Photoreceptors in Plant Photomorphogenesis to Date. Five Phytochromes, Two Cryptochromes, One Phototropin, and One Superchrome[1]

Winslow R. Briggs* and Margaret A. Olney[2]

Department of Plant Biology, Carnegie Institution of Washington, 260 Panama Street, Stanford, California 94305

Plants are bombarded by a myriad of signals, not just from their physical environment, but from friend and foe alike. As a consequence, they have evolved a remarkably sophisticated system of receptors and signal transduction pathways that generate appropriate responses. That light plays a major signaling role in plant development is not surprising. A plant's ability to maximize its photosynthetic productivity depends on its capacity to sense, evaluate, and respond to light quality, quantity, and direction. Likewise, the timing of developmental phenomena, such as flowering or entrance into dormancy, depends on a system of measuring and responding to changes in daylength. This article briefly explores how plant biologists have identified the various photoreceptors and how they have elucidated some of the early events in the transduction of light signals to ultimate plant responses.

A red, far-red-reversible chromoprotein, phytochrome, was the first photoreceptor identified. It is now known that multiple phytochromes exist and sometimes act independently of one another, sometimes redundantly, sometimes antagonistically, sometimes at the same time in development, and sometimes at different times. The first blue-light receptors to be identified were the two cryptochromes, chromoproteins that mediate several responses. More recently, another blue-light-absorbing chromoprotein, phototropin, has been identified as a photoreceptor mediating phototropism. A chimeric photoreceptor, phytochrome 3 (phy3), has been identified that contains both phytochrome and phototropin sequence motifs. For each of these photoreceptors, gene sequences are known, and plant biologists are working toward a greater understanding of their roles in plant development. Let us take a brief look at the events leading to our present knowledge of higher plant photoreceptors.

PHYTOCHROMES

Just over 40 years ago, workers at the U.S. Department of Agriculture laboratories (Beltsville, MD) discovered the first signaling photoreceptor in plants, a photoreversible pigment (9) that they called phytochrome (8). In the following years, photomorphogenesis (a study of the influence of light on plant development) developed as a strong subdiscipline of the field of plant physiology. Within this subdiscipline was a sharp division between those pursuing the phytochromes and those pursuing distinct blue-light receptors. Those studying phytochrome(s) had an enormous advantage in having at their disposal all of the classic phytochrome-mediated responses that were activated by brief pulses of red light interrupting darkness: These include activation of seed germination, inhibition of stem elongation in dark-grown seedlings, induction of leaf expansion, and regulation of flowering. In every case, the effect of red light was negated by subsequent immediate exposure to far-red light. This kind of photoreversibility was regarded as unassailable evidence for the participation of phytochrome. Borthwick et al. (3) had already predicted the existence of a photochromic pigment with red- and far-red-absorbing forms, and it was theoretically a simple matter for the Beltsville group to identify such a pigment. (In reality, it was not simple, as it required the development of some incredibly ingenious spectroscopy.)

During the mid-1970s it was generally assumed that a single phytochrome mediated the many red-, far-red-reversible photoresponses, and frantic efforts were under way in several laboratories to purify it and carry out its biochemical characterization (7). However, it was only in 1983 that both the Quail and Lagarias laboratories reported the purification of undegraded phytochrome and in 1987 reported that the first phytochrome gene sequence was published (see 4). By 1989, we knew that there were at least two different phytochromes in pea (1) and five different phytochromes in Arabidopsis (20). All of these phytochromes show varying degrees of amino acid sequence identity and similarity, and all of them carry a bilitriene chromophore phytochromobilin (14, 22). The Lagarias laboratory recently has provided con-

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[2] Present address: Department of Biology, Colorado College, 14 East Cache La Poudre Street, Colorado Springs, CO 80903–3298.

* Corresponding author; e-mail briggs@andrew2.stanford.edu; fax 650–325–6857.

vincing evidence that phytochrome functions as a photoreceptor kinase (an unusual Ser/Thr kinase with two His kinase-like domains; 25).

Current work based on molecular genetic studies that rely heavily on photomorphogenic mutants has made significant progress in unraveling downstream elements in the various phytochrome signal transduction pathways. These include signaling components such as heterotrimeric G proteins, cyclic GMP, calcium nucleotide diphosphate kinase 2, and calcium, as well as transcriptional regulators. In addition, both phytochrome A and phytochrome B have been shown to migrate into the nucleus under certain conditions, consistent with their proposed action at the transcriptional level in some of the responses they mediate (for phytochrome references, see 16).

After years of frustration, two laboratories have identified potential partners that interact directly with phytochrome. The Quail laboratory has shown that the nuclear basic helix-loop-helix protein PIF3 interacts physically with phytochrome only in its Pfr form and the complex dissociates if the Pfr is transformed back into the Pr form by far-red light (17). Likewise, the Chory laboratory has shown that a phytochrome-binding protein, PKS1, is phosphorylated by phytochrome in a light-dependent manner, with the evidence suggesting that it is a negative regulator of phytochrome B signaling (11).

BLUE-LIGHT RECEPTORS

In 1975, no blue-light receptor had been identified in higher plants. There was considerable controversy as to what the chromophore for a blue-light receptor might be. Somewhat less controversial was the (erroneous) notion that there was probably a single blue-light receptor, commonly designated cryptochrome, just as there was thought to be a single phytochrome. Gressel (13), who coined the term cryptochrome, cautioned against this simplistic interpretation as did Briggs and Iino (6), but it was surprisingly persistent.

At least a partial reason for this failing was that those studying blue-light receptors did not have the elegant photoreversibility assay that those studying the phytochromes had. Most of the action spectra for blue-light-activated responses resembled the absorption spectra of flavoproteins, with bands of activity in the blue and UV-A regions of the spectrum. Although many workers favored flavins as the probable chromophores, one school of thought championed carotenoids. As we shall see below, different photoreceptors have different chromophores, and both carotenoids and flavins (and pterins) serve in this role.

Progress in understanding the basic mechanisms of plant responses to red and far-red light was spectacular following the initial isolation and characterization of a phytochrome. In contrast, progress in understanding events triggered by blue light was severely impeded by the difficulty in identifying the blue-light chromophore(s) and/or receptor(s). Furthermore, plants contain innumerable flavoproteins and carotenoproteins, seriously complicating the quest for the one or the few that might function as blue-light receptors. Those studying phytochrome had no such bewildering array of candidates.

Cryptochromes

It was not until 1993 that Ahmad and Cashmore (2) first reported the discovery of cryptochrome 1 (cry1) in Arabidopsis. It turned out to be a protein with considerable amino acid sequence similarity to prokaryotic DNA photolyases. However, subsequent work showed that the protein had no photolyase activity and contained a C-terminal extension not found in the photolyases. Hypocotyls of mutants at the *CRY1* locus showed greatly reduced sensitivity to blue-light-induced inhibition of growth, and the mutants also showed reduced blue-light induction of the expression of several genes. Recombinant protein, produced in *Escherichia coli*, was subsequently found to bind both FAD and a pterin, methenyltetrahydrofolate, suggesting that like the photolyases cry1 contains two chromophores (see 5). It seem likely that these are the two chromophores bound in planta, but this hypothesis requires testing because earlier sequence studies implicated a deazaflavin (2). The Cashmore group has since identified cryptochrome 2 (cry2); like cry1, it is similar to the photolyases and contains a C-terminal extension (different from that of cry1). Cry2 is also involved in the inhibition of hypocotyl elongation and is involved in flowering as well. At present, little is known about the immediate consequences of photoexcitation of either of the cryptochromes, although given the known photosensitivity of flavins and the known mechanism of action of photolyases, it is likely that they act through some sort of redox-driven reaction. There is evidence that cryptochromes are localized to the nucleus, but to date no interacting partner has been identified (for cryptochrome references, see 5, 10).

Phototropin

In 1988, Gallagher et al. (12) first reported that blue light could activate the phosphorylation of a plasma membrane protein from the growing regions of etiolated seedlings. After extensive biochemical, genetic, and physiological characterization (see 21), there was strong evidence that this protein was not only the photoreceptor and kinase for its own phosphorylation but a photoreceptor for phototropism as well. Originally identified from the Arabidopsis mutant *nph1* (non-phototropic hypocotyl 1), it was subsequently named phototropin. Phototropin contains two PAS domains (domains first identified in the proteins PRE, ARNT, and SIM that are involved both

in protein-protein interaction and ligand binding; see 23) designated LOV domains because they are found in proteins regulating responses to light, oxygen, or voltage. Downstream from the LOV domains is a classical Ser/Thr kinase domain. Each of the LOV domains binds FMN as a chromophore to make the holoprotein (for phototropin references, see 5). Both FMN molecules undergo a photocycle: Light activation leads to the formation of a cysteinyl adduct with the FMN, an adduct that breaks down on a time scale of minutes in subsequent darkness (19).

Adiantum phy3

The story becomes even more fascinating when one looks at a lower vascular plant. Nozue et al. (18) recently identified a hybrid photoreceptor from the fern *Adiantum capillus-veneris*. Its N-terminal 566 amino acids show high homology to phytochrome. Moreover, recombinant protein, expressed in *E. coli*, and reconstituted with a phycocyanobilin chromophore, shows the red-, far-red-reversibility characteristic of phytochrome. However, downstream of a linking domain, the protein shows remarkable similarity to phototropin, containing two LOV domains and a Ser/Thr kinase domain. Hence this single chromoprotein has both phytochrome- and phototropin-like properties, and this author has on occasion referred to it as "superchrome" (Nozue et al. properly designate it phy3). It will be fascinating to learn how this complex three-chromophore photoreceptor functions to mediate some of the many fern light responses.

FUTURE PROSPECTS

The domain organization of the three known classes of plant photoreceptors and a prokaryotic photolyase are illustrated in Figure 1. In addition to our knowledge of the photoreceptors themselves, we are beginning to understand some of the downstream signaling events following phytochrome photoactivation. Photomorphogenic mutants have proved invaluable allies in this process (16). We have some understanding of the early events following photoexcitation of phototropin (19), evidence for an interacting protein (15), and indications that calcium may be involved (see 5). However, we have only untested hypotheses as to how the early photochemistry affects phosphorylation and how that phosphorylation is related to the events that lead to phototropic curvature. We have even less information on events immediately downstream of the cryptochromes.

The list of plant photoreceptors is still incomplete. Studies with Arabidopsis mutants indicate that neither the cryptochromes nor phototropin mediate blue-light-induced stomatal opening and that a carotenoid-based photoreceptor may regulate this response (see 5). To date, the photoreceptor(s) mediating blue-light-activated chloroplast movement are unknown. Likewise, UV-B activates signal transduction pathways leading to synthesis of UV-B-screening compounds (see 24), but the photoreceptor remains unidentified.

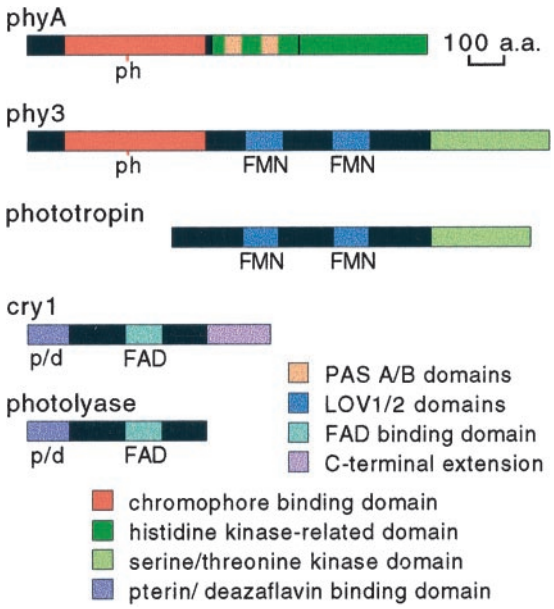

Figure 1. Domain organization of the three classes of known plant photoreceptors and a typical prokaryotic photolyase. PhyA, Arabidopsis phytochrome A; Phy3, *Adiantum capillus-veneris* phy3; Phototropin, Arabidopsis phototropin (nph1); Cry1, Arabidopsis cry1. Chromophores: ph, phytochromobilin; FMN; FAD; d, deazaflavin; p, pterin.

Although much remains to be done, the research of the past 25 years has seen enormous strides. Photomorphogenesis has moved from the physiology of plant light responses and the beginnings of the biochemistry of one photoreceptor to a sophisticated molecular genetic and biochemical knowledge of eight photoreceptors and their signal transduction pathways, with other photoreceptors awaiting discovery.

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