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Photomorphogenesis

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As photoautotrophs, plants are exquisitely sensitive to their light environment. Light affects many developmental and physiological responses throughout plants' life histories. The focus of this chapter is on light effects during 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. Light exposure triggers several major developmental and physiological events. These include: growth inhibition and differentiation of the embryonic stem (hypocotyl); maturation of the embryonic leaves (cotyledons); and establishment and activation of the stem cell population in the shoot and root apical meristems. Recent studies have linked a number of photoreceptors, transcription factors, and phytohormones to each of these events.

INTRODUCTION

As photoautotrophs, plants are exquisitely sensitive to their light environment. Light affects many developmental and physiological responses throughout plants' life histories, including germination (Bentsink and Koornneef, 2008), flowering (Alvarez-Buylla et al., 2010), and direction of growth (Pedmale et al., 2010). In Arabidopsis, there are four major classes of photoreceptors: the phytochromes (phy) acting predominantly in red/far-red wavelengths (Wang and Deng, 2004), the cryptochromes (cry) responding in blue and UVA (Yu et al., 2010; Chaves et al., 2011), the phototropins (phot) responding in blue (Phototropism), and recently identified UVB photoreceptors (Rizzini et al., 2011).

The focus of this chapter will be on light effects during 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 (skotomorphogenesis). In contrast, Arabidopsis seedlings grown in bright light have: short hypocotyls; expanded and photosynthetically-active cotyledons; and self-regulating stem cell populations at root and shoot apices (photomorphogenesis) (Figure 1).

A number of inputs determine 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. In this brief window of time, a plant matures from a seed reserve-dependent embryo to a self-sufficient photoautotroph—correct assessment of the environment is quite literally a matter of life and death. Information about resources and environment must be conveyed across the entire plant to optimally coordinate growth. In the following sections, the focus will be on the major developmental and physiological events specific to seedling exposure to light.

HYPOCOTYL DIFFERENTIATION AND GROWTH INHIBITION

The extent of hypocotyl elongation has been the basis for critical genetic screens identifying key components of photomorphogenetic signaling, as well as the basis for quantitatively classifying mutants from a variety of pathways implicated in light responses. In the dark, extremely rapid growth of the hypocotyl is a strategy to ensure the apex of the plant reaches the light before the seed reserves are exhausted. Hypocotyl growth is driven by cell expansion and is suppressed by less than one minute of blue or a few minutes of sustained red light (Parks et al., 1998; Parks and Spalding, 1999; Wu et al., 2010). Phys, crys and phots are all implicated in inhibition of hypocotyl elongation (Casal, 2000).

Reprogramming of the Genome

A screen for plants with reduced hypocotyl response to light yielded the first photomorphogenetic mutants (Koornneef et al., 1980). In addition to several mutants affecting photoreceptors and showing wavelength-specific hypocotyl defects, one mutant called *long hypocotyl 5* (*hy5*) had an elongated hypocotyl

in all light conditions tested. The gene affected in this mutant was found to encode a basic leucine zipper (bZIP) transcription factor that accumulates in the presence of light, implicating transcriptional changes in the photomorphogenetic response (Oyama et al., 1997). A recent study used a series of ChIP-chip assays to identify more than 9000 promoters as likely HY5 binding sites. Approximately one-third of these genes are differentially expressed in *hy5* mutants (Zhang et al., 2011). Gene Ontology (GO) analysis showed that transcription factors are enriched among the HY5-regulated genes, as well as genes related to auxin, cytokinin, ethylene and jasmonic acid pathways. Not surprisingly, genes related to cell elongation, cell division, and chloroplast development were also among the HY5-regulated target genes (Zhang et al., 2011).

Beyond these transcriptional changes, there is evidence for genome-wide reprogramming following light exposure. An examination of histone modification marks at the *HY5* and the *HY5 HOMOLOG* (*HYH*) loci revealed considerable differences in seedlings grown in the dark versus those experiencing a dark to light transition (Charron et al., 2009). H3K9 acetylation (H3K9ac), a histone mark associated with gene activation, showed a massive peak in the coding region of both *HY5* and *HYH* following transition to light. Additionally, 37% of putative HY5 binding sites identified in an earlier study (Lee et al., 2007) were targeted by H3K9ac in dark grown seedlings and this overlap increased to 52% in seedlings moved from dark to light (Charron et al., 2009).

HY5 is degraded in the dark by association with the ubiquitin ligase CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) (Osterlund et al., 2000). In the light, interaction between COP1 and HY5 is disrupted. This leads to accumulation of HY5 and inhibition of hypocotyl elongation. FIN219, a protein quickly induced by auxin and involved in regulation of jasmonic acid, has been shown to negatively regulate COP1 levels under continuous far-red light (Wang et al., 2011). In the absence of FIN219, COP1 accumulates in the nucleus resulting in an increase of HY5 degradation (Wang et al., 2011). Bimolecular fluorescence complementation, yeast two-hybrid and pull-down assays show that FIN219 can interact directly with the WD40 domain of COP1. Fluorescence experiments indicate that FIN219 can also modulate the subcellular localization of COP1. A *fin219-2/cop1-6* double mutant shows greatly reduced HY5 levels in the dark as well as in far-red light. This suggests that FIN219 may have COP1-independent roles in HY5 stability (Wang et al., 2011).

In addition to HY5, a number of basic helix-loop-helix (bHLH) transcription factors have been implicated in regulation of hypocotyl growth control. Members of the PHYTOCHROME INTERACTING FACTOR (PIF) family of bHLH transcription factors are emerging as hubs of seedling growth control (Leivar and Quail, 2011). Recent examination of a quadruple *pif* mutant (*pif1 pif3 pif4 pif5*, also called *pifq)* has revealed a striking constitutively photomorphogenic (cop)-like phenotype in dark-grown seedlings (Leivar et al., 2008; Shin et al., 2009). In addition to directly promoting hypocotyl growth, PIFs antagonize photoreceptor function by stimulating COP1-catalyzed ubiquitylation and degradation of phyB (Jang et al., 2010). This leads to over-accumulation of phyB and light-hypersensitivity in *pifq* mutants.

LONG HYPOCOTYL IN FAR-RED1 (HFR1), an atypical bHLH, is required for both phy- and cry-dependent light signal transduction in seedlings (Fairchild et al., 2000; Duek et al., 2004). Similar to HY5, HFR1 is degraded in the dark by COP1-catalyzed ubiquitination, while light stabilizes HFR1 in the nucleus to promote photomorphogenesis (Pokhilko et al., 2011). Using ChIP assays, HFR1 was shown to act as a non-DNA binding transcription cofactor directly interacting with PIF4 and PIF5 to inhibit their activation of target genes (Hornitschek et al., 2009). This activity may be antagonized by yet another family of bHLH transcription factors called BANQUO1 (BNQ1), BNQ2, and BNQ3. Seedlings overexpressing any of the *BNQ* genes have elongated hypocotyls in red light (Mara et al., 2010). Overexpression of *BNQ* genes can suppress the short hypocotyl phenotype of seedlings overexpressing HFR1, perhaps through blocking HFR1 interaction with PIF proteins (Mara et al., 2010).

Hormones as Targets of the Photoreceptors

Hypocotyl growth has also been used to identify a large number of mutants involved in hormone biosynthesis and signaling. The pathways most closely associated with proper hypocotyl elongation include auxin, brassinosteroids, gibberellins, ethylene, and cytokinin. Details of these pathways are reviewed elsewhere (Clouse, 2002; Kieber, 2002; Schaller and Kieber, 2002; Michniewicz et al., 2007; Sun, 2008; Stepanova and Alonso, 2009; Argueso et al., 2010; Kim and Wang, 2010; Stewart and Nemhauser, 2010). Here, we focus on a few recent examples of molecular mechanisms connecting light and hormone responses.

Many lines of evidence connect auxin transport and signaling to the seedling light response (Boerjan et al., 1995; Romano et al., 1995; Delarue et al., 1998; Collett et al., 2000; Zhao et al., 2001). For example, blue light acting through cry1 alters the expression of some *AUXIN RESPONSE FACTOR* (*ARF*) genes (Folta et al., 2003). Recently ABCB19, a member of a large family of ABC transporters, was shown to play a role in auxin distribution along the hypocotyl. Overexpression of *ABCB19* led to reduced inhibition of hypocotyl elongation in blue and red light and greater expression of an auxin-responsive reporter. Conversely, mutants in *ABCB19* (*b19-1*) were hypersensitive to high-fluence blue light and loss of ABCB19 strongly suppressed *phyB* and *cry1* long hypocotyl phenotypes. Mutations in either photoreceptor caused a substantial increase in ACBC19 protein, even in the absence of light (Wu et al., 2010). Despite this strong connection to the light response, high-resolution time course analysis of hypocotyl growth indicated that loss or gain of ABCB19 had only a modest effect on the initial 12 hours of hypocotyl growth inhibition by blue light (Wu et al., 2010).

One clue to this surprising result is that co-application of gibberellins with auxin is far more effective than auxin treatment alone in suppressing blue light-mediated growth inhibition (Folta et al., 2003). Defects in gibberellin biosynthesis or response result in light-grown phenotypes in dark-grown seedlings (Alabadi et al., 2004). Gibberellins play a role in photomorphogenesis through the destabilization of the growth repressing DELLA family of transcriptional regulators (Silverstone et al., 1997; Silverstone et al., 1998; Silverstone et al., 2001; Harberd, 2003; Alabadi et al., 2004; Feng et al., 2008; Achard and Genschik, 2009). One hour of light is sufficient to strongly repress expression of genes encoding gibberellin-biosynthesis enzymes, while up-regulating genes involved in gibberellin inactivation (Achard et al., 2007).

Consequently, DELLAs accumulate to higher levels in light-exposed seedlings, resulting in shorter hypocotyls. Following exposure to light, phyB promotes the gradual accumulation of DELLAs in the hypocotyl (Achard et al., 2007). In addition to direct regulation of PIF protein stability, phyB-mediated DELLA accumulation also antagonizes PIF function, as DELLAs directly inhibit PIF3 and PIF4 transcriptional activity (de Lucas et al., 2008; Feng et al., 2008).

Brassinosteroids are yet another class of small molecule hormones whose antagonism of light signaling has been recognized for some time (Li et al., 1996; Song et al., 2009; Li et al., 2010). Plants with defective production or response to BRs show an array of phenotypes specific to light-grown seedlings, even when they are grown in the dark. These phenotypes include a short hypocotyl, expanded cotyledons and expression of light-specific genes (Chory et al., 1991; Li et al., 1996). Brassinosteroid signaling is among the best-understood pathways in plants. Brassinosteroids regulate the activity of BES1 and BZR1 by modulating their phosphorylation status via a kinase cascade (Gampala et al., 2007; Ryu et al., 2010). Phosphorylation of BES1 or BZR1 inhibits DNA-binding and promotes the retention of these proteins in the cytoplasm. In the presence of brassinosteroids, BES1/BZR1 family members are hypophosphorylated and bind to BR-responsive gene promoters (Yin et al., 2005). Loss of brassinosteroid signaling in dark-grown seedlings was recently shown to cause a highly similar transcriptional response as exposure to red light (Sun et al., 2010). Moreover, there is a significant overlap of the BZR1 (Sun et al., 2010) and BES1/BZR2 (Yu et al., 2011) targets with the targets of light-signaling transcription factors PIF1 and HY5, as well as with genes differentially expressed between light and dark conditions (Oh et al., 2009). In addition, BZR1 negatively regulates the expression of the transcription factor GATA2, a positive regulator of photomorphogenesis, by binding to its promoter (Luo et al., 2010).

As more information becomes available describing the interaction of these various hormones in response to light, a complex picture emerges. Hormones affect levels of other hormones, as well as impacting downstream signaling events. For example, both auxin and GA effects on growth appear to converge on HY5, PIFs and DELLAs. As described above, these transcription factors are central to the genomic reprogramming during photomorphogenesis. Additionally, transcription factors regulated by specific hormone pathways may interact on gene promoters. Many brassinosteroid-responsive genes contain Auxin Response Factor binding sites, suggesting a link between auxin and brassinosteroid responses (Vert et al., 2005; Vert et al., 2008).

Outside of the nucleus

Ultimately, the cell wall is the fundamental determinant of cell shape and form. As a result of their dramatic growth potential, cell walls of hypocotyl cells have been extensively analyzed. The cell wall is composed of cellulose microfibrils tethered together by cross-linking hemicelluloses. This fundamental framework lies embedded in a second network of matrix polysaccharides, glycoproteins, proteoglycans and various low molecular weight compounds (Carpita and McCann, 2000). The continuous modification of the cell wall during growth and development requires the hydrolysis and alteration of existing cell wall material, as well as *de novo* synthesis and secretion of cell wall components. During etiolated growth, cells of the hypocotyl undergo rapid cell expansion at right angles to the predominant orientation of cellulose microfibrils. Several lines of evidence support a model where deposition of cellulose is oriented by an interaction between cellulose synthase complexes (CSCs) and microtubules (reviewed in Baskin, 2001). For example, one study used a fluorescently-labeled cellulose synthase subunit to show CSCs delivered to the plasma membrane at sites coincident with cortical microtubules (Gutierrez et al., 2009).

Another indication of the key role of the cytoskeleton in scaffolding growth comes from studies of the MICROTUBULE ASSOCIATED PROTEINS (MAPs) (Lucas et al., 2011). MAPs work alongside a number of other proteins to balance assembly and disassembly of microtubules (Hamada, 2007; Sedbrook and Kaloriti, 2008). A genetic screen for aberrant microtubule patterns identified the temperature-sensitive *mor1* mutant, carrying a mutation in a MAP215/Dis1 family member (Whittington et al., 2001). *mor1* mutants grown at the restrictive temperature show severe morphological abnormalities, including isotropic cell expansion. Mutations in the *MAP65-2* gene cause a modest but significant reduction in the height of dark-grown seedlings. Additional loss of *MAP65-1* leads to a 40% decrease in elongation of dark-grown hypocotyls, as well as defects in hypocotyl growth in light-grown seedlings. Expression of fluorescent *MAP65-1* and *MAP65-2* reporters showed expression in all hypocotyl epidermal cells, cotyledons and root tips. When examined in combination with fluorescently-labeled tubulin, both MAPs localized to regions of structured antiparallel microtubules rather than unbundled microtubules. This association is not absolutely necessary for proper microtubule assembly, as transversely aligned arrays could still be detected in *map65-1 map65-2* double mutants (Lucas et al., 2011).

Temperature is known to increase hypocotyl cell elongation (Gray et al., 1998), and a recent study examined the effects of temperature on the cell wall (Fujita et al. 2011). Higher growth temperatures were found to decrease crystallinity of cellulose, a measure of the overall mechanical properties of cellulose microfibrils. High cellulose crystallinity makes microfibrils inextensible and limits cross-linking by hemicelluloses, a crucial factor for resisting mechanical stress during rapid cell expansion (Chambat et al., 2005). After exposure to only three hours at increased temperature, CSC velocity increased more than 4-fold, suggesting that temperature can stimulate cell growth by increasing the rate of cellulose production. In the temperature-sensitive *mor1* mutant, elevated temperature causes microtubules to shorten and lose parallel order. As a result of disordered microtubules, cellulose crystallinity remains relatively high even at the elongation-promoting higher temperature (Kawamura and Wasteneys, 2008; Allard et al., 2010; Fujita et al., 2011) . By labeling microtubules with another fluorescent reporter, the authors could detect a significant reduction in total area covered by microtubules in *mor1* mutants grown at elevated temperature. Consistent with this finding, *mor1* mutants exposed to 29°C also had an increased proportion of CSCs in microtubule-free domains. Somewhat surprisingly, the microfibril pattern in the *mor1* mutant was consistent with the transverse parallel orientation observed in wild type hypocotyls (Fujita et al., 2011). These findings strongly suggest that

microtubules are not essential for guiding cellulose deposition, although it should be noted that only one cellulose synthase subunit was examined in these studies. Rather than directly affecting cellulose deposition, microtubules might alter the proportion of crystalline and amorphous cellulose by guiding secretion of non-cellulosic polysaccharides or enzymes that modify polysaccharides in the vicinity of the cell wall (Lai-Kee-Him et al., 2002). It is also worth noting that previous studies have demonstrated that increased temperature elevates auxin levels and increases sensitivity to brassinosteroids (Gray et al., 1998; Zhao et al., 2002; Nemhauser et al., 2004). Whether these diverse effects of temperature are related to one another would be an interesting area for future investigation.

A recent observation that growth can be uncoupled from cellulose synthesis provides a possible alternative explanation for the apparent lack of connection between CSC trajectory and microfibril orientation. Hypocotyls of seedlings growing in the dark for 20 hours following germination have been shown to elongate with a velocity of < 0.1 mm h^{-1}. After 40 hours of dark growth, growth rate increases to 0.3mm h^{-1}. This rapid growth is maintained for the following 3 days (Refregier et al., 2004). Surprisingly, growth can be inhibited by a CSC-targeting drug called isohaxben only if seedlings are exposed during the early, slow-growth phase. This differential effect on growth was particularly striking as several lines of evidence indicated that isohaxben was equally able to inhibit cellulose synthesis at both time points (Pelletier et al., 2010).

A microarray analysis of transcriptional changes in dark-grown, isolated hypocotyls during growth acceleration identified nearly 600 differentially regulated genes (Pelletier et al., 2010). Among the overrepresented categories were genes involved in cell wall related processes, including four putative pectin methylesterase inhibitors (PMEIs). Fourier Transformed-Infrared (FT-IR) microspectroscopy of hypocotyl cells undergoing growth acceleration suggests a significant increase in the global amount of cellulose and/or xyloglucan, along with an increase in the degree of de-methylesterified pectin (Pelletier et al., 2010). The degree of pectin methyl-esterification in the cell wall is controlled by pectin methyl-esterases and can be inhibited by PMEIs. De-methylesterified pectins can form Ca^{2+}-crosslinks and lead to wall stiffening (Willats et al., 2001; Willats et al., 2006). The gene encoding PMEI4 shows the strongest growth-associated induction, and when overexpressed causes significantly delayed hypocotyl growth acceleration (Pelletier et al., 2010). Once acceleration is initiated, *PMEI4* overexpressing lines show the same acceleration rate as wild type (Pelletier et al. 2010). This suggests pectin de-methylesterification controls the initiation of the acceleration rather than growth itself. Heterologous expression of the *Aspergillus aculeatus PME1* gene resulted in a clear decrease in degree of esterification and a 20% decrease in hypocotyl length (Derbyshire et al., 2007). Phytohormones may be possible mediators of this cell wall restructuring, as there are clear differences in the degree of esterification in primary cell walls of gibberellin mutants (Derbyshire et al., 2007). Wild-type hypocotyls have a 60% degree of esterification, compared to only 40% in the gibberellin-deficient *ga1-3* mutant. This deficiency can be largely rescued by gibberellin treatment.

COTYLEDON DEVELOPMENT

At the same time that light is inhibiting hypocotyl growth, it is promoting growth of the cotyledons. Many of the same factors control growth in both tissues—often with opposite effects—presenting an intriguing question of how tissue-specific cellular responses are achieved. Recent work has led to a model of seedling photomorphogenesis with two distinct stages of growth dynamics: before and after full opening of the cotyledons (Stewart et al., 2011).

Cotyledon Opening

A young seedling expands its cotyledons to increase the light capturing surface and uses petioles to position blades towards the light source. Similar to hypocotyl growth, many mutants show defects in cotyledon expansion. Among the strongest phenotypes are those of the *cop/det/fus* class (Chory, 2010). The genes affected in these mutants are all negative regulators of response to light. As mentioned previously, *cop1* displays a nearly complete de-etiolated phenotype when grown in the dark as a result of aberrant accumulation of transcription factors such as HY5 and HFR1 (Pokhilko et al., 2011). To degrade HY5, COP1 forms a heterodimeric tetramer complex with SUPPRESSOR OF *phytochromeA* (SPA) proteins (SPA1-4 in Arabidopsis). A quadruple *spa* mutant closely resembles a *cop1* mutant and some single mutants show hypersensitivity to light (Balcerowicz et al., 2011). There is also evidence for some specialization among the SPA family (Ranjan et al., 2011). For example, SPA1 plays additional roles in leaf size, stomatal development and flowering time. Interestingly, SPA1 needed to be expressed in both phloem and mesophyll cells to fully rescue leaf phenotypes, suggesting a possible route of signal transmission through the phloem to achieve coordinated growth across the seedling (Ranjan et al., 2011).

Further evidence for long-distance coordination of growth comes from experiments where expression of phyB is limited to the cotyledon mesophyll (Endo et al., 2005). The long hypocotyl phenotype of *phyB* mutants is completely suppressed in these lines. Similarly, specific inhibition of phy function in cotyledon mesophyll cells is sufficient to cause hypocotyl elongation (Warnasooriya and Montgomery, 2009). Fiber-optic-directed illumination of cotyledons but not hypocotyls has been shown to trigger full de-etiolation in both organs (Tanaka et al., 2002).

Opening of the Apical Hook

Upon emergence from the seed, the upper hypocotyl and immature cotyledons form a hooked structure to protect the shoot apical meristem while the seedling pushes towards the soil surface. Mutant seedlings lacking a hook fail to emerge when their seeds are buried in soil (Gallego-Bartolome et al., 2011). Light triggers complete and irreversible cotyledon opening within six hours (Wu et al., 2010). To fully maintain an apical hook in the light requires near complete loss of cry and phy function (Lopez-Juez et al., 2008).

In the absence of light, the hook must be actively maintained through differential cell growth. Differential growth is established by a gradient of auxin activity and refined by the coordinated action of auxin and ethylene (Lehman et al., 1996; Gallego-Bar-

tolome et al., 2011). Etiolated *hookless1* (*hls1*) mutants show a dramatically reduced expression of an auxin-responsive reporter (Li et al., 2004). *HLS1* encodes a putative aminotransferase and is proposed to cause asymmetric auxin-distribution and/or response. Mutations in a negative regulator of auxin response called *Auxin Response Factor 2* (*ARF2*) partially suppress *hls1* mutants, possibly by restoring balance to auxin response in the hook (Li et al., 2004; Hamaguchi et al., 2008).This is supported by a partial restoration of expression of the auxin-responsive reporter. Auxin moves into the hook cells through regulated transport under the control of a number of proteins (Grunewald and Friml, 2010). Overexpression of the auxin transporter ABCB19 in *cry1* or *phyB* mutants can also affect hook opening, likely by preventing modification of the auxin gradient needed to restore symmetric growth (Wu et al., 2010)

Gibberellins also play a role in hook maintenance. Seedlings treated with the gibberellin biosynthesis inhibitor paclobutazol (PAC) fail to form an apical hook (Gallego-Bartolome et al., 2011) and expression of a stabilized DELLA protein in the endodermis but not the epidermis impairs hook formation (Gallego-Bartolome et al., 2011). Gibberellins promote hook development in several ways, including through transcriptional regulation of several genes in the ethylene and auxin pathways (e.g., *HLS1* and *PIN-FORMED* auxin efflux carriers). Moreover, gibberellins cooperate with ethylene in preventing hook opening, at least in part through interactions with the PIF family (Gallego-Bartolome et al., 2011). PIF5 requires gibberellin-mediated release from the DELLA repressors to bind to the promoter of ethylene biosynthetic genes. *pifq* mutants begin to open their cotyledons immediately upon germination, but this opening can be delayed by gibberellin treatment (Gallego-Bartolome et al., 2011).

Chloroplast Development

As primary sites of seedling photosynthesis, cotyledons have additional developmental programs beyond growth. Light triggers cotyledon greening through conversion of largely undifferentiated etioplasts into photosynthetically active chloroplasts. In preparation for this switch, dark-grown seedlings accumulate the chlorophyll precursor protochlorophyllide (Stephenson et al., 2009). This allows for rapid assembly of functional photosynthetic machinery once exposed to light. Dark-grown *cop1* and *pifq* mutants have partially developed chloroplasts and accumulation of chlorophyll precursor, largely phenocopying wild-type seedlings grown in light (Stephenson et al., 2009). The transcriptome of *pifq* mutants reflects these morphological effects, showing aberrant expression of numerous genes related to the biogenesis of active chloroplasts and metabolic genes required for the transition from heterotrophic to autotrophic growth (Leivar et al., 2009).

One noticeable difference between dark-grown constitutively photomorphogenic mutants and light-grown wild-type seedlings is the absence of greening. Greening is blocked because one of the last steps in chlorophyll synthesis--the conversion of protochlorophyllide into chlorophyllide--is catalyzed by the photon-activated enzyme phrotochlorophyllide oxidoreductase. In Arabidopsis, chlorophyll production is largely independent of the phytochromes. Even in the quintuple phytochrome mutant, a red light pulse increases chlorophyllide levels (Strasser et al., 2010). In contrast, rice mutants lacking phytochrome function are also deficient in chlorophyll (Takano et al., 2009).

The DELLA-PIF interaction acts as a key regulator of chloroplast development. Dark-grown plants with reduced gibberellin levels contain chloroplasts with many similar attributes to those of light-grown wild-type plants (Cheminant et al., 2011). Many of the same light-regulated genes are up-regulated in dark-grown *ga1* (Cheminant et al., 2011) and *pifq* (Leivar et al., 2009) seedlings. Moreover, PIF1 binds in a gibberellin-dependent manner to promoters of several light-induced (*LHB1B1*, *LHCB1* and *2*), photosynthetic (*PSAG* and *PSAE-1*) and chlorophyll biosynthesis (*CAO* and *CHLH*) genes. In particular, DELLA-dependent up-regulation of the photoprotective enzyme protochlorophyllide oxidoreductase in dark-grown seedlings facilitates rapid adaptation upon light exposure (Cheminant et al., 2011).

In addition to genes involved in chlorophyll production and photosystem assembly, genes encoding enzymes needed for carotenoid biosynthesis are strongly up-regulated when seedlings are exposed to light (Rodriguez-Villalon et al., 2009). Carotenoids play a critical photoprotective role by quenching excess excitation energy generated during photosynthesis. Recently, several members of the PIF family have been shown to directly repress expression of phytoene synthase and thereby decrease the accumulation of carotenoids (Toledo-Ortiz et al., 2010). This PIF activity is countered by DELLAs (Cheminant et al., 2011). At least 50 genes show correlated expression with phytoene synthase, including *PIF*s, genes involved in chlorophyll and carotenoid biosynthesis, and genes involved in the production and perception of brassinosteroids, auxins, abscisic acid and jasmonate (Meier et al., 2011). Current models suggest that light-triggered degradation of PIFs leads to a rapid transition to photoautotrophy, at least in part through coordinated production of carotenoids and chlorophyll.

MERISTEM ESTABLISHMENT AND ACTIVATION

While the hypocotyl is elongating and the cotyledons are opening towards the light, stem cells located in the shoot and root apical meristems (SAM and RAM, respectively) are also undergoing significant developmental changes. Both meristems are established during embryogenesis before seedset. In the days following germination, both meristems achieve their mature size and initiate production of new biomass. In the shoot, production of leaves is a direct outcome of the transition to light. Root development is at least initially light-independent, although it cannot continue indefinitely without shoot-derived photosynthate. Appearance of the first true leaves is the endpoint of seedling photomorphogenesis.

Events in the Seedling Shoot Apical Meristem

SAM activity must balance the need for photosynthetic tissue with resources that are frequently in limited supply. In the game “Extinct! Are you smarter than a plant?” (http://www.joecutting.com/extinct.php), the strategy for the seedling is clear and absolute—make enough root biomass to get the nutrients and water to support production of leaves or die early. Of course, without light, there is no point in making leaves at all. Once a seedling

experiences even a short pulse of light, a wave of SAM-specific gene expression is initiated which depends on phys or crys (Lopez-Juez et al., 2008). Microarrays on dissected shoot apices identified nearly 6000 differentially regulated genes within the first six hours after light exposure (Lopez-Juez et al., 2008). Several hypotheses about SAM activation emerged from this analysis. First, cell division is initiated. Genes involved in the cell-cycle and translational machinery are induced early after the dark-to-light transition. Second, hormones need to be re-balanced. Genes associated with auxin and ethylene activity are down-regulated by light, whereas genes associated with cytokinin and gibberellin action are up-regulated. Third, cell walls need to be modified to allow for emergence of lateral organs. A substantial number of genes encoding cell-wall loosening enzymes, as well as cellulose synthases, show increased expression at the later time points when leaf primordia begin expanding.

After activation by light, the SAM grows until it reaches its mature size and is then maintained by a feedback loop comprised of WUSCHEL and the CLAVATA proteins (Barton, 2010). Cytokinin plays a critical role in SAM establishment and is thought to do so through activation of STIMPY/WUSCHEL HOMEOBOX 9 (STIP) (reviewed in Argueso et al., 2010; Skylar et al., 2010). *STIP* expression requires cytokinin receptor function, and *stip* mutants show a similar retarded growth phenotype and cytokinin insensitivity as triple cytokinin receptor mutants. In the triple cytokinin receptor mutant, *STIP* overexpression can partially rescue SAM size and substantially prolong growth (Skylar et al., 2010; Skylar and Wu, 2010). There is also likely a feedback loop between STIP and the cytokinin response pathway. Expression of several negative regulators of cytokinin response called ARABIDOPSIS RESPONSE REGULATORS (ARRs) is reduced in *stip* mutants. However, these genes retain cytokinin response in a *stip* background, suggesting a branched rather than linear pathway. An interesting recent report shows that contrary to the sharp reduction in cytokinin sensitivity of light-grown *stip* mutants, mutants grown in the dark have increased cytokinin sensitivity (Skylar and Wu, 2010). Exogenous cytokinin treatment provoked a severe de-etiolated phenotype in *stip* mutants, a phenotype not observed in wild-type plants. The rescue of *stip* by exogenous sucrose may point to additional connections between photosynthetic activity, cell division, and the balance between division and differentiation (Wu et al., 2005).

Events in the Root Apical Meristem

Several recent studies have led to a provocative and persuasive model for how the RAM reaches its mature size during a critical window three to five days post-germination. While it is interesting that this occurs during the same short time frame as photomorphogenesis, a link between the two processes remains unclear, as all root studies have been performed in light conditions. Over this time, the RAM slows its rate of cell division and arrives at a stable balance between rates of division and differentiation. Expression levels of the gene encoding the auxin-regulated transcriptional co-repressor SHORT HYPOCOTYL 2/IAA3 (SHY2/IAA3) appear to be key to this transition. *SHY2/IAA3* levels rise as the RAM slows its rate of division, and heat-shock induced expression of *SHY2/IAA3* can prematurely arrest RAM expansion (Moubayidin et al., 2010). While no direct link has been made between the maturation of the RAM and photomorphogenesis, it is intriguing that this same timing—five days post germination—is coincident with full cotyledon opening (Stewart et al., 2011).

For the few days immediately after germination, a positive regulator of the cytokinin response called ARR12 weakly induces *SHY2/IAA3* (Moubayidin et al., 2010). Low levels of *SHY2/IAA3* lead to high division rates in the RAM. By day 5, *SHY2/IAA3* expression is induced by both ARR1 and ARR12, causing division rates to slow. The major target of SHY2/IAA3 function appears to be the auxin transporters *PIN1*, *PIN3*, and *PIN7* (Moubayidin et al., 2010). All three are negatively regulated by *SHY2/IAA3* and have increased expression in an *arr12* mutant. A SUMO E3 ligase called AtMMS21/HPY2 provides an additional potential link between cytokinin and auxin signaling in the root. Mutants defective in *AtMMS21/HPY2* display fewer and smaller meristematic cells, as well as a reduced sensitivity to cytokinins and reduced expression of cytokinin-induced genes (Huang et al., 2009; Zhang et al., 2010). AtMMS21/HPY2 has been linked to cell-cycle progression via the PLETHORA (PLT)-dependent auxin signaling pathway. PLTs are AP2-domain transcription factors required to maintain stem cell activity by regulation of polar auxin transport in both RAM and SAM (Galinha et al., 2007; Prasad et al., 2011).

Cytokinin-auxin balance has long been associated with meristem function, but recent work has added gibberellins and brassinosteroids to the mix. Gibberellins act through DELLAs to keep *ARR1* expression low early in meristem development, promoting cell division (Moubayidin et al., 2010). Ubeda-Tomas and colleagues determined that gibberellin biosynthesis mutants have a smaller meristem with fewer cells. This can be rescued by addition of gibberellins or loss of multiple DELLAs. Gibberellin-directed turnover of the DELLAs is required specifically in the endodermis to maintain a wild-type RAM (Ubeda-Tomas et al., 2009) and requires the GRAS transcription factor SCARECROW-LIKE 3 (SCL3). *SCL3* endodermal expression decreases in response to both exogenous gibberellin application and DELLA loss-of-function mutations (Heo et al., 2011). The rate of cell elongation and division in the endodermis may physically dictate what occurs in surrounding tissues.

Brassinosteroids also influence RAM size and maintenance. One mechanism for brassinosteroid action is a feedback loop involving both SHY2/IAA3 and PIN3. Previous work on the root has shown that BREVIS RADIX (BRX) functionally connects brassinosteroid biosynthesis and auxin signaling to promote growth (Mouchel et al., 2006). A recent study revealed that *BRX* is expressed in the developing protophloem of the RAM and is a target of the auxin-regulated transcription factor MP/ARF5 (Scacchi et al., 2010). MP/ARF5 is negatively regulated by SHY2/IAA3 (Weijers et al., 2005). BRX appears to bind to MP/ARF5 to increase its activity, including inducing expression of *SHY2/IAA3*. This ARF5-BRX-SHY2 feedback loop is critical for regulation of auxin transport. *BRX* may play a further integrative role, as cytokinin also induces *BRX* expression, and roots of *brx* mutants are largely cytokinin insensitive (Scacchi et al., 2010). Contrary to gibberellin signaling, the site of brassinosteroid action appears to be the root epidermis (Weijers et al., 2005; Scacchi et al., 2010). Using null and gain-of-function brassinosteroid signaling mutants, two groups found that brassinosteroids control meristem size through regulation of cell-cycle progression (Gonzalez-Garcia et al., 2011;

Hacham et al., 2011). In contrast to the work in BRX, these studies found that brassinosteroid influence on meristem size was not via regulation of expression of *PIN1*, *PIN3* or *PIN7*.

CONCLUSION

Plants are marvelously and maddeningly complex. By analyzing the morphologically simple seedling during the brief window of initial photomorphogenesis, we observe many of the events that will re-occur throughout the rest of the life cycle. These events include: the integration of development and physiology, the rapid reprogramming of the genome, large-scale changes in cellular structure and function, and re-wiring of metabolism. All of these events are coordinated in a tissue and organ-specific manner, yet also coordinated across the entire organism. All green seedlings undergo photomorphogenesis. The spaghetti diagrams of molecular networks from several years ago (Nemhauser and Chory, 2002) are beginning to resolve into maps with major regulatory hubs, like the PIFs. The next challenges will be to test how universal these networks are among flowering plants, and how they are tweaked to fit diverse ecological niches.

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