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Light activates a 46-kDa MAP kinase-like protein kinase in soybean cell culture

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Abstract Light induced rapid and transient activation of a 46-kDa protein kinase in soybean photomixotrophic cell culture. This kinase was designated as LAP kinase (light signal-activated protein kinase). Activation of LAP kinase in response to light was associated with tyrosine phosphorylation of the kinase, and treatment of the kinase with protein tyrosine phosphatase abolished its activity. The LAP kinase efficiently phosphorylated myelin basic protein and histone, but did not phosphorylate casein. Phospho-amino acid analysis indicated that the LAP kinase was a serine/threonine protein kinase. These results indicated that the LAP kinase is related to the MAP kinase family of protein kinases.

Key words: Phototransduction; Protein kinase; Tyrosine phosphorylation; Plant signal transduction; MAP kinase

1. Introduction

Light is an important environmental stimulus controlling plant growth and development, from germination to the regulation of flowering and senescence. Due to their photosynthetic and sessile nature, plants need to particularly adapt to their light environment [1]. Most plant photoresponses require light-dependent modulation of the transcription of specific genes and of enzyme activities, which are mediated by three major classes of photoreceptors: phytochromes (responsive to red and far-red light), blue/UV-A, and UV-B photoreceptors [2]. Of these, the best characterized biochemically and physiologically are the phytochromes [3]. Genetic and biochemical approaches have led to major breakthroughs in the characterization of photoreceptors and the mechanisms of control of gene expression by light, and the signal transduction pathways controlling photoresponses have recently begun to be elucidated [4–7]. Pharmacological approaches, for example, have identified a heterotrimeric G protein, calcium/calmodulin and cyclic GMP as components of phytochrome-mediated light signal transduction pathways [8]. However, cytosolic protein components mediating phototransduction still remain to be elucidated [9,10].

Protein phosphorylation, one of the major mechanisms by which eukaryotic cells transduce extracellular signals to intracellular responses, is likely to be involved in plant phototransduction [11,12]. For example, tyrosine and serine/threonine phosphorylation have been shown to be involved in phytochrome-mediated photoinduction of *chs* (a gene encoding an anthocyanin biosynthetic enzyme, chalcone synthase) and *cab* (a gene encoding a PSII chlorophyll *a*, *b*-binding protein, *lhcb1*1*) [13]. Although it was demonstrated that photoreceptor phytochrome itself functions as a protein kinase [14], the involvement of other protein kinases in the light signal transduction pathway has not been clarified despite their important roles in signal transduction of light.

Here, we report that a 46-kDa protein serine/threonine kinase was activated rapidly and transiently by light. This activation was accompanied by the light-dependent phosphorylation of tyrosine residue(s) in the molecule. The protein kinase, designated as LAP kinase (light signal-activated protein kinase), has characteristics similar to mitogen-activated protein kinases (MAP kinase) in several respects.

2. Materials and methods

2.1. Treatment of soybean cell suspension culture

The *Glycine max* L. (cv. Corsoy) cell culture SB-P [15] was grown photomixotrophically under constant light in KN1 medium [16] containing 5 g/l sucrose at 25°C. 10 to 14 days old cultures, dark-adapted for 2.5 days prior to treatment, were used for all experiments. All manipulations were performed at 25°C under green safe light conditions using two Panasonic FL10G bulbs wrapped in four layers of lemon yellow-colored plastic film, #321C, two layers of cerulean blue plastic film, #521C, and three layers of viridian plastic film, #431C (Nakagawa Chemical Co., Tokyo, Japan). Samples were collected on filters by suction filtration and immediately frozen in liquid nitrogen. For light treatment, cultures were exposed to white light (63 μmol/m^2/s) using six Panasonic FL20SS-EX-D/18 bulbs. UV-A light was provided by three bulbs of blacklight-blue fluorescent lamps (Toshiba FL20S-BLB), which emitted light between 300–410 nm with a λ_{max} of 365 nm.

2.2. Preparation of protein extracts and determination of protein concentration

To prepare extracts from treated cells, cells (~0.2 g) in 500 μl of extraction buffer (10 mM HEPES–NaOH, pH 7.5, 0.1 mM phenylmethylsulfonyl fluoride, 100 mM NaF, 2 mM EGTA, 15 mM EDTA, 10 μg/ml antipain, 10 μg/ml leupeptin, 10 μg/ml chymostatin, 10 μg/ml pepstatin, 100 mM Na_3VO_4) were homogenized with a Physcotron (Nichion Rika, Tokyo, Japan, Type NS-50) for 1 min in 1.5-ml microcentrifuge tubes. After centrifugation at 10000 rpm for 10 min, 100 μl of SDS sample buffer (3×) containing 187.5 mM Tris–HCl, pH 6.8, 6% (w/v) SDS, 30% (v/v) glycerol, and 0.03% (w/v) phenol red was added to 200 μl of the supernatant and boiled for 5 min. Protein concentration was determined using a Bio-Rad protein assay kit with BSA as a standard.

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2.3. Immunoblotting analysis with an anti-phosphotyrosine antibody

Extracts were separated on 12.5% SDS–polyacrylamide gels, and the proteins were transferred onto nitrocellulose membranes (Advantec Co. Ltd., No. A045A330R) by semi-dry electroblotting. The membranes were blocked for 1 h in TBS-T buffer (10 mM Tris–HCl, pH 7.5, 100 mM NaCl, 0.1% (v/v) Tween 20) with 1% (w/v) BSA at room temperature and then incubated with the anti-phosphotyrosine monoclonal antibody horseradish peroxidase conjugate, PY20 (Amersham, RPN2220), in the same buffer for 1 h. The blots were washed with TBS-T buffer for 30 min with three changes of buffer, and the complexes were visualized using an enhanced chemiluminescence kit (Amersham, RPN2209), according to the manufacturer's instructions. Prestained protein markers (New England BioLabs Inc., #7707S) were used to calculate the size of proteins.

2.4. In-gel assay for kinase activity

In-gel kinase assays were performed as described previously [17]. Extracts containing 10 μg of protein were electrophoresed on 12.5% SDS–polyacrylamide gels embedded with 0.5 mg/ml of myelin basic protein (MBP), histone (type III-SS; Sigma) or casein in the separating gel as a substrate for the kinase. After electrophoresis, SDS was removed by washing the gel with buffer A (50 mM Tris–HCl, pH 8.0, 20% (v/v) 2-propanol), buffer B (50 mM Tris–HCl, pH 8.0, 5 mM 2-ME), and then buffer C (50 mM Tris–HCl, pH 8.0, 6 M guanidine HCl, 5 mM 2-ME) twice for 30 min each at room temperature. The kinases were allowed to renature in buffer D (50 mM Tris–HCl, pH 8.0, 0.04% (v/v) Tween 40, 5 mM 2-ME) at 4°C overnight with three changes of buffer. The gels were then incubated at room temperature in reaction buffer A (40 mM HEPES–KOH, pH 7.6, 2 mM DTT, 0.1 mM EGTA, 15 mM $MgCl_2$) with 100 μM ATP plus 3.7 MBq [γ-^{32}P]ATP (1.85 GBq/mmol) for 1 h. The reaction was stopped by transferring the gel into 5% (w/v) trichloroacetic acid (TCA)/1% (w/v) sodium pyrophosphate. Free [γ-^{32}P]ATP was removed by washing in the same solution for at least 5 h with four changes of buffer. Gels were dried on Whatman 3MM paper and exposed to Fuji RX-U film.

2.5. Assay for immunoprecipitated kinase activity

To extracted protein (200 μl), 4 μg of agarose-conjugated PY20 antibody in 20 μl of PBS (Santa Cruz Co. Ltd., #SC-508AC) and 200 μl of 2×immunoprecipitation buffer (20 mM Tris–HCl, pH 7.4, 300 mM NaCl, 2 mM EGTA, 2 mM EDTA, 0.4 mM Na_3VO_4, 0.4 mM PMSF) were added, and the mixture was incubated at 4°C overnight on a rocker. After centrifugation at 16 000×*g* for 10 min, the pellet was washed with 500 μl of immunoprecipitation buffer twice, and 1×SDS sample buffer was added and boiled for 5 min. After centrifugation, the supernatant was electrophoresed on a 12.5% SDS–polyacrylamide gel, and the in-gel kinase assay was performed. Also, the immunoprecipitate was used for the assay of kinase activity toward MBP as described below.

2.6. Assay of kinase activity toward MBP and identification of phosphorylated amino acids in MBP

The immunoprecipitate with the agarose-conjugated PY20 antibody was suspended in 60 μl of reaction buffer B (30 mM Tris–HCl, pH 7.5, 20 mM $MgCl_2$, 2 mM $MnCl_2$, 100 μM ATP) with 1.85 MBq [γ-^{32}P]ATP and 12 μg MBP and incubated at 30°C for 20 min. The labeled MBP was electrophoresed on a 12.5% SDS–polyacrylamide gel and detected by autoradiography.

For identification of phosphorylated amino acids in MBP, aliquots of 0.1 g of SB-P cells exposed to white light for 2.5 min after dark adaptation were used for immunoprecipitation. MBP (25 mg) was labeled by phosphorylation in the presence of 3.7 MBq [γ-^{32}P]ATP (1.85 GBq/mmol) with the suspended immunoprecipitate in the 100 μl of reaction buffer B. After SDS–PAGE and autoradiography, the gel band of phosphorylated MBP was cut out and transferred to a microcentrifuge tube. The gel piece was incubated in 50 mM NH_4HCO_3 at room temperature for 5 min, and then homogenized with a Physcotron. Then, 2-ME (10% (v/v)) and SDS (0.2% (w/v)) were added to the tube and boiled for 3 min. After gentle shaking at room temperature for 90 min, the tube was centrifuged at 7000×*g* for 5 min. The phosphorylated protein was precipitated with 20% (w/v) ice-cold TCA in the presence of BSA (20 μg) as a carrier protein. After washing the pellet with 95% (v/v) ethanol, the protein was hydrolyzed in 6 N HCl for 1.5 h at 110°C in a screw-capped microcentrifuge tube, dried in a Speed-Vac evaporator, and then dissolved in 10 μl of H_2O. After measurement of radioactivity using a liquid scintillation counter, the solution corresponding to 100 cpm was mixed with phospho-amino acid standard (1 mg/ml each of L-phosphoserine, L-phosphothreonine, and L-phosphotyrosine) and separated by high-voltage thin-layer electrophoresis using cellulose plates (Merck), as described [18]. The position of the phospho-amino acid standard was visualized with 0.2% (w/v) ninhydrin and the labeled amino acids were detected by autoradiography.

2.7. Dephosphorylation

The extracted protein from SB-P cells exposed to white light for 2.5 min, and the immunoprecipitate with the agarose-conjugated PY20 antibody, which was prepared from 0.1 g of the same cells, were incubated at 30°C for 20 min in 100 μl reaction buffer C (50 mM Tris–HCl, pH 8.0, 5 mM $MgCl_2$, 150 mM NaCl, 0.1% (v/v) 2-ME, 50 μg BSA) with 150 units of tyrosine-specific protein phosphatase YOP (#539734-S, Calbiochem, San Diego, CA, USA). The protein kinase activities were measured by in-gel kinase assay.

3. Results

3.1. Light promotes tyrosine phosphorylation on a 46-kDa protein

Previous studies have demonstrated that several genes are induced in photomixotrophic soybean SB-P cell cultures exposed to light, and that a tyrosine/histidine-specific protein kinase may be involved in light signal transduction pathway leading to the gene expression [8,13,16]. This finding prompted us to investigate light-dependent tyrosine phosphorylation in SB-P cells. First, we analyzed phosphotyrosine-containing proteins in the extracts of SB-P cells exposed to white light for 2 h by immunoblotting analysis using the phosphotyrosine-specific monoclonal antibody PY20. Several phosphotyrosine-containing proteins were detected in both soluble and insoluble fractions of the cell extracts, i.e. at least eight and three polypeptides were detected in the soluble and insoluble fractions, respectively. Of these proteins, a 27-kDa polypeptide in the insoluble fraction was the predominant species (data not shown). However, most of these phosphotyrosine-containing proteins were also detected in dark-adapted cells indicating that the tyrosine phosphorylation of these proteins was not light-dependent. When the soybean cells were exposed to light for only a few minutes, we found the phosphorylation intensity of a 46-kDa protein in the soluble fraction was markedly increased (Fig. 1A). The tyrosine phosphorylation of the 46-kDa protein was very rapid and transient, peaking within 2.5 min and returning to the dark level in 10 min.

To check the possibility that the observed tyrosine phosphorylation of the 46-kDa protein was due to secondary effects of reactive oxygen species (ROS), which could be generated by light illumination of dark-adapted cells, we investigated the effects of H_2O_2 on tyrosine phosphorylation of the protein. When dark-adapted SB-P cells were treated with 20 mM H_2O_2 in the dark, tyrosine phosphorylation of the 46-kDa protein was not observed (Fig. 1B). In another experiment, UV-A irradiation of dark-adapted SB-P cells also did not induce tyrosine phosphorylation of the 46-kDa protein (data not shown).

3.2. Light activates a 46-kDa protein kinase

To search for protein kinase(s) involved in phototransduction, we used an in-gel assay for kinase activity using MBP as an artificial substrate. Light exposure of the dark-adapted soybean suspension cells caused activation of a 46-kDa kinase (Fig. 2). This kinase was called the LAP kinase (light signal-

activated protein kinase). Activation of the LAP kinase was very rapid and transient, peaking within 5 min. The 46-kDa LAP kinase activity could not be detected in the extract of dark-adapted cells.

3.3. The 46-kDa light-dependent tyrosine-phosphorylated protein is the LAP kinase

To examine the relationship between the 46-kDa light-dependent tyrosine-phosphorylated protein (Fig. 1) and the light-activated 46-kDa protein kinase (Fig. 2), we coupled immunoprecipitation of phosphotyrosine-containing proteins with the in-gel assay for kinase activity. The extracts from soybean cells exposed to light for 2.5 min contained at least three MBP kinases, i.e. 76-, 46- and 34-kDa proteins, where the 46-kDa kinase was predominant (Fig. 3A, lane 1). When phosphotyrosine-containing proteins were first immunoprecipitated from the same cell extracts with the PY20 monoclonal antibody and then subjected to the in-gel assay for kinase activity, the 46-kDa protein kinase was detected as the only major band, demonstrating the specificity of immunoprecipitation, and that among the several kinases only the 46-kDa MBP kinase was tyrosine-phosphorylated (Fig. 3A, lane 2). To confirm these results, immunoprecipitates with the PY20 antibody from total extracts of the soybean cells exposed to light for 0 to 10 min were assayed in solution for MBP kinase activity. As shown in Fig. 3B, MBP kinase activity in the complexes rapidly and transiently increased, peaking at 2.5 min after light exposure of the dark-adapted SB-P cells. Taken together, these results indicated that the 46-kDa light-dependent tyrosine-phosphorylated protein and the 46-kDa LAP kinase were the same protein, and that activation of

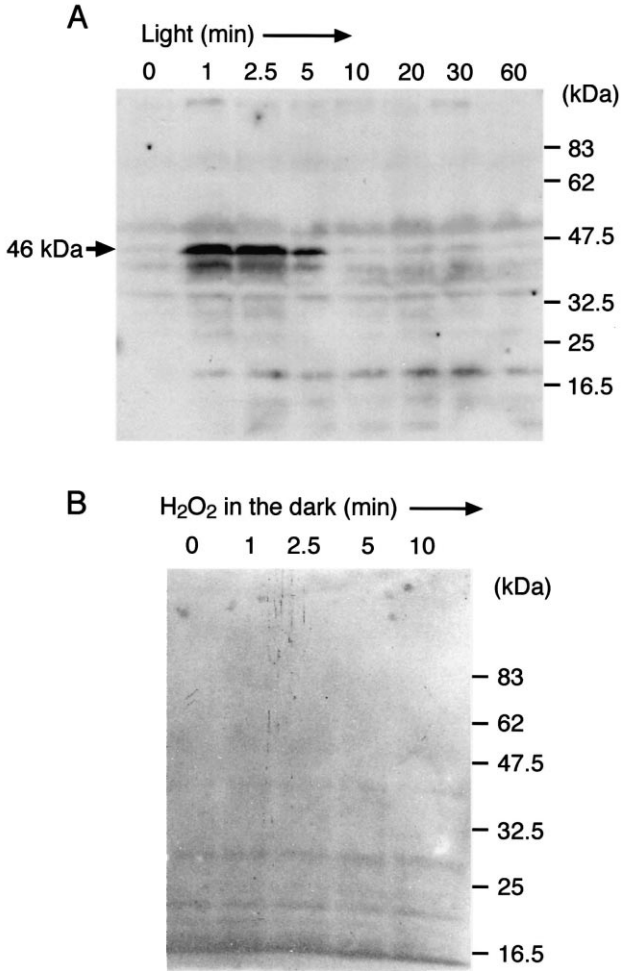

Fig. 1. Light-dependent tyrosine phosphorylation of a 46-kDa protein. Extracts prepared from dark-adapted photomixotrophic soybean SB-P cultures exposed to white light (A) or treated with 20 mM H_2O_2 in the dark for different times (shown in minutes) were separated by 12.5% SDS–PAGE. Proteins were then transferred onto nitrocellulose membranes, and phosphotyrosine-containing proteins were detected by immunoblotting analysis using the phosphotyrosine-specific monoclonal antibody PY20. The positions of molecular mass markers indicated on the right are given in kilodaltons.

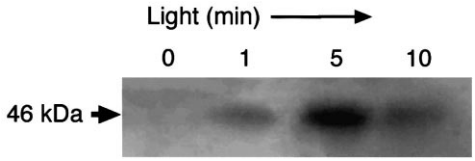

Fig. 2. Light activates a 46-kDa protein kinase. Dark-adapted soybean SB-P cells were exposed to light. Aliquots of the culture were taken at the indicated times, and kinase activity in total cell extracts was tested by an in-gel assay for kinase activity using MBP as a substrate. The mass of the LAP kinase in kilodaltons is indicated on the left.

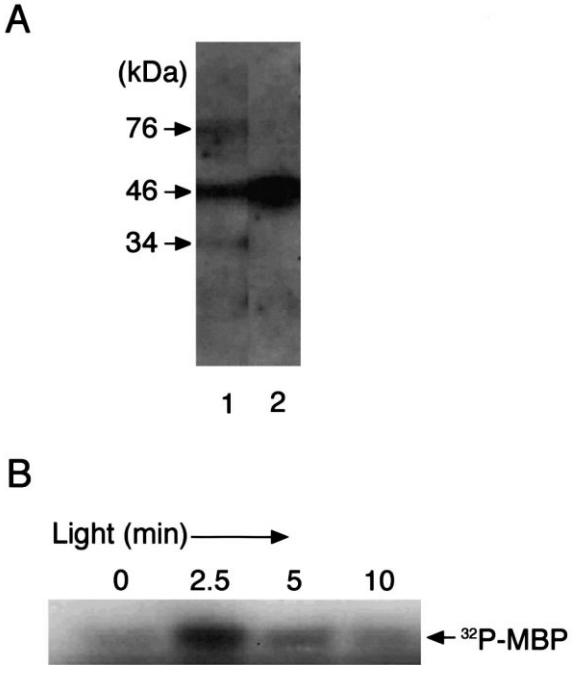

Fig. 3. Activation of the LAP kinase is associated with phosphorylation of tyrosine residue(s) in the kinase. A: Immunoprecipitation coupled with the in-gel assay for kinase activity. Extracts prepared from soybean cells exposed to light for 2.5 min were immunoprecipitated with agarose-conjugated phosphotyrosine-specific monoclonal antibody PY20, and then subjected to the in-gel assay for kinase activity using MBP as the substrate. Lane 1, in-gel kinase assay of the crude extracts (10 μg protein) of cells exposed to light for 2.5 min. Lane 2, in-gel kinase assay of the immunoprecipitate from 50 μl of protein extracts of the cells (ca. 20 μg) exposed to light for 2.5 min. B: MBP phosphorylation by immunoprecipitate. Extracts of soybean cells exposed to light for the indicated times were immunoprecipitated with PY20 antibody as in A. Then, the immunoprecipitates were assayed for MBP kinase activity with [γ-^{32}P]ATP and MBP in solution, after which the labeled MBP was detected by SDS–PAGE and autoradiography.

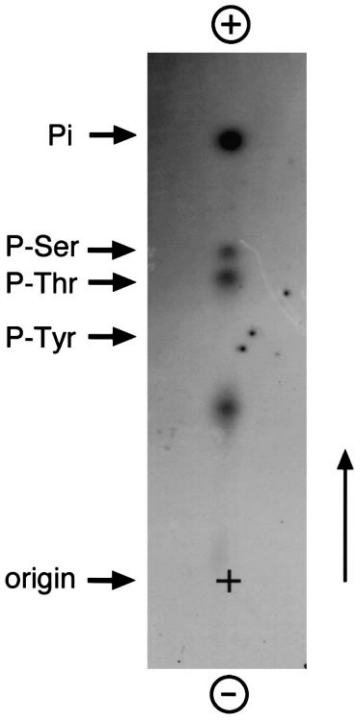

Fig. 4. Analysis of the amino acids phosphorylated in MBP with the LAP kinase. Extracts prepared from soybean cells (0.1 g) exposed to light for 2.5 min were immunoprecipitated with PY20 antibody. To identify the phosphorylated amino acids, MBP was incubated in the presence of [γ-^{32}P]ATP with the suspended immunoprecipitate. After SDS–PAGE and autoradiography, the band of phosphorylated MBP in the gel was cut out and the protein was extracted as described in Section 2. After acid hydrolysis, the dried pellet was dissolved in 10 μl of phospho-amino acid standard solution (1 mg/ml each of L-phosphoserine [P-Ser], L-phosphothreonine [P-Thr], and L-phosphotyrosine [P-Tyr]). The phospho-amino acids were separated by high-voltage thin-layer electrophoresis, as described previously [18]. The positions of each phospho-amino acid were visualized by ninhydrin staining and are indicated by arrows on the left. The labeled amino acids were detected by autoradiography that matched P-Ser and P-Thr visualized by ninhydrin staining. The (+) indicates the origin.

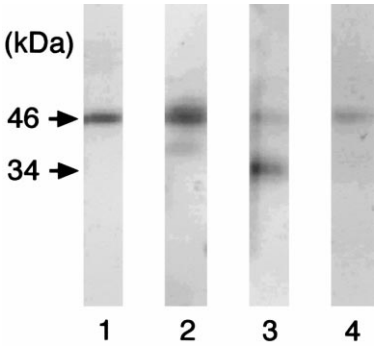

Fig. 5. Substrate specificity of the LAP kinase. The LAP kinase activities in the extracts of the cells exposed to light for 2.5 min were tested with an in-gel kinase assay using MBP (lane 1), histone (lane 2), or casein (lane 3) as the substrate. Lane 4 shows the kinase activity in the absence of substrate. The masses of protein kinases in kilodaltons are indicated on the left.

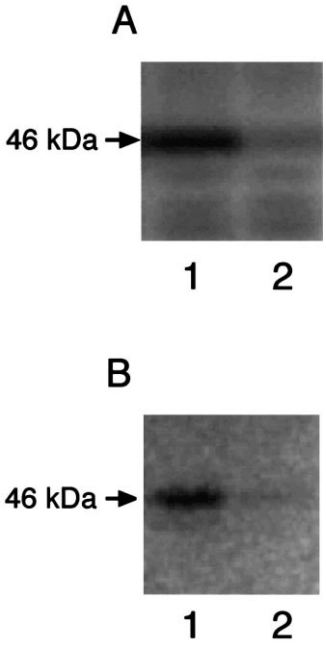

Fig. 6. Inactivation of the LAP kinase by phosphatase. The protein extract from SB-P cells exposed to white light for 2.5 min (A), and the immunoprecipitate with the agarose-conjugated PY20 antibody (B), prepared from 0.1 g of the same cells, were incubated at 30°C for 20 min in the presence (lane 2 in A and B) and absence (lane 1 in A and B) of 150 units of tyrosine-specific protein phosphatase YOP. The kinase activities were then measured by in-gel kinase assay.

the LAP kinase might be associated with the light-dependent phosphorylation of tyrosine residue(s) on the kinase.

3.4. Characterization of the LAP kinase

Phosphotyrosine-containing proteins in total extracts of soybean cells after 2.5 min of light exposure were immunoprecipitated with PY20 antibody, then MBP was phosphorylated with [γ-^{32}P]ATP by the immunoprecipitate. Phospho-amino acid analysis using thin-layer electrophoresis demonstrated that serine and threonine residues but not tyrosine residues were phosphorylated on MBP, indicating that the LAP kinase is a serine/threonine protein kinase (Fig. 4).

The LAP kinase efficiently phosphorylated MBP and histone, but did not phosphorylate casein (Fig. 5). The LAP kinase is likely to have autophosphorylation activity since the phosphorylated protein was detected in the absence of substrate in the gel (Fig. 5, lane 4).

To determine whether phosphorylation of tyrosine residue(s) is required for LAP kinase activation, we treated the kinase with tyrosine-specific protein phosphatase YOP. As shown in Fig. 6, YOP inactivated the LAP kinase.

4. Discussion

Protein phosphorylation and dephosphorylation events are correlated with several light responses in a number of plants [19], and photoreceptor-mediated phosphorylation of a number of mostly unidentified proteins has been demonstrated [20,21]. Recent reports have suggested light-dependent phosphorylation of several transcription factors that interact with promoter elements of light-regulated genes. For example, the

phosphorylation and nucleocytoplasmic partitioning of parsley bZIP transcription factor CPRF2 were shown to be light-regulated [12]. GT-1, a DNA-binding protein that binds to BoxII in pea *rbcS-3A* promoter is likely to act as a molecular switch modulated by Ca^{2+}-dependent phosphorylation and dephosphorylation in response to light signals [22]. Little is known, however, about the contributions of specific protein kinases to the light signal transduction pathway.

In this study, we demonstrated that a 46-kDa protein kinase (LAP kinase) was rapidly activated by light exposure in soybean SB-P cells (Fig. 2). Several lines of evidence suggest that the LAP kinase shares several characteristics with MAP kinases. Activation and inactivation of the LAP kinase were shown to be associated with phosphorylation and dephosphorylation of tyrosine residue(s), respectively, by immunoblotting analysis with a phosphotyrosine-specific antibody (Fig. 1), a coupled immunoprecipitation in-gel assay for kinase activity (Fig. 3), and tyrosine phosphatase treatment followed by the same assay (Fig. 6). In addition, the LAP kinase efficiently phosphorylated MBP and histone but not casein (Fig. 5). Similar substrate specificity had been reported for other plant MAP kinases [17]. Finally, the size of this LAP kinase (46 kDa) was in the range of known members of the MAP kinase family from many organisms (38–55 kDa) [23]. We concluded from its overall properties that the 46-kDa LAP kinase is likely to be a member of the MAP kinase family.

In mammals and yeast, the MAP kinase signaling cascade is one of the major pathways by which extracellular stimuli are transduced into intracellular responses [23,24]. A variety of MAP kinase genes have been isolated from plants, and there is increasing evidence that MAP kinases also play important signaling roles in plants [25]. In *Arabidopsis*, at least nine different MAP kinases have been cloned, indicating that these kinases are involved in multiple signaling pathways [26]. Elevation of MAP kinase activity has been detected in plant cells after exposure to various stresses such as cold, drought, wounding and osmotic shock. Some plant MAP kinases are involved in cell proliferation, and others are activated by bacterial elicitors, pathogen attack and plant hormones [25]. The involvement of MAP kinase in light signal transduction, however, has not been described except for a recent report on the activation of a tomato 48-kDa MBP kinase by UV light [27].

The MAP kinase cascade exhibits a very steep stimulus–response curve, indicating that small changes in the level of stimulus can turn a pathway on or off [28]. Since the activation of LAP kinase by light was also rapid and transient, with activity returning to basal levels within 10 min after stimulation (Figs. 1–3), it is possible that the MAP kinase-like LAP kinase plays a role as a molecular switch for light signal transduction. In yeast and mammalian systems, activation of MAP kinases is brought about by upstream MAP kinase kinase (MAPKK) through phosphorylation of the conserved threonine and tyrosine residues that are located close to kinase domain VIII in all MAP kinases [29], and inactivation of MAP kinases is mediated by dual specificity protein phosphatases (MAP kinase phosphatases), which simultaneously dephosphorylate both the threonine and tyrosine residues of MAP kinases with high efficiency [30]. A given dual specificity MAPKK can only activate a specific MAP kinase. It is important to identify the protein kinase and phosphatase that phosphorylate and dephosphorylate tyrosine residues in the LAP kinase, respectively.

Recently, it has been reported that reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydroxyl radicals ($OH^\bullet$), and hydrogen peroxide (H_2O_2) induced rapid activation of some MAP kinases in plants [31,32]. ROS is an active signaling molecule in plants and is generated in response to different abiotic and biotic stresses [33,34]. The membrane diffusible and comparatively stable molecule H_2O_2 is the most likely candidate for these intracellular signaling roles. ROS is also an unavoidable product of the interaction of molecular oxygen with normal metabolic processes in plants, including the intense electron fluxes associated with photosynthesis. To check whether the observed tyrosine phosphorylation and activation of LAP kinase were due to the secondary effects of ROS generated by light illumination of dark-adapted cells, we investigated the effects of H_2O_2 on tyrosine phosphorylation of LAP kinase. When dark-adapted SB-P cells were treated with 20 mM H_2O_2 in the dark, conditions under which the tobacco MAP kinase was activated [32], tyrosine phosphorylation of the LAP kinase was not observed (Fig. 1B). UV-A irradiation of the dark-adapted SB-P cells also did not induce tyrosine phosphorylation and activation of the LAP kinase (unpublished data). These findings excluded the possibility that the observed tyrosine phosphorylation and activation of the 46-kDa LAP kinase by light was a consequence of the effects of ROS generated by light exposure.

To elucidate the phototransduction pathway in which the LAP kinase participates and the photoreceptor(s) that mediate activation of the LAP kinase, more detailed investigations are required. We are currently investigating the effects of light quality that induces activation of the LAP kinase. Also, we are attempting to purify the LAP kinase from soybean cells and searching for protein substrates that are phosphorylated by the LAP kinase. LAP kinase may directly phosphorylate transcription factor(s) and modulate their functions as reported for CPRF2, which is phosphorylated at serine residue(s) in response to light and subsequently transported from the cytoplasm into the nucleus [12]. Continued study of LAP kinase will reveal novel mechanisms of plant phototransduction pathways and lead to an increased understanding of the roles of protein phosphorylation in them.

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