Phytochrome regulated gene expression

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Light is used by plants as a signal for many physiological and developmental processes. Phytochrome is the most extensively studied family of photoreceptors that plants use to perceive the presence and quality of light in their environment. While the initial action of the phytochrome molecule is not yet known, one important kind of response, changes in the expression of specific nuclear genes, has been intensively investigated. Although phytochrome-regulated promoters are complex and can also respond to other signals, specific DNA elements that are involved in conferring phytochrome responsiveness have been identified. Potential signal transduction pathway components include G proteins, cyclic GMP and Ca$^{2+}$/calmodulin. In addition, the study of transcription factors involved in phytochrome-regulated gene expression has yielded insights into some of the final steps of transcriptional regulation by phytochrome.

Key words: cis-elements / light-regulation / phytochrome / transcriptional regulation

PLANTS HAVE EVOLVED a number of different photoreceptors for perceiving and responding to light signals from the environment. Of the developmentally important photoreceptors, the phytochrome family of chromoproteins has been the most intensively studied; its structure and modus operandi are better characterized than that of other photoreceptors (e.g. blue and ultraviolet light receptors). At least two different genes encoding the phytochrome apoproteins are expressed in Angiosperms. In Arabidopsis five members of the phytochrome gene family have been identified and were shown to be expressed.1a The phytochrome apoproteins combine with a linear tetrapyrrole chromophore to give rise to two different types of the chromoprotein (for a recent review see ref 1, for nomenclature see ref 2). Type I phytochrome (PhyA) is generally more abundant in plants grown in darkness and decreases after exposure of the plants to light. Type II (PhyB) is present at very low levels in both light- and dark-grown plants. Both types of phytochrome molecules can exist in either of two photochemically interconvertible forms, Pr and Pfr. Phytochrome is synthesized in the Pr form, which has its absorption maximum in the red region of the spectrum. The Pfr form absorbs maximally in the far-red region. Brief illumination of dark-grown (etiolated) seedlings with red light (R) causes formation of the active Pfr form and is generally sufficient to elicit a response; the effect of R can be reversed if followed immediately by far-red light (FR), which substantially converts the molecule back to its inactive Pr form. This switch-like mechanism for triggering many phytochrome responses is a function of the absorption spectra of the two forms. The spectra significantly overlap, and an absorbed photon can effect the phototransformation in either direction. Saturating R (665 nm) converts about 80% of the total phytochrome to the Pfr form; saturating FR (730 nm) results in about 97% Pr and 3% Pfr. In light-grown plants, there is a photoequilibrium between Pr and Pfr that depends on the spectral quality of the light, and some phytochrome responses apparently depend on the ratio of the two forms rather than on the absolute amount (see article by H. Smith in this issue, pp 315-325). Thus, phytochrome serves as an exquisitely sensitive detector of light when a newly germinated seedling begins to emerge from the soil and as a sensor of the spectral quality of the light environment during the life of a plant. A diagrammatic representation of the phytochrome response system is shown in Figure 1.

Studies on the mechanism of phytochrome action have been prominent in plant biology research and date back to the discovery of phytochrome and its initial purification over 35 years ago.3 Despite the fact that much is known about the family of phytochrome proteins and the genes that encode them (reviewed in refs 1, 4, 5; see articles by A. Jones and M. Edgerton, pp 295-302, H. Smith, pp 315-325 in this issue), the initial step of signal transduction after the formation of Pfr remains unknown. There have also been extensive studies of the many
developmental and physiological responses that can be regulated by phytochrome, including seed germination, photomorphogenesis, shade avoidance, and flowering (see articles by H. Smith, pp 315-325, J. Reed and J. Chory, pp 327-334, A. Jones and M. Edgerton, pp 295-302, in this issue). It was proposed early in the history of phytochrome investigations that changes in gene expression were involved in such responses. The development of molecular technology, beginning in the 1970s, has allowed increasingly detailed characterization of phytochrome effects on the expression of specific genes (reviewed by refs 7, 8). Because such studies can be so clearly defined at the biochemical/molecular level, they offered the promise of allowing investigators to work back from a specific response to the activation of the signal transduction pathway upon photoconversion of Pfr to Pr. Although there is evidence that phytochrome action may also affect post-transcriptional events (reviewed in ref 8), this article focuses on the recent advances in our understanding of how phytochrome regulates the transcription of nuclear genes.

Much published work has dealt with 'light-regulation' of gene expression without clearly ascertaining which photoreceptor is responsible for the observed effects. Furthermore, experiments have not always been performed in a way that distinguishes the effects of light and dark treatments on development, senescence, photosynthesis or other physiological processes, from a direct effect mediated by phytochrome. For example, the fact that a gene is not expressed in etiolated seedlings but is expressed in mature green plants does not permit the conclusion that that gene is directly regulated by light. In fact, there is abundant evidence that the promoters of genes that are transcriptionally regulated by phytochrome are complex; these promoters also contain regulatory elements that can respond to a variety of signals, including other photoreceptors (e.g. ref 9), the state of development of chloroplasts (reviewed in ref 10), carbon metabolites that are products of photosynthesis (reviewed in ref 11), and other endogenous developmental signals (see ref 12 and references therein). Additionally, there have been many suggestions that the effects of light may interact with or be mediated by plant hormones, including gibberellins (e.g. ref 13; P.W. Morgan, personal communication) and cytokinins (e.g. refs 14, 15).

The question of which type of phytochrome is involved in regulating various responses is currently an active area of research, but is outside the scope of this review. Genes for both type I and type II phytochromes have been cloned. While some responses may depend primarily on phyA or primarily on phyB (see H. Smith, pp 315-325, in this issue), there is also substantial evidence for functional redundancy of the different phytochrome types in eliciting many responses, including the regulation of the transcription of specific genes. Thus far, there is no evidence to suggest that separate regulatory elements in a promoter are involved in
the response of a particular gene to different types of phytochrome.

**Genes regulated by phytochrome**

The abundance of RNA for many genes can be positively or negatively regulated by phytochrome. Not all of these have yet been shown to be regulated at the level of transcription. The early work in this area has been reviewed by Tobin and Silverthorne.  

Those genes for which transcriptional regulation by phytochrome has been demonstrated include genes encoding the small subunit of ribulose, 1,5-bisphosphate carboxylase/oxygenase (rbcS genes), the major light-harvesting chlorophyll a/b-proteins of Photosystem II (Lhcb genes, formerly called cab genes), and phytochrome (PHY genes), as well as genes encoding NADPH:protochlorophyllide oxidoreductase, ferredoxin and phosphoenolpyruvate carboxylase; these studies have used *in vitro* run-on transcription assays, promoter-reporter gene fusions in transformed plants, and transient expression assays. Another set of genes, designated NPR genes, whose transcription can be negatively regulated by phytochrome action, has recently been reported in *Lemna gibba*; the function of the encoded proteins remains to be determined. It is interesting that in the cases of gene families where the expression of individual genes has been examined, not all family members are strongly (if at all) regulated by phytochrome (e.g. refs 35-37).

Many additional nuclear genes have been identified whose mRNA levels can be regulated by phytochrome. Because they have not been shown to be transcriptionally controlled by phytochrome, they will only be briefly mentioned. This group includes glutamine-synthetase, asparagine synthetase, thionins, β-tubulin, a subunit of the oxygen-evolving complex, and a number of pea proteins of unknown function (reviewed by ref 8) as well as genes encoding nitrate reductase, a number of components of the photosynthetic apparatus in addition to Lhcb genes, chalcone synthase, a proline-rich protein, a glycine-rich protein, and a hydroxyproline-rich glycoprotein, small GTP-proteins belonging to the ras superfamily, ferredoxin dependent glutamate synthase, and glyceraldehyde-3-phosphate dehydrogenase. Transcript levels of RNAs encoding two putative homeodomain-leucine zipper proteins, presumed to be transcription factors, have also recently been reported to be induced by FR-rich light, and it was suggested that this effect was mediated by phyB.

Although phytochrome clearly is involved in the regulation of many plant genes, only four kinds of gene families have been closely examined to identify promoter regions involved in conferring phytochrome responsiveness. Investigations of rbcS, Lhcb, PHY and NPR gene promoters from a range of species has begun to provide us with details of the sequences and promoter structures that are involved in phytochrome responsiveness. Studies on each of these gene families are described below.

**rbcS genes**

The phytochrome responsiveness of rbcS genes has been extensively investigated in two plant species, pea and *Lemna gibba*. In both species, studies have delineated promoter regions important for phytochrome responsiveness, which are diagrammed in Figure 2. A 240 bp region of the pea *rbcS* 3A promoter was initially shown to be sufficient to impart phytochrome responsiveness in transgenic tobacco. The phytochrome regulatory elements were further limited to the region of the promoter from -166 to -50 relative to the start of transcription. This region contains two related motifs, termed Box II and Box III; Box II acts as a positive element for expression in white light. Both motifs are *in vitro* binding sites for a protein that has been named GT-1.51,52 Clones of a gene encoding a protein that can bind to these motifs have been isolated, but there is as yet no conclusive evidence that this protein is involved in the phytochrome responsiveness of this gene. Neither the GT-1 binding activity nor the abundance of the RNA encoded by these clones has been found to be altered in response to dark or light treatments. The regions at each end of box III (but not box III itself) have also been found to be important for expression, and each of these regions could bind a different phosphorylated protein; these proteins were called 3AF5 and 3AF3.53

In *Lemna gibba*, the transcriptional responsiveness of the rbcS gene family to phytochrome was first demonstrated by *in vitro* run-on transcription experiments using isolated nuclei. These studies were successfully extended to a single family member, the SSU5B gene, using transient transformation assays. Deletion analysis of the SSU5B promoter localized a region necessary for phytochrome
Figure 2. Phytochrome responsive regions of two rbcS promoters (adapted from refs 31, 50, 51, 55, 56; J. Degenhardt and E.M. Tobin, unpublished work). The striped boxes indicate regions sufficient to impart phytochrome responsiveness. Numbers represent the distance from the start of transcription. (A) The pea rbcS-3A promoter. Two protein binding sites within this region, box II and box III, both of which can bind GT-1 in vitro, are represented by open rectangles, and their sequences are provided above the promoter. Binding factors shown to interact with this region of the promoter, GT-1, 3AF5, and 3AF3, are shown as ovals. See text for details. (B) The Lema gibba rbcS SSU5B promoter. The striped box extends to position +197. The shaded box indicates a region necessary for phytochrome responsiveness. The sequence of the binding site for the nuclear protein LRF-1 is provided, with the I box motif indicated (see text for details).

responsiveness to between −205 and −83 from transcription start. Binding of nuclear proteins to this region was detected by in vitro DNA mobility shift assays. The amount of this binding activity increased after a R treatment of etiolated plants, and the activity was given the name LRF1 (Light Regulated Factor 1). LRF1 that was bound in vitro protected a region of this promoter that extended from −165 to −140 from DNase I digestion. When a fragment including the footprinted region and six additional bp (from −165 to −134) was deleted from a construct consisting of 985 bp of the native promoter fused to a reporter gene, phytochrome regulation of the reporter gene expression was substantially lost (J. Degenhardt and E.M. Tobin, unpublished work). Therefore, LRF1 and its binding site appear to be important for the regulation of this rbcS gene by phytochrome. This region of the promoter does not show sequence similarity to Box II or Box III sequences of the pea rbcS-3A promoter. It does, however, contain a GATAAG motif found in a number of other rbcS gene promoters (reviewed by ref 57); GATA motifs have also been found in Lhcb promoters (see below). The GATAAG motif was originally noted as part of a conserved region in rbcS promoters of several species and named an I box. This region of an Arabidopsis rbcS promoter was shown to be capable of binding, in vitro, a nuclear protein factor named GA-1 that also bound to other regions containing GATA motifs. Recently, a LRF1-like binding activity, which was more abundant in light-grown than in dark-treated plants and which could bind to the I box motif, has been found in tomato extracts. Proteins binding to GATA elements in both Lhcb and rbcS promoters have been observed by a number of groups, but no regulatory role for these GATA-binding activities has yet been established.
**Lhcb genes**

The phytochrome responsiveness of specific *Lhcb* promoters was first shown in tobacco plants transformed with the pea *Lhcb AB80* promoter\(^{25}\) and with the wheat *cab-1* promoter\(^{24}\) fused to reporter genes. The region of this wheat promoter sufficient for phytochrome responsiveness was further limited to between \(-357\) and \(-89\).\(^{63}\) *Lhcb* promoters from *Arabidopsis* and *Lemma* have also been examined for regions involved in phytochrome responsiveness. The results of these studies on *Lhcb* promoters are summarized in Figure 3.

In *Arabidopsis*, two different members of the *Lhcb* gene family have been examined. In the *cab165* gene, a 78 bp fragment, extending from \(-111\) to \(-33\), has been shown to be sufficient to confer both phytochrome and circadian responsiveness to a reporter gene (S.A. Kay, personal communication). For the *cab140* gene, sequences downstream of \(-183\) are sufficient to confer phytochrome responsiveness to a reporter gene (ref 64; D. Kenisgbuch, E.M. Tobin, unpublished work). A protein which can bind to the *cab140* promoter in the CA-rich region from \(-138\) to \(-99\) was also identified and called CA-1.\(^{64}\) This binding activity was of particular interest because it was absent in the *det1* mutant of *Arabidopsis*. This mutant develops a light-grown morphology when seedlings are grown in darkness, and the *Lhcb* genes are expressed at a high level in the dark.\(^{65}\) Thus, CA-1 might function as a repressor of transcription in the dark and could be involved in the phytochrome regulation of this gene. Recently, we have found that mutation of the CA-1 binding region, in the context of a 1.15 kb fragment of the *cab140* promoter, eliminates the phytochrome regulation of a reporter gene in transgenic *Arabidopsis* (D. Kenisgbuch, E.M. Tobin, unpublished work). The exact nucleotides within the footprinted region that are important for phytochrome responsiveness remain to be determined.

Our recent work on the *Lhcb* gene *cab AB19* of *Lemma gibba*\(^{66}\) has precisely established the phytochrome regulatory elements of this promoter.\(^{39,66a}\) The particle bombardment system was used for transient assays of constructs in which portions of this promoter were fused to a reporter gene and introduced into intact *Lemma* plants. Analysis of 5' and internal deletion mutants showed that a region(s) required for phytochrome responsiveness was present between \(-174\) and \(-104\). In addition, although a 171 bp fragment of the promoter (from \(-239\) to \(-69\)) was sufficient to confer phytochrome responsiveness to a minimal ubiquitin promoter, a smaller 72 bp fragment (from \(-170\) to \(-99\)) was unable to do so. This result suggests that either the base pairs from \(-174\) to \(-170\) are essential, or that the regions between \(-174\) and \(-104\) that are necessary for phytochrome regulation require additional elements for normal regulation. DNA mobility shift assays and in vitro footprinting showed that multiple factors in

\[\text{Figure 3.} \] Phytochrome responsive regions of *Lhcb* promoters (adapted from refs 63, 64, 66a; S.A. Kay, unpublished data; D. Kenisgbuch, E.M. Tobin, unpublished data). Striped boxes indicate regions sufficient to impart phytochrome responsiveness, and shaded boxes represent regions necessary for phytochrome responsiveness. Numbers represent the distance from transcription start. CA-1 is a DNA binding activity found in wild-type *Arabidopsis* but not in *det1* mutants. LS5 and LS7 refer to the two regions of the *Lemma* promoter necessary for phytochrome responsiveness (see text for details). Within these regions are conserved CCAAT and GATA elements, designated by C and G, respectively. The locations of these motifs in the wheat and *Arabidopsis* promoters are also shown.
**PHY genes**

The finding that a brief R treatment resulted in a rapid decline in transcription of one or more members of what later was shown to be the *PHYA* gene subfamily was the first demonstration that phytochrome could regulate its own transcription. Since that time, substantial progress has been made in identifying the specific sequences of a *PHY* promoter responsible for this responsiveness.

*PHYA* genes from monocots, which encode type I phytochrome, have thus far been the only *PHY* promoters examined for transcriptional responsiveness to R and FR. Extensive studies have been carried out with the *PHYA3* promoter of oat, and some of the regulatory regions found in this promoter are shown in Figure 4A. The promoter was shown to respond to phytochrome in a transient assay system using particle bombardment of dark grown oat and rice seedlings. Internal deletion and linker scan mutants delimited the phytochrome responsive region to an 11 bp sequence centered at -75. This element was called RE1. It appears to be a negatively acting phytochrome regulatory element since sequence alterations within this region cause de-repression of the *PHYA3* promoter in the presence of high Pfr. Three general positive regulatory regions (PE1, PE2 and PE3) were also identified and occur downstream of -635, with PE3 contiguous to and upstream of RE1. A protein that can bind to PE1 has been cloned. As shown in Figure 4B, seven nucleotides at the 5' end of the RE1 element (CATGGGC) are identical in oat and maize *PHYA* promoters, and the element is highly conserved in all *PHYA* promoters that have been examined to date. The PE3 element also shows substantial conservation among these species. In *vitro* footprinting demonstrated that proteins bound both within the RE1 element and over a portion of the PE3 region. This binding activity was equivalent in extracts from plants treated with R or FR light.

The rice *PHYA* promoter has also been studied, and a factor that binds to a region shown to be critical for its expression has been cloned. The binding site for this protein (the protein has been designated GT-2) is related to the box II motif in the *rbcS-3A* promoter of pea (discussed above). GT-2 appears to function as a transcriptional activator. However, a specific role of GT-2 in the phytochrome regulation of the rice *PHYA* promoter has not been demonstrated, and the motif to which it binds is not present among the positive elements of the oat *PHYA* promoter. The level of mRNA encoding GT-2 was not affected when dark-grown seedlings were treated with R or FR, but decreased when they were transferred to white light. The GT-2 amino acid sequence has regions that are homologous to a protein thought to bind to the box II motif of pea *rbcS*, but it is not known if the regulatory functions of the two proteins are similar.

**NPR genes**

Another set of genes whose expression was decreased in response to phytochrome action were isolated from *Lemna gibba* by screening a cDNA library for clones that encoded RNAs whose levels increased in response...
ABA, and further work is required to determine the relationship between phytochrome and ABA responsiveness of these genes. ABA has been shown in several species to repress transcription of *rbcS* and *Lhcb* genes. Therefore, it may be useful to re-examine the effects of extended dark-treatments (sometimes called ‘dark-adaptation’) for possible effects on ABA levels in other species.

### Signal transduction chain(s)

Although very little is known about the mechanisms by which phytochrome regulates the wide variety of responses that have been reported to be under its control, there are a number of potential signal transduction chain components that have been implicated as important for various of the responses. A review by Deng discusses some of the recent progress in this area, and the isolation and characterization of mutants to identify components involved in phytochrome regulation is presented elsewhere in this issue (see article by J. Reed and J. Chory, pp 327-334, in this issue). Therefore, we will focus on specific molecules that may be part of the phytochrome signal transduction pathways.

Calcium ions have long been known to be required for many events that are triggered by R (see reviews in refs 78, 79). Additional research has also suggested that Ca$_2^+$/calmodulin is a component of phytochrome signal transduction (e.g. refs 80-83). More recently, the involvement of G-proteins in the transduction chain has received experimental support (e.g. refs 84-87). However, the most direct demonstration that both the Ca$_2^+$/calmodulin system and G-proteins are components of the phytochrome signal transduction chain leading to altered gene expression comes from some remarkable experiments with a phytochrome-deficient mutant of tomato. Microinjection of individual hypocotyl cells of seedlings of this tomato mutant was used to test the effect of putative signaling intermediates on the expression of a co-injected reporter gene encoding β-glucuronidase (GUS) fused to a *Lhcb* promoter. The injection of either phytochrome or GTPγS (which activates G proteins) with the reporter gene was able to promote GUS expression. If G protein inhibitors were co-injected with phytochrome, GUS expression was inhibited. Furthermore, injection of Ca$_2^+$ or Ca$_2^+$-activated calmodulin could also stimulate the expression of GUS. A second phytochrome mediated effect, the production of anthocyanin, was stimulated...
by both phytochrome and GTPγS, but not by Ca^{2+} or Ca^{2+} activated calmodulin. Thus, at least two pathways must be involved in phytochrome signal transduction; one requires the activation of heterotrimeric G proteins and does not involve calmodulin (anthocyanin synthesis) while the other involves both G proteins and calmodulin (regulation of the \textit{Lhcb} promoter). Further work\(^9\) has shown that cyclic GMP is a component of the pathway that regulates anthocyanin production and that it is also necessary for the complete development of chloroplasts in this system.

Phytochrome effects on K⁺ channels have been implicated as important for rotation of chloroplasts in the green alga \textit{Mugania}\(^9\) and for leaflet movement in \textit{Samanea saman}.\(^9\) However, these reports provide no evidence of whether such effects are related to changes in gene expression. Phosphoinositides and related compounds have also been suggested to play a role in phytochrome signal transduction, but no causal links have been established (see ref \(92\)).

Another process that has been implicated in some phytochrome responses is protein phosphorylation/ dephosphorylation (reviewed in ref \(93\); more recent papers include ref 94-96). The experiments in this area have not always been easy to interpret (see ref 5 for a discussion of some of the issues), but since phosphorylation is known to affect the binding of many transcription factors, including several that interact with light-regulated genes,\(^35,63,97-99\) it is reasonable to think that phosphorylation events play a role in phytochrome signaling. Lin \textit{et al}.\(^100\) reported that the abundance of RNAs for several protein kinase homologues in pea changed during greening. However, Romero and Lam\(^36\) found that protein kinase inhibitors had no effect on phytochrome-mediated induction of \textit{Lhcb} RNA in soybean cell cultures. The finding that protein phosphatase activity is required for light-inducible gene expression in maize\(^36\) is, perhaps, the most direct evidence to date implicating the importance of the phosphorylation state of proteins in the phytochrome transduction pathway leading to altered gene expression. Also of interest in this regard is a report that a moss phytochrome may itself be a light-regulated protein kinase\(^101\) and the recent report that a protein kinase specific for Pr phosphorylation was co-immunoprecipitated with phytochrome from maize using an oat phytochrome anti-serum.\(^102\)

Finally, some information has been obtained about protein factors that might be involved in the final steps of phytochrome regulated transcription. Regulatory regions of promoters involved in binding such factors and some of the factors that can bind to these regions have already been described. Although DNase footprinting of tomato \textit{rbcS} genes showed no differences in binding between nuclear extracts from dark-grown and light-treated cotyledons,\(^69\) there are a number of DNA binding activities that have been described which differ in abundance between etiolated and green tissue or change in abundance under various light conditions.\(^49,56,60,103-110\) One of these activities, GBF3, has been cloned and is a member of the G-box binding protein family.\(^108\) It is most abundant in dark-grown leaves and in roots, and can form heterodimers with other family members. The GT-2 RNA in rice is also more abundant in dark grown seedlings than after illumination with white light (ref 73; and see section on \textit{PHY} genes). Recently, it has been reported that G-box binding proteins from parsley are transported from the cytoplasm to the nucleus in response to a light treatment.\(^99\) However, thus far, a relationship between factor abundance/activity and R or FR illumination has been observed in only two cases.\(^49,56\) Additionally, it is still unclear whether phytochrome controls binding activities that have been demonstrated to differ in response to light/dark treatments.

**Conclusion**

Although much has been learned in recent years about the regulation of transcription of specific genes by phytochrome, there are still important questions that remain unanswered. Primary among these is the mode of action of the phytochrome molecule itself. Studies of promoters of various families of nuclear genes that are controlled by phytochrome have suggested that there are a number of different sequence elements that may be involved in phytochrome regulation. Those that have been identified occur within 200 bp from transcription start. However, more extensive studies are necessary before general conclusions can be drawn about what exactly is needed to confer phytochrome responsiveness to a promoter. We are only at the beginning of understanding what transcription factors are involved in phytochrome responses, and we know virtually nothing about how phytochrome action regulates their activity or amount. There has been progress recently in identifying some of the components of the signal transduction pathways, and
both biochemical and genetic studies will continue to be important in furthering our knowledge in this area. The extensive research on phytochrome regulation of transcription currently being undertaken in many laboratories should reward us in the near future with a clearer understanding of how this fascinating pigment-protein molecule achieves at least some of its many effects on plant growth and development.

Acknowledgements

We thank Drs Philip Thorner and Arthur Grossman for helpful comments on the manuscript. The work reported from our laboratory, including the unpublished work, was supported by NIH grant GM-23167. D.M.K. is currently supported by an NSF Postdoctoral Research Fellowship in Plant Biology.

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