friedrichsen and Chory, 2001).

Different environmental conditions introduce other parameters for productive growth. For seedlings which are buried, etiolated growth should be fast, energy-efficient, and the cells must be able to withstand the mechanical stress of pushing through soil or other ground cover. Once in the light, cells are exposed to more lateral stresses, such as wind, in addition to greater risk of desiccation. Close morphological examination of *Arabidopsis* hypocotyls reveals several differences between light and dark growth (Gendreau et al., 1997). Epidermal cells of light-grown plants exhibit a distinct differentiation pattern, a mostly uniform growth rate and undergo only two rounds of endoreduplication (resulting in cells containing 2C, 4C, and 8C DNA). On the other hand, epidermal cells on dark-grown plants do not differentiate, exhibit a steep acropetal wave of growth, and undergo an additional round of endoreduplication (resulting in cells containing 2C, 4C, 8C,and 16C DNA). Interestingly, *det1* plants grown in the dark resemble light-grown plants even at this cellular level, suggesting that the differences observed in wild-type plants are not solely derived from effects of functional photosynthetic machinery (Gendreau et al., 1997). Mutants such as *PROCUSTE1*, which has a dark-specific hypocotyl growth defect, provide further support for distinct light and dark growth pathways (Desnos et al., 1996).

Increased cellular DNA content is closely correlated with cell size in many organisms, and this relationship can also be observed in hypocotyl cells of dark-grown seedlings where both DNA content and cell length is increased relative to light-grown tissues. Gibberellins and ethylene have both been shown to promote endoreduplication (Gendreau et al., 1999). Hypocotyl cells of GA-deficient mutants have dramatically reduced rates of endoreduplication and also fail to elongate to the same degree as wild-type cells. Exogenous application of GA can rescue the endoreduplication phenotype at levels 100-fold lower than that required to fully rescue cell length. Ethylene is reported to have a specific role in the last round of endoreduplication in light or dark. Seedlings grown on ACC are able to undergo an additional round of endoreduplication, and the ethylene 'triple response' phenotype is tightly correlated with a cellular DNA content of 32C. Auxin, cytokinin, brassinolide, and abscisic acid also influence the number of rounds of endoreduplication but they only act to shift the proportions of cells in each category of DNA content rather than the absolute ploidy levels (Gendreau et al., 1999). The exact relationship between cell size and DNA content remains a matter of debate. Mutants defective in endoreduplication, like *siamese*, show that the processes of endoreduplication and cell elongation can be uncoupled genetically (Walker et al., 2000). *siamese* seedlings have lower DNA content in the dark but have no hypocotyl length defects, though they do exhibit extreme disruption of trichome development.

Another factor known to act intimately in cellular expansion is the cytoskeleton. Cellulose synthase complex interacts with microtubules in determining the orientation of growth. Alpha and beta tubulin and at least one actin gene (*ACT11*) are transcriptionally repressed by light (Huang et al., 1997; Leu et al., 1995). A close examination of the cell elongation defect in one BR biosynthetic mutant, *bul1-1/dwf7-3/ste1-4* suggests that a major role for BR in cell growth is through reorientation of cortical microtubules and increased expression of tubulin genes (Catterou et al., 2001a; Catterou et al., 2001b). Nuclear localization of PHYA and PHYB, critical for relaying the light signal, also requires an intact cytoskeleton. A T-DNA insertion upstream of PROFILIN1, an actin binding protein, reduces gene expression and causes arrhythmic cotyledon movement, delayed germination, elongated hypocotyls, and an excessive number of root hairs (McKinney et al., 2001). The aberrant light and circadian responses of these mutants, as well as the developmental defects, are proposed to result from excessive actin monomers driving cellular elongation throughout the plant.

HARMONIC CONVERGENCE

In this chapter, a large number of factors have been implicated in controlling the growth habit of the seemingly simple seedling. It is likely that our current knowledge represents only the first glance at a picture that will grow more complex with newly identified molecules and a better understanding of the mechanisms acting in those already identified. It is increasingly apparent that traditional approaches to identify factors in one physiological pathway must be modified to encompass the impact of other pathways. In addition, new and existing mutants can be characterized in relationship to the entire network of pathways, rather than in isolation. Perhaps most importantly, the complexity observed in *Arabidopsis* seedlings likely is echoed in many other plant processes, though often in ways which are more difficult to dissect. In this way, attempts to unravel the network of factors acting in seedlings may lay the groundwork for understanding crosstalk throughout the plant.

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