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Photoregulation of a phytochrome gene promoter from oat transferred into rice by particle bombardment

(autoregulation/transient expression assay/mRNA levels/regulatory photoreceptor)

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ABSTRACT **The regulatory photoreceptor phytochrome controls the transcription of its own *phy* genes in a negative feedback fashion. We have exploited microprojectile-mediated gene transfer to develop a rapid transient expression assay system for the study of DNA sequences involved in the phytochrome-regulated expression of these genes. The 5′-flanking sequence and part of the structural region of an oat *phy* gene have been fused to a reporter coding sequence (chloramphenicol acetyltransferase, CAT) and introduced into intact dark-grown seedlings by using high-velocity microprojectiles. Expression is assayable in <24 hr from bombardment. The introduced oat *phy*–CAT fusion gene is expressed and down-regulated by white light in barley, rice, and oat, whereas no expression is detected in three dicots tested, tobacco, cucumber, and *Arabidopsis thaliana*. In bombarded rice shoots, red/far-red light-reversible repression of expression of the heterologous oat *phy*–CAT gene shows that it is regulated by phytochrome in a manner parallel to that of the endogenous rice *phy* genes. These data indicate that the transduction pathway components and promoter sequences involved in autoregulation of *phy* expression have been evolutionarily conserved between oat and rice. The experiments show the feasibility of using high-velocity microprojectile-mediated gene transfer for the rapid analysis of light-controlled monocot gene promoters in monocot tissues that until now have been recalcitrant to such studies.**

The regulatory photoreceptor phytochrome controls numerous aspects of plant development at all phases of the life cycle (1, 2). Much has been learned about the properties and biogenesis of the molecule, and there is abundant evidence that in performing its regulatory function the photoreceptor controls the transcription of a number of nuclear genes (1–5). Despite the remarkable progress that has been made, the molecular mechanism by which phytochrome transmits its regulatory signal to nuclear genes remains to be determined.

The phytochrome molecule is a cytoplasmically localized dimeric chromoprotein with each subunit consisting of a single chromophore covalently bound to a 116- to 127-kDa polypeptide (2, 6). The photoreceptor functions as a molecular switch by its capacity to exist in two photointerconvertible forms: the inactive red light-absorbing form Pr and the active far-red light-absorbing form Pfr. The molecule is synthesized in the Pr form and accumulates exclusively in this form in dark-grown tissue. Red light converts ≈86% of the phytochrome to Pfr, whereas far-red light reconverts or retains ≥99% of the molecules in the Pr form. Thus, tissue exposed to light and returned to darkness contains a residual pool of Pfr, the size of which can vary over two orders of magnitude depending on the wavelength of the final irradiation. Because the Pfr form turns over rapidly ($t_{1/2} \approx 60$ min) in the cell, the duration of the presence of this active form in a postirradiation dark period also depends on the final irradiation. This behavior of the phytochrome molecule in the cell is the basis for the observation that a large array of plant responses to light can be induced by red and abrogated by subsequent far-red irradiation (1, 2). In addition, this behavior accounts for the observation that certain very sensitive responses are maximally induced by far-red light alone, indicating that ≤1% Pfr is sufficient to saturate these responses (2).

Some phytochrome-regulated genes are induced by Pfr, whereas others are repressed (3–5, 7). The cis-acting sequences and trans-acting factors responsible for modulation of the expression of these genes are under intensive investigation. Deletion analysis in transgenic plants and *in vitro* DNA-binding protein assays have led to the identification of an array of conserved motifs shown variously to possess enhancer-like properties, to modulate expression positively or negatively in response to light/dark signals, and to bind nuclear factors (4, 8–10). Nevertheless, the molecular components of the transduction pathway are yet to be identified.

We have been investigating the autoregulation of *phy* gene expression because these genes display a very rapid and sensitive response to Pfr. The transcription of oat *phy* genes is repressed within 5 min of Pfr formation and occurs in the presence of protein synthesis inhibitors, suggesting that all necessary transduction pathway components pre-exist in the cell before phytochrome photoconversion (11). Moreover, transcription of oat and rice *phy* genes is fully repressed by far-red light alone in the period immediately after irradiation, indicating that initial repression is saturated by ≤1% Pfr (11, 12). On the other hand, transcription rates recover in the dark much sooner after far-red than after red pulses, indicating that derepression occurs upon depletion of Pfr and that the duration of transcriptional repression is a function of the size of the initial Pfr pool (11).

Our initial attempts to analyze promoter sequences involved in the autoregulated expression of *phy* genes by established gene-transfer techniques were unsuccessful. Because routine stable transformation procedures for monocots are unavailable, we originally tried a transient expression assay approach using electroporation (13) of oat protoplasts (14). This strategy failed apparently because the osmotic stress inherent to the procedure inhibited light-induced changes in gene expression in these cells (14). *Agrobacterium*-mediated stable transformations of tobacco also failed to produce detectable transcripts from an introduced oat *phy* gene in light- or dark-grown plants (ref. 15; W.B.B., unpublished data). Moreover, in contrast to monocots, the endogenous *phy* genes of the dicots that we have examined lack strong autoregulation (14, 16, 17). To circumvent these problems we have used the recently developed high-velocity

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Abbreviations: CAT, chloramphenicol acetyltransferase; Luc, luciferase.

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microprojectile procedure (18) to introduce an oat *phy* promoter/chloramphenicol acetyltransferase (CAT) reporter gene construct into intact seedlings and have examined light regulation of this fusion gene. We demonstrate that the oat *phy* fusion gene is properly regulated in a heterologous rice system in a manner similar to endogenous *phy* genes, making this system useful for further studies involving the autoregulation of these genes in monocots.

MATERIALS AND METHODS

Plant Material. Barley (*Hordeum vulgare* cv. Himalaya) and dehusked rice [*Oryzae sativa* M101 (19)] and oat (*Avena sativa* cv. Garry) were imbibed for 4 hr at room temperature, surface-sterilized, and then incubated at 26°C in the dark on 0.8% H_2O/agarose plates for 48 hr. Tobacco (*Nicotiana tobacum* SR1) and cucumber (*Cucumis sativa* cv. Marketeer) seeds were imbibed for 12 hr, surface-sterilized, and then incubated at 26°C in the dark on 0.8% H_2O/agarose plates for 3 days. After the dark incubation, 18 seedlings of the barley, rice, oat, and cucumber were placed on degassed 0.8% H_2O/agarose plates so that the shoots of the monocots and the cotyledons of the cucumber were parallel to the agarose surface for maximal exposure to the bombardment. *Arabidopsis thaliana* (Columbia ecotype) seeds were surface-sterilized and placed on 0.5× MS salts (GIBCO)/0.7% H_2O/agarose followed by incubation in the dark for 4 days at 4°C. The *A. thaliana* seeds were given 20 min of white light to induce germination after which they were grown in the dark for 2 days at room temperature before bombardment. Approximately 50 mg of tobacco seeds and 55 mg of *A. thaliana* seeds were used for each bombardment.

***phy*–CAT (pGP403 and pGP228) and pAHC18 Construction.** To construct the *phy*–CAT fusion gene, pGP403, a pUC19:CAT vector was first generated by converting the *Eco*RI site in pUC19 to an *Xho* I site by linker addition. This step was to facilitate the subcloning into the pUC19 vector of the pGA-582 *Hin*dIII–*Sal* I fragment (20), which consists of the coding region and the transfer-DNA transcript-6 poly(A) addition site. A *Hin*dIII–*Stu* I fragment of the phytochrome type 3 (*phy-3*) gene from position −1004 base pairs (bp) to +1372 bp (21) was cloned into the *Hin*dIII–*Hpa* I site of the pUC19:CAT vector. The resultant pGP403 plasmid consists of a fragment of the oat *phy-3* gene containing 1 kilobase pair (kbp) of 5′-flanking region, the first exon, the first intron, and 75 bp of the second exon (including the first five amino acids) fused to a CAT–transcript-6 poly(A) addition site (20) resident in pUC19. Construction of pGP228, a 5′ deletion of clone pGP403, involved BAL-31 digestion from the *Cla* I site (−493 bp in the 5′-flanking region of the *phy* gene) to position +10 bp followed by *Bam*HI linker addition. The 35S–CAT construct was made by subcloning the *Hin*dIII–*Bam*HI fragment containing the promoter of the 35S gene of cauliflower mosaic virus from pDO432 (22) into the same site of the pUC19:CAT vector.

A maize ubiquitin-promoter/luciferase (*ubi*/Luc) fusion gene, pAHC18, was used as an internal reference for monocots and was included with each bombardment. pAHC18 was constructed using a *Pst* I fragment from a genomic clone (λ7.2b1) of a maize ubiquitin gene (A.H.C., unpublished data). pAHC18 consists of 900 bp of 5′-flanking region, the first exon, and the intron of the ubiquitin gene ligated into a vector containing a Luc coding sequence derived from pDO432 (22), and a nopaline synthase 3′ poly(A) addition site. For dicots, pDO432 was included as an internal reference with each bombardment.

Bombardment, Irradiation, and Enzyme Assays. Two- and three-day-old etiolated seedlings were bombarded with *phy*–CAT plasmid constructs combined with an internal reference plasmid, pAHC18 or pDO432. Six micrograms of pGP403 and 2 μg of pAHC18 were mixed and precipitated together onto tungsten particles (average diameter of 1.2 μm), as described (18). The plasmids pGP228/pAHC18 and pGP403/pDO432 were also coprecipitated onto the particles in separate tubes in the same 3:1 molar ratio and in amount similar to that of the pGP403/pAHC18 DNA mix. The etiolated seedlings were bombarded two times under a partial vacuum by using a particle gun (18) under laboratory white light (1.9×10^2 $\mu W/cm^2$, cool white fluorescent bulbs). Exposure time to the laboratory white light was standardized at 3 hr. All samples were then irradiated with 5 s of far-red [>720-nm cutoff filter (CS7-69 Corning type) 6.1×10^5 $\mu W/cm^2$]. Immediately after the far-red irradiation some samples were placed directly into the dark (designated far-red), whereas other samples were given either 5 s of red [660-nm interference filter (Baird) 1.2×10^4 $\mu W/cm^2$] (designated far-red/red), or 5 s each of red followed by far-red (designated far-red/red/far-red), before being placed in the dark. Yet other samples were placed in continuous white light (fluorescent bulbs, 3.6×10^2 $\mu W/cm^2$) (designated far-red/white). Postirradiation incubations were for the periods indicated at 26°C (22°C for *A. thaliana*). Shoots of monocots, cotyledons of cucumber, and whole seedlings of tobacco and *A. thaliana* were harvested and ground in a 1.5-ml microcentrifuge tube with a pellet pestle (Kontes) in 0.2 M potassium phosphate buffer, pH 7.8/1 mM dithiothreitol to a concentration of 2 ml/g of fresh weight. The extract was clarified at 14,000 × *g* for 5 min at 4°C in a microcentrifuge. Protein concentrations of the supernatants were determined by using the method of Bradford (23). CAT assays were performed as described (24). Luc assays were performed in duplicates as described (22), except that glycylglycine was omitted from the Luc assay buffer and 0.5 mM luciferin was used to initiate the reaction. Light units are proportional to the accumulated number of photons generated over a period of 30 s at 25°C. Raw values for Luc assays were corrected for background values generated from extracts bombarded with tungsten particles containing no DNA.

RNA Isolation and Analysis. Total RNA was isolated from rice seedlings after various periods of dark incubation and growth and handling in a manner similar to that used for bombarded seedlings. The shoots of the rice seedlings were harvested and frozen in liquid N_2 under dim green light. Total RNA was isolated from the frozen tissue as described (25), except that 50 mM Tris·HCl, pH 8.3/150 mM NaCl/10 mM EDTA/1% lauroylsarcosine (Sigma) was used as the extraction buffer. Six micrograms of total RNA was loaded in each lane of a formaldehyde agarose gel, fractionated, and transferred to GeneScreen (DuPont). A *Kpn* I–*Sac* I fragment from an oat *phy* cDNA clone pAP3.2 (26) was labeled using an oligo-primer labeling kit (Pharmacia/LKB) and was hybridized to the filters as described (16). The filters were successively washed with 2× wash solution (17) at room temperature and 1× wash solution at 65°C for 1 hr. Filters were exposed to x-ray film for 16 hr at −80°C with intensifying screen. Relative transcript levels were determined by densitometer measurements of Northern blots from two independently grown tissue samples.

RESULTS

***phy*–CAT Expression in Homologous and Heterologous Plants.** To assess the activity of the oat *phy* promoter after microprojectile-mediated transfer into intact seedlings, we originally surveyed several monocot and dicot species, in addition to the homologous oat system, for their capacity to support oat *phy*–CAT expression. These experiments were also used to explore some basic parameters of the experimental system. Initially, because of the high degree of uncontrollable variability inherent to the bombardment pro-

cedure, substantial variations in CAT activity were seen between replicate samples, thus making precise quantitative comparisons difficult. We therefore included an internal reference *ubi*–Luc (pAHC18) or 35S–Luc (pDO432) fusion construct with each bombardment in subsequent experiments (Fig. 1). The manipulations associated with the microprojectile procedure necessitated exposing etiolated seedlings to white light, which is known to repress the levels of *phy* transcription in oats (5, 14). We therefore standardized the white light period at 3 hr and routinely administered a far-red irradiation immediately after this period in all experiments so that the existing pools of Pfr were established at <1%. The seedlings were then incubated as indicated and assayed for CAT and Luc activity. The various light treatments did not significantly affect the expression of the *ubi*–Luc or 35S–Luc constructs. For example, average activity levels of 4.5×10^5 ($\pm 8.0 \times 10^3$) and 4.8×10^5 ($\pm 5.0 \times 10^3$) light units per mg of protein·min^{-1} were seen for the *ubi*–Luc construct in bombarded rice tissue exposed to far-red and far-red/red, respectively. Likewise, the 35S–Luc internal reference construct resulted in 1.2×10^6 ($\pm 2.4 \times 10^5$) and 0.94×10^6 ($\pm 3.4 \times 10^4$) light units per mg of protein·min^{-1} in bombarded dicots for far-red and far-red/white irradiations, respectively.

Fig. 2 shows *phy*–CAT expression in the different plant species relative to the internal Luc standard. In each monocot the CAT activity in seedlings maintained in continuous white light is repressed to levels 10–50% of the far-red control (Fig. 2*A*). The *phy*–CAT gene is expressed strongly in far-red-treated barley and rice but is nearly 7-fold lower in oats. This lower level appears to be due to a high degree of damage to the oat seedlings caused by bombardment, as these seedlings subsequently grew very poorly, and the expression of the internal reference (*ubi*–Luc) gene was only ≈10% of the activity in barley or rice.

The activity of the oat *phy*–CAT fusion gene when transferred into the three dicots is essentially at background levels (Fig. 2*B*). For comparison, Fig. 2*C* shows the activity of the 35S–CAT fusion gene transferred into these dicot seedlings as well as into rice seedlings. These data demonstrate that the bombardment process was effective in delivering DNA to the dicot tissue. The expression level of the oat *phy*–CAT gene in rice is ≈50-fold greater than the 35S–CAT gene in rice (Fig. 2 *A* and *C*). On the other hand, the expression level of the 35S–CAT is much lower in rice than that in the dicots.

These data indicate that rice or barley would be the most suitable for introduction and analysis of the regulation of the *phy*–CAT gene. Rice was chosen for further studies primarily because of the opportunities for future comparisons with stable transformants as the result of recent success in the development of stable transformation procedures for this species (27, 28).

Regulation of Endogenous *phy* mRNA Levels in Rice. Preliminary experiments indicated that the endogenous rice *phy*

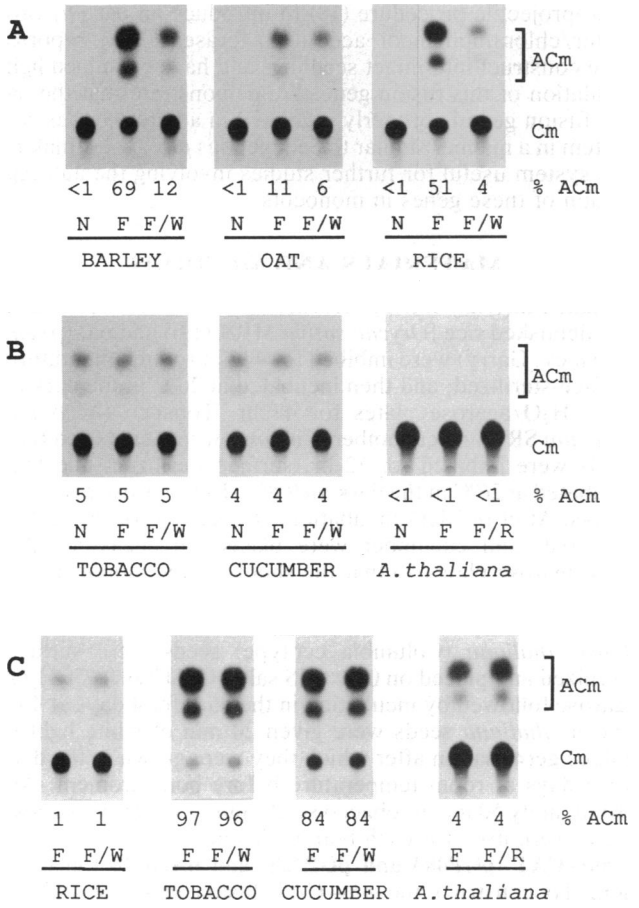

FIG. 2. Expression of *phy*–CAT and 35S–CAT constructs in various plants. (*A*) Etiolated 2-day-old barley, oat, and rice seedlings bombarded with pGP403:pAHC18 mix. (*B*) Etiolated 3-day-old tobacco and cucumber and 2-day-old *A. thaliana* bombarded with pGP403:pDO432 mix. (*C*) Rice seedlings bombarded with 35S–CAT:pAHC18 mix; dicots bombarded with 35S/CAT:pDO432 mix. N, samples bombarded with internal reference plasmids only; after bombardment, all samples were exposed to a 5-s pulse of far-red light followed by either incubation in the dark (N and F), a 5-s pulse of red and then incubation in the dark (F/R; *A. thaliana* only), or incubation in continuous white light (F/W) for 24 hr. Autoradiograms are from TLC plates exposed for 3 days (*A. thaliana* for 9 days). ACm, 1- and 3-acetylchloramphenicol; Cm, chloramphenicol. Lanes F represent the CAT activity present in 60 μl (30 mg of fresh weight equivalent) of extract for the monocots and 180 μl (90 mg of fresh weight equivalent) of extract for the dicots. The other CAT values have been normalized separately for each plant species to the internal reference Luc activity observed in the far-red-treated samples (lanes F) for that species. CAT activities are quantitated as % ACm (percentage of chloramphenicol conversion to acetylated forms) below each track of the autoradiograms.

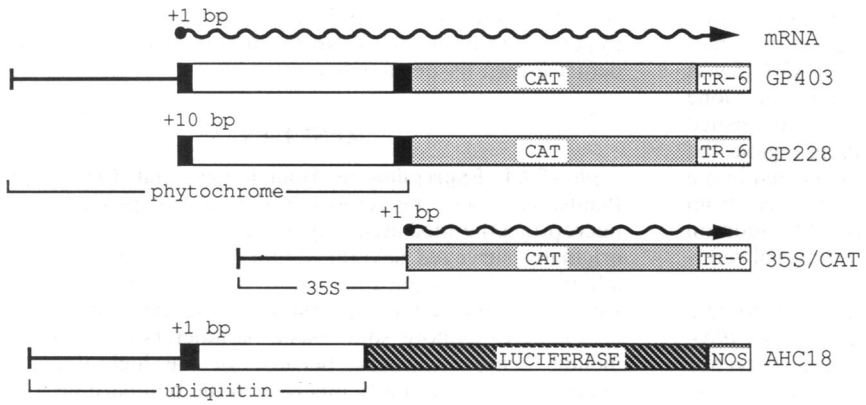

FIG. 1. Schematic depicting *phy*–CAT, 35S–CAT, and ubi–Luc fusion constructs. Single lines indicate the 5′-flanking region of the oat *phy-3* (17), 35S (22), and maize *ubi-1* (A.H.C., unpublished data) genes. Black boxes represent exon 1 and part of exon 2 (containing the start of translation), whereas white boxes represent intron 1 of the oat *phy-3* and maize *ubi-1* genes. Stippled and striped boxes indicate the coding regions of the CAT and Luc genes, respectively. The 3′-untranslated regions containing the poly(A) addition site of the transferred DNA transcript 6a (TR-6) and nopaline synthase (NOS) genes are indicated.

genes are regulated by phytochrome in 3- to 4-day-old etiolated seedlings in a manner similar to that well-established for oats (5, 11). However, because the microprojectile procedure chosen necessitated exposing 2-day-old seedlings to a standard 3-hr period of white light at the time of bombardment, with a subsequent dark period to allow expression of the introduced gene, we examined the expression of the endogenous rice *phy* genes under the same conditions. The kinetics of the recovery in steady-state levels of *phy* mRNA in darkness after 3 hr of white light and terminal pulses of far-red or far-red/red were monitored by Northern analysis (Fig. 3). The levels of *phy* transcripts in far-red-pulsed tissue increase rapidly to a plateau nearly 8-fold above the level detected at 1 hr after bombardment. In contrast, transcript levels in far-red/red-pulsed tissue show a lag before increasing to a level approaching the far-red-treated tissue by 24 hr.

Photoregulation of the Oat *phy*–CAT Gene in Rice. In exploratory experiments, we determined that the expression of *phy*–CAT activity in bombarded rice seedlings increased linearly with the concentration of input *phy*–CAT DNA from 0.5 to 12 μg, the highest level tested. These data indicate that the expression of the introduced *phy* promoter is not rate-limited by cellular factors. As a result we used 6 μg of *phy*–CAT DNA for each subsequent bombardment to ensure that expression is DNA-limited.

Fig. 4 shows the time course of expression of pGP403 relative to the internal *ubi*–Luc reference construct after particle-mediated transfer into 2-day-old etiolated rice shoots. A red pulse after the initial far-red pulse at the start of the dark period represses CAT accumulation 3- to 4-fold relative to the far-red-pulsed tissue alone, and a far-red pulse immediately after the red pulse reverses the repressive action of red light. Continuous white light strongly represses CAT accumulation (Fig. 4, F/W).

DISCUSSION

The data presented here show that the oat *phy* promoter is transcriptionally active after direct transfer into homologous and heterologous monocot seedling tissue. Moreover, the expression of the introduced chimeric *phy*–CAT construct in rice is regulated by light in a manner parallel to that of the endogenous rice *phy* genes. The pattern of recovery in steady-state levels of endogenous *phy* mRNA in rice seedlings in darkness after a 3-hr white light exposure and terminal pulses of far-red or far-red/red light (Fig. 3) is similar to that in oat seedlings (5) and is indicative of autoregulation of rice *phy* genes under these conditions. The faster recovery in far-red-pulsed tissue is attributed to the earlier derepression of *phy* gene transcription resulting from the more rapid depletion, by intracellular turnover, of the smaller residual Pfr pool (<1%) established by this irradiation (11). Conversely, the slower recovery in red-irradiated tissue is attributed to the longer period of repression of *phy* gene transcription caused by the longer time required to deplete the larger pool of residual Pfr (86%) established by the terminal red irradiation at the start of the dark period (11).

The far-red/red/far-red-reversible repression of oat *phy*–CAT transcription after microprojectile-mediated transfer to rice seedlings (Fig. 4) establishes that the oat *phy* promoter is also negatively regulated by the endogenous phytochrome system of rice cells. Possible reasons for the apparent slower recovery in the level of CAT activity (Fig. 4) compared with that of the endogenous *phy* mRNA (Fig. 3) in far-red/red-irradiated tissue, might include a lag between CAT mRNA and CAT enzyme accumulation and/or slower depletion of initially established Pfr pools in cells perturbed by microprojectile entry. The strong repression of CAT accumulation by

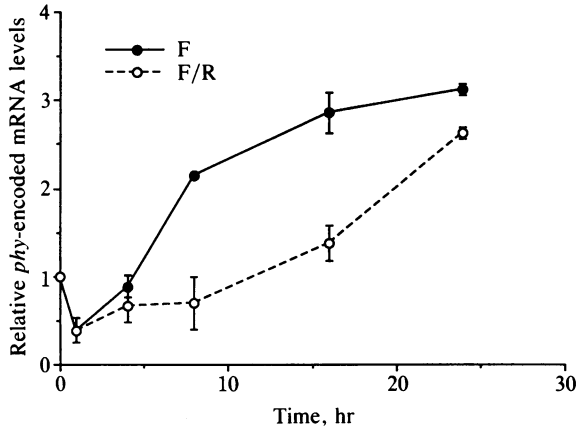

FIG. 3. Relative endogenous *phy* gene transcript levels in rice seedlings under conditions simulating the microprojectile delivery protocol. Two-day-old dark-grown rice seedlings were exposed to 3-hr white light and then irradiated with 5 s of either far-red light (F) or far-red immediately followed by red light (F/R). Total RNA was isolated after various periods of darkness following the irradiations. Relative transcript levels are an average of values from densitometer measurements of Northern (RNA) blots from two independently grown tissue samples. Error bars represent the SDs in data from two independently grown tissue samples.

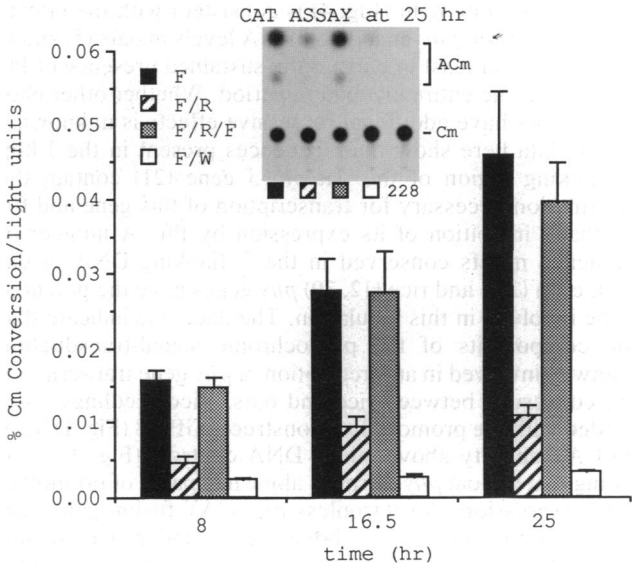

FIG. 4. Expression of *phy*–CAT in bombarded rice tissue subjected to various light treatments. Two-day-old etiolated rice seedlings were cobombarded with tungsten particles coated with either pGP403:pAHC18 or pGP228:pAHC18 and given irradiations as follows. All samples were given a 5-s far-red pulse immediately after the 3-hr white light period and then divided into four groups as indicated. One group was placed in the dark for various times with no further irradiation (F). A second group was given a 5-s red pulse and then placed in the dark (F/R), whereas a third group was given a red pulse followed by a second far-red pulse and then placed in the dark (F/R/F) for various times. The fourth group was placed in continuous white light (F/W). The shoots were extracted after the indicated incubation times, and both CAT and Luc activities were measured. CAT activity is expressed as a ratio of % chloramphenicol conversion (to 1- and 3-acetylchloramphenicol) to light units of Luc activity in the same extract. All values are an average of at least four independently grown tissue samples and have been corrected for pGP228 (promoterless construct) activity. Error bars represent the SEMs. The inset autoradiogram (exposed for 36 hr) of a TLC plate shows representative CAT activities (loaded on a per Luc activity basis) from bombarded rice exposed to the various light treatments and from the promoterless construct pGP228 at 25 hr after bombardment. ACm, 1- and 3-acetylchloramphenicol; Cm, chloramphenicol.

continuous white light (Fig. 4) is consistent with the effects of white light on *phy*-encoded mRNA levels in oats (5) and is attributable, at least in part, to the sustained presence of Pfr throughout the entire incubation period. Whether other photoreceptors have additional repressive effects is unknown.

The data here show that sequences present in the 1-kbp 5′-flanking region of the oat *phy-3* gene (21) contain the information necessary for transcription of this gene and for feedback inhibition of its expression by Pfr. A number of sequence motifs conserved in the 5′-flanking DNA of oat (21), corn (29), and rice (12, 30) *phy* genes have the potential to be involved in this regulation. The data also indicate that the components of the phytochrome signal-transduction pathway involved in autoregulation of *phy* gene transcription are conserved between rice and oats. Rice seedlings bombarded with the promoterless construct pGP228 (Fig. 1) have no CAT activity above minus DNA controls (Fig. 4), indicating that the oat *phy* intron is alone incapable of promoting CAT expression. An intronless *phy*–CAT fusion gene also produced no CAT activity above background in rice (unpublished data), suggesting that the intron may play an important role in *phy* expression analogous to strong modulating effects of introns observed for a maize *Adh* gene (24).

The absence of oat *phy*–CAT expression after microprojectile-mediated transfer into etiolated dicot seedling tissue (Fig. 2*B*) is consistent with data from stable transformation experiments. The same oat *phy* gene used to construct pGP403 was not expressed at detectable levels in stable transformants of tobacco (ref. 15; W.B.B., unpublished data). One possible explanation is that the *phy* intron in pGP403 is inefficiently spliced in dicot cells in a manner similar to that reported for a wheat *rbcS* gene in tobacco (31). However, we have tested an intronless *phy*–CAT fusion gene in bombarded and stably transformed tobacco and an intronless *phy*–β-glucuronidase fusion construct in stably transformed *A. thaliana* (W.B.B., M. Boylan, and P.H.Q., unpublished data) and were not able to detect enzyme activity above background levels. Therefore, a more plausible explanation is that the oat *phy* promoter is monocot-specific and is not active at detectable levels in dicots. A report that transcripts from the oat *phy* promoter fused to a phytochrome cDNA were not detected in stably transformed tobacco (15) is also consistent with this latter explanation. We note that, whereas the 35S construct activity vastly exceeded any *phy* construct activity in the dicots, the converse was true of rice (Fig. 2). The oat *phy* construct was 50 times more active than the 35S construct in dark-grown rice seedlings.

The principal significance of the present results is twofold. (*i*) They provide an opportunity to perform *in vivo* functional analysis of cis-acting elements involved in autoregulation of *phy* gene transcription in monocots. The *phy* genes are thus far the most thoroughly characterized gene system negatively regulated by Pfr in plants. (*ii*) Of more general impact, these investigations establish a facile transient expression assay system for rapid functional analysis of light-regulated promoters after gene transfer into readily available intact monocot tissue. This approach circumvents some of the major remaining obstacles to the analysis of regulated monocot promoters without the need for protoplasts or transgenic dicot plants. Other promoters, such as those regulated by hormones (32), heat shock (33), and anaerobiosis (34) are also likely to be amenable to this approach.

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