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# Regulation of Phytochrome Gene Expression

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# Regulation of Phytochrome Gene Expression

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In etiolated oat seedlings exposure to red light results in a decrease in the transcription of the phytochrome genes, the abundance of phytochrome mRNA, and the level of phytochrome protein. Phytochrome itself serves as the photoreceptor for the response of decreased mRNA and transcription levels. The decrease in phytochrome mRNA is sensitive to low levels of Pfr. Even green "safelight" is capable of inducing a decrease in phytochrome mRNA abundance. Barley phytochrome mRNA abundance is also dramatically down-regulated in response to red light but other plant species vary in their ability to decrease phytochrome mRNA abundance after red light treatment. Kinetic analysis and protein synthesis inhibitor data indicate that the abundance of phytochrome mRNA in oat seedlings may be regulated in part at the post-transcriptional level. Phytochrome mRNA may provide a useful model system for the investigation of post-transcriptional regulation of plant gene expression.

INDEX DESCRIPTORS: Phytochrome, gene expression, oats, mRNA, post-transcriptional regulation.

Phytochrome is a photoreceptor that plays an important role in regulating plant development at all stages of the life cycle (Kendrick and Kronenberg, 1986). The photoreceptor is present in two forms in plants. A red light absorbing Pr form ($\lambda max = 666$ nm) and a far-red light absorbing Pfr form ($\lambda max = 730$ nm). Phytochrome is reversibly interconvertible between the two forms by sequential irradiation with red and far-red light. Based on the available data, the Pfr form is thought to be the active form of the photoreceptor (Kendrick and Kronenberg, 1986).

Phytochrome is synthesized in the Pr form and accumulates, as Pr phytochrome, to relatively high levels in etiolated seedlings. Production of Pfr with red light results in a substantial decrease in the amount of phytochrome protein that is present in etiolated seedlings. The decrease in phytochrome protein level is due, at least in part, to rapid degradation of the Pfr form of the photoreceptor (Quail et al., 1973; Pratt et al., 1974). Recent evidence indicates that Pfr phytochrome is degraded via the ubiquitin pathway (Shanklin et al., 1987 Speth et al., 1987).

Although the mechanism by which phytochrome regulates plant development is unknown, it has become clear that phytochrome is capable of regulating gene expression. (For reviews see: Thompson et al., 1985; Harpster and Apel, 1985; Kuhlemeier et al., 1987; Silverthorne and Tobin, 1987, Watson, 1989). For example, during greening of etiolated seedlings Pfr stimulates the expression of the chlorophyll a/b binding protein (cab) gene (Silverthorne and Tobin, 1984), while decreasing the expression of the genes encoding protochlorophyllide reductase (Mösinger et al., 1985) and asparagine synthetase (Tsai and Coruzzi, 1990). We have been investigating the ability of phytochrome to regulate the expression of its own genes (Colbert, 1988; Quail et al., 1986).

## Regulation of Phytochrome mRNA abundance

Etiolated seedlings of oat (Colbert et al., 1983; Colbert et al, 1985), pea (Otto et al., 1984; Tomizawa et al., 1989), corn (Christensen and Quail, 1989), and rice (Kay et al., 1989) respond to red light treatment by inducing rapid decreases in phytochrome (*phy*) mRNA abundance. Etiolated barley seedlings also respond to red light by dramatically decreasing the abundance of *phy* mRNA (Fig. 1). In oat seedlings the decrease in *phy* mRNA abundance is preceeded by a lag of 15 min after exposure to red light (Colbert, 1988). Etiolated seedlings of other plant species exhibit moderate (zucchini, Lissemore et al., 1987; cucumber, Cotton et al., 1990), or weak (tomato, Sharrock et al., 1988; *Arabidopsis thaliana*, Sharrock and Quail, 1989) down-regulation of *phy* mRNA abundance in response to red light. Although red light is ineffective, the predomin-

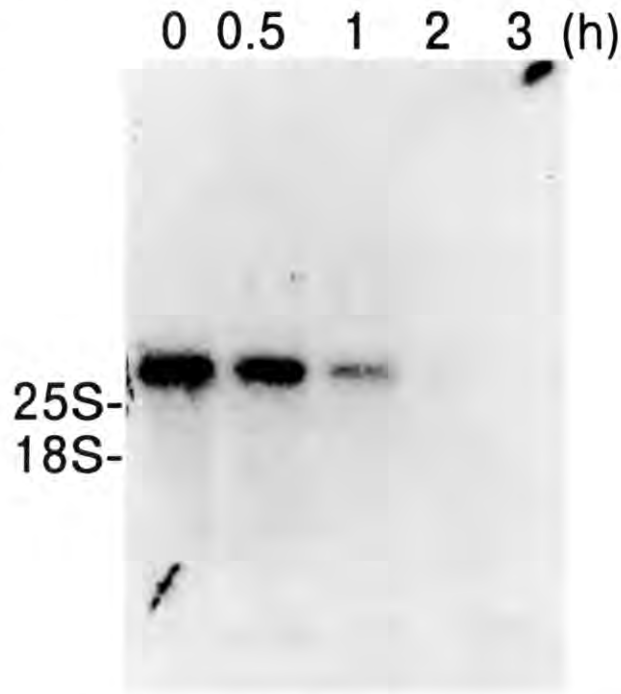


Fig. 1. Down-regulation of barley *phy* mRNA in response to red light. Four-day-old etiolated barley seedlings were either harvested at time zero or exposed to a saturating pulse of red light and returned to dark growth for 30 min, 1 h, 2 h, or 3 h. Poly $(A)^+$ RNA was isolated from the seedlings (Dean et al., 1985; Lissemore et al., 1987) and 1 μg from each sample was electrophoresed through a 1% agarose / 3% formaldehyde gel. The RNA was transferred to a nylon membrane and probed with $^{32}$P-labeled pAP3.2 (Hershey et al., 1985). Hybridization was carried out in 50% formamide 1M NaCl, 10% dextran sulfate 1% SDS and 100 μg/ml denatured salmon sperm DNA at 42°C with constant agitation. Final wash of the blot was in 0.1X SSC, 0.1% SDS at 60°C. Autoradiography was at −80°C for 10 h. The positions of oat 25S and 18S rRNA are indicated.

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ant *phy* mRNA species in *Arabidopsis* is substantially decreased in abundance after exposure of etiolated seedlings to continuous white light (Sharrock and Quail, 1989). The significance of the variability among plant species with regard to regulation of *phy* mRNA abundance is unknown. While *phy* mRNA abundance in some plant species is not dramatically decreased upon red light treatment, in the species that have been investigated, for example zucchini (Quail et al., 1973), *phy* protein levels do decrease dramatically after red light treatment.

In oats, peas and rice red/far-red reversibility experiments have demonstrated that the decrease in *phy* mRNA abundance in response to red light is regulated by phytochrome itself (Colbert et al., 1983; Colbert et al., 1985; Otto et al., 1988; Tomizawa et al., 1989; Kay et al., 1989). Treatment with far-red light alone also induces a decrease in *phy* mRNA abundance in oats (Colbert et al., 1983; Colbert et al., 1985) pea (Otto et al., 1984; Tomizawa et al., 1989) and rice (Kay et al., 1989). These observations indicate that down-regulation of *phy* mRNA abundance is, in part, a very low fluence response (Kaufman et al., 1984; Watson, 1989). The far-red light source used in the oat experiments converts about 1% of the phytochrome population from Pr to Pfr (Colbert et al., 1983) and consequently is equivalent to irradiation with a very low fluence (<1 μmol photons/$m^2$) of red light. Recently, we have observed that even green "safelight" (Westinghouse FG40 green fluorescent bulbs wrapped with Roscolene No. 877 and No. 874 colored plastic film (Musson Theatrical); Bolton and Quail, 1981), providing <0.01 μmol photons/$m^2$ of green light, is capable of inducing, but not saturating, the down-regulation of *phy* mRNA abundance in etiolated oat seedlings (Fig. 2).

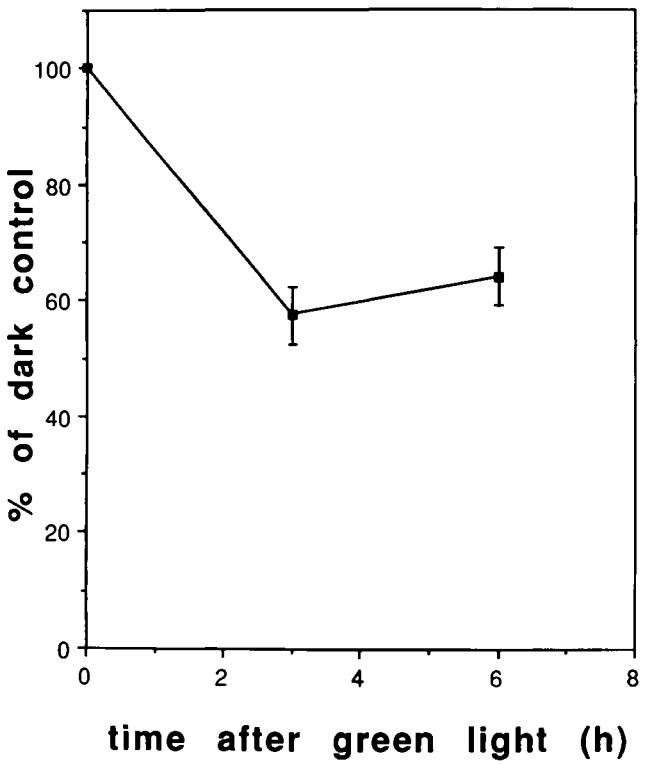


Fig. 2. Regulation of *phy* mRNA abundance in response to green light. Four-day-old etiolated oat seedlings were either harvested at time zero or exposed to 5 min of green light (Bolton and Quail, 1981) and returned to dark growth. Green light treated seedlings were harvested at 3 and 6 h post-irradiation. Poly(A)$^+$ RNA was isolated (Dean et al., 1985; Lissemore et al., 1987) from each sample and immobilized (0.5 μg) on a nylon membrane using a slot blot apparatus as described (Dean et al., 1985). Each poly (A)$^+$ RNA sample was immobilized in triplicate on three separate slot blots. The relative amount of *phy* mRNA was determined by hybridization with a $^{32}$P-labeled RNA probe derived from the 1.7 kbp PstI fragment of a pAP3.2 (Hershey et al., 1985). Hybridization was carried out as described in Fig. 1 except at 63°C. After hybridization the slots were excised from the nylon filter and the amount of hybridized probe determined by liquid scintillation spectroscopy. These data are the means from three independent experiments. The error bars indicate the standard error of the mean.

### Regulation of Transcription of the Phytochrome Genes

The observation that production of Pfr induces a dramatic decrease in the abundance of oat phytochrome mRNA raised the question of whether transcription of the phytochrome genes was also affected. Preliminary experiments employing isolated nuclei in *in vitro* transcription assays (Quail et al., 1986; Fig. 3) showed a rapid (<5 min after red light) decrease in transcription of the oat phytochrome genes. In this set of experiments transcription of the phytochrome genes was observed to decrease by about a factor of three after either a red light pulse or after transfer to continuous white light.

Subsequent experiments have repeated the observation of a rapid decrease in phytochrome transcription after red light treatment, but have revealed a more substantial 10 to 20-fold decrease in oat phytochrome transcription (Lissemore and Quail, 1988; Fig. 3). The reason for the quantitative discrepancy between these two data sets is unknown. However a difference in the experimental protocols that

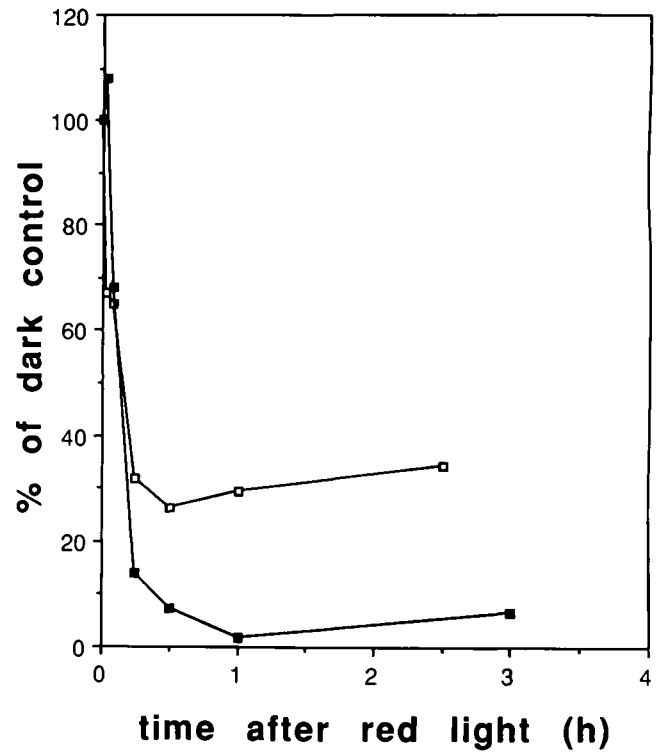


Fig. 3. Regulation of *phy* transcription is response to red light. Four-day-old etiolated oat seedlings were either harvested at time zero or exposed to a saturating pulse (5 to 10s) of red light and returned to dark growth. Nuclei were isolated (Beach et al., 1985) and *in vitro* run-on assays were performed as described (Quail et al., 1986; Lissemore and Quail, 1988). One set of experiments employed double-stranded pAP3.2 plasmid DNA as a probe for *phy* transcripts (□), while another set of experiments employed a single-stranded DNA probe derived from the 3.0 kbp KpnI/SacI fragment of pAP3.2 (■). Data points are from Quail et. al. (1986;□) and Lissemore and Quail (1988;■).

were employed may provide a clue. The initial experiments (Quail et al., 1986; Lissemore and Quail, 1988) employed double-stranded pAP3.2, a pBR322-based plasmid containing a 3.4 kbp phytochrome cDNA insert (Hershey et al., 1985), as a probe, while the subsequent experiments (Lissemore and Quail, 1988) employed a 3.0 kbp single-stranded DNA derived exclusively from the pAP3.2 insert as a probe. It is possible that the extra 400 nucleotides present in the insert of the pAP3.2 probe hybridize to a non-phytochrome RNA species which is not down-regulated by red light. It is also possible that vector sequences present in pAP3.2, but lacking in the 3.0 kbp single-stranded probe, result in red-light-independent non-specific binding to radiolabeled RNA molecules produced in the *in vitro* transcription reaction. Given that the data of Lissemore and Quail (1988) were obtained using a probe containing DNA sequence capable of hybridizing only to phytochrome RNA, it appears likely that the quantitative decrease in phytochrome transcription is 10 to 20-fold.

Recent work has shown that transcription of the rice phytochrome genes is decreased about 6-fold by 15 min after a red light pulse (Kay, 1989). No data are yet available regarding the effect of red light on the transcription of phytochrome genes in other plant species.

### Post-transcriptional Regulation of Phytochrome mRNA Abundance.

Investigation of potential regulation of phytochrome gene expression at the post-transcriptional level is of interest from two perspectives. First, relatively little is known about the mechanism and significance of post-transcriptional regulation of gene expression in plants. Recent evidence has indicated that one post-transcriptional event, control of cytoplasmic mRNA stability, may be a widespread and important level of control of gene expression (for reviews see Ross, 1989; Shapiro et al., 1986; Brawerman, 1987; Raghow, 1987). Most of the available data are from animal or bacterial systems although some evidence for post-transcriptional regulation of plant genes is available (Colbert, 1988; Gruissem et al., 1988). Second, in order to achieve the maximum benefit from the ability to introduce genes into crop plants, it will be necessary to precisely specify the pattern of expression of the foreign genes. Understanding how plant cells regulate mRNA stability will help in achieving appropriate expression of the introduced genes. Phytochrome may provide a useful model system in which to increase our understanding of the regulation of mRNA stability by plant cells.

Although the quantitive extent of the decrease in transcription of the oat *phy* genes (10-to 20-fold) and the decrease in *phy* mRNA abundance (10-fold) after exposure to red light is similar, these data do not establish whether the dramatic decrease in transcription would lead to the observed *rate* of decrease in *phy* mRNA abundance. Kinetic analysis (Quail et al., 1986; Colbert, 1988) suggests either that a substantial increase in the rate of degradation of *phy* mRNA occurs after exposure of etiolated oat seedlings to red light, or that *phy* mRNA is relatively unstable both in etiolated and light-treated seedlings. Other investigators (Kay et al., 1989) have also proposed that rice *phy* mRNA may be an exceptionally unstable mRNA species, even in etiolated seedlings. However, the half-life of *phy* mRNA in etiolated seedlings of any plant species remains to be determined. We are currently trying to distinguish between the possibilities that *phy* mRNA is inherently unstable and that *phy* mRNA is destabilized in response to production of Pfr.

The decrease in transcription of the oat phytochrome genes occurs in less than 5 min after red light (Quail et al., 1986; Lissemore & Quail, 1988), while the decrease in phytochrome mRNA abundance does not begin for at least 15 min after red light (Colbert, 1988). The difference in lag time, by itself, does not provide support for distinct transcriptional and post-transcriptional events. However, the 15 minute lag could potentially be sufficient time for a new protein, which functions in decreasing *phy* mRNA abundance, to be expressed.

To investigate the possibility that red light induces the production of a protein that functions in degrading *phy* mRNA we have employed the protein synthesis inhibitor cycloheximide. Inhibition of protein synthesis with cycloheximide does not prevent the red-light-induced decrease in phytochrome transcription (Lissemore and Quail, 1988; Fig. 4). However, preliminary experiments indicate that inhibition of protein synthesis decreases the ability of oat seedlings to down-regulate phytochrome mRNA abundance (Fig. 4). These data, together with the observed 15 min lag, could be interpreted to mean that oat *phy* mRNA abundance is regulated both by transcriptional events that are not dependent on protein synthesis, and by post-transcriptional events that are dependent on protein

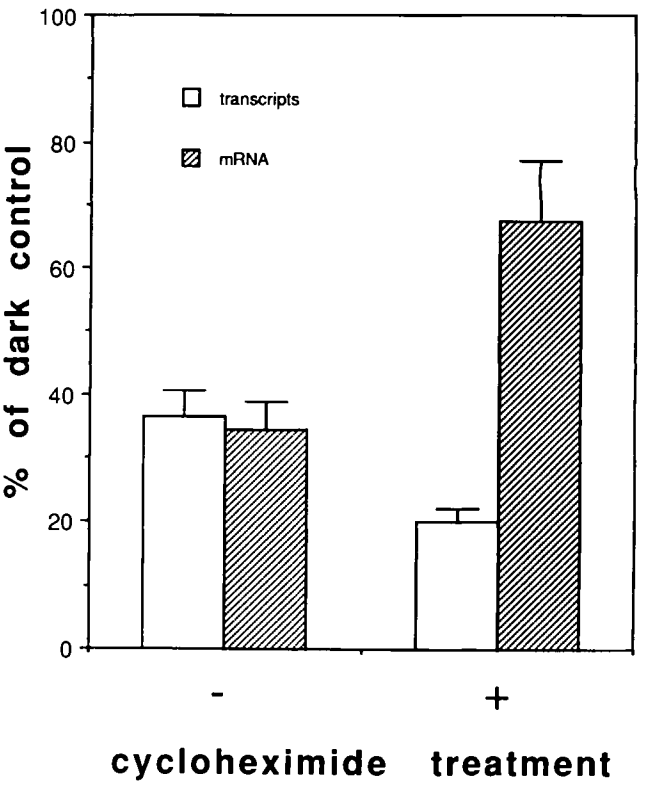


Fig. 4. Effect of cycloheximide on the regulation of *phy* transcription and the regulation of *phy* mRNA abundance. Four-day-old etiolated oat seedlings were excised just below the mesocotyl node. Subsequently the tip of the coleoptile was removed and excised sections were placed, in an upright position, into incubation buffer with (+) or without (−) 10 μg/ml cycloheximide and 50 μg/ml chloramphenicol for 1 h. Under these conditions protein synthesis in the excised section is inhibited by 90 to 95% (Lissemore et al., 1988). After 1 h the excised sections were irradiated with red light and returned to dark growth for 15 min (open bars) or 120 min (shaded bars). The data points showing the effect of red light on transcription of the *phy* genes are from Lissemore and Quail (1988). The effect of red light on *phy* mRNA abundance was measured in isolated total RNA (10μg) using a $^{32}$P-labeled RNA derived from the 1.7 kbp Pst I fragment of pAP3.2 (Hershey et al., 1985) as a hybridization probe in either northern (experiment 1) or slot blot (experiments 2 and 3) analysis. Hybridization was carried out as described in Fig. 1 except at 63°C. Phytochrome mRNA was quantitated by excising the portions the blots containing the phytochrome bands and determining the bound radioactivity using liquid scintillation spectrometry. All values were corrected for background. Error bars indicate the standard error of the mean of the three independent experiments.

synthesis. The putative new protein would be rapidly induced by red light and could be envisioned to be either a selective ribonuclease or a tagging molecule that signals a more general ribonuclease to degrade *phy* mRNA. However, the cycloheximide experiments could also be interpreted to mean that translation must be occurring for *phy* mRNA to be degraded. Translation is required for selective degradation of histone mRNA (Graves et al., 1987) and tubulin mRNA (Yen et al., 1988). In addition, *in vitro* mRNA degradation experiments have provided additional evidence that selective mRNA degradation occurs on polysomes (Ross, 1989). Given the uncertainty in interpretation, the cycloheximide experiments, unfortunately, do not allow discrimination between the two possible explanations for the rapid decrease in *phy* mRNA abundance after red light treatment of etiolated oat seedlings.

Another approach to investigating the possibility that oat *phy* mRNA abundance is regulated at the post-transcriptional levels would be to produce transgenic plants containing an oat phytochrome gene fused to a constitutive promoter (e.g. the CAMV 35S promoter; Kuhlemeir et al., 1987). Transcription would, therefore, be unaffected by light treatment allowing direct investigation of post-transcriptional events. Oat phytochrome genes have been isolated (Hershey et al., 1987) and production of transgenic tobacco plants containing a chimeric oat phytochrome gene would be possible using the *Agrobacterium tumefaciens* system (Klee et al., 1987). However, sequence conservation between monocot and dicot phytochromes is surprisingly low (Sato, 1988; Sharrock et al., 1986; Sharrock and Quail, 1989). In addition, solanaceous dicots (e.g. tomato; Sharrock et al., 1988), unlike oats, exhibit little change in *phy* mRNA abundance in response to red light. Therefore, easily transformable species like tomato or tobacco may not be a useful model systems in which to study potential post-transcriptional regulation of oat *phy* mRNA abundance.

Consequently, we chose to attempt transformation of a plant species that shares a high degree of phytochrome sequence homology with oats and that regulates *phy* mRNA abundance in a fashion similar to oats. The plant species we selected was barley (Fig. 1). We attempted to transform barley using the floral injection method. Transgenic rye plants (de la Peña et al., 1987) and rice plants (Croughan et al., 1988) have been reported to be produced using this approach. As a model transformation system we injected the plasmid pCAMVNEO (Fromm et al., 1986) into developing floral tillers of barley and screened over 3,100 progeny seeds for the resistance to kanamycin conferred by pCAMVNEO. We did not obtain any transformed barley seedlings. If more efficient means to produce transgenic barley plants become available (e.g. Klein et al., 1988; Luo and Wu, 1988), production of barley plants containing chimeric oat phytochrome genes would provide a means to assess the importance of post-transcriptional events in the regulation of *phy* mRNA abundance.

### Biological Significance of the Regulation of Phytochrome Gene Expression.

Light decreases the expression of the phytochrome genes in most plant species which have been investigated. In some plant species (e.g. zucchini) the regulation of *phy* gene expression appears to be principally at level of *phy* protein turnover. Other plant species (e.g. oats) regulate *phy* gene expression at the transcriptional, mRNA, and protein levels. The importance of decreased phytochrome expression to plant development is unclear. However, recent experiments involving the over-expression of oat phytochrome in transgenic tobacco (Keller et al., 1989) and tomato (Boylan and Quail, 1989) have resulted in the production of developmentally abnormal plants. It appears likely that the high level expression of phytochrome in etiolated seedlings must be decreased to allow normal plant development to proceed.

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