The Plant Cell, Vol. 1, 765–773, August, 1989

Oat Phytochrome Is Biologically Active in Transgenic Tomatoes

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To determine the functional homology between phytochromes from evolutionarily divergent species, we used the cauliflower mosaic virus 35S promoter to express a monocot (oat) phytochrome cDNA in a dicot plant (tomato). Immunoblot analysis shows that more than 50% of the transgenic tomato plants synthesize the full-length oat phytochrome polypeptide. Moreover, leaves of light-grown transgenic plants contain appreciably less oat phytochrome than leaves from dark-adapted plants, and etiolated R1 transgenic seedlings have higher levels of spectrally active phytochrome than wild-type tomato seedlings in direct proportion to the level of immunochemically detectable oat polypeptide present. These data suggest that the heterologous oat polypeptide carries a functional chromophore, allowing reversible photoconversion between the two forms of the molecule, and that the far-red absorbing form (Pfr) is recognized and selectively degraded by the Pfr-specific degradative machinery in the dicot cell. The overexpression of oat phytochrome has pleiotropic, phenotypic consequences at all major phases of the life cycle. Adult transgenic tomato plants expressing high levels of the oat protein tend to be dwarfed, with dark green foliage and fruits. R1 transgenic seedlings have short hypocotyls with elevated anthocyanin contents. We conclude that a monocot phytochrome can be synthesized and correctly processed to a biologically active form in a dicot cell, and that the transduction pathway components that interact with the photoreceptor are evolutionarily conserved.

INTRODUCTION

Phytochrome is a regulatory protein that controls many aspects of plant development in response to light, including seed germination, stem elongation, and flowering (Kronenberg and Kendrick, 1986). The regulated expression of genes involved in some of these morphological responses has been intensively investigated (Tobin and Silverthorne, 1985; Kuhlemeier et al., 1987). The purified phytochrome molecule has been extensively characterized biochemically; its biogenesis is understood in molecular terms, yet the primary mode of action of the photoreceptor remains unknown.

Phytochrome is a soluble, cytoplasmic protein (Pratt, 1986) consisting of a linear tetrapyrole chromophore covalently attached to a cysteine residue in the N-terminal domain of the polypeptide chain (Vierstra and Quail, 1986). The native protein behaves as a homodimer in solution (Jones and Quail, 1986), with subunits of 120 kD to 127 kD, depending on the species (Vierstra et al., 1984). Phytochrome exists in two photoreversible forms: Pr (λ_{max} = 666 nm) and Pfr (λ_{max} = 730 nm). Absorption of red light by Pr converts the molecule to Pfr, the biologically active form; subsequent irradiation with far-red light changes phytochrome back to the inactive Pr form. Phytochrome is synthesized as Pr in dark-grown plant tissues (Quail, Schafer, and Marme, 1973a). The amount of phytochrome present in light-grown plants is only 1% of the dark value (Hunt and Pratt, 1979) because Pfr is proteolytically degraded at a much faster rate than Pr (Quail, Schafer, and Marme, 1973b).

We are interested in identifying the regions of the phytochrome polypeptide responsible for its structural properties and diverse biological activities, including sequences involved in chromophore interaction, dimerization, differential turnover of the Pr and Pfr forms, and the regulatory action of the photoreceptor. Mutations induced in phytochrome structural genes would be valuable for this purpose. Phytochrome-deficient mutants have been identified in tomato and *Arabidopsis* (Koornneef, Rolff, and Spruit, 1980; Koornneef et al., 1985) but, unfortunately, none of these appears to be due to lesions in the phytochrome polypeptide itself (Sharrock et al., 1988; Parks et al., 1989). Moreover, the probable existence in all angiosperms of multiple phytochrome genes, with possible overlapping functions (R.A. Sharrock and P.H. Quail, unpublished data), raises the possibility that selection for phytochrome-deficient mutants using existing phenotypic criteria may not detect lesions in individual members of the gene family.

Two of the principal strategies that can be exploited as

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alternatives to classical genetic approaches for dissecting the functional domains of the phytochrome molecule are dominant negative mutations (Herskowitz, 1987), and antisense/complementation experiments (Smith et al., 1988). In the dominant negative mutation approach, a cloned gene is altered in vitro and introduced into a wild-type cell. This approach has the potential for generating a dominant negative phenotype if the mutant protein can inhibit the function of the wild-type gene product. In addition, the effects of the alterations on the behavior of the phytochrome molecule can easily be monitored if the mutated gene product is distinguishable from the wild-type product. The second strategy involves the use of antisense RNA to block wild-type gene activity, in conjunction with the expression of an introduced in vitro mutagenized sequence. The successful implementation of either strategy relies on the correct expression of introduced phytochrome sequences in transgenic plant cells. In this study, we examine the expression of a full-length oat phytochrome polypeptide sequence in transgenic tomato and describe the effects of phytochrome overexpression on the phenotype of these plants.

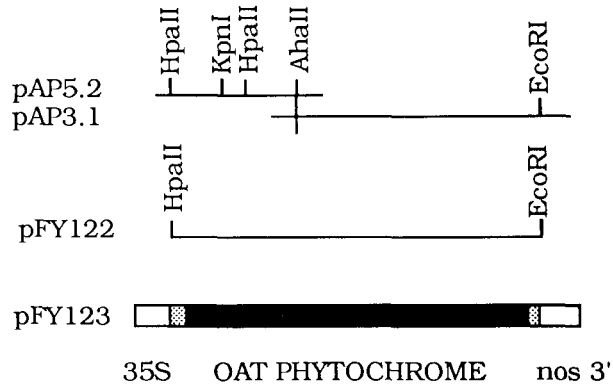

Figure 1. Construction of the Chimeric Gene Fusion Used To Express Oat Phytochrome in Transgenic Tomato.

A 1.4-kb HpaII/AhaII fragment from pAP5.2 was fused to a 2.0-kb AhaII/EcoRI fragment from pAP3.1 to generate the complete oat phytochrome coding sequence contained in pFY122. The hybrid cDNA was introduced into the intermediate vector pMON316 (Rogers et al., 1987) between the 35S promoter element (35S) and the nopaline synthase terminator (nos 3') to form pFY123. The stippled boxes (□) indicate the untranslated sequences flanking the 1124 amino acid coding portion (solid box, ■) of the chimeric oat phytochrome construct.

RESULTS

Construction of a Full-Length Oat Phytochrome cDNA

Several phytochrome cDNA clones were isolated previously in our laboratory from etiolated oat tissue, but none of these was a full-length sequence (Hershey et al., 1985). Figure 1 shows a complete oat phytochrome coding sequence, assembled by joining two overlapping cDNA clones at their common AhaII restriction site to form pFY122. The amino-terminal half of the resulting full-length cDNA is a "type 5" phytochrome, with 70 bp of untranslated sequence preceding the ATG, while the carboxy-terminal portion is a "type 3" phytochrome, extending 50 bp beyond the termination codon. Because the type 3 and type 5 sequences are 98% identical at the amino acid level, the chimeric polypeptide differs by only 3 amino acids from that of an authentic type 3 phytochrome. The hybrid phytochrome was cloned into the polylinker of the plant expression cassette vector pMON316 (Rogers et al., 1987) to form pFY123.

Detection of Oat Phytochrome in R0 Transgenic Tomatoes

Agrobacterium cells, containing a pFY123::Ti plasmid co-integrate, were used to infect approximately 600 cotyledons of tomato cv VF36. Within 4 months, 84 independent kanamycin-resistant tomato plants were regenerated, rooted, and transferred to soil. After 6 weeks of growth under greenhouse conditions, the plants were transferred to continuous darkness for 4 days to let phytochrome levels reaccumulate. Vegetative shoot tips were then removed, and extracts were subjected to immunoblot analysis to monitor phytochrome content. A representative sample of these results is shown in Figure 2.

The 84 transgenic plants fell into distinct categories of oat phytochrome expression when immunoblots were probed with a monocot-specific, anti-phytochrome monoclonal antibody (Figure 2A). Authentic oat phytochrome is 124 kD (lane 1). No immunoreactive protein was detected in a wild-type tomato extract (lane 2), demonstrating that the monoclonal antibody could distinguish monocot from dicot phytochrome. Thirty-four of the transgenic plants contained no detectable oat phytochrome (e.g., 59, lane 3). The other 50 plants displayed a range of values extending from very low (22, lane 4) to very high (28, lane 6).

A monoclonal antibody that cross-reacts with both monocot and dicot phytochromes was used to visualize the endogenous tomato photoreceptor in these extracts (Figure 2B). Tomato phytochrome has an apparent molecular mass of 116 kD (lane 2), and was therefore easily resolved from the 124-kD oat protein (lane 1). Each of the transgenic plants (lanes 3 through 7) contained about the same amount of tomato phytochrome as in wild-type tissue (lane 2). Expression of endogenous tomato phytochrome did not vary among the different transgenics, regardless of the level of oat phytochrome (undetectable, low, or high). As a result, in plants containing particularly high amounts of oat phytochrome (28, lane 6), this protein accumulated to levels in excess of its endogenous tomato homologue.

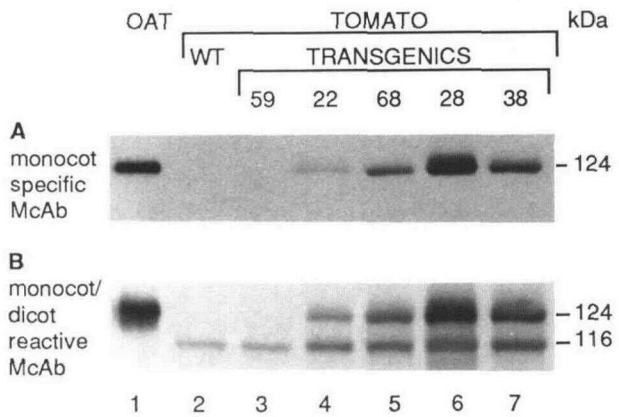

Figure 2. Oat Phytochrome Expression in Transgenic Tomato.

(A) Immunoblot probed with a monoclonal antibody (McAb) that recognizes oat phytochrome. Crude extracts were prepared from either dark-adapted wild-type (WT) (lane 2) or transgenic (lanes 3 through 7) tomato plants. Aliquots (25 μL) were added to sample buffer and analyzed by immunoblotting. The transgenic tomato plants are identified by the numbers above lanes 3 through 7. Lane 1 contains 25 ng of purified 124-kD oat phytochrome.
(B) Immunoblot probed with a monoclonal antibody that recognizes both oat and tomato phytochromes. The lanes contain the same samples described in **(A)**. The apparent molecular mass of tomato phytochrome is 116 kD (Parks et al., 1987).

In Vivo Degradation of Oat Phytochrome in R0 Transgenic Tomatoes

Our results demonstrate that the apoprotein for oat phytochrome is synthesized and accumulated stably in dark-adapted tomato tissue. If chromophore were attached to the oat polypeptide, the molecule would be in the proteolytically sensitive Pfr form in light-grown plants, resulting in decreased amounts of immunologically detectable phytochrome. To explore this possibility, we returned the dark-adapted transgenics to the greenhouse for 2 weeks and then assayed the phytochrome content of vegetative shoot buds by immunoblotting, as shown in Figure 3.

Analysis with the monocot-specific monoclonal antibody (Figure 3A) demonstrated that the dark-adapted transgenics (lanes 4 and 6) contained appreciably more oat phytochrome than was detected following transfer to the light (lanes 5 and 7), suggesting that the monocot protein did indeed undergo photoconversion from Pr to Pfr. As expected, the 116-kD tomato polypeptide also underwent Pfr-specific degradation (Figure 3B). Although the tomato protein was undetectable on immunoblots of extracts of both wild-type (lane 3) and transgenic (lanes 5 and 7) light-grown tissues, measurable quantities of the 124-kD oat polypeptide remained. This was readily apparent for transgenic plants such as 28 (lane 7), which had a high oat phytochrome content in the dark (lane 6). However, immunoblots developed for longer times to enhance sensitivity showed that this was also true for all of the other R0 plants we analyzed (data not shown), including low expressors such as 22 (lane 5). These data show that the heterologous oat protein persisted at much higher levels than its endogenous tomato homologue following transfer of the plants to light.

Quantitative Analysis of Phytochrome Levels

The light-induced decrease in oat phytochrome observed on immunoblots suggests that the protein was photochemically active in the R0 transgenic tomatoes. To verify this conclusion, we compared the amount of spectrophotometrically detectable phytochrome in wild-type seedlings with that found in the R1 progeny of the transgenic plants, as shown in Figure 4. The changes in phytochrome content measured by spectral assay (Figure 4C) were closely correlated with the phytochrome levels observed on immunoblots (Figures 4A and 4B).

Phytochrome spectral activity in dark-grown transgenic seedlings from the high-level expressor 28 (Figure 4C, lane 5) was 1.7-fold higher than that of wild-type material (Figure 4C, lane 2). Immunoblot analysis showed that the increased spectral signal was reflected by almost equiva-

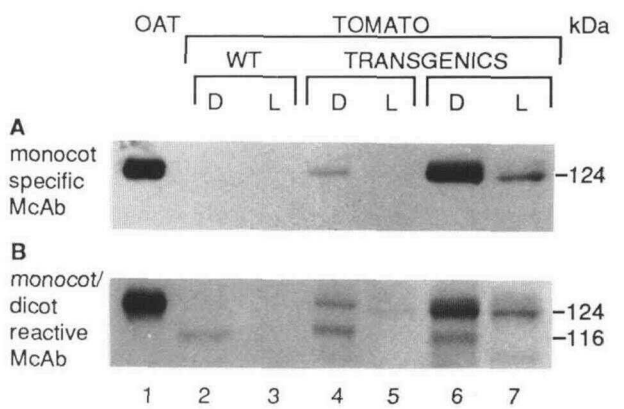

Figure 3. Light-Induced Decrease of Oat Phytochrome in R0 Transgenic Tomato.

(A) Immunoblot probed with a monoclonal antibody that recognizes oat phytochrome. Extracts were prepared from either dark-adapted (4 days) tomato plants (lanes D), or from the same plants after they had been returned to the light for 2 weeks (lanes L). Aliquots (25 μL) were added to sample buffer and analyzed by immunoblotting. Lane 1 contains 50 ng of purified oat phytochrome; lanes 2 and 3 contain extracts from wild-type tomato; lanes 4 and 5 contain extracts from a transgenic tomato expressing low levels of oat phytochrome (22 in Figure 2); lanes 6 and 7 contain extracts from a transgenic plant expressing high levels of oat phytochrome (28 in Figure 2).
(B) Immunoblot probed with a monoclonal antibody that recognizes both oat and tomato phytochromes. The lanes contain the same samples described in **(A)**.

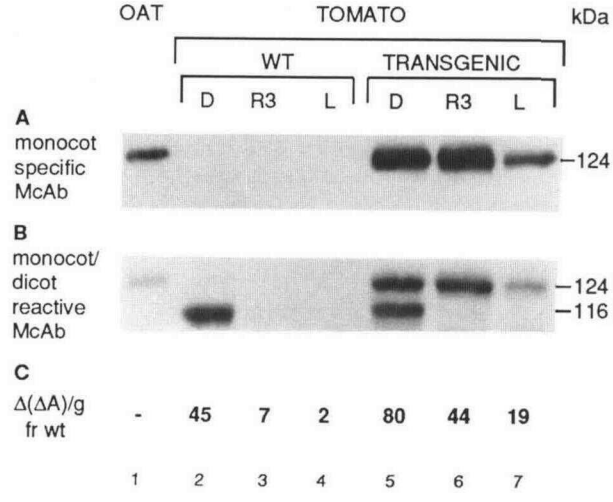

Figure 4. Phytochrome Levels in R1 Transgenic Tomato Seedlings.

(A) Immunoblot probed with a monoclonal antibody that recognizes oat phytochrome. Seedlings were grown in the dark for 6 days and then either kept in the dark (lanes D) or exposed to red light for 3 hr (lanes R3). Another group of seedlings was grown in continuous white light for 6 days (lanes L). Aliquots of crude extracts (25 μL) were mixed with sample buffer and analyzed by immunoblotting. Lane 1 contains 25 ng of purified oat phytochrome; lanes 2 through 4 contain extracts from wild-type seedlings; lanes 5 through 7 contain extracts from the R1 progeny of transgenic plant 28.

(B) Immunoblot probed with a monoclonal antibody that recognizes oat and tomato phytochromes. The lanes contain the same samples described in **(A)**.

(C) Spectral analysis of phytochrome levels. The phytochrome content of the extracts was measured using a dual-wavelength spectrophotometer and is expressed as $\Delta(\Delta A)/g$, fresh weight. Extracts from light-grown seedlings were treated with polyethyleneimine to remove chlorophyll prior to spectral assay. The lanes contain the same samples described in **(A)**.

lent amounts of oat and tomato phytochrome in the etiolated R1 seedlings (Figure 4B, lane 5). We would expect the actual phytochrome content of a plant homozygous for oat phytochrome expression to be even higher than this since the pooled R1 seedlings used in this experiment were a segregating population, with a ratio of 3 oat phytochrome expressors to 1 nonexpressor (see below). Etiolated wild-type and R1 seedlings contained similar amounts of tomato phytochrome (compare lanes 2 and 5, Figure 4B), indicating that the increased spectral signal was due to the expression of the 124-kD oat protein.

Irradiation of etiolated wild-type tomato seedlings for 3 hr with red light reduced the phytochrome spectral activity sixfold (Figure 4C, lane 2) to levels that were undetectable on immunoblots (Figure 4B, lane 2). Surprisingly, spectral activity in transgenic seedlings decreased only twofold following similar irradiation (Figure 4C, lane 6). Immunoblot analysis showed that, while the tomato polypeptide had virtually disappeared from the R1 seedlings (Figure 4B, lane 6), the amount of oat protein was unaffected by this treatment (Figure 4A, lane 6). This result indicates that the vast majority of the photoreversible spectral activity present in the transformants after 3 hr in red light was due to photochemically active oat phytochrome. Moreover, the relative strengths of the spectral and immunochemical signals are consistent with most, if not all, of the oat phytochrome having a spectrally active chromophore. This observation provides an easy way to estimate directly the spectral signal due to oat phytochrome in these plants.

These data indicate that the Pfr form of the oat phytochrome molecule in etiolated R1 transgenic seedlings does not undergo the rapid degradation process experienced by the endogenous tomato photoreceptor. However, R1 seedlings grown in continuous white light for 6 days contained less oat protein than did the etiolated seedlings (Figures 4A and 4B, lane 7), consistent with our observations on the R0 plants (Figure 3). Spectral activity in the light-grown R1 transgenic plants (Figure 4C, lane 7) was 10-fold higher than in the wild-type (Figure 4C, lane 4), demonstrating that the oat phytochrome present in the light was photochemically functional.

Phenotypic Consequences of Excess Phytochrome

As summarized in Table 1, oat phytochrome was detected in more than 50% of the kanamycin-resistant tomatoes. Adult R0 plants could be differentiated phenotypically based on the amount of oat phytochrome they contained, as determined from immunoblotting. Transgenic plants classified as either nulls or low-level expressors resembled wild-type tomato plants. By contrast, expression of high levels of oat phytochrome had pleiotropic effects on plant morphology. As shown in Figure 5, transgenic plants in this category tended to be dwarfed, with deep green foliage and fruit.

The R1 progeny of the primary transgenic tomatoes analyzed so far segregated 3:1 for oat phytochrome expression, as illustrated in Table 2, indicating that the

Table 1. Classification of R0 Transgenic Tomatoes

Oat Phytochrome Expression[a]	Number of Plants	Dwarfs	Normal
Null[b]	34	0	34
Low levels[c]	22	0	22
High levels[d]	27	21	6
Truncated (80-kD) polypeptide[e]	1	0	1

[a] Oat phytochrome expression was based on the level of immunologically detectable 124-kD oat protein.
[b] e.g., 59 in Figure 2.
[c] e.g., 22, 68, and 38 in Figure 2.
[d] e.g., 28 in Figure 2.
[e] One plant contained an 80-kD polypeptide that reacted with the monoclonal antibody.

Table 2. Segregation of the Oat Phytochrome Gene in the R1 Progeny of Transgenic Tomatoes

Transgenic Plant[a]	Oat^{+} [b]	Oat^{-} [c]	χ^{2} [d] 3:1
19	42	16	0.21
28	25	7	0.04
39	20	10	1.16

[a] R0 plants 19, 28, and 39 were high level oat phytochrome expressors; 19 and 39 were of normal height, 28 was dwarfed.
[b] Oat^{+} = R1 progeny expressing oat phytochrome.
[c] Oat^{-} = R1 progeny not expressing oat phytochrome.
[d] χ^{2} values were calculated with Yates correction factor.

monocot sequence was inserted at a single locus in these plants. Figure 6 and Table 3 show that the hypocotyls of light-grown seedlings expressing oat phytochrome were only half as long as those of their nonexpressing siblings. The fresh weight of the seedlings was unaffected, indicating that the decreased height was not due to less vigorous growth (Table 3). The short hypocotyls of the oat phytochrome expressors were noticeably darker than normal, and had 3 times the anthocyanin content of wild-type seedlings (Table 3). Inheritance of the T-DNA insert was also monitored by nopaline assay. Nopaline co-segregated with both the hypocotyl phenotype and oat phytochrome expression in the R1 generation. Oat phytochrome-expressing seedlings displayed the short hypocotyl phenotype regardless of whether the height of the parent plant was normal or dwarfed (see Table 1). Dark-grown R1 seedlings were indistinguishable from wild-type in all respects, indicating that phenotypic expression was strictly dependent on Pfr formation.

DISCUSSION

Several pieces of evidence indicate that oat phytochrome was correctly synthesized and processed to a biologically active form when the monocot polypeptide sequence was introduced into tomato. First, monocot-specific, anti-phytochrome monoclonal antibodies detected a single polypeptide, of the expected size (124 kD), in extracts of transgenic tissue. Second, the behavior of this polypeptide in response to light mimicked, at least qualitatively, that of endogenous tomato phytochrome. Third, the transgenic plants contained elevated levels of phytochrome spectral activity that were closely reflected by changes in the amount of immunologically detectable oat polypeptide. Finally, expression of oat phytochrome was associated with striking phenotypic consequences, both at the seedling stage and in adult plants.

The quantitative variation in oat phytochrome expression exhibited by the transgenic plants could be due to either the copy number or the position of the T-DNA insert

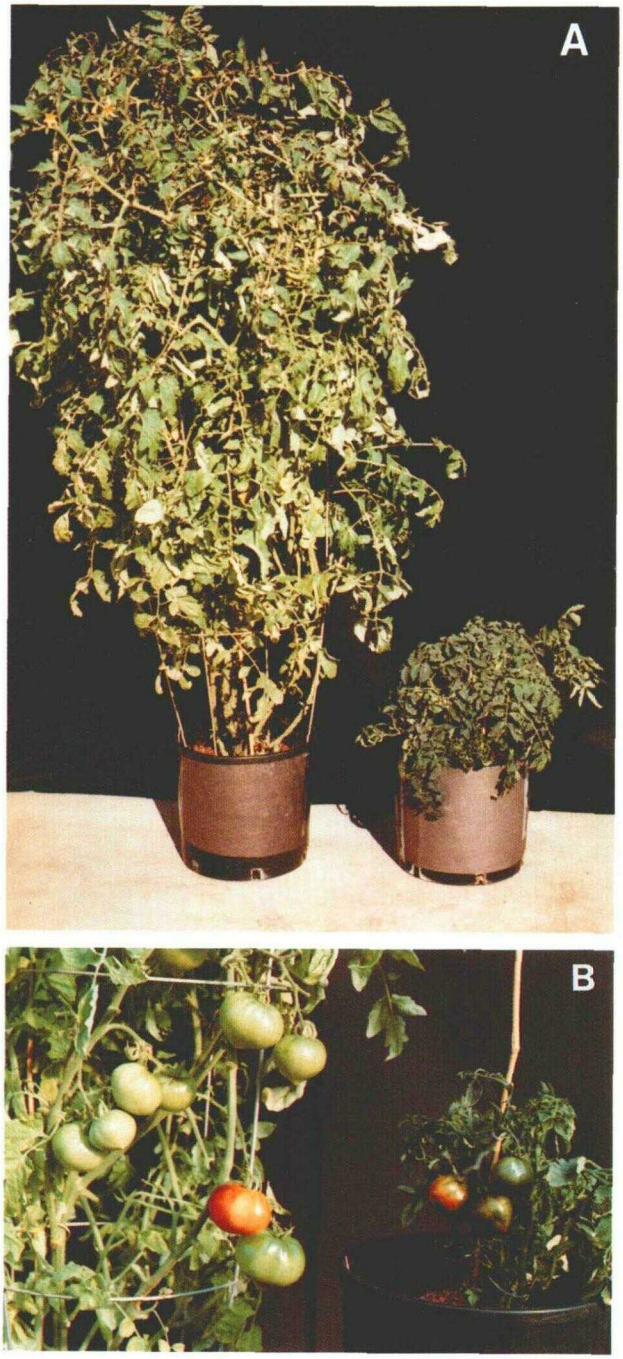

Figure 5. Adult Phenotype of R0 Transgenic Tomatoes Expressing Oat Phytochrome.

(A) Two transgenic plants demonstrate the influence of phytochrome overexpression on plant height and color. The transgenic plant on the left is classified as a null in terms of oat phytochrome expression (59 in Figure 2); the darker, dwarf plant on the right contains high amounts of oat phytochrome (28 in Figure 2).
(B) Comparison of the intensely pigmented foliage and fruits of a dwarf phytochrome overexpressor (right, 28) with the light green color of an oat phytochrome null (left, 59). The phenotype of the null individuals is indistinguishable from wild-type. Note that the fruits of the phytochrome overexpressor ripen normally.

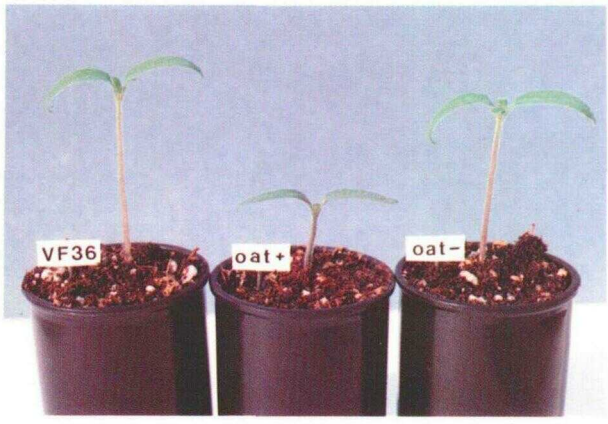

Figure 6. Phenotype of R1 Transgenic Tomato Seedlings Expressing Oat Phytochrome.

One-week-old, light-grown tomato seedlings. The seedling on the left is wild-type (cv VF36), the seedling in the middle is one of the oat phytochrome-expressing progeny of transgenic plant 28, the seedling on the right is one of its nonexpressing siblings. R1 plants were tested for oat phytochrome expression when they were 1 month old by subjecting leaf extracts of light-grown plants to immunoblot analysis. All of the seedlings with short, darkly pigmented hypocotyls contained oat phytochrome; their normal-sized siblings did not. The ratio of oat phytochrome expressors to nonexpressors was 25:7 (see Table 2).

in the tomato genome (McCormick et al., 1986). In contrast, the amount of tomato phytochrome did not vary among the different classes of transgenic plants (nulls, low-level expressors, high-level expressors). In all cases, the level of endogenous phytochrome was comparable to that of wild-type plants, leading to a net increase in phytochrome content in oat polypeptide expressors, as shown by immunoblot and spectral analysis. Formation of a functional phytochrome molecule requires synthesis of both chromophore and polypeptide, but the degree of coordination between these two pathways is unclear (Jones et al., 1986; Parks et al., 1989). Our data suggest that most, if not all, of the oat polypeptides present in the tomato tissue carried a spectrally functional chromophore. Thus, the results indicate that the amount of chromophore is not a limiting factor in phytochrome production, either because of a constitutive excess of chromophore or because the tomato cells can adjust the level of chromophore biosynthesis to meet the demands of increased phytochrome accumulation.

Recent attempts to explore the mechanism of chromophore attachment have led to the surprising conclusion that phytochrome can self-assemble with chromophore in vitro in a nonenzymatic process to yield a photoreversible product (Elich and Lagarias, 1989; Lagarias and Lagarias, 1989). Thus, the apoprotein itself may be the major determinant for correct phytochrome assembly in plant cells. Our data demonstrate that the critical sequences involved in the recognition, covalent attachment, and correct conformational arrangement of the chromophore are conserved between monocot and dicot phytochromes. In this respect, it is noteworthy that, although monocot and dicot phytochromes share only 65% amino acid homology, sequences postulated to be involved in chromophore interaction have been highly conserved (Sharrock, Lissemore, and Quail, 1986).

Reduced levels of both tomato and oat phytochrome were found in the transgenic plants following exposure to white light, consistent with the photoconversion of Pr to Pfr and its subsequent degradation. This result indicates that the molecular components responsible for the selective turnover of Pfr, as well as the target sequence(s) in the phytochrome polypeptide, have been conserved between oat and tomato.

The reasons for the quantitative differences in the rate and extent of degradation of the Pfr forms of tomato and oat phytochromes in the transgenic plants are unclear, as illustrated in Figures 3 and 4. Whereas the level of tomato phytochrome declined dramatically as expected in 3 hr of red light, the heterologous oat polypeptide was apparently unaffected (Figures 4A and 4B). This effect was not due to a lack of chromophore because the oat molecule was fully photoreversible (Figure 4C). Moreover, the oat polypeptide was susceptible to degradation, at least over more prolonged periods of irradiation, in both R0 plants (Figure 3) and R1 seedlings (Figures 4A and 4B), indicating the absence of complete resistance to the normal degradative process in the tomato cell. Factors that could contribute to these differences might include a slower rate of turnover of oat Pfr and/or a higher rate of oat phytochrome transcription driven by the constitutive 35S promoter than for

Table 3. Comparison of 7-Day-Old, Light-Grown, Wild-Type Seedlings with the R1 Progeny of Transgenic Plant 28 Shown in Figure 6

		Transgenic	
	Wild-Type	Short	Tall
Hypocotyl length, mm	18 ± 5 [a]	9 ± 1	20 ± 5
Fresh weight, mg[b]	26 ± 8	25 ± 6	27 ± 9
Anthocyanin, A_{535}[c]	0.47	1.44	0.40
Nopaline[d]	–	+	–
Oat phytochrome[e]	–	+	–

[a] Standard deviation determined from sample sizes of 20 seedlings.
[b] Fresh weight is that of the intact seedling.
[c] Anthocyanin = spectrophotometrically determined anthocyanin content of the hypocotyls of 20 seedlings.
[d] – = absence, + = presence of nopaline in the cotyledons of the seedlings measured for anthocyanin.
[e] Oat phytochrome = immunochemically detectable 124-kD oat protein in leaf buds of 1-month-old, light-grown plants; – = absence, + = presence of the oat polypeptide.

the endogenous tomato polypeptide. Slower turnover of the oat polypeptide could result from intrinsic sequence or structural differences between monocot and dicot phytochromes or from the 35S promoter-driven expression of the oat sequence in cells not normally equipped for expression and efficient degradation of the photoreceptor. Regardless of the mechanism(s), it is clear that overproduction of oat phytochrome results in the persistence of this protein in white light-grown plants, such that high expressors contained up to 10-fold more total phytochrome in fully light-grown tissue than their wild-type counterparts (Figures 3 and 4).

The expression of high levels of oat phytochrome in tomato had pleiotropic, phenotypic consequences extending to at least three stages of development [seedling, vegetative, and reproductive (Figures 5 and 6)], thereby establishing that a monocot photoreceptor can regulate biological processes in a dicot cell. Thus, it appears that the sequences critical to the regulatory activity of phytochrome, as well as the primary reaction partner(s) with which the photoreceptor interacts, have been conserved between the two major groups of angiosperms. Moreover, the data demonstrate that "type 1" or "etiolated-tissue" phytochrome (Tokuhisa and Quail, 1989) can be biologically active in fully green tissue. This observation has implications for the relative roles of type 1 and type 2, or "green-tissue," phytochromes in regulating plant development (Pratt and Cordonnier, 1987; Tokuhisa and Quail, 1989).

The phenotypic changes we have observed suggest that the amount of phytochrome in a light-grown, wild-type tomato cell is poised at a level that is rate-determining in such well-documented, phytochrome-controlled responses as inhibition of stem elongation (Smith, 1986), enhanced chlorophyll biosynthesis (Briggs, Mosinger, and Schafer, 1988), and anthocyanin accumulation (Beggs, Wellman, and Grisebach, 1986). Artificial elevation of these phytochrome levels then would appear to amplify the extent of these responses. The physiological basis of the observed phenotype is unknown, but obvious candidates include altered hormone levels and changes in hormone sensitivity. The distinctive syndrome described here presents an easy visual screen for identifying potential phytochrome overproducing mutants that may already exist in tomato and other species.

The short, dark, hypocotyl phenotype we observed in the R1 generation is consistent with the role phytochrome plays during the de-etiolation of tomato seedlings. Phytochrome interacts with blue light receptors to inhibit the elongation of tomato hypocotyls (Thomas and Dickinson, 1979) and also to stimulate anthocyanin synthesis in the subepidermal cells of the hypocotyl (Drumm-Herrel and Mohr, 1982). Phytochrome deficiency in tomato, caused by lesions at the aurea locus (Koornneef et al., 1985), has the opposite effect. Aurea mutants have an abnormally long hypocotyl with reduced anthocyanin content in the light. In addition, chlorophyll levels are lower in these mutants, resulting in yellow-green foliage in adult plants. Thus, aurea, wild-type, and overexpressors represent a continuum of phenotypic expression in response to increasing levels of phytochrome. It is interesting to note that the transgenic seedlings segregated for the short hypocotyl trait even when the parent plant was a normal height, suggesting that hypocotyl elongation is more sensitive to changes in phytochrome content than is stem extension.

Overexpression of phytochrome increased the pigmentation of transgenic tomato fruits, raising the possibility that this trait could be used to manipulate flavor and processing qualities. Carotenoid biosynthesis during tomato fruit ripening is mediated by phytochrome (Khudairi and Arboleda, 1971). In addition, tomato lines carrying the recessive *hp* (high-pigment) gene produce exceptionally dark green fruits, similar to those we observed (Peters et al., 1989). The enhanced pigmentation is correlated with higher levels of lycopene and vitamin C, as well as increased fruit firmness, making high-pigment varieties especially useful in tomato breeding programs.

Since this work was completed, an article has appeared describing the expression of oat phytochrome in transgenic tobacco (Keller et al., 1989). Observations similar to ours on the behavior of the heterologous phytochrome molecule in the dicot tissue are reported. However, differences in the phenotype of the transgenic plants of the two species are apparent. Keller et al. (1989) reported the recovery of a total of only four transgenic plants, apparently because of low efficiency of regenerating mature tobacco plants expressing the oat photoreceptor. Although the transgenic tobacco also displayed decreased height and smaller, dark green leaves relative to wild-type, the severity of the phenotype appears to be less than in tomato, and no effects on other parameters, such as anthocyanin production, were reported.

The demonstration that our chimeric phytochrome sequence is correctly expressed in readily regenerable, transgenic tomato provides the opportunity to analyze the structure, behavior, and biological action of the photoreceptor by introducing constructs that have been mutated in vitro. This approach will help define the regions of the protein responsible for such features as (1) chromophore attachment and dimerization, (2) the differential stabilities of the Pr and Pfr forms, and (3) the regulatory properties of the photoreceptor.

METHODS

Construction of a Full-Length Oat Phytochrome cDNA

A 1.0-kb KpnI/AhaII fragment containing most of the 5′ region of a type 5 phytochrome cDNA was isolated from the plasmid pAP5.2 (Hershey et al., 1985); a 2.0-kb AhaII/EcoRI fragment containing the 3′ region of a type 3 phytochrome cDNA was isolated from

the plasmid pAP3.1 (Hershey et al., 1985). These two fragments were cloned into KpnI/EcoRI-digested pUC19 to generate pFY121. A 0.8-kb HpaII fragment containing the remaining portion of the 5′ phytochrome coding region was isolated from pAP5.2, the ends were filled in with Klenow, and the fragment was digested with KpnI. The digestion mixture was ligated with SmaI/KpnI-digested pFY121 to generate pFY122. The phytochrome cDNA insert from pFY122 was cloned as a BamHI/EcoRI fragment into the BglII/EcoRI sites of pMON316 (Rogers et al., 1987) to generate pFY123. pFY123 was transferred into *Agrobacterium* by triparental mating as described (Rogers, Horsch, and Fraley, 1986).

Tomato Transformation

Seeds of tomato (*Lycopersicon esculentum* cv VF36) were germinated aseptically on 1/2 × MS salts (GIBCO) containing B5 vitamins, 3% sucrose, 0.8% agar, pH 5.8. The cotyledons of 14-day-old seedlings were infected with *Agrobacterium* cells containing the expression plasmid as described (McCormick et al., 1986). The terminology of McCormick et al. (1986) is used: primary transgenic plants were designated the R0 generation, and the first-generation progeny obtained by selfing the R0 plants were designated R1.

Immunoblotting

Vegetative shoot tips of R0 plants were tagged above the uppermost leaf that was ≥ 3 cm long. The plants were irradiated with far-red light (12 W m^{-2}) for 10 min and placed in the dark for 4 days (26°C). Tagged buds were excised under dim-green safelight (2 to 3 buds/plant). The plants were returned to the greenhouse, and light-grown bud tissue was removed 2 weeks later. Preparation of crude extracts, SDS-PAGE, and immunoblotting were performed according to Parks et al. (1987). Protein was visualized by using monoclonal antibodies developed against oat (*Avena sativa*) phytochrome. One antibody was monocot-specific and recognizes an epitope at the amino terminus of the phytochrome molecule (Daniels and Quail, 1984). The other antibody reacted with both monocot and dicot phytochromes and recognizes an epitope in the middle of the polypeptide (Shanklin, 1988).

Wild-type and R1 seedlings were grown on moist vermiculite for 6 days in darkness or white light (26°C). One group of dark-grown seedlings was irradiated with red light (9 W m^{-2}) for 3 hr (26°C) prior to harvest. Extraction of entire seedlings and immunoblot analysis were performed as described above.

Spectrophotometric Measurements

Seedlings were grown, harvested, and extracted as described above. The phytochrome content of the crude extracts was measured in a dual wavelength spectrophotometer (measuring beams 730 nm/800 nm) using $CaCO_3$ as a scattering agent. Extracts from light-grown seedlings were brought to 0.1% polyethyleneimine by the addition of 10 μL of 10% polyethyleneimine/mL of extract, stirred for 15 min, and centrifuged at 48,000 *g* for 10 min to remove chlorophyll prior to measurement (Tokuhisa, Daniels, and Quail, 1985).

Nopaline Assays

The cotyledons of 7-day-old, light-grown tomato seedlings were assayed for nopaline according to Rogers, Horsch, and Fraley (1986).

Anthocyanin Measurements

Wild-type and R1 tomato seedlings were grown on moist vermiculite for 7 days in white light (26°C). R1 seedlings were first tested for nopaline, which co-segregated with oat phytochrome expression, as described above. The hypocotyls of 20 seedlings (nopaline +, nopaline −, or wild-type) were then assayed for anthocyanin according to Schmidt and Mohr (1981), using 5 mL of extraction media/g of tissue, fresh weight.

ACKNOWLEDGMENTS

We thank Dr. Sheila McCormick for advice with tomato transformation, Ms. Anne Spelletich for carefully tending our greenhouse plants, Dr. Brian Parks for his help with immunoblot procedures and for supplying us with purified oat phytochrome, Drs. John Shanklin and Richard Vierstra for the generous gift of the monocot/dicot reactive monoclonal antibody, and Dr. Stephen Rogers (Monsanto) for providing *Agrobacterium* vectors and strains. This work was supported by National Science Foundation grant DMB-8796344 and U.S. Department of Agriculture competitive grant 85-CRCR-1-1578 to P.H.Q.

Received June 7, 1989.

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