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Rice Phytochrome Is Biologically Active in Transgenic Tobacco

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To investigate the mechanisms of phytochrome action in vivo, we have overexpressed rice phytochrome in transgenic tobacco plants. A full-length rice phytochrome cDNA was fused to the cauliflower mosaic virus 35S promoter and transferred to tobacco. The progeny of some of the transgenic plants contain large amounts of rice phytochrome mRNA in green leaves. Extracts prepared from overexpressing plants contain twofold to fivefold more spectrophotometrically detectable phytochrome than extracts from control plants. Species-specific, anti-phytochrome monoclonal antibodies were used in immunoblots to discriminate between rice and tobacco phytochrome apoproteins in fractions eluted from a DEAE-Sepharose column. Red minus far-red difference spectra of the partially purified rice phytochrome from the transgenic plants indicate that the rice phytochrome assembles with chromophore and is photoreversible. Analysis of the circadian pattern of *Cab* mRNA levels in transgenic plants versus controls demonstrates that the overproduction of rice phytochrome extends the duration of the free-running rhythm of *Cab* gene expression. The rice phytochrome is, therefore, biologically active in the transgenic tobacco plant, which establishes a system for in vivo functional analysis of phytochrome.

INTRODUCTION

Plants utilize light both as an energy source to drive photosynthesis and as a stimulus to trigger a series of developmental events ranging from germination to flowering (Kendrick and Kronenberg, 1986). Higher plants grown in the dark are termed etiolated. They lack chlorophyll, have elongated stems, and are arrested in normal development. Illumination of etiolated plants results in a shift to normal growth, chlorophyll synthesis, and, hence, greening. The light-dependent development of plants is termed photomorphogenesis, which is a complex process resulting from the combined action of several photoreceptors (Shropshire and Mohr, 1983; Kendrick and Kronenberg, 1986). The best characterized of these is the red light photoreceptor, phytochrome.

Phytochrome is a chromoprotein consisting of an apoprotein (monomer 118 kD to 125 kD) and a linear tetrapyrrole attached to the N-terminal domain (Vierstra and Quail, 1986; Furuya, 1987), and exists as a dimer in solution (Jones and Quail, 1986; Tokutomi et al., 1989). Phytochrome is synthesized in the dark as the red absorbing form, Pr, that is physiologically inactive. Absorption of red light by Pr converts the molecule to the far-red form Pfr, which initiates the biological response to light. Pfr can be converted back to the Pr form by absorbing far-red light. Phytochrome, therefore, serves as a biological switch, being activated by red light and attenuated by far-red light. Under natural conditions of solar radiation, the relative amounts of Pr and Pfr are indicative of the ratio of red/far-red light to which the plant is exposed. This information influences a variety of developmental programs; for example, to detect shading and induce stem elongation, to promote seed germination, or to sense daylength and determine the shift from vegetative growth to flowering (Shropshire and Mohr, 1983; Kendrick and Kronenberg, 1986). It has recently been shown that at least two physically distinct forms of phytochrome exist in green tissues of pea and oat (Abe et al., 1985; Shimazaki and Pratt, 1985; Tokuhisa, Daniels, and Quail, 1985). Type I or "light-labile" phytochrome is abundant in etiolated tissue and declines upon illumination; type II phytochrome is poorly characterized, but appears not to be depleted by light (Konomi, Abe, and Furuya, 1987). One of the most interesting problems concerning phytochrome regulation is the assignment of specific functions to distinct molecular species.

The underlying basis for phytochrome-regulated plant development is the modulation of gene expression patterns by phytochrome (Cuozzo, Kay, and Chua, 1988; Nagy, Kay, and Chua, 1988a). Our laboratory and others have previously shown that specific *cis*-acting elements

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and *trans*-acting factors are essential in mediating light-regulated transcription (Kuhlemeier, Green, and Chua, 1987; Nagy, Kay, and Chua, 1988a). At the other end of the transduction pathway, much is known about the physical properties of phytochrome *in vitro* (Kendrick and Kronenberg, 1986; Furuya, 1987). In contrast to the knowledge on phytochrome and light-responsive gene expression, almost nothing is known about the transduction pathways and their components. Although a number of mutants have been isolated that are depleted in active phytochrome (Adamse et al., 1988), transduction mechanisms remain a mystery.

We have previously proposed (Nagy, Kay, and Chua, 1988a) that one valid approach toward elucidating the mode of phytochrome action in vivo is the generation of dominant mutations in the phytochrome response by overexpression of wild-type and mutant phytochrome molecules in transgenic plants. This should exaggerate the normally subtle effects exerted by the photoreceptor, thus revealing its mechanism of action for specific responses. This approach will also allow the study of structure-function relationships to be made in vivo, as well as dissecting the spatial and temporal components of phytochrome regulation by the judicious choice of promoters. As an initial step toward these goals, we have chosen to overproduce type I rice phytochrome in transgenic tobacco by using the 35S promoter of cauliflower mosaic virus (CaMV; Odell, Nagy, and Chua, 1985). Rice phytochrome was used, as this allows us to discriminate between the transgenic phytochrome and the endogenous molecule by using monoclonal antibodies. Overproduction of rice phytochrome mRNA was observed in a number of transgenic plants, as was the authentic rice apoprotein. The rice apoprotein assembles with chromophore to produce a photoreversible phytochrome. Overexpression of the rice phytochrome results in altered expression patterns of the endogenous tobacco *Cab* genes.

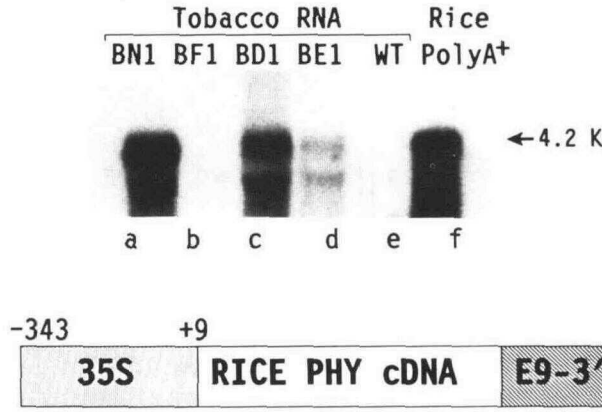

Figure 1. RNA Gel Blot Analysis of Total RNA Isolated from Transgenic Tobacco Plants Containing the 35S Rice Phytochrome cDNA.

Tracks a to d, total RNA (25 μg) from leaves of four independent transgenic tobacco plants grown in the light. Track e, total RNA (50 μg) from wild-type (WT) tobacco. Track f, poly(A$^+$) RNA (5 μg) isolated from etiolated rice leaves. The blot was probed with a 250-bp cDNA fragment specific for rice phytochrome mRNA. KB, kilobases.

RESULTS

Overproduction of Rice Phytochrome mRNA in Transgenic Tobacco

A rice type I phytochrome cDNA (Kay et al., 1989a, 1989b) containing the entire protein coding sequence, 3′-untranslated region, and 50 base pairs (bp) of 5′-untranslated leader was fused to the CaMV 35S promoter. A 3′ polyA addition site derived from the pea *rbcS-E9* gene was placed at the extreme 3′ end. Figure 1 shows the entire chimeric construct. The construct was transferred to tobacco (var SR1), and primary transformants were grown to maturity and selfed. The transgenic F_1 seeds were germinated on kanamycin and grown for 6 weeks in a growth chamber. At this time, leaves were harvested from several plants and RNA was isolated and analyzed by RNA gel blots using a rice phytochrome cDNA probe. As controls, RNA from wild-type tobacco was included as well as poly(A$^+$) RNA isolated from etiolated rice leaves. Tracks f and e in Figure 1 show a signal that corresponds to the authentic 4.2-kilobase rice phytochrome mRNA in the rice poly(A$^+$) RNA, whereas no hybridization is detected with wild-type tobacco total RNA (track e), demonstrating the specificity of the rice cDNA probe. We have analyzed a total of 20 independent transgenic plants and only results from four of them are shown. The transgenic tobacco RNAs (tracks a to d) contain various levels of rice phytochrome RNA, with BN1 being the highest expressor (track a) and BF1 the lowest (track b). BN1 transgenic F_1 plants were, therefore, chosen for further study.

BN1 Transgenic Tobacco Contains Increased Levels of Phytochrome

To investigate whether the high level of rice phytochrome mRNA in the BN1 transgenic tobacco results in an increase in the amount of phytochrome, extracts were prepared from light-grown plants that were either dark-adapted for 3 days, or were harvested at the end of a 12-hr light cycle. The crude extracts were fractionated on DEAE-Sepharose, and the fractions containing spectrophotometrically detectable phytochrome were pooled. Red minus far-red difference spectra were recorded for pooled fractions from both BN1 and wild-type extracts to measure the amount

of photoreversible phytochrome present. The results of this analysis are presented in Table 1. In extracts prepared from plants at the end of the photoperiod, BN1 contains about twice as much extractable phytochrome as the wild-type. However, when the plants are transferred to darkness for 3 days, the phytochrome level preferentially increases in the BN1 plant. Thus, in extracts prepared from dark-adapted leaves, BN1 contains about fivefold the amount of phytochrome compared with the wild-type. This presumably reflects the greater rate of resynthesis of rice phytochrome in the dark, which is under the control of a strong, constitutive promoter. However, to demonstrate unequivocally that the increase in phytochrome level is due to the transgene, the plants were analyzed further.

BN1 Plants Contain Rice Phytochrome Apoprotein

To determine whether the rice phytochrome mRNA results in the production of authentic rice phytochrome apoprotein, protein extracts prepared from dark-adapted BN1 and wild-type plants were fractionated on DEAE-Sepharose, and individual fractions were analyzed by spectrophotometry and immunoblotting. Figure 2 shows the elution profile of a typical fractionation. The total protein content of the elution profiles for both BN1 and wild-type is very similar. In contrast, the spectrophotometrically detectable phytochrome content is about fivefold higher in BN1 than in wild-type. Note that the peak of eluting phytochrome in the BN1 extract precedes that of the wild-type extract by one or two fractions.

To discriminate between rice and tobacco phytochrome apoproteins, we made use of monocot-specific and dicot-specific monoclonal antibodies. Figure 3 shows the immunoblot analysis of DEAE-Sepharose fractions that contain phytochrome. Fractions from the BN1 or wild-type extract were analyzed by SDS-PAGE and immunoblotting using either the anti-pea phytochrome monoclonal antibody, which detects tobacco phytochrome (Figure 3A), or the anti-rye phytochrome monoclonal antibody, which stains rice phytochrome (Figure 3B). Tobacco phytochrome is present in both the BN1 and wild-type fractions and is most abundant in fraction 9 of both extracts (Figure 3A, tracks c to j). Tracks a and b contain purified samples of rice and tobacco phytochrome, respectively, and demonstrate that the anti-pea phytochrome monoclonal antibody does not cross-react with rice phytochrome. The standards in Figure 3B demonstrate the specificity of the anti-rye monoclonal antibody for rice phytochrome. The small ladder of bands beneath the largest fragment in each of the standards is due to degradation of the purified phytochrome samples during preparation and storage. Figure 3B (tracks c to f) shows that a polypeptide of about 125 kD is stained by the anti-rye monoclonal antibody, and this band is most abundant in fractions 7 and 8. Conversely, no staining is observed with this antibody with the same fractions from the wild-type extract (tracks g to j). Taken together, these results clearly demonstrate that BN1 transgenic plants contain authentic rice phytochrome apoprotein, which can be partially separated from the tobacco phytochrome protein.

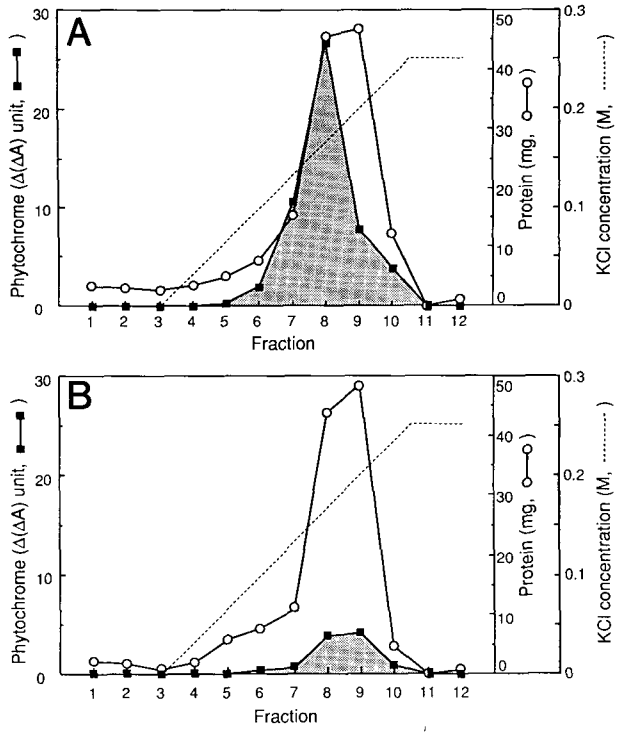

Figure 2. Protein Profile and Phytochrome Elution Profile of DEAE-Sepharose Chromatography of Extracts from Dark-Adapted Tobacco Leaves.

(A) BN1 transgenic tobacco extract. Forty grams of leaves, fresh weight, were extracted as described in Methods and fractionated on DEAE-Sepharose by elution with a 0 M to 0.25 M KCl gradient. Fractions were assayed for protein content and phytochrome by spectrophotometry.

(B) Wild-type tobacco extract. Wild-type tissue was extracted and assayed by procedures identical to those in **(A)**.

Table 1. Phytochrome Content in Green Leaves of Dark-Adapted or Light-Grown Transgenic (BN1) and Wild-Type (WT) Tobacco Plants

Treatment	Plant	Phytochrome Content
		Δ(ΔA) unit/g, fresh wt
Light-grown	BN1	0.199
	WT	0.110
Dark-adapted	BN1	0.930
	WT	0.195

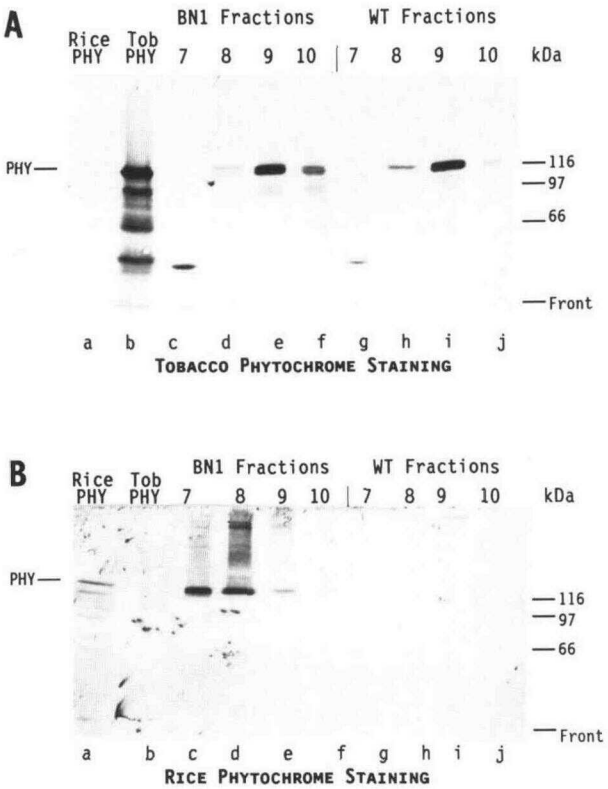

Figure 3. Immunoblot Analysis of DEAE Fractions of BN1 and Wild-Type Extracts.

(A) Detection of tobacco phytochrome apoprotein. The blot was stained with mAP5. Lane a, 1.5×10^{-2} $\Delta(\Delta A)$ units of rice phytochrome. Lane b, 1.5×10^{-2} $\Delta(\Delta A)$ units of tobacco (Tob) phytochrome. Lanes c to f, 1/400 aliquot of fractions 7 to 10 of BN1 extract. Lanes g to j, 1/400 aliquot of fractions 7 to 10 of wild-type (WT) extract.

(B) Detection of rice phytochrome apoprotein. The blot was stained with mAR14. Lane a, 5.9×10^{-2} $\Delta(\Delta A)$ units of rice phytochrome. Lane b, 5.9×10^{-2} $\Delta(\Delta A)$ units of tobacco phytochrome. Lanes c to f, 1/200 aliquot of fractions 7 to 10 of BN1 extract. Lanes g to j, 1/200 aliquot of fractions 7 to 10 of wild-type extract.

Rice Phytochrome Assembles with Chromophore in Transgenic Tobacco

To demonstrate directly that the rice phytochrome polypeptide assembles with chromophore and is photoreversible, it is necessary to record difference spectra. Fraction 8 of the DEAE-Sepharose column was chosen for both extracts, as this is the peak fraction of the rice phytochrome in BN1 and contains only a small amount of the tobacco phytochrome. Figure 4 shows red minus far-red difference spectra for the concentrated fractions. Although equivalent amounts of protein were used for both the BN1 and wild-type fractions, the former contains about 5 times to 10 times more photoreversible phytochrome than the latter. Therefore, this result demonstrates that, despite the documented structural differences between monocot and dicot phytochromes (Cordonnier, Greppin, and Pratt, 1984; Sharrock, Lissemore, and Quail, 1986), rice phytochrome apoprotein is able to assemble with the chromophore in tobacco cells to produce a spectrally active photoreceptor.

Rice Phytochrome Is Biologically Active in Transgenic Tobacco

Having shown that rice phytochrome mRNA, polypeptide, and photoreversible holoprotein can be produced in tobacco, we wished to determine whether it is biologically active. As our primary goal is to characterize the pathways and transduction components of phytochrome-regulated gene expression, we chose to investigate whether overproduction of rice phytochrome alters the expression of endogenous tobacco genes. For this analysis, two possible sources of tissue could be used: leaves from either etiolated or light-grown plants. In etiolated tissue, there are large endogenous pools of phytochrome, whereas, in leaves from light-grown plants, the endogenous tobacco phytochrome is greatly depleted. We therefore chose to use leaves from light-grown plants, as the overproduction

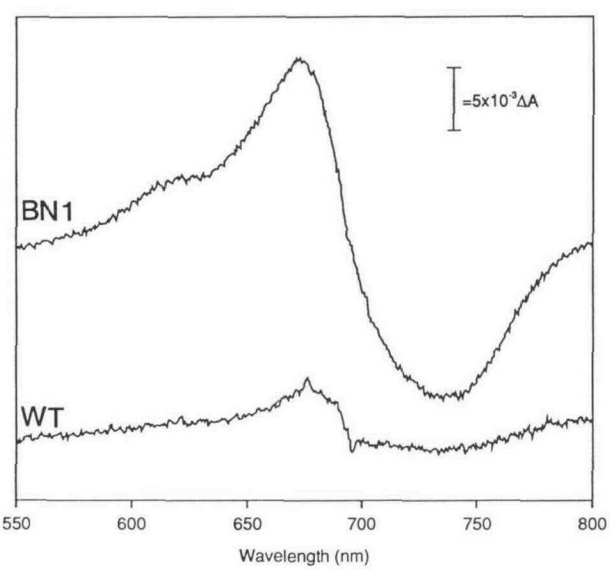

Figure 4. Difference Spectra of Phytochrome Extracted from BN1 or Wild-Type (WT) Plants.

Fraction 8 of the DEAE-Sepharose column (Figure 3) was concentrated by ammonium sulfate precipitation, and red (660 nm) minus far-red (730 nm) difference spectra were recorded.

of type I rice phytochrome would raise the normally low level of type I phytochrome in this tissue. Although most studies on phytochrome-regulated gene expression have been done with etiolated tissue, we have previously demonstrated that phytochrome controls the level of *Cab* gene transcription in leaves of light-grown plants by interacting with a circadian clock (Nagy, Kay, and Chua, 1988b). Thus, *Cab* mRNA can be detected in the morning but not in the evening. Phytochrome appears to exert its influence by regulating the *abundance* of *Cab* mRNA but not the *timing*. When plants are transferred from light-dark cycles to continuous darkness, the *Cab* mRNA level continues to oscillate, but the level of expression decreases over 1 day or 2 days. We have proposed that this decrease is due to the degradation of phytochrome during the dark period (Nagy, Kay, and Chua, 1988b). We chose this system to test whether the overproduction of rice phytochrome alters tobacco *Cab* gene expression.

BN1 F_1 transgenic plants and control transgenic plants that do not express rice phytochrome (BF1, see Figure 1) were grown from seed for 6 weeks in a growth chamber under a 12-hr light/12-hr dark cycle as described in the schematic diagram shown in Figure 5. Day 0 is designated as the last light/dark cycle before transferring the plants to continuous darkness. Leaves were harvested at 10 AM and 10 PM on day 0, and on days 1 and 2 in darkness. Total RNA was isolated and analyzed for *Cab* mRNA level in slot-blot assays (Figure 5). In the control plants, the *Cab* RNA level is high at 10 AM and barely detectable at 10 PM on day 0, and rapidly decreases in level on days 1 and 2 in darkness. In contrast, the *Cab* RNA level in BN1 plants cycles on each day, but dampens much less rapidly than in the control. Identical slot-blots were also probed for the mitochondrial β-ATPase mRNA, which is a constitutively expressed housekeeping gene (Boutry and Chua, 1985), and demonstrates that equal amounts of RNA were loaded in each slot. The same effect has been observed in the homozygous F_2 progeny of BN1, demonstrating that this molecular phenotype segregates with the transgene; we have also observed a similar effect in the F_1 progeny of BD1, an independent transgenic plant (S. Kay and M. Deak, unpublished results). The observation that *Cab* mRNA continues to cycle longer in the BN1 plants versus the controls demonstrates that the rice phytochrome is biologically active in the transgenic tobacco and is recognized by the transduction chain that transmits the light signal to the genome.

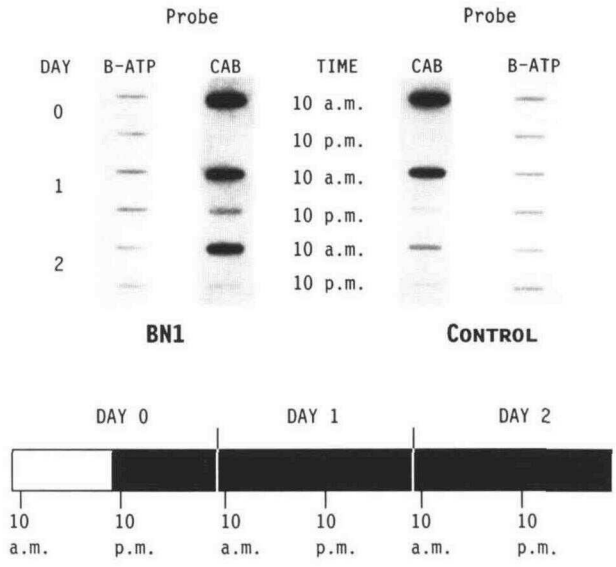

Figure 5. Analysis of *Cab* mRNA Cycling in BN1 and Control Transgenic Tobacco.

Total RNA was isolated from plants under the regime shown. Slot-blots were performed with 5 μg of RNA per slot and probed for either *Cab* mRNA (CAB) or β-subunit of the mitochondrial ATPase (B-ATP).

DISCUSSION

We have recently reported the isolation and characterization of the gene encoding rice type I phytochrome (Kay et al., 1989a, 1989b). To study the mechanisms of phytochrome function in vivo, we chose to introduce the rice phytochrome cDNA under the control of the constitutive 35S promoter into tobacco plants. The expression of the monocot phytochrome in the dicot background has the advantage that monoclonal antibodies can discriminate between phytochrome molecules from the two species. This strategy resulted in the successful synthesis and assembly of spectrally active rice phytochrome in the transgenic tobacco plants. Overproduction of rice phytochrome in transgenic tobacco has allowed us to address questions regarding phytochrome-regulated *Cab* gene expression in vivo, as we can demonstrate that the damping of the rhythm in *Cab* mRNA level can be attenuated by increasing the cellular level of phytochrome. It should now be feasible to introduce mutant forms of the rice phytochrome molecule and measure the effects on endogenous tobacco gene expression. This will provide valuable information on the structure-function relationships for the photoreceptor in planta. The manipulation of rice phytochrome in transgenic tobacco, therefore, provides a powerful tool for investigating the in vivo mechanisms of phytochrome-regulated gene expression.

We can also address the kinetics of synthesis and degradation (Schafer, 1978) of both the transgenic rice and tobacco phytochromes. In this respect it is interesting to note that the rice phytochrome present in BN1 plants is degraded in the light. Fivefold higher levels of phytochrome

are observed in the leaves of dark-adapted BN1 plants versus leaves harvested in the light. It is therefore likely that the Pfr-specific degradation apparatus of tobacco recognizes the rice phytochrome as a substrate. We tentatively predict that Pfr degradation is the primary control mechanism regulating phytochrome levels in the light, as the large overexpression of rice phytochrome mRNA in light grown leaves (Figure 1) is not reflected in the level of spectrophotometrically detectable phytochrome in this tissue (Table 1). More detailed analysis, using transgenic plants that express different levels of rice phytochrome mRNA, is underway to investigate the relative contribution of mRNA abundance and Pfr degradation to regulating the levels of active phytochrome.

Keller et al. (1989) have recently expressed functional oat phytochrome in transgenic tobacco. Interestingly, the progeny of the plants that overexpressed high levels of oat phytochrome exhibited morphological phenotypes, such as semi-dwarfism and reduced apical dominance. No observations were presented on the expression of endogenous tobacco genes in these plants. It is therefore striking that, in our population of transgenic plants, no morphological phenotype was observed that segregated with the transgene in a statistically significant way. Three possible explanations may account for this difference in our observations: (1) BN1 plants have not overproduced sufficient amounts of rice phytochrome; (2) the transgenic rice phytochrome is an aberrant molecule, or rice phytochrome is less active than oat phytochrome in tobacco plants; (3) different cultivars of tobacco exhibit differential sensitivity to transgenic phytochromes.

The first explanation is unlikely because, although Keller et al. (1989) observed 20-fold more phytochrome in overproducers versus controls, the absolute levels of phytochrome produced in BN1 (per gram, fresh weight) are similar to those in the transgenic tobacco plants containing oat phytochrome. The difference in ratio is explained by the fact that we have partially purified phytochrome before spectral analysis, allowing us to detect the low level of phytochrome in green leaves of control plants harvested in the light. This directly affects the calculated ratio of detectable phytochrome between overproducers and controls. The rice phytochrome present in BN1 has identical molecular weight, immunoreactivity, and spectral properties of authentic rice phytochrome, and is therefore highly likely to be authentic. This is corroborated by the observation that the rice phytochrome alters *Cab* gene expression and is therefore functional. It is possible that BN1 does not exhibit morphological phenotypes due to the particular cultivar of tobacco used in our experiments (SR1) versus that used in the previous study (xanthi). We are currently investigating whether various tobacco hosts display differential sensitivity to the overproduction of rice phytochrome.

We have previously proposed (Nagy, Kay, and Chua, 1988b) that the damping of the *Cab* mRNA rhythm in extended darkness is due to degradation of Pfr in the dark. The observation that BN1 plants exhibit an extended *Cab* free-running rhythm supports this hypothesis. However, as the level of phytochrome in BN1 is twofold higher than that in controls, and the normal half-life of monocot Pfr in oats is only 60 min (Vierstra and Quail, 1986), the magnitude of the effect over 2 days is surprising. It is possible that the degradation rate of rice Pfr is slower than that of the endogenous tobacco molecule in the dark. Alternatively, the overproduced type I rice phytochrome may be interfering with the regulation of *Cab* gene expression by the endogenous type I or type II tobacco phytochrome.

These results raise two interesting points concerning the biological function of phytochrome. The first is that the introduced type I rice phytochrome is functional in green leaves, a tissue in which it is normally greatly depleted. This notion is further supported by observations in the tomato *aurea* mutant that is depleted in type I "light-labile" phytochrome (Adamse, Kendrick, and Koornneef, 1988). Light-grown *aurea* plants are deficient in *Cab* gene expression (Sharrock et al., 1988) and chlorophyll accumulation, but not in classical "light-stable" phytochrome responses such as end-of-the-day far-red stimulation of stem elongation (Adamse et al., 1988). The overproduction of transgenic phytochrome, therefore, provides direct evidence for the function of type I phytochrome in green leaves. The second point is the possibility for interaction between functionally distinct forms of phytochrome. We would have predicted that the *Cab* expression rhythm is controlled by "light-stable" phytochrome (Adamse, Kendrick, and Koornneef, 1988) that has a much longer Pfr half-life. However, the effect of the type I rice phytochrome on the *Cab* gene free-running rhythm demonstrates that type I phytochrome is able to affect a classical "type II" response. These experiments have taken the first step toward elucidating the interaction between phytochrome and the endogenous circadian clock in regulating *Cab* gene expression.

METHODS

Plant Material

Tobacco plants (var SR1) were maintained in sterile culture on MS medium to provide leaves for transformation experiments. Regenerated transgenic tobacco were maintained in a growth chamber under an 18-hr light/6-hr dark regime, grown until maturity, and selfed. F_1 seeds were germinated on MS medium containing kanamycin, and resistant seedlings were transferred to pots and placed in a growth chamber. For extraction of phytochrome, leaves were harvested from light-grown (12-hr light/12-hr dark) plants at the end of the photoperiod or from similar plants irradiated with 5 min far-red light (Nagatani et al., 1989) at the end of the photoperiod and then kept in the dark for 72 hr.

One week prior to *Cab* gene analysis, the plants were placed in a growth chamber on a 12-hr light/12-hr dark cycle.

Constructs and Transformation Experiments

A full-length cDNA clone was constructed by fusing a cDNA clone (*cphy*5, +411 to +3691, relative to translation initiation) with the 5′ fragment (−50 to +410) derived from the genomic clone *phy*18 (Kay et al., 1989a, 1989b). This was then cloned into the SmaI site of pMON530-E9 (Cuozzo et al., 1987). The resulting construct was transferred to tobacco via *Agrobacterium*-mediated leaf disc transformation (Horsch et al., 1988).

Phytochrome Extraction and Analysis

All procedures for phytochrome partial purification, extraction, and detection were performed as described by Nagatani et al. (1989). Phytochrome was partially purified from leaves of light-grown transgenic plants by DEAE-Sepharose chromatography. Immunoblot analysis was performed using the anti-rye phytochrome monoclonal antiserum mAR14, which was prepared as described in Nagatani et al. (1983), and the anti-pea phytochrome monoclonal antibody mAP5 (Nagatani et al., 1984). Phytochrome was measured with a recording spectrophotometer (Hitachi model 3410). One $\Delta(\Delta A)$ unit is defined as the amount of phytochrome that shows 0.001 photoreversible absorbance-difference change between 660 nm and 730 nm when dissolved in 1 mL in a 1-cm path-length cuvette.

RNA Extraction and Analysis

Total RNA was extracted from leaves and analyzed in RNA gel or slot-blots as described previously (Nagy, Kay, and Chua, 1988c; Kay et al., 1989a). The rice cDNA probe used in Figure 1 was a 250-bp PstI-EcoRI fragment derived from the 3′ end of the *cphy*5 rice phytochrome cDNA clone (Kay et al., 1989a). Coding sequence probes were used from the wheat *Cab-1* gene (Lamppa, Nagy, and Chua, 1986) and the β-subunit of the mitochondrial ATPase (Boutry and Chua, 1985).

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