A possible tyrosine phosphorylation of phytochrome

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Abstract Red/far-red light signal transduction by the phytochrome family of photoreceptors regulates plant growth and development. We investigated the possibility that tyrosine kinases and/or phosphatases are involved in phytochrome-mediated signal transduction using crude extracts of oat seedlings that are grown in the dark. We found that a 124 kDa protein was tyrosine-phosphorylated as determined by Western blotting with a phosphotyrosine-specific monoclonal antibody. The 124 kDa protein was recognized by the anti-phosphotyrosine antibody in anti-phytochrome A immunoprecipitates. The level of anti-phosphotyrosine antibody binding to the 124 kDa protein(s) in phytochrome immunoprecipitates that had been treated with red light prior to immunoprecipitation decreased relative to dark controls. These results suggest that either phytochrome from dark-grown seedlings is tyrosine phosphorylated or that it co-immunoprecipitates with a phosphotyrosine-containing protein of the same molecular weight. The implications of these results in the regulation of (a) the putative Ser/Thr kinase activity of the photoreceptor and (b) the binding of signaling molecules, such as phospholipase C to phytochrome, are discussed.

Key words: Phosphorylation; Signal transduction; Phytochrome; *Avena*

1. Introduction

The phytochromes are a family of homologous photoreceptors that detect and transduce red/far-red light signals into a diverse array of developmental and morphogenic responses, including the induction of seed germination, gravitropism, growth, flowering and senescence (reviews [1–3]). The most extensively characterized member of this family, phytochrome A, contains a covalently linked tetrapyrrole chromophore bound to an ~124 kDa soluble apoprotein. This chromoprotein, which is present in the leaves, stems and roots of higher plants, is synthesized in the dark in what has traditionally been referred to as the 'inactive' Pr form. Exposure to red and/or white light induces isomerization of the chromophore, changes in the conformation of the protein to the 'active' far-red absorbing Pfr form, and ultimately leads to alterations in gene expression and biochemistry. Although the phytochrome signal transduction pathways remain largely enigmatic, recent evidence suggests that light signals are transduced by the regulated interaction of GTP-binding proteins, calcium-calmodulin, and protein kinases and phosphatases (for initial studies and reviews, see [2–8]).

A large number of protein-tyrosine kinases and phosphatases are present in animal systems. These proteins play crucial roles in the regulation of growth, development and differentiation [reviewed by [9–12]. For example, it has been estimated that the genome of the nematode *C. elegans* contains about 115 genes encoding protein-tyrosine phosphatases. The human genome may encode as many as 500 such proteins [10]. It is likely that these organisms possess twice as many protein-tyrosine kinases. The protein-tyrosine kinases identified thus far in animals include the transmembrane tyrosine kinase receptors that undergo autophosphorylation or transphosphorylation at tyrosine residues in response to the binding of growth factors, insulin, and lymphokines, and the JAK family of protein-tyrosine kinases that are responsible for the phosphorylation of the cytokine receptor superfamily in response to the binding of certain polypeptide ligands (review [13]). The activation of these receptors results in the rapid induction of tyrosine phosphorylation in a number of cellular proteins, including the mitogen activated protein kinase (MAPK) that lies downstream of the ras GTP-binding proteins, phospholipase C, phosphatidylinositol 3-kinase, *src*-like protein-tyrosine kinases, ras GTPase-activating proteins (GAPs) and certain transcription factors.

In contrast to animal systems, relatively little is known regarding the abundance or physiological roles of protein-tyrosine kinases and phosphatases in plants. Recent reports, however, suggest that these proteins are indeed present in plants and are physiologically relevant. For example, a potential tyrosine phosphatase has been cloned from the cyanobacterium *N. commune* [14] and tyrosine phosphatases have been isolated from wheat embryos and seedlings, as well as from pea nuclei [15–17]. There have been reports that a mitogen-activated protein Ser/Thr/Tyr kinase (MAPK) is activated in response to plant hormones in *Arabidopsis* cell cultures [18] and that a raf-like kinase, which is an upstream regulator of MAPKs in mammals, participates in ethylene signal transduction [19]. Furthermore, it has been demonstrated that a tyrosine kinase inhibitor blocks the expression of at least one phytochrome-regulated gene [20]. The activity of RUBISCO may also be regulated by tyrosine phosphorylation [21]. Phosphotyrosine in *Cicer* RUBISCO was detected by chromatographic separation of ^{32}P-labelled phosphoamino acids and confirmed by treatment with phosphotyrosyl protein phosphatase [21].

While investigating the possibility that protein tyrosine kinases and/or tyrosine phosphatases are components of the phytochrome signal transduction pathway, we observed a reduction in the binding of a monoclonal antibody specific for

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Abbreviations: BSA, bovine serum albumin; ECL, enhanced chemiluminescence; GAP, GTPase-activating protein; MAPK, mitogen-activated protein kinase; PBS, phosphate-buffered saline; PBST, phosphate-buffered saline containing 0.05% Tween 20; Pfr, far-red absorbing form of phytochrome; Phy, phytochrome; PI3-kinase, phosphatidylinositol 3-kinase; PLC, phospholipase C; PMSF, phenylmethanesulfonyl fluoride; Pr, red-absorbing form of phytochrome; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Dedicated to Professor Harry Smith on his 60th birthday.

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phosphorylated tyrosine residues to a protein of approx. 124 kDa following illumination of dark-grown crude oat extracts with red light. To test the hypothesis that this ~124 kDa protein was indeed phytochrome, anti-phytochrome A and anti-phosphotyrosine immunoprecipitates were probed with both anti-phytochrome and anti-phosphotyrosine-specific monoclonal antibodies.

2. Materials and methods

2.1. Materials

Avena sativa L. (cv Garry oats) seeds were obtained from Agricul-ver (Trumansburg, NY) and grown in darkness as described by [22]. The seedlings were harvested 3.5 days after planting under a dim green safety light and were stored at −70°C until use. The anti-phytochrome monoclonal antibody, oat 22 [23], was a generous gift from Professor Lee Pratt (University of Georgia, Athens, GA). The monoclonal anti-phosphotyrosine (clone IG2) and the ECL chemiluminescence kit were purchased from Amersham. Nitrocellulose membranes (0.22 μm) and electrophoresis reagents were from Bio-Rad Laboratories. The anti-mouse IgG HRP conjugate was from Promega. The molecular weight markers were from Pharmacia and the protein G-plus/protein A-agarose was from Oncogene Science. The other reagents were purchased from Sigma.

2.2. Irradiation of plant extracts

The oat extracts were irradiated with a Fiber-Lite fiber optic illuminator (Model 190, Dolan-Jenner Industries) equipped with either a far-red cutoff filter (Ealing 26-4457; 1.8 kW/m^2) or a 660 nm interference filter (Oriels C572-6600; fluence rate, 8.3 W/m^2).

2.3. Preparation of plant extracts

The dark-grown seedlings were homogenized in a blender with 50 mM Tris-HCl, pH 8.3, containing 10 mM EDTA, 15 mM 2-mercaptoethanol, 2 mM sodium orthovanadate, and 2 mM PMSF at a 1:1 (w/v) ratio under green safety light. The homogenate was squeezed through four layers of cheesecloth and the resulting filtrate was centrifuged at 16000×g for 20 min at 4°C.

Experiments investigating the rate of phytochrome tyrosine dephosphorylation used extracts prepared as described above, except that sodium orthovanadate, a tyrosine phosphatase inhibitor [24], and EDTA were omitted from the extraction buffer.

2.4. Immunoprecipitations

The 1 ml aliquots of extract, prepared as described above, were added to Eppendorf tubes containing either (1) 25 μl protein G-plus/protein A agarose and 10 μl oat 22 antibody or (2) 25 μl protein G-plus/protein A agarose and 60 μl (6 μg) anti-phosphotyrosine antibody. Control mixtures were composed of (1) 25 μl protein G-plus/protein A agarose, 10 μl oat 22 antibody or 60 μl anti-phoshotyrosine antibody, and 1 ml of extraction buffer and (2) 25 μl protein G-plus/protein A agarose and 1 ml of plant extract. The preparations were incubated in the dark on a rocker platform for 2 h at 4°C. The immunoprecipitates were collected by centrifugation in an Eppendorf microcentrifuge at 2500 rpm for 15 min. The supernatants were removed and the pellets were resuspended in 1 ml of phosphate buffered saline (PBS; 9.1 mM K_2HPO_4, 1.7 mM $KHPO_4$, 150 mM NaCl, pH 7.4) and centrifuged again at 2500 rpm for 15 min. This washing step was repeated four times. The final pellets were resuspended in 20 μl of 4×SDS sample buffer (100 mM Tris-HCl, pH 6.8, 200 mM 2-mercaptoethanol, 4% SDS, 20% glycerol and 0.2% bromophenol blue) and were placed in boiling water for 3 min.

For experiments investigating the rate of tyrosine dephosphorylation, 20 ml of the supernatant, prepared as described above, was irradiated with red light and occasional stirred. At 1, 3, 5, 10 and 15 min intervals, 1 ml of the extract was withdrawn and added to Eppendorf tubes containing the protein G/A agarose and antibody mixtures. 40 μl of 50 mM Tris, pH 7.4, containing 40 mM sodium orthovanadate was added per tube and the immunoprecipitations were performed, as previously described.

2.5. Immunoblotting

Unless indicated otherwise, 20 μl of the immunoprecipitates were loaded onto a 7% gel and electrophoresed according to the method of [25]. Following electrophoresis, the proteins were transferred to nitrocellulose membranes in a buffer containing 25 mM Tris-glycine, pH 8.3, 20% methanol and 0.03% (w/v) SDS for 1 h at 250 mA. The membranes were blocked in PBST (PBS plus 0.05% Tween 20) containing 5% (w/v) dried skim milk for 1 h at room temperature. After cutting the membranes into strips, they were incubated with either oat 22 (1 μl/ml PBST/5% dried skim milk) or anti-phosphotyrosine antibody (5 μg/ml PBST/5% dried skim milk) for 1 h at room temperature. The membranes were then washed three times for 7 min each with PBST before incubation with anti-mouse IgG-horseradish peroxidase (0.1 μg/ml PBST containing 5% skim milk) for 30 min at room temperature. Following incubation with the secondary antibody, the membranes were washed four times for 5 min each with PBST and one time for 5 min with PBS. They were then incubated in Amersham ECL reagents and exposed to hyperfilm as described by the manufacturer.

3. Results

Western blot analyses of the anti-phytochrome and anti-phosphotyrosine immunoprecipitates are shown in Fig. 1. A broad band at approx. 124 kDa was detected in anti-phytochrome immunoprecipitates Western blotted with either the anti-phytochrome oat 22 monoclonal antibody or the anti-phosphotyrosine antibody (Fig. 1A,B). A 124 kDa protein was also visualized in anti-phosphotyrosine immunoprecipitates probed with either oat 22 or the anti-phosphotyrosine antibody (Fig. 1C,D). No bands were present at 124 kDa in control precipitates in which the oat extract was replaced by extraction buffer (Fig. 1E–H) or in precipitates in which the antibodies were omitted (Fig. 1I,J). Furthermore, bands were not observed at 124 kDa in the control experiments where primary antibodies were omitted during Western blotting (Fig. 2B). The addition of 5 mM phosphotyrosine to the anti-phosphotyrosine antibody preparation before Western blotting significantly reduced the binding of the antibody to the 124 kDa protein (Fig. 2C). These experiments were repeated more than 10 times. The results were similar each time. Coomassie blue-stained gels of the immunoprecipitates revealed few bands other than phytochrome and the IgG chains (data not shown). Lanes A and C of Fig. 1 contain a high molecular weight band. High molecular weight bands are sometimes observed in western blots of phytochrome preparations. Pratt et al. [23] detected a high molecular weight band in phytochrome preparations blotted with Oat 22 and other anti-phytochrome antibodies. Most likely the high molecular weight band was a small amount of aggregated phytochrome. Although this band was not observed in lanes B and D it is may be present but below the detection limits. The amount of protein in this band is lower than that of the 124 kDa band. Only a portion of the phytochrome would be expected to contain phosphotyrosine, and merely a fraction of the tyrosine phosphorylated protein would bind the anti-phosphotyrosine antibody. The low molecular weight band found in lanes A–H of Fig. 1 is the heavy chain of the antibody used during the immunoprecipitation procedure.

The effects of red light treatment on the binding of the anti-phosphotyrosine antibody to Western blots of anti-phytochrome immunoprecipitates are shown in Fig. 3. Irradiation of the extract prior to immunoprecipitation produced a decrease in the signal detected at 124 kDa (Fig. 3A) relative to the dark control (Fig. 3C). A gradual narrowing of the 124 kDa band occurs when the extract was irradiated for up to 15 min (Fig. 3A vs. C). A 5 min irradiation with far-red light,

after the red light treatment, failed to produce an increase in the intensity of the signal detected. These observations suggest that if the phosphate transfer is reversible, it may require a protein or co-factor that was lost during the initial homogenization or centrifugation step.

Although phytochrome becomes more susceptible to proteolysis following photoconversion from the Pr to the Pfr form [2], it is unlikely that the decrease in tyrosine phosphorylation detected by the anti-phosphotyrosine antibody is solely a function of decreased native phytochrome in the extract. When the antibodies, bound to the immunoblot shown in Fig. 3A, were removed by incubating the membrane in stripping buffer (62.5 mM Tris-HCl, pH 6.7, containing 100 mM 2-mercaptoethanol and 2% SDS) for 30 min at 70°C and the membrane was re-probed with oat 22 (Fig. 3B), the amount of phytochrome detected was comparable to that observed in the dark controls. Relatively little change in the binding of anti-phosphotyrosine, or oat 22 antibodies, was observed in control experiments in which the supernatant was kept in the dark for 1, 3, 5, 10 or 15 min prior to immunoprecipitation (Fig. 3C,D). The small time-independent variations in phytochrome levels detected during the time courses of Fig. 3B–D can probably be attributed to experimental error.

4. Discussion

Oat 22, an anti-phytochrome monoclonal antibody, recognized a 124 kDa protein from anti-phosphotyrosine immunoprecipitates, while an anti-phosphotyrosine antibody recognized a 124 kDa protein from anti-phytochrome immunoprecipitates. The level of detectable tyrosine phosphorylation decreased in response to red light treatment. Although we cannot completely eliminate the possibility that phytochrome co-immunoprecipitates with a separate 124 kDa protein that is tyrosine phosphorylated, this possibility seems unlikely considering the fact that the immunoprecipitates were extensively washed with buffer containing 150 mM NaCl. Furthermore, when 1% Triton X-100, 0.5% deoxycholate, and 0.1% SDS was added to the PBS used to wash the immunoprecipitates, similar results were obtained (Fig. 4). These results suggest that if the 124 kDa protein, that is detected by the anti-phosphotyrosine antibody, is a co-immunoprecipitating contaminant other than phytochrome, then it must be tightly associated with phytochrome by high-affinity interactions.

From Fig. 3A there appears to be more than one band around 124 kDa. The slightly lower molecular weight band(s) seem to disappear first, with irradiation, thus narrowing the

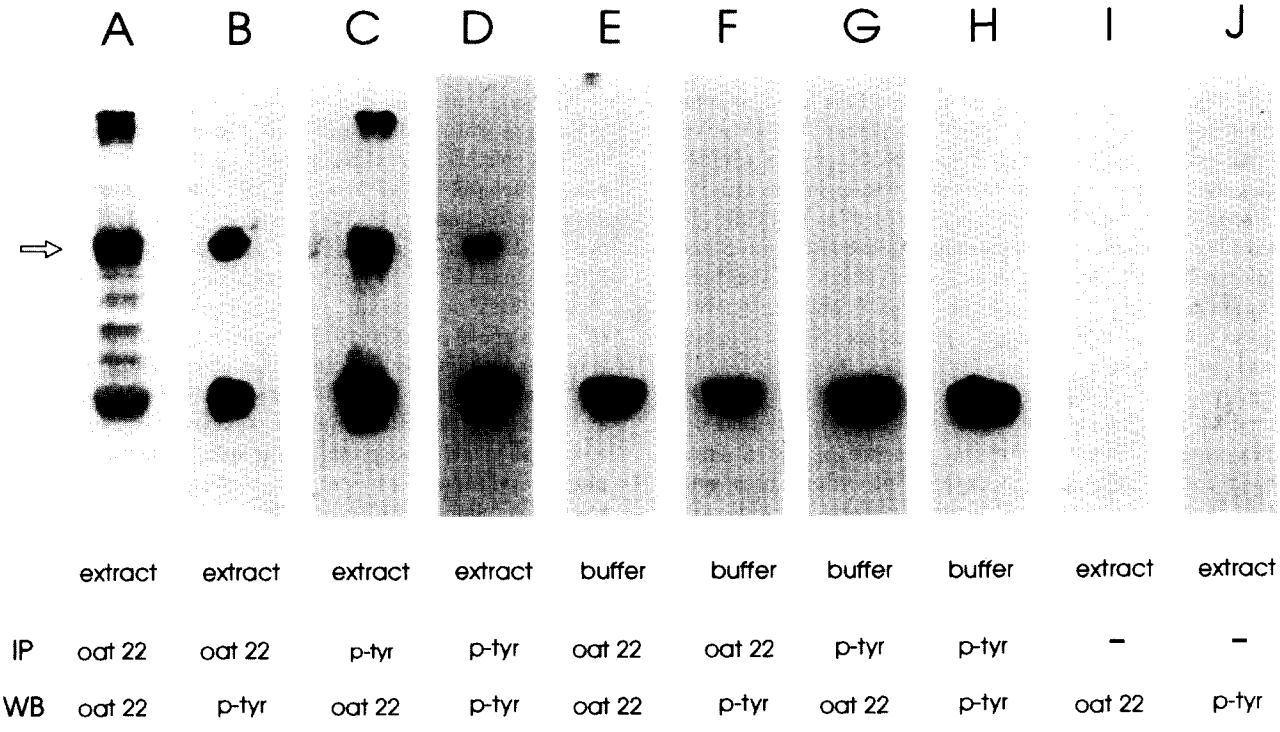

Fig. 1. Western blot (WB) analyses of anti-phytochrome and anti-phosphotyrosine immunoprecipitates (IP). (A) Anti-phytochrome immunoprecipitates Western blotted with the anti-phytochrome antibody; (B) anti-phytochrome immunoprecipitates Western blotted with the anti-phosphotyrosine antibody; (C) anti-phosphotyrosine immunoprecipitates Western blotted with the anti-phytochrome antibody; (D) anti-phosphotyrosine immunoprecipitates Western blotted with the anti-phosphotyrosine antibody; (E) non-extract control. 25 μl of protein G/A agarose and 10 μl anti-phytochrome antibody were incubated with 1 ml of extraction buffer for 2 h as described under Section 2 and Western blotted with oat 22; (F) non-extract control. 25 μl of protein G/A agarose and 10 μl of the anti-phytochrome antibody were incubated with 1 ml of extraction buffer as described above prior to Western blotting with the anti-phosphotyrosine antibody; (G) non-extract control. 25 μl of protein G/A agarose and 60 μl of the anti-phosphotyrosine antibody were incubated with 1 ml of extraction buffer prior to Western blotting with anti-phytochrome; (H) non-extract control. 25 μl of protein G/A agarose and 60 μl of the anti-phosphotyrosine antibody were incubated with 1 ml of extraction buffer prior to Western blotting with anti-phosphotyrosine; (I,J) 1 ml of supernatant was incubated with 25 μl of protein G/A agarose in the absence of antibodies. The precipitates were then Western blotted with anti-phytochrome and anti-phosphotyrosine antibodies, respectively. The arrow indicates the position of phytochrome.

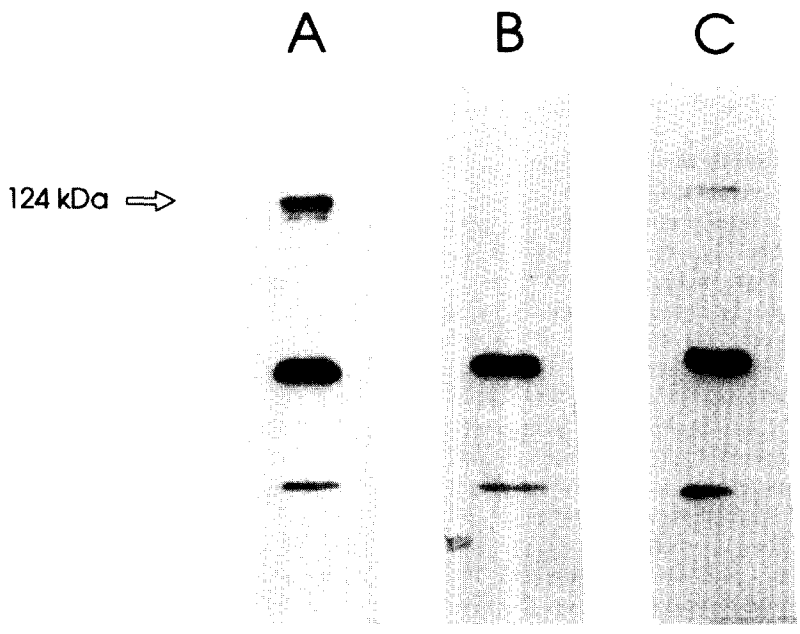

Fig. 2. Control and competition binding experiments. 1 ml of crude supernatant was added to an Eppendorf tube containing 25 μl protein G/A agarose and 10 μl oat 22. Following immunoprecipitation, the pellet was resuspended in 80 μl of 4×SDS sample buffer. 5 μl aliquots were loaded onto a 7% gel. Following electrophoresis and transfer of the proteins to nitrocellulose, the membranes were probed with (A) anti-phosphotyrosine antibody; (B) the secondary antibody only (i.e. the primary antibody was omitted); and (C) the anti-phosphotyrosine antibody plus 5 mM phosphotyrosine.

band centered at 124 kDa. The sharpened band at 15 min irradiation appeared to persist even beyond 15 min of irradiation, albeit at gradually diminishing signal level (data not shown). There are several possible explanations for the apparent heterogeneity of the band centered around 124 kDa. (1) Different phytochrome species (for example, phytochrome A and B, are present) and the phosphotyrosine in the latter is more sensitive to red light treatment. (2) Proteolytically degraded phytochrome(s) are represented in the lower molecular weight part of the band. (3) Phosphorylation of the same protein occurs, but with different tyrosine phosphorylation sites, the lower molecular weight species being more sensitive to red light treatment.

The apparent lack of bands, other than that of 124 kDa, detected by the anti-phosphotyrosine monoclonal should not be interpreted as evidence for the presence of just a single phosphotyrosyl protein. The current procedure uses only 1 ml of extract and the levels of other phosphotyrosine containing proteins were probably below the detection limits. Indigenous phosphatases and proteases may also affect levels of phosphotyrosine containing proteins [26]. Also, anti-phosphotyrosine antibodies have been shown to bind only a fraction of the total phosphotyrosine containing proteins in some cell extracts [27].

Less than 1% of the total phosphoprotein in normal mammalian cells is tyrosine phosphorylated [28]. The amount of phosphotyrosine in plant cells is expected to be similar. Therefore, a method for identifying phosphotyrosyl containing proteins must be able to selectively detect a scarce amount of phosphotyrosine without cross-reacting with abundant phosphoserine and phosphothreonine. The anti-phosphotyrosine monoclonal antibody used here (IG2 clone) is similar to that produced by Frackelton et al. [29] and can distinguish phosphotyrosine from phosphoserine and phosphothreonine even when the latter are present at more than 100 times that of phosphotyrosine. These antibodies have been used to identify phosphotyrosine containing proteins and as a method of receptor purification [26,27,29]. Non-specific binding to phytochrome in the present work can also be ruled out. When the antibodies, bound to the immunoblot shown in Fig. 3A, were removed by incubating the membrane in stripping buffer (62.5 mM Tris-HCl, pH 6.7, containing 100 mM 2-mercaptoethanol and 2% SDS) for 30 min at 70°C and the membrane was re-probed with oat 22 (Fig. 3B), there was little change in the amount of phytochrome detected. If the binding were non-specific, one would expect that the strength of the signals detected after irradiation would remain constant. Although non-specific interaction with phytochrome and cross-reactivity with phophoserine and phosphothreonine are extremely doubtful, some anti-phosphotyrosine monoclonals also bind phosphohistidine [29]. Therefore, histidine phosphorylation of phytochrome cannot be ruled out as one possible explanation of our data.

One possible interpretation of our results is that phytochrome A may possess tyrosine kinase activity. Based on sequence analyses of phytochromes from various species, it has previously been proposed by Schneider-Poetsch and colleagues [30,31], and more recently by Thümmler et al. [32], that phytochrome may function as a protein-tyrosine kinase. Schneider-Poetsch's proposal was based on the observation that a C-terminal portion of phytochromes from various species share an approx. 25% sequence identity with the transmitter modules of bacterial sensor proteins. In the presence of certain environmental stimuli, these bacterial proteins autophosphorylate at a conserved histidine residue before transferring the phosphate to a receiver molecule. Since this catalytic histidine residue is conserved only in oat and rice phytochrome A and *Arabidopsis* phytochrome C (not in dicot phytochrome A, phytochrome B, or in phytochromes from lower plants) the hypothesis was extended to suggest that a nearby

tyrosine residue that is conserved in all phytochromes sequenced may functionally replace the bacterial histidine residue. However, biochemical support in favor of this hypothesis has not been forthcoming and mutational analyses of the conserved histidine present in oat phytochrome A, as well as mutagenesis of other nearby residues that are conserved between all known phytochromes and the bacterial sensor proteins, failed to produce altered phenotypes when expressed in transgenic *Arabidopsis* [2]. The more recent proposal of Thümmler et al. [32] notes limited sequence homologies between the C-terminal domains of various phytochromes and the catalytic domains of eukaryotic histidine, serine/threonine and tyrosine kinases, but does not present experimental verification of this proposal. Although our results neither prove nor refute these hypotheses, they do suggest that further investigations are warranted.

An alternative proposal concerning the possible physiological significance of phytochrome tyrosine phosphorylation is that it may participate in the regulation of the putative serine/threonine kinase activity of the photoreceptor. It has been reported that purified oat phytochrome is capable of undergoing serine phosphorylation and that it possesses intrinsic polycation-stimulated kinase activity [33–35]. Recently, protein kinase activity has been observed in maize anti-phytochrome immunoprecipitates [36]. Although it has not been adequately resolved if phytochrome functions as a serine/threonine kinase, this putative activity could be regulated by tyrosine phosphorylation. Several yeast and mammalian protein kinases contain both serine/threonine and tyrosine regulatory sites. Their kinase activity depends on which of these residues is phosphorylated. For example, cdc2 (a protein-serine kinase) is inhibited by the phosphorylation of a tyrosine residue, while the EGF receptor protein-tyrosine kinase is inhibited by serine phosphorylation. On the other hand, mitogen-activated protein kinase (MAPK) requires phosphorylation of both threonine and tyrosine regulatory sites for activation (reviews [10,37]). These regulatory motifs have been found in higher plants. Treatment of tobacco cells with a fungal elicitor initiates tyrosine phosphorylation of a 47 kDa serine/threonine protein kinase. This phosphorylation event results in the kinases rapid transient activation [38]. Moreover, the characteristics of the elicitor activated kinase resemble those of MAPK. The existence of the MAP kinase/extracellular signal-regulated protein kinase family has already been demonstrated in several plant species [39–43].

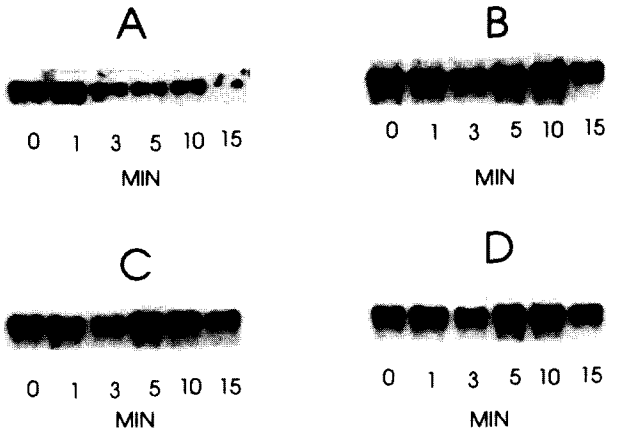

Fig. 3. Effects of red light treatment on the binding of anti-phosphotyrosine antibody to phytochrome immunoprecipitates. Prior to immunoprecipitation of phytochrome using oat 22, the extract was either irradiated with red light for 1, 3, 5, 10, and 15 min, or was kept in the dark for the same time period. Following immunoprecipitation, the pellets were resuspended in 80 μl 4×SDS sample buffer. 5 μl/lane was loaded onto a 7% gel. (A) Red-light irradiated samples probed with anti-phosphotyrosine; (B) the antibodies were stripped from the membrane used in (A) and it was re-probed using oat 22; (C) dark control Western blotted with anti-phsophotyrosine; (D) the antibodies were stripped from the membrane used in (C) and it was re-probed with oat 22.

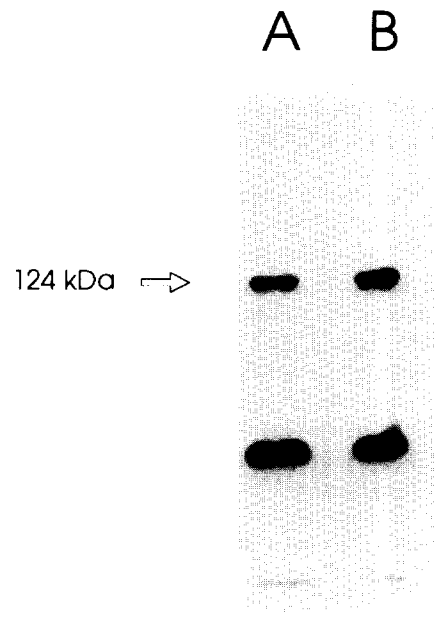

Fig. 4. Detergent vs. non-detergent washed immunoprecipitates. Phytochrome was immunoprecipitated by incubating 1 ml of crude supernatant with 25 μl protein G/A agarose and 10 μl oat 22. Following immunoprecipitation, the pellets were washed four times with either PBS (A) or PBS containing 1% Triton X-100, 0.5% deoxycholate and 0.1% SDS (B). The final pellets were resuspended in 80 μl 4×SDS sample buffer. After electrophoresing 5 μl/lane on a 7% gel, the proteins were transferred to nitrocellulose and the membrane was Western blotted with the anti-phosphotyrosine antibody.

It may also be possible that tyrosine-phosphorylated residues within the phytochrome molecule serve as binding sites for proteins containing SH2 (*src* homology) domains. These domains, present in signaling molecules such as PI3-kinase, rasGAP, phospholipase C and the src family of tyrosine kinases, stably bind to tyrosine-phosphorylated residues in receptors in a sequence-specific manner (reviews [12,44]). The specificity of these interactions is largely determined by the presence of a negatively charged residue N-terminal to the phosphotyrosine as well as by the three residues immediately carboxyl to the phosphorylated tyrosine residue. Most SH2 proteins recognize one of two categories of sequence motifs: (1) pTyr-hydrophilic-hydrophilic-hydrophobic or (2) pTyr-hydrophobic-X-hydrophobic [45]. Of the 21 tyrosine residues present in oat phytochrome A [46], there are 6 which are immediately N-terminal to one of these motifs. They are Tyr-167, -235, -263, -378, -496 and -682. However, only Tyr-167 and -235 are conserved in all phytochromes A thus far sequenced. Intriguingly enough, the sequence around Tyr-235, Glu-Pro-Tyr-Leu-Gly-Leu, is homologous (except for the proline) to the proposed binding site for phospholipase C in

the human growth factor receptor ErbB2 (Glu-Tyr-Leu-Gly-Leu) [45]. This motif is conserved among the 28 phytochromes sequenced thus far, with the exception that in eight phytochromes, mostly phytochromes B, Leu-236 is replaced with an isoleucine and in Ceratodon phytochrome this Leu is a Met.

Although the possible roles of phytochrome tyrosine phosphorylation are highly speculative, and admittedly without experimental foundation, they do suggest possible new avenues for phytochrome research. In this context, we are currently attempting: (1) to identify which tyrosine residue(s) is phosphorylated (initial attempts were unsuccessful due to insufficient amounts of the protein) and to what extent; (2) to determine whether or not the phosphorylation is autocatalytic; and (3) to determine what role phytochrome tyrosine phosphorylation/dephosphorylation plays in red-light mediated signal transduction.

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