The Red Side of Photomorphogenesis[1]

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The importance of light to normal plant growth and development cannot be overstated. As sessile photoautotrophs, plants depend on efficient light capture to compete and reproduce successfully within a relatively restricted geographical realm. For this purpose, these organisms have evolved very sophisticated sensory networks for monitoring the status of several important features of their illuminated surroundings including light intensity, duration, quality, and direction (Kendrick and Kronenberg, 1994). Response to these light signals in the form of altered plant growth and development is termed photomorphogenesis, a process that is distinct from that of photosynthesis, where far greater quantities of light serve as a source of energy for the fixation of carbon. The entire photomorphogenic process rests upon a set of specialized photochromic sensory receptors falling into at least three distinct known classes: phytochromes, cryptochromes, and phototropins. Structural properties of these photoreceptors essentially restrict spectrally important regions for photomorphogenesis to the UV-A and -B, blue, and red portions of the electromagnetic spectrum.

Historically, most progress toward understanding the molecular and cellular processes that underlie photomorphogenesis has come from studies of red-light sensing, which spans a relatively broad spectral region (approximately 600–750 nm) to include both red and far-red (approximately 700–750 nm) light. In terms of photomorphogenesis, this region is unique because phytochrome is the only known photoreceptor that absorbs light here exclusively for photosensory purposes. And as a consequence, this characteristic of red-light sensing provides photobiologists with a unique capacity to probe the mechanics of phytochrome-regulated development in isolation from other photosensory systems. However, as an *Update* on red-light sensing, the intent here is not to provide a comprehensive review of phytochrome because several excellent and quite recent articles are already available to serve this purpose beautifully (Neff et al., 2000; Smith, 2000; Fankhauser, 2001b; Nagy and Schäfer, 2002; Quail, 2002; Wang and Deng, 2003). Rather, this *Update* is intended to provide new students and educators with a primer to photomorphogenic research in plants. Specifically, it will use both historical and more recent contexts of phytochrome research to describe how our understanding of red-light sensing has evolved over time as well as the challenges that must be successfully addressed for continued progress in the future.

A FUNDAMENTAL FRAMEWORK FOR THE PHOTORECEPTOR

It has been just over 50 years since the discovery of phytochrome by Borthwick et al. (1952), the result of their stunning and now classic finding that light-regulated lettuce (*Lactuca sativa*) seed germination operates through a red/far-red photoreversible process. This unique photosensing feature greatly facilitated all subsequent physical characterization of phytochrome and the photophysiology that it controls. And over this time, phytochrome research has continued to advance through the exploitation of technological achievements in several areas including spectroscopy, biochemical purification, immunochemistry, and molecular genetics (Kendrick and Kronenberg, 1994). What general picture of phytochrome has emerged from these past decades of research, and where is the field currently poised to uncover it further?

In plants, where the majority of physical characterization has been performed, phytochrome is a relatively large dimeric chromoprotein, with each monomeric unit being approximately 125 kD (Kendrick and Kronenberg, 1994). Its light-absorbing entity, the chromophore, is a linear tetrapyrrole (phytochromobilin) that is covalently attached autocatalytically via a thioether linkage to a Cys residue of the polypeptide located about one-third the length along the polypeptide sequence from the amino terminus. The photoreceptor can exist in either of two photointerconvertible conformations—a biologically inactive Pr form ($\lambda_{max} \sim 670$ nm) and a biologically active Pfr form ($\lambda_{max} \sim 730$ nm). Phytochrome is synthesized as Pr and is localized initially in the cytosol. Absorption of light by either form induces a conformational change to the other spectral form, thus enabling a flipping between inactive and active states. Analysis of mutant forms of phytochrome has demonstrated that the amino-terminal portion of the polypeptide is critical to the photosensory properties of the holoprotein, whereas regions within the carboxy-terminal two-thirds of the molecule are important to the regulatory function of the photoreceptor.

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Is there only one type of phytochrome in plant cells? No. Molecular cloning and sequence analysis have shown that phytochrome is encoded by a small multigene family in higher plants, likely evolving from ancient origins as indicated by the recent discovery of phytochrome in certain bacteria (Hughes et al., 1997; Yeh et al., 1997; Bhoo et al., 2001). In Arabidopsis, where the body of recent and current molecular research is proceeding, phytochrome is represented by five members labeled A through E (Clack et al., 1994). Sequence analysis of these plant phytochromes suggests that they arose through four primary duplication events during plant evolution, with the initial split producing the A and B major groups about the time of seed plant appearance (Mathews and Sharrock, 1997). Further separations occurring later in evolution led to the five established family members recognized presently.

Most biophysical and functional characteristics for the phytochromes are known for phytochromes A and B (phyA and phyB, respectively; Quail et al., 1994). The relative amounts of these phytochrome types vary according to the ambient light conditions. In this regard, phyA was found to comprise the great majority of photoreceptor type in dark-grown (etiolated) tissue, whereas light-grown green tissue showed this balance shift toward phyB. This dramatic change in photoreceptor proportion results from differences in the expression and stability of the different phytochrome types. PhyA as Pfr is relatively light labile compared with its Pr conform and suppresses its own expression. In contrast, phyB is constitutively expressed and relatively stable as Pfr (Kendrick and Kronenberg, 1994).

The molecular confirmation that plants contain distinct and differentially regulated phytochrome types strongly indicated that they might possess shared and/or unique regulatory functions during normal development. This possibility was specifically addressed and validated with the isolation and subsequent analysis of photomorphogenic mutants deficient in one or more phytochrome types (Nagatani et al., 1991, 1993; Somers et al., 1991; Parks and Quail, 1993; Whitelam et al., 1993). Numerous such studies, both older and recent, have now confirmed that a variety of functional relationships exist among the phytochromes, including shared, distinct, and even seemingly antagonistic signaling programs (Whitelam and Devlin, 1997; Devlin et al., 1998, 1999; Franklin et al., 2003b). One major outcome of these works found that phyA controls many processes associated with de-etiolation occurring during seedling emergence and dominates responsiveness to far-red-enriched conditions (termed the far-red high-irradiance response; Smith, 2000; Quail, 2002). PhyB also appears to regulate many of the same processes associated with de-etiolation, such as the inhibition of stem elongation, but it is more important to conditions rich in red light and for sustained developmental progress under longer term natural lighting conditions including adult adaptive responses such as shade avoidance (Smith, 2000; Quail, 2002). That red and far-red light appeared to affect the same processes in a similar manner but through different photoreceptor types, gave the impression that phyA and phyB act in opposition to each other under a single common light regime. But it now appears that this is actually an evolved adaptation to extend the regulatory capacity of the phytochromes over a broader spectral range (Smith et al., 1997). Of the other phytochrome types found in Arabidopsis, more recent mutant studies indicate that, in general, the roles for phyD and phyE appear to be partially redundant to those of phyB in seedling establishment and flowering and may represent an evolved adaptive means to adjust to varying changes in microenvironment (Franklin et al., 2003b). And recent reports describing phyC-deficient mutants have tested ideas concerning possible roles for this photoreceptor that were suggested by earlier studies of phyC-overexpressing transgenic plants and photoreceptor mutants deficient in all other phytochrome types (Qin et al., 1997; Franklin et al., 2003a, 2003b; Monte et al., 2003).

FORMULATING SIGNALING NETWORKS

Primary Signaling

A central and persistent goal of phytochrome research has been to identify and arrange the cellular and molecular steps underlying the photoreceptor's ability to regulate photomorphogenesis. Early focus was directed toward determining a primary mechanism for photoreceptor function. Yet a clear answer here is still not available. In general, two fundamental views for phytochrome action have been considered. One hypothesis argues that phytochrome-regulated responses are the direct outcome of light-signaled alterations in gene expression. The other view sees many light responses as a manifestation of biochemical/biophysical changes occurring in the cell through pre-existing signaling components. In fact, the reality is probably a complicated combination of physiological processes and alterations in gene expression. Numerous reports of phytochrome-regulated changes in gene expression have supported the former hypothesis for many years (Kuno and Furuya, 2000). And recent evidence demonstrating that phytochrome moves from the cytoplasm into the nucleus upon phototransformation to Pfr clearly implicates a functional role for phytochrome in the regulation of nuclear gene expression (Sakamoto and Nagatani, 1996; Kircher et al., 1999; Yamaguchi et al., 1999; Nagy and Schäfer, 2002). In this regard, a very tantalizing collection of work demonstrating that phyA and phyB can directly associate with a transcriptional complex to alter expression of developmentally important genes indicates that the signaling

cascade can be extremely short (Ni et al., 1999; Martinez-Garcia et al., 2000; Zhu et al., 2000).

But these observations supporting such a rudimentary signaling cascade do not necessarily invalidate the possibility for other distinct functions of phytochrome in the processing of light information. One large class of proteins important to many aspects of photomorphogenesis is the COP/DET/FUS family (Schwechheimer and Deng, 2000). This group of nuclear-localized factors appears to affect photomorphogenesis through the selected proteolytic degradation of important downstream-signaling elements (Osterlund et al., 2000; Schwechheimer et al., 2001; Schwechheimer and Deng, 2001). Evidence indicates that the phytochromes (and possibly the cryptochromes) alter the activity of this group by controlling their nuclear abundance (Osterlund and Deng, 1998). How this occurs is still not known. There is now also strong evidence to support the long-proposed idea that phytochromes can act as protein kinases to regulate development (Yeh and Lagarias, 1998; Fankhauser, 2001a). The identification of PKS1 as a kinase substrate and putative phytochrome-signaling partner supports the hypothesis that phytochromes might control photomorphogenesis by regulating phosphorylation status (Fankhauser et al., 1999). That this factor is cytoplasmically localized leaves open the question of whether phytochrome can control photomorphogenesis through means not wholly centered on regulated nuclear gene expression. Some responses controlled by phytochrome are still best understood by invoking a role for the photoreceptor in the modulation of cellular ionic balances (Fondeville et al., 1966; Smith and Jackson, 1987; Bossen et al., 1988). Therefore, in the face of these present data, it seems that the functional capacity of the phytochromes is probably not singular.

Downstream Signaling

The primary molecular processes that occur between the phytochromes and their immediate reaction partners represent just the first step in a series of likely separate and interdependent signaling networks that together embody photomorphogenesis. Describing the full list of components necessary to complete these processes and understanding how they are ordered and regulated within these signaling cascades is one of the great challenges that faces the plant photobiology field today. That phenotypic responses can require minutes to days to develop indicates that signaling pathways can be relatively short or quite long. How extensive and varied are these cellular processes? Currently, the most logical and direct way to address this question is to organize systematically the many pathways, with their included signaling elements, into a time-ordered sequence. This is being done in two general ways. The first represents a stepwise approach proceeding along a given pathway originating with the photoreceptor. Information gained concerning how individual phytochromes function (e.g. mutant studies demonstrate that only phyA mediates responses under continuous far-red high irradiance conditions) is used to design traps or screens for targeting components that are particular to a given phytochrome or shared between them. This information is then used subsequently to design strategies whereby the next component in the pathway can be exposed. The other method for identifying downstream elements, made possible by the recent completion of the Arabidopsis genome sequence, involves global expression surveys to highlight en masse all entities that have changed within a specified time frame as the result of phytochrome signaling. The clear assumption in this approach is that many photomorphogenic responses occur through mechanisms that necessitate altered patterns of gene expression.

Forward genetic approaches used to screen for specific photomorphogenic mutants or modifiers, including suppressors and enhancers of previously described signaling mutants, have been used successfully to identify many gene products important to phytochrome-regulated development. The growing understanding of what responses the different phytochrome types regulate under specified conditions has allowed investigators to identify signaling elements both specific and shared between different photoresponses and phytochrome types. The screening strategy used to identify most but not all of these phytochrome signaling mutants hinges on finding individuals that display aberrant stem growth characteristics under specific light conditions compared with the balance of the population. The current number of different genes identified in this manner is about 20, and they are described more specifically elsewhere (Quail, 2002; Wang and Deng, 2003).

Although this more conventional phenotypic screening approach has been quite productive, it also suffers from limitations that inevitably restrict its overall utility. First, because phenotypic screens can only be conducted on known phytochrome-controlled responses, it therefore follows that knowledge of all responses regulated by the phytochromes would be required to design phenotypic screens that could potentially encompass all signaling components. This limitation, sufficiently imposing in itself, is further compounded by several additional drawbacks. Knowing that a particular phenotype is controlled by a given phytochrome type does not necessarily mean that a phenotypic screen can be easily devised to expose abnormally responding individuals. For example, experimental conditions that were previously thought necessary to reveal an aberrant phenotype may be insufficiently defined. The significance of this point is revealed by recent work demonstrating the effect of photoperiod and temperature on the hierarchy of functional roles for the different

phytochrome types (Halliday and Whitelam, 2003). It is also best, but not mandatory, that the monitored phenotype is detectable during a time in the life-cycle that is permissive to a large screening throughput. In this instance, screens of young seedlings can lend themselves better than those of adults because of a greater overall developmental uniformity among individuals and larger available population density. Any successful screening strategy would also require that redundancy does not exist within otherwise separate signaling networks that could affect this same phenotype under the particular experimental screening conditions used. For example, if one gene product affects leaf expansion under certain conditions, but a different gene product also controls this response through this signaling cascade or an alternate one, then it is possible that the aberrant phenotype would not surface in the presence of a mutation in either gene alone. And finally, a discrete developmental window for the screen may exist to restrict the time during which any aberrant individual could be readily identified. Taken together, it would appear unreasonable to expect that classic forward genetic screens can be used to identify all the necessary signaling components involved in photomorphogenesis. What other approaches can be exploited that avoid some of these limitations?

Yeast two-hybrid screens have been used to identify entities that associate directly with phytochrome or known downstream components important to the regulation of photomorphogenesis. This molecular genetic approach avoids any need for a photomorphogenic response as part of the screening strategy and is designed to target specifically the immediate partner to the photoreceptor or a known downstream signaling intermediate. It has been used successfully to identify prospective intermediates in light-signaling through the phytochromes (Ni et al., 1998; Choi et al., 1999; Fankhauser et al., 1999). However, this approach is limited by characteristics that preclude its use as a tool for unlocking significant portions of downstream signaling networks. To construct an individual screen for functional interacting partners, one of the components must be predetermined. As such, the progress in identifying downstream components necessarily proceeds slowly in a single stepwise fashion along a given signaling sequence. This approach also necessitates subsequent control tests (e.g. insuring that interaction is not indicated in plate assays where either the known "bait" or newly identified "prey" proteins are not expressed) to validate the authenticity of the interaction (McAlister-Henn et al., 1999).

A new and promising means to identify and catalog a large number of signaling components important to phytochrome-regulated development involves the use of specifically designed genome-wide expression surveys. This approach has been used very successfully in global scans for putative elements downstream of phyA specifically (Tepperman et al., 2001). It has also served as a means to examine the expression profiles displayed in genetic backgrounds that are mutant in previously identified components downstream of phyA signaling (Wang et al., 2002). The clear advantage of this approach over others mentioned rests on its ability to expose, in one "snapshot," an extensive number of genes potentially important to light-regulated development in plants. It represents a broad and unbiased genome-wide survey for genes expressed differentially as a result of environmentally induced conditions or through differences in genetic backgrounds. The number of genes identified by these surveys that are potentially important to light signaling is quite substantial. A general comparison of separate surveys for Arabidopsis genes regulated by long-term ($\geq$24 h) far-red-light treatment (phyA-exclusive photoregulation conditions) indicates that approximately 10% of the genes represented on these microarrays (total genes represented number about 8,000, or nearly one-third of all Arabidopsis genes) see a 2-fold or greater change in expression (Tepperman et al., 2001; Wang et al., 2002). Interestingly, over the initial hour of far-red-light treatment, the percentage of genes changing is less than 1% and is substantially skewed toward genes encoding known or putative transcriptionally related proteins (Tepperman et al., 2001). This result lends significant favor to functional models for phytochrome signaling that ascribe a major role to altered gene expression. Comparisons of expression profiles for different signaling mutants found that many changes were the same, thereby indicating that these mutations affect common signaling pathways (Wang et al., 2002). Additionally, the timing (early versus late) for the action of these mutationally defined downstream elements was inferred from the relative number of genes affected in a given signaling mutant background (Wang et al., 2002). The task remaining is to determine which of these identified genes possess authentic roles in light-signaling processes and where they fit precisely in the timing sequence of the developmental program. One important caveat of this general method is that these surveys can only address instances in which altered expression states are a necessary feature of the signaling process. Any signaling step that does not depend on changes in transcript abundance, such as protein phosphorylation or degradation, among many others, of course would not be detected by an expression profiling approach. These deficiencies could be addressed by using alternative profiling surveys, including proteomics and phosphorylomics. By comparison, however, such strategies still require much further development.

TERRA INCOGNITA

The Importance of Timing

The level of understanding that research is attempting to achieve with regard to plant photomorphogenesis is reflected in the degree of signaling detail that has been uncovered recently. The clear goal is to attain a state of comprehension that grants the capacity to predict how a plant would respond to a given light regime through precisely defined and networked signaling cascades. Such a capacity embodies two fundamental characteristics of a signal response pathway that have been inferred above: the identity of the components involved and the order in which they are arranged along a stepwise sequence.

As the previous discussion shows, our understanding of phytochrome-regulated light sensing has advanced considerably since its beginning in the early 1950s. The evolving picture reveals a family of photoreceptors displaying shared and unique functions, each controlling complex sensory networks that encompass both cytosolic and nuclear processes. Recent attempts to gauge the extent of these networks in restricted terms of altered gene expression alone now indicate, not so unexpectedly, that the volume of downstream signaling steps is very extensive. As such, further emphasis will need to be placed on describing their time-ordered placement within the signaling pathway as a means to uncover functional relationships in the scheme of a developmental program.

The need to order newly identified signaling entities within photomorphogenic response pathways underscores the importance of knowing the kinetics of a given response. The utility of response kinetics analysis to research on photomorphogenesis was first recognized long ago (Meijer, 1968). In current settings, response timing can be used in conjunction with other previously acquired data to arrange prospective signaling elements into a logical stepwise progression and/or to test the validity of preexisting photosensory models. As a basic example, comparing normal response kinetics with those of a mutant altered in the same response can be used to determine when the mutation affects the overall response process.

We have used this kinetic approach to study specifically the timing of phytochrome-regulated stem growth inhibition. And this single application of kinetic analysis to this conspicuous photomorphogenic response has permitted us to draw important conclusions regarding the details of the growth response to both blue- and red-region illumination (Parks et al., 1998; Parks and Spalding, 1999; Folta and Spalding, 2001a, 2001b). Figure 1 is a diagrammatic representation of how this process provides a more discriminating view of light-induced growth inhibition. Here are shown phyA- and phyB-deficient mutants along with their parent wild type as they appear after several days of growth in red light. On the basis of the photograph and accompanying bar graph displaying the resultant hypocotyl lengths for these seedlings, one would conclude that only phyB controls response to light under this condition because it is the only mutant with an abnormally long hypocotyl. A graphic model of wild-type response kinetics, shown adjacent to the bar graph, reveals how rapidly growth inhibition ensues and is sustained through time after the onset of illumination. How would the long-hypocotyl phyB-deficient mutant respond temporally under these same conditions? At least three different response kinetic profiles for this mutant could yield the same overall mutant phenotype (Fig. 1, bottom). The top example shows a seedling that responds with the same pattern as the wild type but more weakly. The middle portrays a seedling that initially responds the same as wild type, but that is released from inhibition beyond a certain time after the onset of illumination. And the final graph is for a mutant that is insensitive to light throughout the treatment period, only gradually decreasing in growth rate days later when growth simply can no longer be sustained. It is important to stress that each one of these possible kinetic profiles would be interpreted differently. In fact, our laboratory has performed this analysis of growth for phyA- and B-deficient mutants grown under continuous red illumination and found that the response profile of the phyB-deficient mutant is like that shown in the second example (Parks and Spalding, 1999).

Our interpretation was that a normal response to red light seen over the first 3 h of illumination suggested that a photoreceptor other than phyB was controlling the initial response to light. It turns out that this photoreceptor is phyA, because a mutant of this phytochrome type gave a response profile exactly reciprocal to that of the phyB mutant. This demonstrated that phyA and phyB act coordinately and sequentially to control stem growth in response to red light. Therefore, the absence of a long-hypocotyl phenotype for phyA mutants grown in red light (Fig. 1) does not result from an inability of phyA to inhibit growth under these conditions, but rather because the short time over which it regulates growth precluded its detection in final end-point determinations (Fig. 1, photo and bar graph). An additional important result of this study was the confirmation that phyA can control growth rate under the same red-light conditions where phyB normally dominates. Original mutant analysis of phytochrome-deficient seedlings led to the general conclusion that phyA only significantly regulates hypocotyl elongation in far-red-enriched environments, whereas phyB controls the dynamics of this process under conditions that are more red enriched. This first analysis gave the somewhat confusing indication that these two phytochromes normally act in opposition to each other under a single common light regime. It seems,

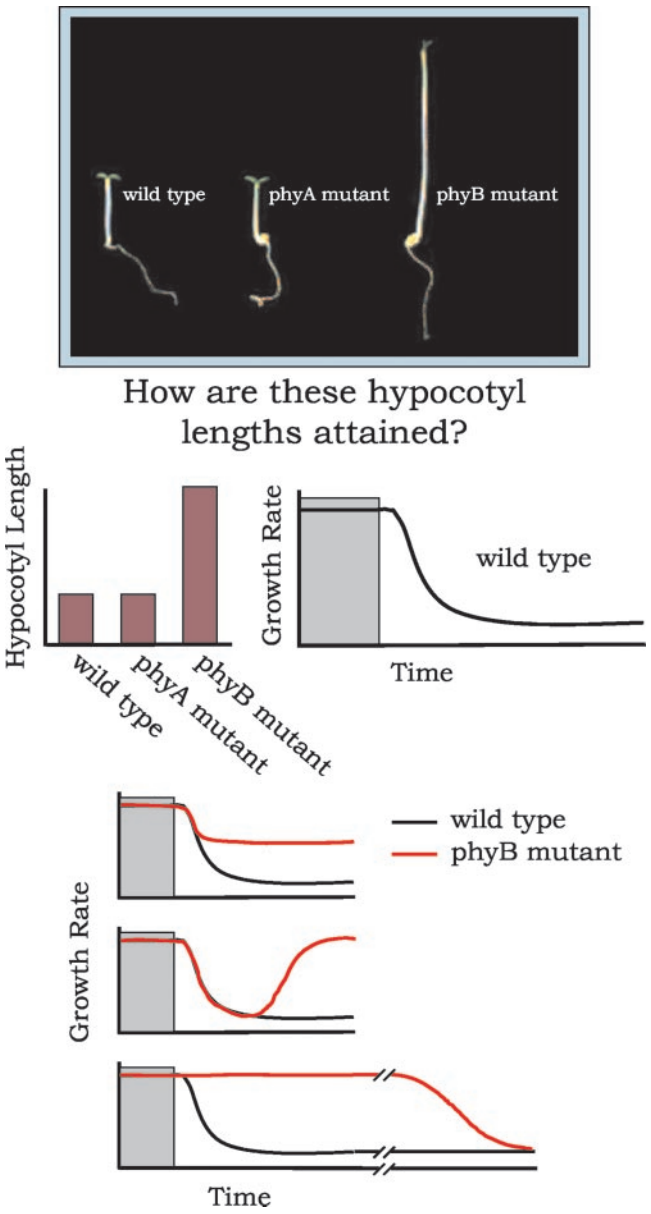

Figure 1. Light-regulated hypocotyl growth in Arabidopsis. The photograph at top shows Landsberg *erecta* wild type, phyA-deficient mutant (*phyA-201*), and phyB-deficient mutant (*phyB-1*), all as described previously (Devlin et al., 1999). These seedlings were grown for 4 d under continuous red (670-nm) light (25 μmol m^{-2} s^{-1}) supplied by a bank of light-emitting diodes (QB1310S-670-735, Quantum Devices, Barneveld, WI). The bar graph represents the resultant hypocotyl lengths measured after this growth period. The growth rate curve for the wild type (black line) is a simulated representation of the general growth response kinetics seen for wild-type seedlings grown in continuous red light for approximately 6 h. For this curve and others following, the shaded area represents an initial period of growth in darkness. The unshaded portion of the growth rate plot represents the time when seedlings are exposed to continuous red light. The grouping of three possible response kinetic profiles for the phyB-deficient mutant (red line) is shown at the bottom of the figure. The wild-type response is shown in each profile for comparison. The top representation describes a system in which at least two photoreceptors (one being phyB) act with identical timing to control growth rate. The middle shows the response profile that actually occurs (Parks and Spalding, 1999) in which phyB acts in sequence after a different photoreceptor (phyA). The final response, shown at the bottom, is for a system in which only phyB would control growth until it can no longer be sustained by the seedling after days of growth in continuous red light.

however, that this intriguing photobiological feature of the two phytochromes results, in part, from light-dependent differences in photoreceptor-type abundance for a given illumination quality. Kinetic analysis of the growth response to red light further resolved this apparent contradiction by showing that phyA and phyB both actually contribute the red-light-induced growth response but to differing degrees and with distinct temporal profiles. Similar kinetic studies of the red-light-regulated growth responses of downstream signaling mutants have helped in the analysis of stem growth dynamics by describing the window of time over which a given signaling component is important to the growth response (Parks et al., 2001). And a novel application of response kinetics has been used recently to enhance phytochrome-regulated root phototropism, thereby facilitating subsequent studies concerning the roles played here by this photoreceptor (Kiss et al., 2003). Future kinetic analyses of phytochrome-regulated responses might also be applied to studies that could address the potential ramifications of known circadian oscillations in photoreceptor expression and nucleocytoplasmic partitioning on signaling dynamics (Bognár et al., 1999; Tóth et al., 2001; Kircher et al., 2002).

The importance of timing in signaling analysis is further demonstrated by the kinetic profiles included as a central feature of recent genome-wide expression surveys. The incorporation of a time course into this expression analysis yielded valuable information with respect to when the expression of certain prospective gene product types was most dynamic (Tepperman et al., 2001). This aspect of the research provided the most compelling argument to date that a very substantial portion of initial phytochrome signaling is dedicated to altered gene expression, because the majority of genes whose expression changed early likely encode transcriptionally related proteins. Yet even though this portion of the survey represented the initial hour of phytochrome signaling, it is not known whether all such protein types identified were the result of primary signaling. This could be resolved by either conducting additional expression surveys over shorter illumination periods or by separate and possibly kinetic studies of the transcriptional events that gave rise to the altered expression profiles.

The continued analysis of response timing, measured either as a change in phenotype or gene expression, should provide at least one more tool toward ordering the sensing networks important to red-region sensing regulated by the phytochromes.

The utility of kinetic analysis has been established for one important developmental program, the control of hypocotyl growth. It should be possible to extend this method to the analysis of other downstream elements important to light-regulated growth that have been identified previously by mutation. In addition, the analysis of response kinetics could also be applied to other conspicuous light-regulated responses. For example, would it be possible to design a means to monitor the kinetics of phytochrome-controlled leaf expansion or greening? Comparisons of the time-dependent generation of phenotypic response profiles to the kinetics of expression for potentially important genes revealed in genome-wide surveys could help to direct future work designed to determine what gene products and signaling elements are important to particular response pathways.

Complications of Photoreceptor Co-Action

This discussion has been purposefully biased by focusing on red-region sensing in the process of photomorphogenesis. As stated initially, plants use the UV (both UV-A and UV-B), blue, and red regions of the solar spectrum to monitor and respond developmentally to their illuminated environment. In contrast to red-region sensing that occurs exclusively through the phytochromes and describes a narrowly defined experimental system, the near-UV and blue spectral regions are accessible to all known sensory receptors, including the phytochromes. As a result, a clear danger accompanying photobiological studies of red-light sensing is that, by design, they do not regard the potential for interaction between the known diverse families of developmentally important photoreceptors. In nature, plants never experience ambient light conditions that are deficient in all spectral regions other than red. This means that there are no practical instances during the normal growth and development of a plant where only phytochrome is operating to control development. And so, even though the capacity to study phytochrome in "photobiological isolation" exists, it is important to note that this photoreceptor normally functions in concert with the other classes of photosensory receptors to yield a given photomorphogenic program in a given light environment. Co-action of photoreceptors is a necessary element of photomorphogenesis that has been proposed and investigated for years (Casal, 2000; Nagy and Schäfer, 2002). And there are clear examples in recent literature across diverse photobiological responses to show that the phytochromes, cryptochromes, and phototropins interact in various signaling networks (Devlin and Kay, 2000; Folta and Spalding, 2001a, 2001b). Several examples are even available to suggest direct physical interaction between the photoreceptors (Ahmad et al., 1998; Más et al., 2000; Jarillo et al., 2001). But for the purpose of the present discussion, the point here is to stress that any degree of signaling complexity found through studies of red-light sensing will probably be compounded under normal lighting conditions where photoreceptor co-action could occur. And as such, all models for signaling deduced from studies of red-light sensing will require validation under the illumination conditions represented in natural settings.

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