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The levels of two distinct species of phytochrome are regulated differently during germination in *Avena sativa* L.

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Abstract. The abundance and molecular mass of phytochrome in germinating embryos of *A. sativa* (oat) grown in light or darkness have been monitored using immunoblot and spectrophotometric assays. Immunoblot analysis shows that imbibed but quiescent embryos have two immunochemically distinct species of phytochrome with monomeric molecular masses of 124 and 118 kDa (kdalton). The 118-kDa species has the properties of the 118-kDa phytochrome extracted from fully green oat tissue (J.G. Tokuhisa, S.M. Daniels, P.H. Quail, 1985, Planta 164, 321–332), whereas the 124-kDa polypeptide appears similar to the well-characterized photoreceptor of etiolated tissue. The capacity of antibodies directed against etiolated-oat phytochrome to immunoprecipitate the 124-kDa species but not the 118-kDa species has been exploited to quantitate the levels of each separately over a 72-h time course of germination and seedling development. The abundance of the 124-kDa molecule increases at least 200-fold in etiolated seedlings over 72 h whereas in light-grown seedlings the level of this molecule is relatively constant. In contrast, the amount of the 118-kDa species increases only twofold in both dark- and light-grown seedlings over the same period of time. These data indicate that whereas the abundance of 124-kDa phytochrome is regulated at the protein level by the well-documented, differential stability of the red- and far-red-absorbing forms in vivo, the 118-kDa molecule is present at a low constitutive level, presumably reflecting no such difference in the stability of the two spectral forms.

Key words: *Avena* (phytochrome) – Enzyme-linked immunosorbent assay (ELISA) – Phytochrome from green tissue – Seed germination.

Introduction

Recent data show that a phytochrome molecule different from the well-characterized photoreceptor in etiolated-oat tissue predominates in green-oat tissue (Shimazaki et al. 1983; Thomas et al. 1984; Tokuhisa et al. 1985). Green-oat phytochrome, extracted in nondenaturing buffers, when compared with etiolated-oat phytochrome, exhibits an altered difference spectrum and lower immunochemical activity per unit phytochrome absorbance when measured by enzyme-linked immunosorbent assays (ELISA). Immunoblot analysis of these green-oat extracts indicates the presence of two different molecular species of phytochrome which can be separated by immunoprecipitation using antibodies directed against etiolated-oat phytochrome (Tokuhisa et all. 1985). One species, etiolated-like in that it has a monomeric molecular mass of 124 kDa and is immunoprecipitable, represents about 30% of the total spectrally detectable phytochrome. The other species, representing the remaining spectral activity, is a distinct phytochrome in that it has a monomeric molecular mass of 118 kDa, and is not immunoprecipitated by our existing antibody preparations under nondenaturing conditions, although it is recognized in denatured form upon immunoblot analysis (Tok-

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Abbreviations: ELISA = enzyme-linked immunosorbent assay; Ig = immunoglobulin; kDa = kilodalton; Pfr, Pr = far-red-absorbing and red-absorbing forms of phytochrome, respectively; SDS-PAGE = sodium dodecyl sulfate-polyacrylamide gel electrophoresis

uhisa et al. 1985). The abundance of this distinct green-tissue-type phytochrome corresponds to only 1% of the total spectral activity observed in etiolated tissue.

The existence of a second small but biologically active population of phytochrome has been invoked to explain several phenomena described in the phytochrome literature. The biphasic kinetics of phytochrome destruction in red-irradiated, etiolated tissue of a number of plant species (Jabben and Holmes 1983) indicates a pool of phytochrome resistant to degradation in the far-red absorbing form (Pfr). Observations on the red-light inhibition of stem elongation in preirradiated pea stems ("*Pisum* paradox") are difficult to rationalize in terms of one large pool of photoreceptor, but are compatible with the notion of a small active pool of phytochrome with very low turnover (see Hillman 1967). The biphasic kinetics of phytochrome appearance, and the resistance of phytochrome as Pfr to in-vivo degradation in germinating embryos, led to the concept of "dry-seed" phytochrome, present in germinating embryos, in addition to a more abundant "seedling" phytochrome (Spruit and Mancinelli 1969).

The predominant phytochrome species from green-oat tissue (Shimazaki et al. 1983; Tokuhisa et al. 1985) exhibits the basic characteristics of this proposed additional pool of phytochrome, namely the low level, relative to that in etiolated tissue and the resistance of the Pfr form to destruction. However, the behavior of this distinct photoreceptor in a developmental context is not known. For example, it is not known whether the molecule is present in etiolated tissue nor how its abundance is regulated; the levels of green-tissue-type phytochrome may be tissue specific, and light-regulated or constitutive.

A convenient system for investigating these possibilities is the germinating embryo. We reasoned that the relatively low levels of phytochrome present in the mature embryo might permit detection and quantitation of the 118-kDa species over a sufficient time course to determine if there is net accumulation before it is obscured by the synthesis of the more abundant 124-kDa species. Furthermore, the similarity in the morphology of young dark- and light-grown seedlings permits comparison of similar tissue types.

Hilton and Thomas (1985) have also approached the question of whether seed phytochrome may be equivalent to the stable phytochrome that remains after the rapid degradation that occurs in etiolated tissue placed in the light and to the spectrally active phytochrome that is not detected immunochemically in extracts of green tissue by ELISA (Shimazaki et al. 1983; Thomas et al. 1984). Using an ELISA, they found lower immunochemical activity per unit phytochrome spectral activity with freshly imbibed and with red-light-treated embryos than with older, dark-germinated embryos. They concluded that the phytochrome detectable by ELISA corresponds with the labile pool of phytochrome and that the pool undetectable by ELISA is stable in the light.

In the work reported in this paper, we monitor phytochrome activity in embryos/seedlings of oats from imbibition to 72 h in darkness or continuous light. In this analysis we have exploited the capacity of our polyclonal antisera to immunoprecipitate 124-kDa and not 118-kDa phytochrome (Tokuhisa et al. 1985) to separate physically the two molecular species for the purpose of spectral and immunochemical quantitation of the two polypeptides.

Material and methods

Chemicals. Reagent-grade chemicals were used in all experiments. Ethylene glycol was from Eastman Kodak (Rochester, N.Y., USA); $(NH_4)_2SO_4$, ultrapure special enzyme grade, from Schwarz-Mann (Orangeburg, N.Y.). Poly(ethyleneimine) (PEI) was purchased from Eastman-Kodak or from Sigma Chemical Co. (St. Louis, Mo., USA). Nitrocellulose (HAHY 304 FO) was from Millipore Corp., Bedford, Mass., USA. Sodium dodecyl sulfate (SDS), acrylamide and methylene bis-acrylamide were obtained from BioRad Laboratories (Richmond, Cal., USA). The alkaline-phosphatase substrate 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and the coupling dye nitro blue tetrazolium were from Sigma.

Plant material. Oat caryopses (*Avena sativa* L. cv. Garry; Olds Seed Co., Madison, Wis., USA) (lemma and palea removed) were sown in plastic boxes (2700 per 19·28-cm^2 box) on top of an ice water-saturated bedding consisting of successive layers of vermiculite, adsorbent paper (Kimpak; Kimberly-Clark Corp., Neenah, Wis.) and cheesecloth. The caryopses were covered with another layer of cheesecloth, placed in the dark at 4° C for 12 h to allow imbibition without germination or new synthesis of phytochrome (Hilton and Thomas 1985), and then transferred to one of two different growth regimes. To obtain etiolated seedlings, the boxes were irradiated for 5 min with far-red light (11 $W \cdot m^{-2}$; LI-550B Printing Integrator; LI-COR, Lincoln, Neb., USA) to maximize for the level of the red-absorbing form of phytochrome (Pr), covered with foil, and placed in darkness at 25° C. At various times, seedlings were removed and placed on ice under green safelight (Bolton and Quail 1982). Chlorophyllous seedlings were obtained by covering the box with plastic wrap and placing under continuous light from fluorescent white lamps (F96T12/CW/SHO; Westinghouse Electric, Pittsburgh, Pa., USA) supplemented with light from incandescent lamps (total fluence, 11 $W \cdot m^{-2}$) at 25° C. At various times, seedlings were removed under laboratory lighting and placed on ice. Separation of the embryo from the endosperm tissue was done under green light for the etiolated embryos, and under laboratory lighting for light-grown embryos. Tissue samples obtained at various time points for

both growth conditions consisted of the excised embryo with the scutellum. Roots protruding more than 1 mm from the coleorhizae were removed. Harvested tissue was frozen in liquid nitrogen, lyophilized, and then pulverized with a pestle.

Buffers. Extraction Buffer consisted of a stock solution of 50% ethylene glycol, 100 mM 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris)-HC1, 150 mM $(NH_4)_2SO_4$, and 10 mM tetrasodium ethylenediaminetetraacetic acid (Na_4EDTA) adjusted to 100 mM $NaHSO_3$ and pH 8.5 (4° C) diluted with an equivalent volume of ice and adjusted to 10 mM iodoacetamide and 2 mM phenylmethylsulfonyl fluoride (PMSF; 200 mM stock in anhydrous 2-propanol). Resuspension Buffer was identical to Extraction Buffer without $(NH_4)_2SO_4$ and at pH 8.2 (4° C). Tris-buffered saline (TBS) contained 20 mM Tris-HCl, 150 mM NaCl and 0.02% NaN_3, pH 7.8 (20° C).

Extraction procedure. All manipulations were performed under dim green light with samples maintained at ice temperatures unless stated otherwise. The lyophilized, pulverized tissue was mixed with Extraction Buffer at a ratio of 1:15 (g DW:ml Extraction Buffer). The brei was adjusted to 0.5% poly(ethyleneimine) with a 10% stock solution and centrifuged at 48000 *.g* for 20 min. The supernatant was mixed with 0.25 g of powdered $(NH_4)_2SO_4$ per 1 ml of supernatant and centrifuged as before. The pellet was resuspended to 20% of the original volume with Resuspension Buffer and clarified by centrifugation. The extraction procedure differs from the previous procedure (Tokuhisa et al. 1985) in the inclusion of iodoacetamide, higher concentration of $NaHSO_3$, and the omission of red-light irradiation of the extract. The inclusion of iodoacetamide in the buffers minimizes proteolytic degradation of phytochrome and an accompanying shift in the difference-spectrum absorbance maximum of Pfr from 730 to 724 nm (Tokuhisa 1986). Iodoacetamide does not affect the difference spectrum of green- or etiolated-oat phytochrome and does not differentially affect the immunochemical properties of green-oat phytochrome either as Pr of Pfr (data not shown).

Phytochrome spectral measurement. Phytochrome was measured in a custom-built, dual-wavelength spectrophotometer (L.H. Pratt, University of Georgia, Athens, USA; Pratt et al. 1985) using $CaCO_3$ as a scattering agent (Butler and Norris 1960) as described previously (Tokuhisa et al. 1985). Absorbance and difference spectra of phytochrome-containing samples were obtained using $CaCO_3$ as a scattering agent with measurement in a Perkin-Elmer 557 spectrophotometer (Perkin-Elmer Co., Norwalk, Conn., USA) in the double-beam mode.

Antibodies. Two antibody preparations directed against etiolated-oat phytochrome were used. The rabbit antiserum 4032, directed against 124-kDa etiolated-oat phytochrome was characterized previously on immunoblots of extracts from etiolated-oat tissue (Vierstra et al. 1984) and from green-oat tissue (data not shown). Monospecific antiphytochrome A/S 7.1 was prepared from an antiserum directed against 114/118-kDa degraded etiolated-oat phytochrome using an affinity column of 114/118-kDa etiolated-oat phytochrome. The antibody preparation was made by P.H.Q. in the laboratory of W.R. Briggs (Carnegie Institute of Washington, Stanford, Cal., USA) with the initial assistance of L.H. Pratt (University of Georgia, Athens, USA).

Phytochrome immunoprecipitation. Immunoprecipitations were performed as described in Vierstra and Quail (1983). The samples were incubated with "A/S 7.1" for 60 min at 4° C. Aliquots of 30% (v/v) formalin-fixed, heat-treated *Staphylococcus aureus* Cowan I suspension in Extraction Buffer were added to the samples as an immunoadsorbent at 1 1:1 g immunoglobulin (Ig), incubated for 30 min, and then precipitated by centrifugation for 5 min at 4° C (microfuge B; Beckman Instruments, Fullerton, Cal.). The extracts, supernatants and washed precipitates were assayed for phytochrome spectral activity and prepared for immunoblot analysis.

Immunoblot analysis. The procedure, including sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), electroblotting and antibody detection, was performed as before (Tokuhisa et al. 1985) with modifications as described below. The second and third antibody incubations were for 1.5 h and washes were for 45 min with three changes of buffer. The enzyme substrate was prepared according to Blake et al. (1984). The enzyme substrate BCIP was dissolved in dimethyl sulfoxide (150 $mg \cdot ml^{-1}$) and stored at −20° C. Nitro blue tetrazolium was dissolved in H_2O (7.5 $mg \cdot ml^{-1}$) and stored at 25° C in the dark. The reaction mixture for one 14·16-cm^2 blot was prepared by mixing 2 ml of nitro blue tetrazolium solution with 100 ml of Color Buffer (0.1 M Tris-HCl, 0.1 M NaCl, 5 mM $MgCl_2$, pH 9.0, 25° C) followed by 50 µl of the BCIP stock solution. The blots were rinsed sequentially in H_2O and Color Buffer and then incubated in the substrate-containing Color Buffer from 10 min to 24 h. The reaction was terminated by rinsing the blot in 0.02% (w/v) sodium azide. The developed blots were stored in 0.02% (w/v) NaN_3 or wrapped in plastic film.

Densitometry. Photographic negatives of the immunoblots were scanned on a densitometer (E-C Apparatus Corp., St. Petersburg, Fla., USA). The electronic output was transcribed onto chart recorder paper and the area under the curve corresponding to each individual band was cut out and weighed.

Results

Spectrophotometric assay of phytochrome levels. Both etiolated and light-grown seedlings have similar general morphology up to 72 h, with the leaves still enclosed in the coleoptile, although the light-grown seedlings have detectable chlorophyll in the enclosed leaves. Extracts of embryos at 0 h have low but detectable levels of phytochrome spectral activity per embryo (Fig. 1). This phytochrome is considered to represent that already present in the mature embryo of the dry seed, since its appearance in spectrally active form occurs upon rehydration in the absence of protein synthesis (Hilton and Thomas 1985). Embryos of caryopses exposed to continuous light show about a twofold increase in phytochrome spectral activity per seedling all occurring within the first 24 h (Fig. 1 A). In contrast, this spectral activity increases 250-fold per seedling in 72 h for embryos of caryopses transferred to 25° C in the dark (Fig. 1 B). At 72 h the etiolated-seedling extracts have 100-fold more phytochrome per seedling than the extracts of light-grown seedlings.

Immunochemical assay of phytochrome levels. The embryo extracts, which were quantitated for spec-

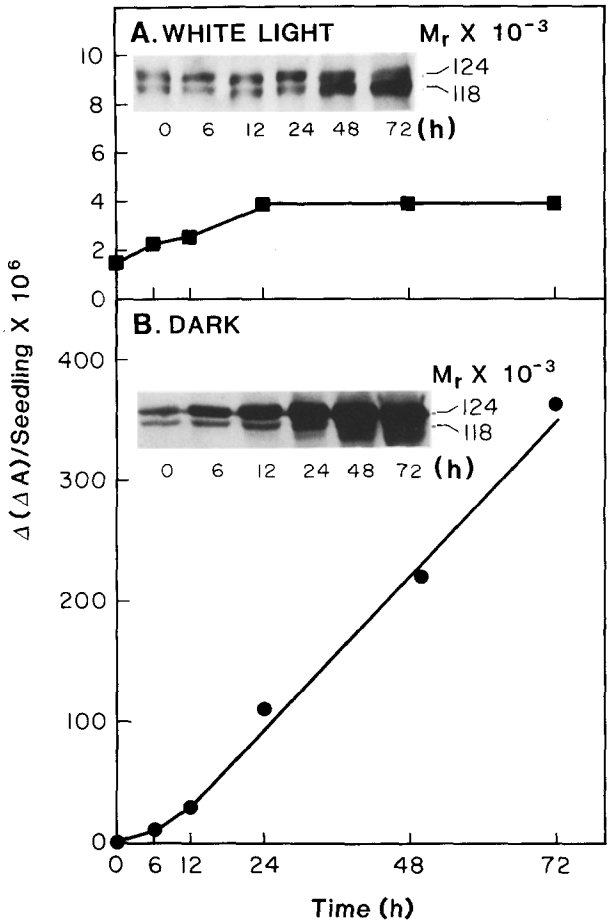

Fig. 1 A, B. Spectral and immunoblot analysis of phytochrome in extracts of germinating light- and dark-grown oat embryos/seedlings. Oat caryopses were imbibed at 4° C for 12 h in the dark and transferred to 25° C (0 h) for germination and growth in either continuous white light (**A**) or darkness following 5 min of far-red irradiation (**B**). Embryos were harvested under the growth regimes at the indicated times and lyophilized in the dark. The lyophilized tissue was pulverized and extracted as described in *Materials and methods*. The extracts were measured for phytochrome spectral activity (*curves*) using $CaCO_3$ for amplification of the absorbance signal and prepared for immunoblot analysis (*insets*). Extract equivalent to two seedlings at each time point was subjected to SDS-PAGE in a 5.3% acrylamide gel. The separated polypeptides were blotted onto nitrocellulose and probed successively with antiserum 4032, polyclonal rabbit Igs directed against 124-kDa etiolated-oat phytochrome, goat-antirabbit IgG, and rabbit-antigoat IgG linked to alkaline phosphatase

tral activity, were also subjected to either (a) immunoblot analysis using 4032, a rabbit antiserum directed against 124-kDa, etiolated-oat phytochrome, or (b) to initial immunoprecipitation followed by spectral and immunoblot analysis of the derived supernatant and precipitate fractions. Immunoblots of the total, unfractionated extract (Fig. 1, inset) clearly resolve, at early time points, two bands with mobilities of 118 and 124 kDa. These bands are obscured at later time points as a result of loading the gel lanes on a per-embryo basis.

Immunoprecipitation from the embryo extracts using "A/S 7.1", an antiserum directed against degraded (114- and 118-kDa) etiolated-oat phytochrome purified on a phytochrome affinity column was used as a test of whether the 118-kDa band had properties similar to those of green-tissue phytochrome. This antibody preparation can immunoprecipitate 114-, 118- and 124-kDa phytochrome from etiolated oats (Vierstra and Quail 1982) but not the 118-kDa phytochrome from green tissue (Tokuhisa et al. 1985). Immunoblot analysis of the resultant fractions from extracts of both light- and dark-grown embryos shows separation of the 124-kDa and 118-kDa species to the precipitate and supernatant fractions, respectively (Fig. 2).

Immunoblot analysis of the immunoprecipitates from the extracts of light-grown embryos shows a 124-kDa band that appears to increase in intensity up to 24 h and decrease subsequently to initial levels by 72 h (Fig. 2, immunoprecipitate), while the 118-kDa band in the supernatant fractions increases steadily in intensity over 72 h (Fig. 2, supernatant). Despite these changes, the total amount of spectrally detectable phytochrome per light-grown seedling is constant from 24 h to 72 h (Fig. 1 A), apparently because the increase in the amount of the 118-kDa species is offset by a compensatory decrease in the amount of the 124-kDa molecule (Fig. 2, total).

Spectrophotometric and immunochemical analysis of the extracts of etiolated seedlings shows that the substantial increase in phytochrome over 72 h is associated almost exclusively with the 124-kDa species (Fig. 1 B; Fig. 2, immunoprecipitates). A 400-fold increase in the level of spectral activity that is immunoprecipitated from these extracts (data not shown) corresponds with the total accumulation of spectrally detectable phytochrome. Immunoblot analysis of the supernatant fractions from the extracts of etiolated seedlings demonstrates the presence of the 118-kDa species at all time points during germination in darkness. However this species constitutes a minor fraction of the total phytochrome at later time-points (Fig. 2).

To compare the levels of the 118-kDa species in light- and dark-grown seedlings, the intensities of bands on the immunoblot were quantitated by densitometry (Fig. 3). From standard curves it was established that, under our assay conditions, the area under the densitometric scan curve is linearly proportional to the amount of phytochrome up to $15 \cdot 10^{-6}$ $\Delta(\Delta A)$ per gel lane, which corresponds

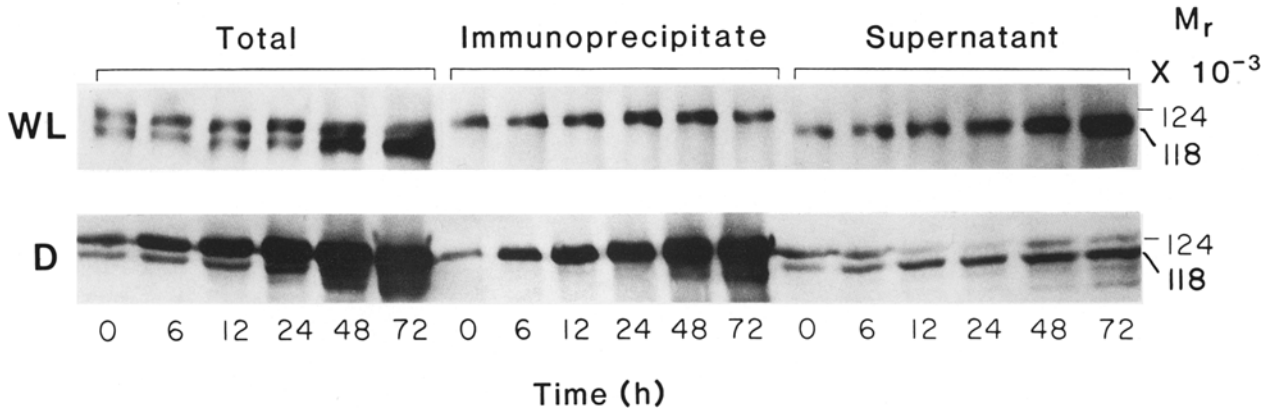

Fig. 2. Immunoblot analysis of the fractions obtained from immunoprecipitation of oat embryo/seedling extracts. Extracts prepared as described in *Materials and methods* were mixed with antibody preparation A/S 7.1, rabbit Igs directed against degraded (114/118-kDa) etiolated-oat phytochrome for 1 h followed by heat-killed, formalin-fixed *Staphylococcus aureus* Cowan I for 0.5 h, and subjected to centrifugation. The proportions of the components were 1 g phytochrome to 17 g Igs to 17 l 30% (v/v) *S. aureus*. The supernatants and washed precipitates were prepared for immunoblot analysis as described in Fig. 1

to 0.03 arbitrary units in Fig. 3 (data not shown). Since all the band intensities for the 118-kDa species fall within this range the data in Fig. 3A provide a direct quantitative comparison of the levels of this species in dark- and light-grown tissue over the complete time-course. A two- to threefold increase in the amount of the 118-kDa polypeptide occurs over 72 h, with no difference between the two tissues. The levels of the 124-kDa polypeptide in light-grown tissue also fall within the linear range of the assay. There is a transient two- to threefold increase in the abundance of this species peaking at 24 h (Fig. 3B). The levels of the 124-kDa molecule in etiolated seedlings exceed the linear range of the immunoblot assay for the time points after 12 h (Fig. 3B). As a result the increase in this species appears to be only about 10-fold over 72 h as determined from the densitometric scans, rather than the 400-fold difference known to have been applied to the gel from spectral measurements.

Difference spectrum of phytochrome in light-grown seedlings. Light-grown seedlings were harvested at 36 h, lyophilized and extracted as described in *Materials and methods*. The extract was concentrated sufficiently by $(NH_4)_2SO_4$ precipitation to obtain a difference spectrum of phytochrome using $CaCO_3$ as a scattering agent (Fig. 4). The absorbance maxima are at approx. 655 and 730 nm with an isosbestic point at 684 nm.

Discussion

Our data indicate that mature *Avena sativa* embryos imbibed in the cold to prevent germination and germinating embryos/seedlings grown in either darkness or light contain two distinct molecular species of phytochrome. These phytochrome species have properties consistent with those of the phytochrome species that predominates in either etiolated or green tissue from *Avena* (Tokuhisa et al. 1985). One polypeptide has a molecular mass of 124 kDa and is precipitated with antibodies directed against etiolated-tissue phytochrome. The other polypeptide has a molecular mass of 118 kDa and is not precipitated by the same antibodies which can immunoprecipitate degraded (114/118-kDa) etiolated-oat phytochrome (Vierstra and Quail 1982). We have provided evidence that the 118-kDa species from green tissue is different from the 124-kDa etiolated-tissue phytochrome (Tokuhisa et al. 1985) and is not derived as a degradation product of a larger species during handling or extraction of the tissue (Tokuhisa 1986).

The difference-spectra maxima of 655 and 730 nm and the isosbestic point of 684 nm (Fig. 4) for a phytochrome-containing extract prepared from 36-h-old, light-grown embryos with about equal proportions of the 118- and 124-kDa bands (Figs. 1, 2), are consistent with the notion of two populations of spectrally distinct phytochrome contributing to the overall difference spectrum. Etiolated-oat phytochrome has an absorbance maximum in the red region at 667 nm and an isosbestic point at approx. 688 nm whereas green-oat phytochrome has a maximum at 654 nm and an isosbestic point of 681 nm (Tokuhisa et al. 1985; Vierstra and Quail 1983). A composite difference spectrum of two phytochrome populations would be expected to have a broad peak in the red region

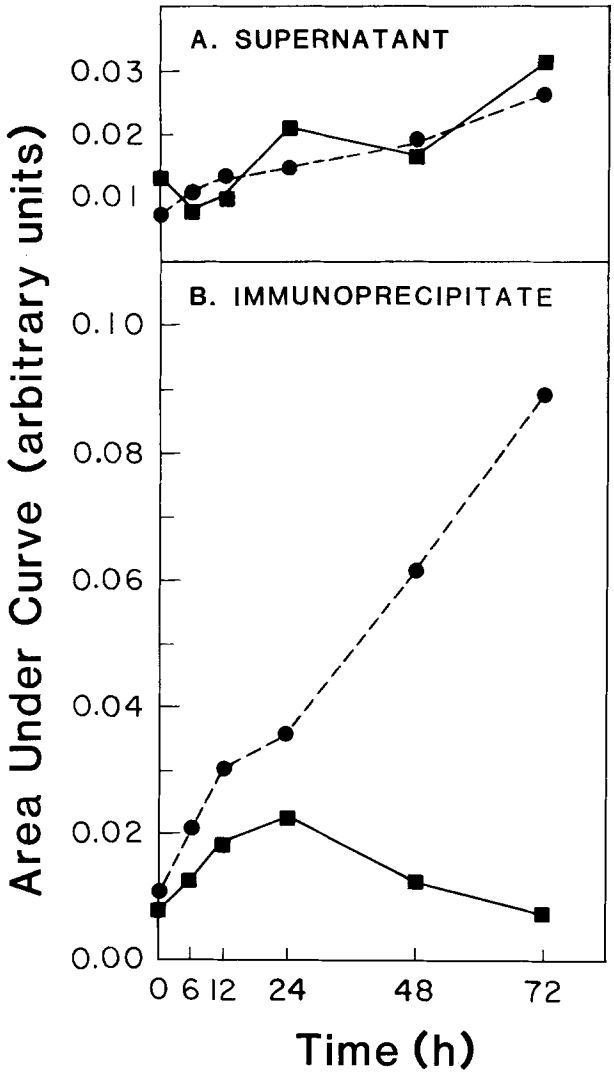

Fig. 3A, B. Quantitation of 118- and 124-kDa phytochrome bands after immunoblot analysis of extracts of light- (■) and dark- (●) grown oat seedlings. Immunoblots of the fractions obtained following immunoprecipitation of phytochrome from extracts of light- and dark-grown embryos shown in Fig. 2 were scanned with a densitometer. The densitometer output was recorded on chart paper, and the area under the curve representing each individual band was excised and weighed

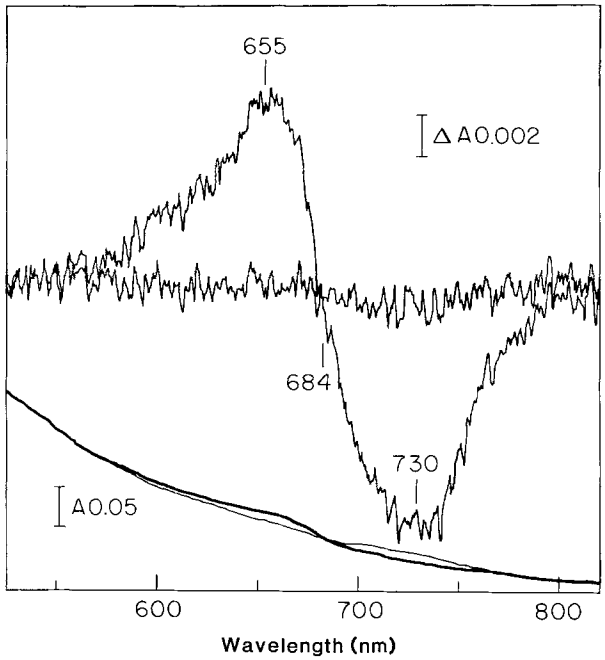

Fig. 4. Difference spectrum of phytochrome in an extract of light-germinated oat embryos. Caryopses were imbibed for 12 h at 4° C followed by 36 h in white light at 25° C. Isolated embryos were lyophilized, pulverized and extracted as described in *Materials and methods*. The extract was mixed with $CaCO_3$ to amplify the absorbance signal. The absorbance spectra (*lower curves*) were measured at 2° C after saturating red (*thin*) or far-red (*thick*) light. The difference spectrum (*upper curve*) was determined by electronic subtraction of the Pfr spectrum from that of Pr

with an isosbestic point shifted to a higher wavelength, as shown here. The absorbance maximum of 730 nm in the difference spectrum (Fig. 4) is identical to that obtained previously and is indicative of the spectral integrity of the phytochrome in these extracts (Tokuhisa et al. 1985). Phytochrome in green-tissue extracts incubated at 20° C for 2 h in the absence of iodoacetamide exhibits altered spectral properties correlated with a proteolytically induced reduction of apparent molecular mass similar to that observed for degradation of 124-kDa phytochrome from etolated tissue (Tokuhisa 1986).

Quantitation of the immunochemical and spectral activity of the phytochrome in the precipitate and supernatant fractions after immunoprecipitation from the two extracts indicates that the abundance of the 118-kDa species is constitutive whereas that of the 124-kDa species is light regulated (Figs. 1–3). The levels of the 118-kDa species increase only two- to threefold per seedling over the time course and at the same rate in dark- and light-grown seedlings (Fig. 3A). This relative increase corresponds with an approximately twofold increase in dry weight of the seedlings. The slow and similar rate of accumulation of the 118-kDa species contrasts with the differential increase in the level of the 124-kDa species in dark- versus light-grown embryos apparent as early as 6 h after imbibition (Figs. 2, 3B). The 124-kDa phytochrome in light-grown seedlings shows a slight transient increase in abundance at 24 h (Fig. 3B) similar to that observed by Hilton and Thomas (1985) and Konomi et al. (1985). This observation may reflect a developmental regulation of the 124-kDa species in addition to light regulation, or the loss of phyto-

chrome from the excision of root tissue in seedling samples beyond 24 h.

Research on phytochrome from other plants grown in the light indicates similar but not identical results to those obtained with *Avena*. Abe et al. (1985) have identified two immunochemically distinct populations of phytochrome in extracts of light-grown *Pisum sativum*. However in contrast to *Avena* the *Pisum* phytochrome is not spectrally distinct from that in etiolated tissue. In work similar to that of Hilton and Thomas (1985), Konomi et al. (1985) have characterized phytochrome with different immunochemical activity in embryonic axes of *Pisum* germinated in either light or darkness.

We conclude that there are two distinct molecular species of phytochrome in *Avena* whose levels are regulated differentially in tissue grown in darkness or in light. Whereas the abundance of the 124-kDa molecule is regulated in light versus darkness by the well-documented differential turnover rates of Pr and Pfr as well as the abundance of phytochrome mRNA (Colbert et al. 1985; Frankland 1972), the 118-kDa molecule does not turn over differentially in the two forms, and consequently, appears to be constitutively expressed in the tissues and stages of development thus far examined. The stable, "seed" phytochrome described by Hilton and Thomas (1985) appears comparable to the 118-kDa molecule, while the labile, "seedling" phytochrome is analogous to the 124-kDa molecule. Overall, the evidence supports the notion that the total level of phytochrome appears to be modulated in transitions between light and dark by the levels of 124-kDa phytochrome above a stable background level of the 118-kDa species (Hilton and Thomas 1985; Hunt and Pratt 1980). The presence of the 118-kDa species in vegetative as well as embryonic tissues of *Avena* may indicate the potential for the 118-kDa species to have a regulatory function throughout the life cycle (Brockmann and Schäfer 1982).

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