# 2

# The Guiding Force of Photons

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## 1. Introduction

In a book titled *Photosynthesis* it is easy to forget that light is not simply the energy driving plant metabolism. Light also is the central environmental factor that affects plant size, shape and development. In fact, light activation of photomorphogenic signaling pathways sets the stage for photosynthesis and ensures the maintenance of the apparatus. The effects of specific wavebands of light exert their influence on plant biology from the molecular level all the way up to the higher morphological level, and even contribute to the canopy form as a whole. The wide influence is based on the fact that the light wavelengths that optimally activate photosynthesis also strongly modulate mechanisms that control plant morphology, such as the length of internodes, expansion of leaves or even leaf position. The same light qualities also guide the development, activity and maintenance of the chloroplast, as the demands of light-driven autotrophy require specialized communication and coordination between the plastid and nucleus to ensure full function of the organelle. Contrastingly, the light qualities that lend relatively little power to photosynthesis provide important information about the ambient environment as well that lead to adaptive adjustments in physiology.

Clearly, integrating information from the light environment is an important prerequisite of photosynthesis, as it sets the stage for photosynthetic activity and later maintains the core apparatus. Precise regulatory mechanisms guide the non-photosynthetic plastid, the etioplast, toward photosynthetic competence. Sensing of the first photons of light sparks a rapid cascade of events that shift the role of the plastid from a warehouse of essential materials to a dynamic center of metabolism. The control of gene expression associated with the conversion of etioplast to chloroplast has been well described, and is a central theme of this chapter.

Nuclear genes are required for photosynthetic competence. Studies of Ribulose bisphosphosphate carboxylase-oxygenase small subunit (*Rbcs)* and Chlorophyll a/b binding protein (*cab*; synonymous with Light-harvesting, chlorophyll-binding protein or *Lhcb*) transcript accumulation have been models of photomorphogenic gene expression going back almost three decades. The current literature describes the transition in the parlance of genome-wide changes, and a complementary proteomics literature adds additional understanding of how the dark-state of the plastid matures rapidly into a light-harvesting sub-cellular machine.

The first major heading of this chapter will cover the qualities of light and the receptors that sense them. The approach will be more historical and provide an understanding of how each receptor system delivers a signal and some of the processes that are controlled. Development and competence of the plastid is the second area of emphasis, describing events that transform the etioplast to the chloroplast. The final portion of the chapter will discuss the communication between the chloroplast and the nucleus. These separate compartments must be in constant and precise communication to ensure coordinated gene expression that fulfills the requirements of the plastid for new proteins. While many of the proteins required for photosynthesis are encoded in the plastid itself, a subset of important genes reside in the nucleus, and their precise expression is required for normal chloroplast operation. Many of these are subunits of chloroplast protein complexes that are non-functional in the absence of nuclear encoded subunits. Careful communication between these compartments has been the subject of interest for decades, and recent findings have illuminated how antero- and retrograde mechanisms might mediate this critical network.

## 2. Connecting light to gene expression and development

### 2.1 Not all wavebands are created equal

Back in seventh grade I was introduced to the Spectronic 20, or "Spec 20" for short. For those readers that are unfamiliar, it is essentially a tan breadbox with two dials, an analog meter, a dial to determine the wavelength transmitted, and a chamber for introducing a sample in a test tube. The device was originally made by Bausch & Lomb back in 1954, and even modern iterations reflect the basic, industrial, sturdy simplicity of the original model.

Back in 1980 we were given the charge to determine which wavelengths chlorophyll absorbed best. I don't remember how we purified our sample, I just know that I zeroed the machine using a blank filled with water using the dial on the left, lowered the sample into the chamber, closed the lid, and then recorded the values for light absorption as we marched across the dial—from the UV to past the red wavelengths. From these readings we'd build a graph that would reflect an absorption spectrum for chlorophyll, absorbing light in the blue and red most efficiently, while offering little to no absorption in the green, yellow, orange and far-red regions of the spectrum.

Some basic hypotheses could have been constructed from these findings. Certainly the qualities of light that excite chlorophyll must provide information to the plant as well. In my third year of college I learned that this was so. I learned that red and blue light would trigger photomophogenic development. We discussed effects in a variety of plants, from peas to mung beans, to tobacco, to *Lemna* as well as studies of chloroplast orientation in the green alga *Mougeotia*. There were even studies in a strange plant called *Arabidopsis thaliana*. All showed developmental effects of light, but mostly blue and red wavelengths. The correlation between the wavelengths that stimulated development and drove light-regulated metabolism was no surprise. Some wavelengths impart valuable information to the plant that promotes growth, development and photosynthetic capacity. Other wavelengths, like far-red and green, are not so important for metabolism but they are not benign- they shape plant processes in other ways that optimize light capture and adaptation.

### 2.2 The light sensor collection

Plants interact with the ambient light environment through a series of light sensors. These specialized molecules capture photons of discrete wavelengths and initiate downstream signaling events that ultimately lead to changes in gene expression, development, and/or morphology. It is important to remember that plants rely on these environmental cues to drive, or in some cases constrain, their development. In the context of photosynthesis, light signaling is important to consider in two general contexts. The transition from etiolate to autotrophic growth is driven by light. As the developing seedling meanders through the soil, sensitive light receptors are in place and prepared to ignite a downstream flow of events upon capture of a photon. These signaling events in many cases prepare the plastid, shifting its function from that of an etioplast to the metabolic center of the chloroplast.

The sensory networks that transduce information starting with the capture of a photon into a suite of downstream responses are well known. Historically these pigments were postulated to control various aspects of plant growth and development, particularly germination, phototropic movements and the transition between vegetative and reproductive growth. Long before the genes and proteins were identified and characterized, a tremendous body of work produced evidence of their activities and effects on physiology. Light quality effects on germination were examined by Lewis H. Flint where he demonstrated the promotive effects of red light (Flint, 1936). These studies were expanded through collaboration with E.D. McAlister, a physicist that utilized a spectrograph to split the spectrum and illuminate seeds with discrete wavelengths. Together Flint and McAlister generated elegant action spectra that illustrated how red light promoted germination, while blue, green and far-red light were inhibitory (Flint and McAlister, 1937). Work E.S. Johnson followed earlier studies by Blaauw that implicated that shorter wavelengths of blue light were more effective in generating phototropic responses in oat coleoptiles (Johnson, 1937). Monochromatic light studies of this period are well documented in the book, *Pigment of the Imagination*, by Linda Sage (1992). The text documents the quest for higher fluence rates of pure monochromatic light, noting barriers like countless blown circuit breakers and generation of deadly gases, along with the use of all kinds of light sources from arc lamps and 200W incandescent filaments. One interesting passage describes the development of a large spectrograph, cobbled together from parts obtained from streetcars, movie theatres and other sundry sources, assembled in a windowless wine-racking room at the USDA laboratories in Beltsville, MD. This spectrograph projected a 14 m rainbow of light onto an adjacent wall powered by a 10,000 W cabon-arc lamp. This large spectral projection allowed great advances in understanding how specific light qualities affected discrete plant processes. Clearly different parts of the spectrum had unique abilities to spur developmental or morphological changes—and even plants placed outside of the visible spectrum exhibited treatment effects, indicating that plants responded to a wider span of wavelengths than the human eye.

In discussion of photoreceptor families it is important to define the nomenclature, as first presented in a Plant Cell Letter to the Editor (Quail et al., 1994). In this report the notation is as follows: wild-type gene: *PHY, PHYA, PHYD* mutant gene: *phy, phyA, phyD* apoprotein: PHY, PHYA, PHYD chromoprotein (apoprotein + chromophore) phy, phyA, phyD.

In the following pages the discussion on photosensory systems is broken down into sections on discovery, mechanism, and associated physiology.

### 2.2.1 The phytochromes

#### 2.2.1.1 Discovery

The USDA spectrograph and tools like it led to the discovery that red and far-red light presented opposing effects on biological processes. Within a short time the red/far-red reversibility of Grand Rapids lettuce seed germination was described (Borthwick et al., 1952), and the floodgates of phytochrome research were open. Within several decades a series of light-sensing mutants were obtained from mutagenized *Arabidopsis thaliana* collections (Koornneef et al., 1980). Ultimately several of these would be shown to encode light-signaling components. The *hy3* mutation was shown to be a lesion in the phytochrome B receptor (Somers et al., 1991). Soon after, a separately-isolated mutant called *hy8* was shown to encode phytochrome A (Parks and Quail, 1993). These genetic studies now attached genes and their cognate proteins to processes controlled by red and far-red light.
Additional phytochromes were isolated, a total of five in Arabidopsis, phyA-phyE (Clack et al., 1994). Phytochromes may be grouped by their stability in light. They Type I phytochromes are light-labile while the Type II's are light stable. In Arabidopsis phyA is the only Type I phytochrome, and it is also thought of as the dark phytochrome because of its abundance (Jordan et al., 1997). The other phytochromes are stable in light, where phyB makes up the majority of phytochrome in the cell (Chen et al., 2004; Franklin and Quail, 2010). The individual phytochromes form functional hetero- and homodimers (Sharrock and Clack, 2004; Clack et al., 2009).

#### 2.2.1.2 Signaling mechanism

The hallmark photoreversiblity is achieved from switching phytochromes between two conformational states. In darkness, phytochromes exist in a form known as Pr. This form is biologically inactive and has an absorption peak of approximately 660 nm. When illuminated the Pr form converts to the Pfr form, which initiates biological activity (for review see Chen et al., 2004). The Pfr form may be photoconverted back to the Pr form immediately by illumination with far-red light, or over a longer period of time in darkness. Both conformations maintain some overlap in their spectral absorption profiles while maintaining their distinct sensitivities. When illuminated with light and an equilibrium is established between Pr and Pfr.
After conversion to Pfr phytochromes travel from the cytosol to the nucleus. The phyA receptor moves quickly to the nucleus. The phyA::GFP fusion proteins are detected only minutes after illumination, while the phyB receptor moves with different kinetics, showing up hours after light treatment (Kircher et al., 1999; Hisada et al., 2000). The timing of movement to the nucleus matches well with the earliest detected responses to phytochrome activation. Using high-resolution imaging and phytochrome mutants, Parks and Spalding (Parks et al., 1998) demonstrated that phyA and phyB activity control inhibition of stem elongation by red light with similar kinetics. The phyA receptor exerts a transient influence within minutes while phyB involvement is evident later, but persists longer. Here localization kinetics overlap impeccably with physiological events, suggesting that rate of nuclear localization is directly influencing plant growth and development. Later, regulated nuclear import of phyB using a steroid-inducible system demonstrated that both light and nuclear localization were required for phyB to induce its effects (Huq et al., 2003).
Once in the nucleus phytochromes interact with a suite of other proteins. Some of these were first identified in interaction screens using the C-terminal PHYB as bait (Ni et al., 1998).

Interactors were termed PHYTOCHROME INTERACTING FACTORS, or PIFs (for review Castillon et al., 2007; Leivar and Quail, 2011). Analysis of PIF function would prove complex. For instance, PIF3 (a bHLH protein) binds phyA or phyB upon illumination, yet through separate domains and with distinct affinities (Leivar and Quail, 2011). PIF3, PIF1 and PIF5 have been shown to be rapidly phosphorylated and degraded via a ubiquitin-dependent process, with half lives between 5-20 min. One interpretation is that PIFs repress photomorphognesis in darkness and they are degraded rapidly upon light exposure to initiate developmental responses (Leivar et al., 2008).

#### 2.2.1.3 Associated physiology

Phytochromes are relevant to just about all aspects of light-mediated development because they absorb well in red, blue, far-red and UV portions of the spectrum. The sum of molecular and physiological processes controlled is too extreme to list here. The most relevant roles to applied phy biology are in regulation of plant stature. Phytochromes repress stem elongation, promote leaf expansion and alter plant body form in response to crowding. The phytochromes also contribute to flowering. In Arabidopsis phyA has a role in promoting flowering in response to far-red signals whereas phyB works against it after absorbing red (Valverde et al., 2004).

The role of phytochrome in establishing a platform for photosynthesis is clearly observed during photomorphogenic development. During this time there is a substantial contribution of phy to chloroplast developmental processes. Phytochromes regulate the accumulation of transcripts encoding CAB (LHCB) proteins required for anchoring the photosynthetic apparatus (Kaufman et al., 1985; Karlin-Neumann et al., 1988), as well as the small subunit of RUBISCO (Kaufman et al., 1984). Global analysis of gene expression shows that many transcripts encoding proteins destined for the plastid are induced within minutes to hours of phy activation (Tepperman et al., 2001; Tepperman et al., 2004). The major role of phy in plastid development is in de-repression of the PIF-mediated constraint of transcription and will be discussed later in this chapter. The effect is strong as developing seedlings treated with far-red light can actually be permanently disabled from greening and chloroplast development (Barnes et al., 1996).

### 2.2.2 The cryptochromes

#### 2.2.2.1 Discovery

While the participation of phytochromes defined a mechanism for red light effects in many plant processes, there were clear effects of blue light that could not be easily ascribed to phytochromes. In fact, phototropic curvature, had been described as blue-favored by Charles Darwin (Darwin, 1897), complementing a battery of blue light responses characterized in the early part of the century (Briggs, 2006). Analysis of plant actions in response to red and/or blue light provided clear evidence that more than one photosensory pigment was involved in light responses. Throughout the 20$^{th}$ century there was considerable discussion about the nature of the pigment, fueled by experimentation in plants and fungi. Analysis of countless action spectra drove speculation that the receptor was based on a carotenoid, a flavin, or a pterin, since there were general peaks at 450, 475 and 420 nm that supported these possibilities, yet fine structure of action spectra left the absolute identification of the chromophore(s) ambiguous.

In 1979 Jonathan Gressel gave a name to the illusive photoreceptor controlling plant and cryptogam form and function in blue light, appropriately, *cryptochrome* (Gressel, 1979). In his review he notes that the name was "despised by many". Yet his moniker was quite accurate, as blue light responses would later be shown to be transduced by a series of receptors (including phytochromes) some requiring phytochrome co-activation. These ambiguities would hide the genetic nature of the cryptochrome gene for another fourteen years. Gressel also contended that the cryptochrome receptor would be the single blue-light receptor.

#### 2.2.2.2 Molecular structure

The actual structure of the cryptochrome receptor was eventually elucidated in 1993, yet the path to its characterization was laid with a series of plants that grew long and tall under blue light in a 1980 report. A screen for light sensing mutants in a mutagenized *Arabidopsis thaliana* population revealed a seedling that failed to suppress elongation in light. This particular seedling, noted as *hy4* (the fourth of the hypocotyl elongation mutants), showed an especially strong presentation of the long-hypocotyl phenotype under blue light, moreso than red, green, far-red, or white light (Koornneef et al., 1980). These findings suggested a lesion in the blue-light sensing pathway. Later, several T-DNA mutants with long hypocotyls under blue light revealed the first sequence identity of the HY4 protein- a sequence that matched convincingly with the long-wavelength microbial DNA photolyases. DNA photolyases are chromophore-bound proteins that catalyze repair of pyrimidine dimers in DNA (REF). Later studies would show that the HY4 protein (later renamed to CRY1 for CRYPTOCHROME1) was the receptor controlling these blue light responses. The receptor maintains two chromophores—flavin adenine dinucleotide (FAD) and methenyltetrahydrofolate (MTHF), with the photon exciting MTHF and shuttling the excitation energy to FAD to initiate the signaling process (Cashmore et al., 1999).

#### 2.2.2.3 Various types of cryptochromes

The cry proteins are distinguished by two domains that underlie its diverse functions (Lin and Shalitin, 2003). The first is an N-terminal photolyase related (PHR) domain. The other domain is a C-terminal extension. This latter domain is variable between the different cryptochromes and defines the function. While exhibiting variation in this extension there are short islands of conserved sequence. These motifs (from N to C) are DQXVP, an acidic region high in D and E, and a STAES sequence followed by GGXVP. Because their order and sequences are so highly conserved they are noted in the literature together as a DAS domain. The DAS organization has been conserved from the most rudimentary mosses to angiosperms, so cryptochromes date back approximately 400 million years. The DAS domain also dictates cry localization and interaction that defines how individual crys contribute to physiology. A comprehensive report on cry structure and function is presented by Lin and Shalitin (2003).

In Arabidopsis there are three cryptochromes. The CRY1 and CRY2 proteins are translated and then localized to the nucleus upon activation by light. The third cryptochrome is called cry3 or cry-DASH. This member is localized to the chloroplast and performs DNA repair, much like prototypical photolyases (Kleine et al., 2003). No other signaling role has been proposed.

A late flowering mutant (known at the time as *fha1*) would connect the cry2 receptor to control of the flowering transition (Guo et al., 1998; Mockler et al., 2003). The contribution to

flowering time is probably the cryptochrome's most agriculturally relevant attribute. The transition is controlled by blue light activation of cry2, followed by its nuclear localization and enhanced stability (Valverde et al., 2004). The cry2 receptor contributes to seedling height under certain fluence rates (Lin et al., 1998), yet has potent effects on stem elongation during early development (Folta and Spalding, 2001). Perhaps the most well-studied output of cryptochrome activation is the modulation of gene expression. A number of reports have examined the role of cryptochromes using genomic-level analyses. Studies during blue light induced de-etiolation show that crys alter gene expression associated with the plant hormone gibberellic acid (Folta et al., 2003; Zhao et al., 2007), providing a means to connect light and cryptochromes to growth responses.

**2.2.2.4 Signaling mechanism**

What is the mechanism of cryptochrome action? Great steps were made to pinpoint the transduction mechanism when the c-terminus of the CRY1 protein was overexpressed in transgenic plants (Yang et al., 2000). Such transgenic seedlings, known as CCT for CRY C-Terminus, exhibited *cop*-like phenotypes, meaning that that were presenting light-grown phenotypes even in darkness. This finding was exciting because it potentially linked the COP1 protein, a regulator known to repress the light response in darkness, to cry function. Two hybrid interactions and co-immunoprecipitation analysis in vitro would confirm the interaction between COP1 and the cryptochrome light sensors (Wang et al., 2001).

The mutant *hy5* locus was isolated as part of the original screen of Arabidopsis photomorphogenic mutants (Koornneef et al., 1980). The mutant exhibited light-insensitivity symptoms especially under blue light. Later it was observed that *HY5* exhibited epistatic interactions with *COP1* (Ang and Deng, 1994), a gene encoding a ring-finger E3 ubiquitin ligase that shows a constitutive photomorphogenic phenotype. *HY5* transcripts and proteins accumulated rapidly after illumination, presenting the hypothesis that they were causal to development. It was demonstrated that *HY5* was transcribed and translated in darkness, yet the protein did not accumulate (Osterlund et al., 2000). The lack of accumulation could be reversed with the application of proteosome inhibitors, indicating that HY5 was likely being degraded via a ubiquitin-mediated mechanism. Moreover, the protein also accumulated in the *cop1* mutant. The stage was set- a positive regulator of photomorphogenesis, HY5, was destabilized when it was not needed and mutation or pharmacological block of the degradation system caused hyperaccumulation. It was possible to infer a mechanism. Now how to connect it with the light sensor?

Studies soon after tested the possibility that the cry receptor itself interacted with the COP1 degradation system. Examination of COP1-cry interaction showed that the receptor did interact with COP1 through the CCT domain, and interaction between the receptor and COP1 would interrupt ubiquitin-mediated degradation of the positive regulator HY5 (Wang et al., 2001). The effect appears to take place predominantly in the nucleus.

While this mechanism is supported by many lines of evidence it is important to remember that crys also have effects outside of the nucleus. Constructs that exclude cry from the nucleus show physiological function (Wu and Spalding, 2007) and events at the depolarization events at the cell membrane seconds after illumination (Folta and Spalding, 2001) suggest that crys are indeed functional in other contexts.

The flavin chromophore of the cryptochromes, when activated, opens new absorption properties and signaling states for cryptochrome receptors. When treated with blue light the chromophore takes on a different oxidation state that absorbs in the green, yellow and into

the red. Several lines of evidence show that the treatment of plants with green light can reverse cryptochrome mediated responses (Banerjee et al., 2007; Bouly et al., 2007), including anthocyanin accumulation, hypocotyl elongation and flowering. In this way the cry responses to blue light may be attenuated much like the red/far-red responses of phytochromes.

The cryptochromes are a stellar example of why examination of plant processes can have large-scale impacts. Cryptochromes were first identified in plants, yet since have been shown to have central positions in the animal circadian oscillator, and in magnetoperception that guides bird migration. Fungal cryptochromes have been identified, yet their precise functions remain elusive for the most part, and understood members bind DNA reminiscent of the cry3 (CRY-DASH) proteins of Arabidopsis. The cryptochrome receptors clearly control a great swath of responses relevant to all eukaryotes.

### 2.2.3 The phototropins

#### 2.2.3.1 Discovery

Characterization of the cryptochromes gave plant science discrete receptors for red, far-red and blue light responses. A number of lines of evidence indicated that the effects of cryptochrome activation were distinct from those that regulated phototropism (Liscum et al., 1992; Liscum and Briggs, 1996; Lasceve et al., 1999), suggesting the existence of an additional blue light receptor class. A report in *Nature* showed that the *cry1cry2* double mutant was deficient in first-positive phototropism (Ahmad et al., 1998). Yet the results of this work did not bear out with further tests.

The pursuit for the blue-light photosensor controlling phototropism was heating up in concert with the characterization of the first cryptochrome receptor in the years leading up to 1993. Several independent research tracks were racing toward receptor identification that would ultimately converge in an Arabidopsis mutant with defects in the receptor. One approach was an attempt to identify the receptor genetically using the Arabidopsis system. The photophysiological characteristics of curvature were well understood in this species (Steinitz and Poff, 1986), and formed a sound basis for a mutant screen. Two non-complementing mutants with defects in phototropic curvature (JK224 and JK218) were isolated (Khurana and Poff, 1989). JK224 was defective in first-positive curvature, requiring substantially higher fluences to induce measurable change. The JK218 mutant also showed resistance to phototropic curvature, only bending after long treatments with unilateral blue light. Both mutants were perfectly gravitropic, suggesting that they were sensory mutants and not simply unable to respond to stimuli that induce differential growth (Khurana and Poff, 1989).

With a separate approach a team of scientists working under the direction of Winslow R. Briggs used biochemical methods to characterize the blue light sensor for phototropism. A 120 k-Da phosphorylated protein was identified in association with plasma membranes of pea epicotyls (Gallagher and Ellis, 1982; Short and Briggs, 1990; Short et al., 1993; Short et al., 1994). When biochemistry and physiology were compared through time and space, some important correlations were uncovered. The threshold and saturation in the phototropic fluence response (Baskin, 1986) mirrored the parameters of light induced phosphorylation (Short and Briggs, 1990; Short et al., 1992). The regions of the seedling that exhibit the strongest phototropic response show the highest degree of phosphorylation (Short and Briggs, 1990). Both responses obeyed the Bunsen-Roscoe Law of Reciprocity, and the

phosphorylation reaction is complete just prior to the development of phototropic curvature. Such correlations were observed in other species as well (Palmer et al., 1993a; Palmer et al., 1993b).

The Arabidopsis mutants and the phosphorylation activity would become linked. Reymond et al. (1992) tested the diminutive Arabidopsis plants for the phosphorylation activity detected in the epicotyls of peas, zucchini, tomato and sunflower hypocotyls, and the coleoptiles of maize, barley and oat coleoptiles. The non-phototropic JK224 mutant exhibited low levels of phosphorylation upon illumination, suggesting that JK224 was in fact the receptor. On the other hand, JK218's levels were not significantly altered. This reported tied the autophosphorylation to phototropic curvature.

With a mutant genotype possessing defects in biochemistry and phenotype it would seem simple to move to the process of gene discovery. In the early 90's the Arabidopsis system was emerging as a tractable genetic system (Konieczny and Ausubel, 1993), it would be possible to map the gene if the phenotypes were robust. This was the problem. Poff's mutant phenotypes were solid, yet subtle, as first-positive phototropism would bend a seedling only 6-10 degrees. Furthermore, the defective seedlings did eventually bend over time. Because of the subtle differences, and the fact that these were not null mutants, it would have been extremely difficult to screen reliably in large populations suitable for genetic mapping.

Liscum and Briggs also performed a screen in the Arabidopsis system, yet they resorted to fast-neutron treated seeds in an attempt to find strong non-phototropic alleles (Liscum and Briggs, 1995, 1996). Four loci were identified. The *nph1* mutant was allelic to JK224 and the *nph3* mutant proved to correspond to JK218. The *nph1* mutant had no detectable phosphorylation of the 120 kDa protein, leading to the hypothesis that it was locus encoding the receptor for phototropism. The gene was eventually cloned (Huala et al., 1997).

#### 2.2.3.2 Phototropin structure

The NPH1 protein contains two highly similar domains reminiscent of LOV (Light, Oxygen and Voltage) PAS domains. These domains bind flavins (FAD) and perform a variety of functions relative to environmental sensing from bacterial aerotaxis to modulating K+ currents in *Drosophila*. NPH1 also possessed a serine-threonine kinase domain in the C-terminus. The NPH1 protein was shown to preferably bind FMN as a chromophore, and the absorption spectrum for the purified receptor mirrored that of phototropism (Christie et al., 1998). These findings prompted a functionalized name change from the locus *NPH1* to the gene encoding the receptor, *PHOT1.*

Based on sequence homology the *NPH-LIKE* (*NPL1*) gene was soon identified (Jarillo et al., 1998). This sequence has a similar topology to that of *NPH1*, with the same conserved kinase region and LOV domains. The individual LOV domains (LOV1 and LOV2) have distinct roles in phototropin action. Mutation of Cys39 of the LOV domain in the LOV1 domain has no effect on phototropism, whereas this mutation in the LOV2 domain abolishes curvature. The LOV2 domain also is the critical domain for promoting leaf expansion (Cho et al., 2007). There is evidence that the role of the LOV1 domain is to attenuate LOV2 effects by acting as a site for dimerization.

Separating the LOV domains from the kinase domain is an alpha-helical hinge that holds LOV domains in proximity to the kinase domain. Upon activation with light, the protein opens around this hinge region, allowing the kinase to be phosphorylated (Tokutomi et al., 2008). This is the basis for the phototropin signaling mechanism.

#### 2.2.3.3 Signaling mechanism

A tremendous wealth of information has arisen concerning the photocycle of the LOV domains and how it is translated into receptor function. The field of LOV domain receptors exploded from two labs in 2000 to at least 42 by 2004 (Letter to Plant Physiol. October 2010, Vol. 154, p. 1,). There are literally hundreds today.

The most progress has been made on understanding the light induced activation of the photosensor itself. The phot proteins associate with the plasma membrane. Here a photon of blue light is captured by the FMN chromophore bound to the LOV2 domain of the receptor. This excitation establishes a covalent bond between the FMN and the aforementioned Cys39 of the LOV domain. A conformational change occurs and an adjacent alpha helix (termed the J-domain) opens access to the kinase domain. The protein then autophosphorylates on multiple serine residues. These events are a simple sketch of how the receptor begins to excite the downstream events mediated by phototropins.

The mechanism that controls phototropism toward unilateral blue light relies on a simple starting point—the plant must transform a gradient of blue light into a chemical gradient capable of inducing differential growth. A framework for inter-molecular signaling in phototropic curvature was deduced from the members of the original genetic screen. The *nph1* (*phot1*), *nph3* and *nph4* mutants were all defective in phototropic curvature (Liscum and Briggs, 1996). As mentioned earlier, *NPH1* encodes the phototropin receptor. NPH3 is a phot1 interacting protein thought to be an adapter or scaffold protein (Motchoulski and Liscum, 1999). The action of NPH3 has remained unclear for the last decade, but recent studies show that members of a family that are likely involved in ubiquitination of substrates. The NPH3 protein is phosphorylated in darkness and upon light activation of phot1 it is dephosphorylated (Pedmale and Liscum, 2007).

Recent studies have shown that two of the PHYTOCHROME KINASE SUBSTRATE proteins, PKS1 and PKS2, co-immunoprecipitate with phot1 and phot2 (de Carbonnel et al., 2010). PKS1 binds to both phototropin1 and NPH3, and is required for phototropic curvature (Lariguet et al., 2006). These proteins have roles in the phot transduction to leaf position and flatness, but do not affect chloroplast relocation (de Carbonnel et al., 2010). A specific isoform of the 14-3-3 protein class also binds to phot1, but does not interact with phot2 (Sullivan et al., 2009). A growing list of proteins have been confirmed as interactors (reviewed in Inoue et al., 2010).

One confirmed interactor ties phototropin to redistribution of auxin. The auxin efflux carrier ABCB19 was shown to interact with phot1, and is a substrate for its kinase activity (Christie et al., 2011). The phosphorylated carrier fails to translocate auxin it accumulates in the cells, leading to lateral efflux by PIN3, developing the onset of phototropic curvature.

A variety of other downstream signaling events may be required for phot resposnes. Various reports have implicated calcium release from endomembranes or insolitol phosphates as potential links in phot signal transduction. These studies involved the use of pharmacological agents or reporters, so while coincident with phot signaling events, it is unclear how they precisely contribute to the processes.

#### 2.2.3.4 Associated physiology in plants

Photosynthesis is constantly tuned on the biochemical and molecular level, yet many other adjustments happen at a level that one may witness simply with the naked eye and time. Phototropins dictate the position of plant organs and organelles to optimize light intercept.

They regulate guard cells that gate gas exchange may be restricted or opened to admit carbon dioxide. At the molecular level the transcripts of specific genes necessary for photosynthetic activity accumulate and decay in light-dependent ways. All of these diverse processes share phototropins as primary photoreceptors. All of these responses have the potential to affect on optimizing photosynthesis, which appears to be the major codifying theme for the phototropins.

The physiology regulated by phot1 and phot2 may be broken down into responses that have contrasting fluence thresholds, time courses and areas of action. The phot receptors have been implicated in phototropism (Christie et al., 1998), chloroplast relocation (Jarillo et al., 2001a; Kagawa et al., 2001), stomatal opening (Kinoshita et al., 2001), leaf expansion (Sakamoto and Briggs, 2002), control of stem elongation (Folta and Spalding, 2001), inflorescence, stem and petiole positioning (Kagawa et al., 2009), leaf positioning (Inoue et al., 2008), growth responses in low-light environments (Takemiya et al., 2005) and post-translational stability of transcripts encoding chloroplast-targeted proteins (Folta and Kaufman, 2003).

Its isolation as a genetic mutant proved the importance of phot1 to phototropism in Arabidopsis. The null *phot1* mutants show severe defects in phototropic curvature. There is some evidence of redundant function between the two phot receptors. For instance, while *phot1-5* is a null mutant, it eventually will bend toward unilateral light based on compensatory activity of phot2 (Sakai et al., 2001).

While the phot2 receptor has a clear role in phototropism in response to higher fluence blue light at lengthy time course, the receptor controls the predominance of other functions. The control of stomatal opening is also mediated by redundant function of the two phot receptors (Kinoshita et al., 2001). Both receptors are capable of modulating the response with similar light sensitivity and time course. The phot2 receptor also controls the accumulation of chloroplasts into a plane perpendicular to low fluence rate light. The chloroplasts move in the cell to orient themselves to optimize position for photosynthesis. This is known as the accumulation response. In times of low light the chloroplasts will align themselves to intercept incoming light. When light is extreme, the chloroplasts retreat to positions perpendicular to incoming light, shielding themselves essentially by hiding behind other chloroplasts. This is known as the avoidance response. It has been demonstrated that both phot1 and phot2 contribute to the accumulation response to low light, but the avoidance responses are controlled by phot2 (Jarillo et al., 2001a; Kagawa et al., 2001).

The phot1 receptor solely mediates the first phase of hypocotyl growth inhibition in response to blue light. Upon first illumination hypocotyl growth slows significantly within minutes. This primary, sensitive and early response is due to phot1 (Folta and Spalding, 2001) Sustained effects are cry dependent. The phot1 receptor also controls the stability of the *Lhcb* transcript in response to a short, single pulse of blue light. Whereas the accumulation from low fluence blue light requires the plant g-protein and the GCR1 receptor (Warpeha et al., 2007), the transcript is destabilized in a manner that requires phot1 (Folta and Kaufman, 2003). Phots have also been shown to control leaf expansion (Sakamoto and Briggs, 2002), leaf and petiole position (Kagawa et al., 2009), and will probably be shown to control solar tracking (Briggs and Christie, 2002). All of these responses, from molecular to macroscopic, utilize the phot system to optimize the position and content of the hardware for photosynthesis.

### 2.2.4 The other LOV domain photosensors

The central flavin-binding, photocycling domain of the phototropin receptor, the LOV domain, has emerged as a recurrent theme in many proteins spanning many species. Their direct connection to processes germane to photosynthesis is limited at this point, but their existence merits discussion. LOV domain proteins have been identified in non-vascular plants, several types of algae, in fungi and bacteria. They are found within transcription factors, kinases, phosphatases, and proteins with undefined function. Their function outside of plants is diverse, with LOV domain proteins regulating processes as ranging from plant light signaling to virulence in *Brucella* (Swartz et al., 2007), to transcriptional changes in fungi (Ballario et al., 1998).

In Arabidopsis three non-phot, LOV domain proteins reside in the genome. These same genes were identified in genetic screens for defects in the circadian clock and flowering time. These are ZEITLUPE/ADAGIO (Somers et al., 2000; Jarillo et al., 2001b), FKF1 (Nelson et al., 2000), and LKP2 (Schultz et al., 2001). All three undergo a photocycle that mirrors that of the phototropin LOV domains (Salomon et al., 2000). The three proteins share a common role in using light to coordinate the stability and accumulation of regulatory proteins.

The other main class of LOV domain proteins comes from studies in *Adiantum*. In these organisms phototropic curvature and chloroplast relocation, canonical blue light responses in plants, are induced by red light (Kawai et al., 2003). Genetic analysis of phototropic deficient mutants showed that the fern receptor is a hybrid between the red/far-red sensor phytochrome and the LOV-domain sensors. The receptor is a fusion between two receptor types that has adapted to exploitation of the understory.

### 2.2.5 A UV-B receptor

#### 2.2.5.1 Discovery, structure and physiology

The light from the sun presents the plant with a double-edged problem. While necessary for photosynthetic growth, the mixture of light energies contain parcels of poison that could impart damage to DNA to the detriment of the organism. Plants being anchored to the earth by a root must therefore have means to detect ultraviolet (UV) light energies and tailor appropriate physiological and molecular countermeasures to combat the problems associated with UV exposure. Growing evidence to support this hypothesis has mounted for decades and recently resolved in the elucidation of a UV-B (280-320 nm) photosensor.

Observation of many plant physiological and molecular responses pointed to the existence of this receptor (for review, Ulm and Nagy, 2005; Jenkins, 2009). A suite of plant responses to UV-B were reported, including increases in intercellular calcium (Frohnmeyer et al., 1999), strong effects on hypocotyl growth inhibition (Shinkle et al., 2004; Shinkle et al., 2005), induction of genes associated with disease (Green and Fluhr, 1995), as well as patterns of global gene expression that differ from those observed from activation of cryptochromes or phytochromes (Ulm et al., 2004). Effects on stomatal opening have also been observed (Eisinger et al., 2003), and synergistic interactions with phytochromes have been long documented (Yatsuhashi and Hashimoto, 1985).

The quest for identification of the UV-B receptor followed a trail established from studies of other light sensors. As mentioned earlier, interaction between receptors and the ubiquitin E3 ligase COP1 is a regulatory node of light signaling. Additionally, photoreceptors have been shown to move to, or reside in, the nucleus upon illumination. The same patterns were

observed for the protein UV RESISTANCE LOCUS 8 (UVR8; Kaiserli and Jenkins, 2007), and the results were UV-B specific (Favory et al., 2009). Mutations in UVR8 that abolished UV-B induced photomorphogenesis also impaired interaction with COP1, and interaction in yeast was UV-B dependent (Rizzini et al., 2011) presenting support for the hypothesis that UVR8 was the UV-B receptor. The mechanism of action was shown to be dependent on UVR8 dimers splitting to monomers when specific aromatic amino acids were activated by UV-B radiation. The UVR8 protein is constitutively expressed throughout the plant (Kaiserli and Jenkins, 2007; Favory et al., 2009), allowing all cells to maintain a system to respond to potentially damaging wavelengths.

### 2.2.6 Hypothetical green light receptors

A quick glance at the current receptor collection shows that the visible light spectrum is well blanketed with the absorption spectra of photosensors to receive it. As noted, the sensor collection extends plant signal perception clearly into the UV and far-red. Are there truly responses that cannot be account for by the current set of receptors? Are there likely to be more ways that a plant can sense the light environment? A series of green light responses that persist in the absence of known sensors suggest that there are additional players in the plant sensorium.

Green light can excite phytochrome, cryptochrome and phototropin responses, depending of course on fluence rate and time of illumination. Green wavebands can induce phytochrome-mediated germination (Shinomura et al., 1996), several effects via cryptochromes as discussed earlier (Banerjee et al., 2007; Bouly et al., 2007; Sellaro et al., 2011), and even phototropic curvature (Steinitz et al., 1985) that in retrospect must be phototropin dependent. Green light has also been shown to be transmitted efficiently within the plant body and efficiently drive photosynthesis in deeper layers of the leaf (Terashima et al., 2009).

However, examination of the literature presents a suite of green-light-dependent phenomena that cannot easily be described as the action of cryptochromes, phytochromes or LOV domain receptors. These actions are induced specifically by green wavebands (~500-540 nm) and tend to oppose those of red and blue light (for review, Folta and Maruhnich, 2007). Some of the first evidence was noted when plants were grown under white light, or the same light source with various parts of the spectrum filtered to skew the quality of illumination. In early studies Frits Went observed that tomato seedlings grown under white light (red, blue and green) had a lower dry mass than tomato plants grown under red and blue light alone (Went, 1957). The effect was observed across fluence rates, so it was not simply an effect of limiting photosynthetic capacity. It was as if the presence of green wavebands contradicted the effects of red and blue.

Later, similar "reversal" effects in plant growth and the performance of tissue cultures were observed (Klein et al., 1965; Klein and Edsall, 1967). A curious blue-green reversal of stomatal open was described in Arabidopsis (Frechilla et al., 2000), sunflower (Wang et al., 2011a), and other species (Talbott, 2002). During experiments testing Arabidopsis stem growth kinetics in response to blue and red light, effects of green illumination were observed that were quite unusual. Unlike the inhibition caused by other wavebands, green light caused an increase in stem elongation rate. This finding was surprising because the etiolated elongation rate was always presumed to be the most rapid. The response was analyzed for its photophysiological and genetic parameters (Folta, 2004) and the results

indicated that the green light induced stimulation of hypocotyl growth rate was not likely mediated by known photosensors.

Based on the results of this study a microarray experiment assessed the state of the transcriptome in green light treated, etiolated seedlings. Surprisingly, a dim pulse of green light, far below "safelight" energies, excited large-scale changes in the transcriptome. The most conspicuous difference observed as the lower abundance of transcripts associated with the chloroplast, especially those playing a role in photosynthesis. Green light incuded reduction of steady-state transcripts encoding (among many others) the large subunit of RUBISCO (RbcL), psaA, and psbD was observed (Dhingra et al., 2006). This response was shown to be excited by low fluence pulses of light, occur within minutes, happen only in response to green light, and persist in the suite of photoreceptor mutants tested. These findings also indicated that a response to dim green light could drive a series of counterintuitive adaptive responses.

Additional observations now show that the addition of green wavebands to a background of red and blue light can attenuate light responses. Green light can induce shade avoidance phenotypes (Mullen et al., 2006; Zhang et al., 2011) and directly antagonize the effects of red light on stem growth inhibition, but not blue light (Y. Wang and K. Folta, unpublished). These effects point to the presence of a yet-to-be-characterized green light sensor that works in concert with other light sensing systems to optimize plant physiology in low-light environments.

## 3. The transition to photosynthetic competence

### 3.1 The plastic plastid

As mentioned previously, the plastid housed within the cells of the etiolate seedling is simply a structure poised to rapidly mature into a center of light-driven metabolic activity. In darkness the etiolated plastid, or etioplast, maintains a process known as skotomorphogenesis, or the developmental state occurring in the absence of light. The etioplast should not be considered simply a default, ground state. When we consider that the plastid has evolved from an endosymbiont that was photosynthetic (Margulis, 1970), the etioplast must be a derived state, a structure that provides a selective advantage for the emerging plant. This interpretation is supported by the observation that etioplasts are specialized. They feature a unique arrangement of thylakoid membrane precursors into a highly-ordered pro-lamellar body (Selstam and Widell-Wigge, 1993). This structure contains a storehouse of lipids and proteins (Selstam and Widell-Wigge, 1993; Kleffmann et al., 2007) required for the greening process. The prolamellar body's paracrystalline matrix also contains carotenoids, chlorophyll precursors and NADPH:protochlorophyllide oxidoreductase (Rosinski and Rosen, 1972 ; Selstam and Sandelius, 1984; Masuda and Takamiya, 2004). In angiosperms, POR is the central enzyme required for the production of chlorophyll via a light dependent reaction (Lebedev and Timko, 1998). In the etioplast POR complexes with protochlorophyllide which is immediately (within 2 ms) converted to chlorophyllide upon activation with light (Heyes and Hunter, 2005). POR is responsible for the majority of chlorophyll synthesis as the prolamellar body with unstacked prothylakoids transitions to the mature thylakoids of photosynthetically active chloroplasts (Solymosi et al., 2007). Not only is POR activity light dependent, but the expression of POR-encoding genes has also been shown to be driven by light. Here a handful of photons steers the competence of the developing chloroplast by generating chlorophyll. While necessary for

photosynthesis, it is certainly not the sole entity that is required for the process. A cast of additional factors must be recruited to the rapidly developing plastid to facilitate photosynthetic functions. Their coordinated manufacture and assembly underlie maturation of the chloroplast during the transition to the light environment.

### 3.2 Molecular control of plastid development

Two of the phytochrome interacting factors (PIF1 and PIF3) have been generally shown to limit chloroplast development, primarily by interacting with the promoters of target genes. Their repression is lifted by activation of phytochrome as the PIFs are degraded by ubiquitin-mediated proteolysis

Its counterpart, PIF1, is generally regarded as a negative regulator of phytochrome activity. It also has been shown to be an active repressor of blue light response It also has been shown to repress chlorophyll biosynthesis (Huq et al., 2004) by binding to the G-box in genes associated with chlorophyll synthesis (Moon et al., 2008), as well as limit carotenoid biosynthesis by binding directly to the *PHYTOENE SYNTHASE* promoter (Toledo-Ortiz et al., 2010).

The phytochrome interacting bHLH protein PIF3 has been described to promote chloroplast development (Monte et al., 2004). Other reports examined early chloroplast development in *pif* mutants, unveiling clear roles as repressors of chloroplast development (Stephenson et al., 2009). The *pif1pif3* mutant exhibited a constitutively photomorphogenic phenotype in the dark. The plants accumulates protochlorophyllide, and showed more evidence of thylakoid stacking. Genes associated with chlorophyll and heme synthesis were also mis-regulated in the mutant, permitting accumulation of protochlorophyllide in darkness. The transition from darkness to light is phytochrome mediated, but the precise mechanisms that control the cross talk between compartments are now being elucidated. This is the subject of the next section of this chapter.

## 4. Biochemical communication between compartments

The hardware of photosynthesis is composed of many components that are encoded in the nucleus. Some of these components are labile, requiring a constant reloading of the plastid from parts transcribed in the nucleus, translated in the cytosol and located to the chloroplast. How do these two separate intracellular entities coordinate activities to ensure efficient interaction?

Earlier in this chapter there was a discussion of the etioplast and its light-driven transition to the chloroplast. This transition is critical, the timing is important, and the requirements of the plastid tax the cell as a whole. These organellar demands increase with the maintenance of autotrophy, as many proteins required for chloroplast function are encoded by genes in the nucleus. These two retrograde mechanisms have been referred to as developmental control and operational control, respectively (Pogson et al., 2008). To satisfy these demands, lines of careful biochemical coordination network the chloroplast and nucleus. This feedback between cellular genomes come as no surprise, as at some point (or probably many points) there was genetic exchange between the genes of the endosymbiont and the new resident cell. Evidence of this is rich, with islands of plastid genes present in the nucleus, likely benefiting from a finer control of gene expression, splicing and useful economic properties of the nuclear environment.

The cell requires the chloroplast to be in harmony with the nucleus- the two compartments working in concert with great precision. The chloroplast contains genome fewer than one-hundred open reading frames, yet function of the chloroplast requires over three thousand proteins. Regulatory steps that communicate demands to the nucleus to start construction of these proteins need to be precise. There are challenges to fluid exchange of signals between chloroplasts and the nucleus, in particular the presence of membranes that block passage of the vast majority of ions, peptides or other small molecules. The co-evolution between the endosymbiont and the plant cell had to involve a way to bypass these barriers.

There are many lines of evidence that show evidence of communication between chloroplast and nucleus. Pharmacological disruption of transcription in the plastid transcription or chlorophyll synthesis results in aberrant nuclear gene expression. Using tagetitoxin (a potent phytotoxin that inhibits select RNA polymerases including the plastidic one) to repress transcription in the chloroplast, Rapp and Mullet (1991) illustrated that the nuclear *cab* (now *Lhcb*) and *RbcS* transcripts failed to normally accumulate when the chloroplast was impaired. Other nuclear-encoded transcripts like actin responded normally, and the plants grew in a typical fashion. Disruption of plastid translation with lincomycin or disruption of carotenoid biosynthesis with norflurazon, an inhibitor of phytoene desaturase, also inhibits the normal accumulation of *Lhcb* and *RbcS* transcripts (Gray et al., 2003). The same treatments do not affect mitochondrial gene expression patterns. The chloroplast specificity was demonstrated through the use of erythromycin in peas, a compound that inhibits plastid translation but does not affect translation in the mitochondrion (Sullivan and Gray, 1999). The use of thujaplicin arrests the production of protochlorophyllide, causing a back-accumulation of Mg-ProtoIX and Mg-ProtoIXme. The treatment also hinders *Lhcb* accumulation (Oster et al., 1996), a result that is important to underscore, as chlorophyll biosynthetic mutants will later show similar effects when this step is interrupted.

While these pharmacological studies had utility, the introduction of nucleus-chloroplast signaling mutants brought new illumination to the processes of intra-compartmental feedback. The barley genotypes *albostrians* and *Saskatoon* fail to accumulate chlorophyll in various sectors. Analysis of nuclear gene expression indicated that *RbcS* and *Lhcb* gene expression levels were repressed in these regions (Hess et al., 1991; Hess et al., 1994). Early studies in other plants, including maize (Mayfield and Taylor, 1984) and mustard (Oelmüller et al., 1986), showed that plants deficient in carotenoid synthesis similar breakdowns in nuclear gene expression. There is also evidence that the redox state of photosynthetic electron transport affects the expression of these transcripts (Pfannschmidt et al., 2001; Masuda et al., 2003; Brautigam et al., 2009). Other evidence suggests that an accumulation of nuclear encoded proteins that fail to localize to the chloroplast is a retrograde signal (Kakizaki et al., 2009).

Together the observation that *Lhcb, RbcS,* and other nuclear genes are repressed when the chloroplast is not functioning correctly is significant because these transcripts accumulate rapidly in response to phytochrome activation. Active repression of these transcripts indicated that some factor reflecting the state of the chloroplast was overriding the normal response. The repression was selectively affecting specific nuclear genes, impairing activity of those required for chloroplast function. In times of internal dystrophy the plastid is instructing the nucleus that there is no need for various gene products.

The understanding of chloroplast-nuclear communication was accelerated with studies in *Arabidopsis thaliana*. By exploiting the powerful genetics of this system a series of mutants

were isolated that affected retrograde signaling. It was well demonstrated that application of norflurazon treatment actively repressed *Lhcb* accumulation by disrupting chlorophyll synthesis. Therefore, mutagenized plants with lesions in the repressing pathway should be allow expression of a reporter from an *Lhcb* promoter in the presence of the norflurazon. Susek et al. (1993) utilized this approach, using a truncation of the Arabidopsis *CAB3* promoter to drive hygromycin resistance and the *uidA* (GUS) gene. Seedlings growing on hygromycin and norflurazon would be candidates for genotypes possibly deficient in the plastid to nuclear signal. Results could be confirmed using the colormetric detection. The results of this screen identified non-complementing alleles called *gun* (for genomes uncoupled) mutants—*gun1*, *gun2* and *gun3*. These mutants were deficient in *Lhcb* and *Rbcs* repression, indicating that nuclear gene expression could be uncoupled from the chloroplast, allowing light-mediated changes in gene expression while the chloroplast remained undeveloped (Susek et al., 1993). Three other loci, *gun4, gun5* (Mochizuki et al., 2001) and *gun6* (Woodson et al., 2011), were later isolated.

Analysis of *GUN1* shows it to encode a pentatricopeptide repeat protein localized to the chloroplast (Koussevitzky et al., 2007). Analysis of promoters affected by *gun1* (and also *gun5*) mutation presented a suite of genes that shared an abscisic acid response element in the promoter, suggesting a role for ABA in retrograde signaling. The *gun2* and *gun3* mutants were shown to possess lesions in heme oxygenase and biliverdin reducase, respectively. Later it was shown that *GUN4* encodes a protein required for normal Mg chelatase activity, while *GUN5* encodes a required subunit of the Mg chelatase enzyme (Mochizuki et al., 2001). The *gun2*-*gun5* loss-of-function mutants disrupt genes essential for tetrapyrrole metabolism. Genetic evidence shows that they participate in the same signaling pathway, supporting the hypothesis that accumulation of a precursor could be the retrograde signal. *GUN6*-1D is a gain-of-function mutant that overexpresses a plastidic ferrochelatase (Woodson et al., 2011). Its overexpression leads to the hyper-accumulation of heme that could serve as a retrograde signal. In addition to the *GUN* genes, the *GOLDEN2-LIKE* (*GLK*) genes also have been shown to control similar sets of genes relevant to chlorophyll synthesis and antenna binding (Waters et al., 2009), playing central roles in communication between plastid and nucleus (Fitter et al., 2002).

When considered together the mutants and pharmacological treatments demonstrate that blocks in tetrapyrrole and/or heme synthesis may cause accumulation of precursor compounds that would leave the plastid (or trigger another mobile signal) leading to repression of plastid-associated, nuclear-encoded genes. While attractive, several lines of evidence reject this hypothesis. Mainly, there is no observed difference in Mg-ProtoIX or Mg-ProtoIXme is detected in plants with disrupted signaling responses (Mochizuki et al., 2008). Using sensitive LC/MS methods to identify chlorophyll precursors in norflurazon treated plants, it was shown that there was no effect on MgProtoIX when chlorophyll synthesis was disrupted (Moulin et al., 2008).

There is an undeniable chemical communication link between the chloroplast and nucleus. Genetic and biochemical tools suggest that chlorophyll precursors and/or heme play a part in the process, yet clearly it is not as simple as over-accumulation of a compound like MgProtoIX. While many careers and high-profile publications frame this question, there are answers to be resolved before a complete picture of retrograde signaling is understood.

## 5. Conclusions

The last two decades have brought tremendous resolution about how the guiding force of photons shapes plant biology, especially processes germane to photosynthesis. The 1990's produced a wellspring of genetic tools that would define several major classes of photosensors and their contiguous protein transduction partners. The last decade brought the utility of genomics tools that would help define the mechanisms and targets of light signal transduction events. New methods in imaging and improved reporter genes have allowed researchers to monitor small changes in plant growth and development, as well as localization and interaction between proteins in vivo. The challenge of the next decade will be to apply these basic discoveries in meaningful ways that escape the models. Here the rules that integrate light signals, change gene expression, alter development, and shape plant form may be manipulated to improve the production of food with less environmental impact.

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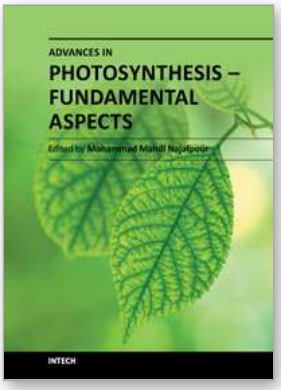


**Advances in Photosynthesis - Fundamental Aspects**
Edited by Dr Mohammad Najafpour

ISBN 978-953-307-928-8
Hard cover, 588 pages
**Publisher** InTech
**Published online** 15, February, 2012
**Published in print edition** February, 2012

Photosynthesis is one of the most important reactions on Earth. It is a scientific field that is the topic of many research groups. This book is aimed at providing the fundamental aspects of photosynthesis, and the results collected from different research groups. There are three sections in this book: light and photosynthesis, the path of carbon in photosynthesis, and special topics in photosynthesis. In each section important topics in the subject are discussed and (or) reviewed by experts in each book chapter.



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