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Guanine nucleotide binding protein involvement in early steps of phytochrome-regulated gene expression

(signal transduction/soybean cells/*cab*/cholera toxin/pertussis toxin)

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ABSTRACT **The transmission process of light signals from plant photoreceptors to target cellular events is largely unknown. In the present work, we show that treatment of dark-adapted soybean cells (SB-P) with cholera toxin or pertussis toxin uncouples phytochrome-dependent gene expression. Addition of as little as 10 ng of toxin per ml is sufficient to activate expression of genes encoding the major chlorophyll a/b-binding protein (*cab*) in the dark. Significant levels of cab transcript accumulation are detected within 0.5 h after addition of the toxins and expression of these genes is desensitized to further light treatments. Treatment of SB-P cells with the calmodulin antagonist *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride (W-7) prevents induction of the photoregulated gene by phytochrome or bacterial toxins. These results indicate the involvement of guanine nucleotide binding protein(s) in phytochrome-mediated *cab* gene activation. A likely site of action for this step is between the photoreceptor and a downstream W-7-sensitive effector.**

Photoregulation of genes plays a critical role in plant development. Many light-responsive genes are regulated at the transcriptional level and important progress has been made in the analysis of the promoter of light-responsive genes (1). However, relatively few studies have characterized the early events involved in the signal transduction pathway. Although phytochrome is the best characterized plant photoreceptor (2), how it regulates signal transduction is still largely unknown. In recent years, two approaches have been taken to study this process. One is the identification and characterization of photomorphogenic mutants, especially in *Arabidopsis thaliana*, that display clear alterations in developmental pathways that are normally light-dependent. Three well-characterized loci are defined by the *det1*, *det2*, and *cop1* mutations (3, 4). A second approach is to study intermediates involved in light-mediated responses by searching for plant homologs of well-known enzymes or second messengers involved in animal signal transduction processes. Guanine nucleotide binding proteins (G proteins) play critical roles in signal transduction processes in many organisms where they have been studied. One class, the heterotrimeric G proteins, has especially well-characterized structural and functional properties (5). A common feature of these proteins is that a receptor-mediated stimulus induces the exchange of GTP for protein-bound GDP. The active protein (GTP-bound) then interacts with an effector molecule to trigger a cascade of amplification and transmission of the signal to downstream targets. An endogenous GTPase activity hydrolyzes the GTP to GDP, thus switching off the process. The α subunit of many heterotrimeric G proteins ($G\alpha$) can be modified by bacterial toxins. Some members of this family, such as those that belong to the stimulatory $G\alpha$ subfamily $G\alpha_s$, are ADP-ribosylated by cholera toxin at a conserved arginine residue that inhibits or reduces the GTPase activity of the protein. In contrast, pertussis toxin catalyzes ADP-ribosylation of the inhibitory $G\alpha$ subfamily $G\alpha_i$ at a conserved cysteine residue close to the C terminus, preventing the activation of the protein (5–7). The fact that cholera and pertussis toxins are able to penetrate into cells has made possible their use for *in vivo* studies of G-protein functions in animal signal transduction pathways.

Several reports of GTP-binding proteins in different plant species have been published in the last few years (8–11) and a cDNA encoding a putative $G\alpha$ protein has been cloned from *A. thaliana* (12). Although the function of G proteins in the regulation of K^+ channel and light-mediated responses has been suggested (10, 13, 14), the roles of G proteins in plant signal transduction events are largely unknown. We have used a homogeneous cell suspension system that responds to phytochrome for the characterization of light-dependent signal transduction pathways (15). With this cell suspension, we have studied the involvement of G proteins in phytochrome-dependent gene expression by using bacterial toxins as modulators of G-protein activity.

MATERIALS AND METHODS

Growth of SB-P Cell Cultures. Photoautotrophic soybean cell cultures (SB-P) were grown under mixotrophic conditions as described (15). The cultures were dark-adapted by wrapping the flasks with two layers of aluminum foil and shaking for 4 days. For light treatment, the flasks were irradiated in a dark room as described (15).

Inhibitor and Toxin Treatments. Cholera toxin and pertussis toxin (GIBCO/BRL) were dissolved and thiol-activated before each experiment according to the company instructions. The toxins were used at 1 μg/ml unless otherwise indicated. The inhibitors *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride (W-7; Sigma), KN-62 (Seikegaku America, St. Petersburg, FL), and K-252a (Calbiochem) were dissolved in dimethyl sulfoxide before use. The toxins or the drugs were added to dark-adapted cell cultures in the dark under a dim green safelight. The cultures were then wrapped again with aluminum foil and shaken for 6 h before RNA isolation.

Isolation of RNA and Northern Blot Analysis. Isolation of RNA from SB-P cells and Northern blot analyses were performed as described (15). ^{32}P-labeled DNA probes were prepared by random-primer labeling of the isolated cDNA inserts of soybean *cab* and *hsp75* and maize 17S rRNA for detection of the soybean 18S rRNA (16–18). The relative intensities of the autoradiograms were measured by densitometric analysis using a charge-coupled devise (CCD) video camera (DataVision model 261; ITM, Waltham, MA) and the

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Abbreviations: G protein, guanine nucleotide binding protein; $G\alpha_s$ and $G\alpha_i$, stimulatory and inhibitory G-protein α subunits, respectively.

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IMAGE Version 1.4 gel scanning routines (National Institutes of Health) for processing of the data with a Macintosh IIsi computer. The linear range of intensity measurement was determined by Northern blots with a dilution series from 0 to 25 μg of total RNA (Fig. 1A). The blots were hybridized sequentially with the *cab* and 17S rRNA probes and the intensities of the signals were measured and processed with the video system. As shown in Fig. 1A, relative intensities of 3000 units or less are within the linear range of our system. Thus, for quantitation of our autoradiograms, we adjusted exposure of our Northern blots to keep the relative intensities of the resultant bands to <2500 units. The levels of cab mRNA were measured relative to that of the 18S rRNA with each Northern blot and the ratios are then normalized to that obtained with RNA from a light-grown SB-P culture, which is assigned as 100%. All experiments were repeated at least two times and the results from a single representative experiment are presented.

***In Vivo* ^{32}P-Labeling of SB-P Cells.** Dark-adapted SB-P cells were collected by centrifugation, resuspended in phosphate-free KN-1 medium, and returned to the shaker for 24 h. Aliquots of cells (100 μl) were preincubated with the toxins or the drugs for 30 min and then 50 μCi of [^{32}P]orthophosphoric acid (8000–9000 Ci/mmol; 1 Ci = 37 GBq) was added. After 10 min of incubation, the cells were frozen in liquid nitrogen. The samples were then thawed and washed several times with 0.5 M sodium phosphate (pH 7.5). After the last wash, 50 μl of phosphate-buffered saline and 50 μl of 2× SDS/PAGE sample buffer (10) were added to the pellet and boiled for 10 min. The same amounts of proteins were added to GF/C filters (Whatman) and washed consecutively with 2 ml of 10% (vol/vol) and 5% trichloroacetic acid, ethanol, and acetone. The filters were air-dried and then radioactivity was measured.

RESULTS

Toxin Activation of *cab* Expression in Dark-Adapted SB-P Cell Suspensions. The expression of *cab* in SB-P cells is tightly regulated by phytochrome and is quantitatively similar to that observed in young soybean seedlings (15). To examine whether G proteins are involved in phytochrome-regulated gene expression, we studied the effect of cholera and pertussis toxins on *cab* expression. As demonstrated before, dark adaptation of light-grown SB-P cell cultures resulted in a drastic reduction of cab mRNA (Fig. 1*B*). The expression of *cab* was activated by phytochrome, as demonstrated by red light induction and far-red light reversal. Incubation of dark-adapted cells with activated cholera toxin resulted in the stimulation of *cab* expression after 6 h in the dark. This effect was readily detectable at a toxin concentration of 1 μg/ml but induction was observed at cholera toxin concentrations as low as 10 ng/ml (data not shown). Similar results were observed when SB-P cells were incubated with pertussis toxin (Figs. 1*B* and 2*A*).

To test whether the expression of *cab* remains coupled to the photoreceptor phytochrome after treatment with the bacterial toxins, we illuminated the dark-adapted SB-P cells with either red light alone or red light followed immediately by far-red light. Fig. 1*B* shows that red light illumination of toxin-treated SB-P cells no longer showed any dramatic effects on *cab* expression, in contrast to nontreated cells. Far-red light irradiation was also unable to affect the toxin-dependent induction of cab mRNA accumulation. Both observations indicate that modification of G proteins by bacterial toxins can uncouple phytochrome-dependent gene expression from the light stimulus. One interesting difference between the activation by phytochrome and the bacterial toxins was the maximal level of cab transcript that could be induced. We routinely found that activation by toxin treatments resulted in maximal cab mRNA levels that were ≈25% of that observed with red light and this level did not increase significantly after light treatment (Fig. 1*B*).

To further characterize the induction process, we have compared the kinetics of cab transcript accumulation after activation by red light or treatment with bacterial toxins. We found that cab mRNA was undetectable 0.5 h after red light irradiation. At 2 h after illumination, a significant amount of transcript was then observed (≈30%). Maximal level of cab accumulation was achieved between 6 and 12 h after red light treatment. In contrast, either cholera or pertussis toxin treatment caused a faster induction of *cab* expression with

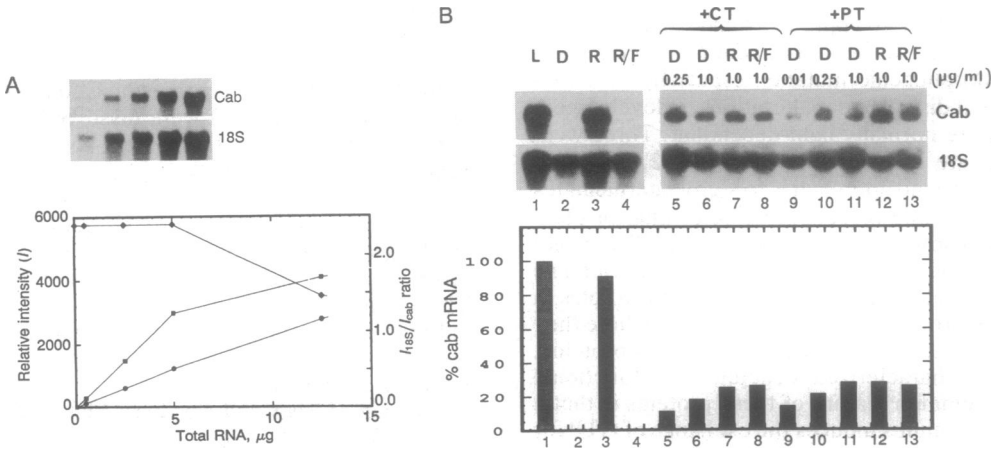

FIG. 1. (*A*) Quantitation of mRNA level. A dilution series consisting of 1.25, 2.5, 5.0, 12.5, and 25.0 μg of total RNA was used for the determination of the linear range of cab mRNA quantitation by Northern blots. The intensity of the bands was measured by densitometric analysis and plotted against the concentration of RNA. The ratio between 18S rRNA and cab mRNA remains constant in the same range where the intensities of both signals showed linearity relative to the amount of RNA. ◆, Ratio of 18S rRNA to *cab*; ■, 18S rRNA; ●, cab mRNA. (*B*) Effects of cholera and pertussis toxins on the expression of *cab*. Two-day dark-adapted soybean cells (D) were irradiated with red light (R) or consecutive pulses of red and far-red light (R/F), and the levels of cab mRNA and 18S rRNA were determined 6 h later by Northern blot analysis. The expression level in cells that have been grown under continuous white light (L) is also shown. Where indicated, dark-adapted cells were incubated in darkness with cholera toxin (+CT) or pertussis toxin (+PT) for 1 h at the concentrations indicated. The cells were then either kept in the dark (D) or were irradiated with red (R) or red plus far-red light (R/F), and the level of cab mRNA and 18S rRNA was determined 6 h later. To keep the measured intensities within the linear range, the level of cab mRNA and 18S rRNA was quantitated from autoradiograms of lower exposure time than was used for photography. The level of cab mRNA was normalized to the level of the 18S rRNA obtained for each sample, with the value obtained with the light-grown SB-P cells set as 100%. The numbers of the bars and lanes correspond.

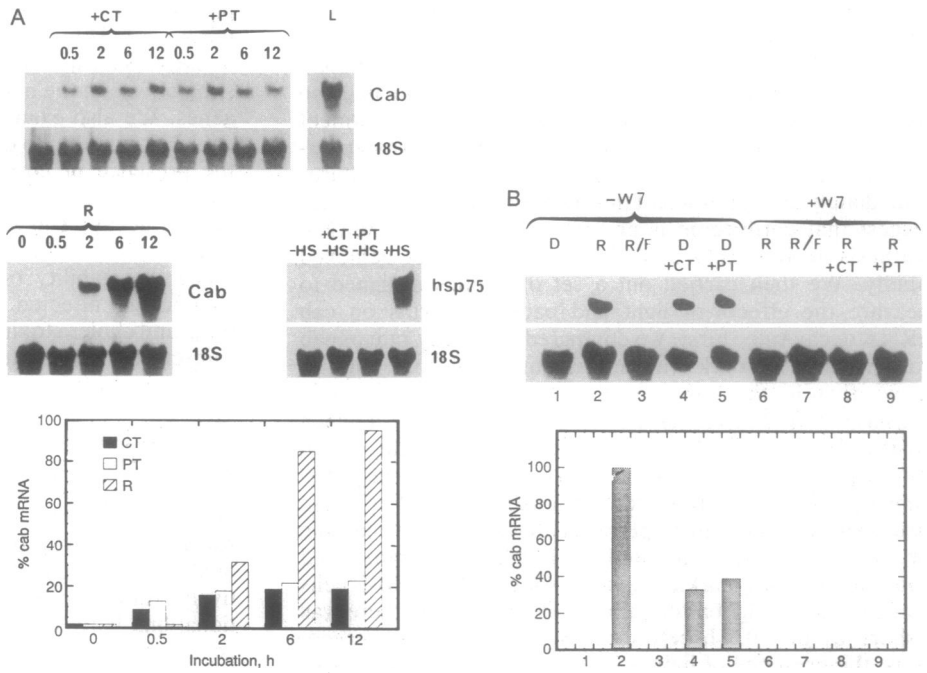

FIG. 2. (*A*) Time course of *cab* induction after red light and toxin treatment. Dark-adapted SB-P cells were irradiated with red light (R) or incubated with cholera toxin (+CT) or pertussis toxin (+PT) at 1 μg/ml for 0.5, 2, 6, and 12 h in darkness, before RNA isolation. The level of cab mRNA and 18S rRNA is shown. Quantitation of cab mRNA expression is shown after normalization to the 18S rRNA as in Fig. 1. The level of cab transcript in light-grown SB-P cells (L) is set as 100%. As a control for gene activation specificity, dark-adapted cells were incubated at 25°C (−HS) with cholera or pertussis toxin for 1 h and the level of hsp75 mRNA was determined by Northern blot analysis. RNA from SB-P cells treated at 42°C for 1 h (+HS) without toxins is also shown for comparison. (*B*) Inhibition of the toxin-dependent activation of *cab* by the calmodulin antagonist W-7. Dark-adapted cells were preincubated for 30 min with cholera toxin (1 μg/ml), pertussis toxin (1 μg/ml), or W-7 (0.2 mM) and irradiated with red (R) or red followed by far-red light (R/F). The level of RNA was analyzed 6 h after the various treatments. Quantitation of *cab* expression is shown after normalization to the 18S rRNA. The numbers of the bars and lanes correspond.

the maximal level reached between 0.5 and 2 h (Fig. 2*A*). As a control, treatment of SB-P cells with either toxin did not affect the expression of the heat-inducible gene *hsp75*, suggesting that cab accumulation is not a result of general transcriptional activation (Fig. 2*A*). We have also examined whether blue light is involved in the activation of *cab* in SB-P cells. We found that illumination of dark-adapted SB-P cultures for 10 min with blue light (λ_{max} = 499 nm, fluence = 350 $\mu mol \cdot s^{-1} \cdot m^{-2}$) was unable to activate *cab* expression to any significant levels (data not shown).

Inhibition of Toxin-Stimulated *cab* Expression by W-7. It has been shown (15) that phytochrome-dependent *cab* expression in SB-P cells may involve a calmodulin-dependent step. To examine whether the activation of phytochrome-dependent genes by bacterial toxins may involve the same signal transduction intermediates that are important during light induction, we tested the effects of W-7 on toxin-dependent *cab* expression. Fig. 2*B* shows that treatment with 0.2 mM W-7 completely abolished phytochrome activation of *cab*. Furthermore, induction of *cab* expression by either cholera toxin or pertussis toxin was also completely suppressed. These observations suggest that toxin- and phytochrome-dependent *cab* activation may require a similar, if not identical, intermediate step that is sensitive to W-7 treatment.

We have also reprobed the Northern blot shown in Fig. 2*B* with a cDNA clone of pea *rbcS* (19). The pattern of expression observed was essentially the same pattern found with *cab* (data not shown). This shows that toxin activation of light-dependent genes may be a general phenomenon for light-activated genes and not specific to *cab*.

The Role of Phosphorylation in Light-Dependent Gene Expression. Calmodulin is well known to modulate activities of various classes of protein kinases (20). Our observation with W-7 prompted us to examine whether phosphorylation may also play a critical role in the activation of *cab* by phytochrome. If light activation of gene expression requires protein phosphorylation, we would expect potent kinase inhibitors such as KN-62 (specific for calmodulin-dependent kinase II) and K-252a (a general protein kinase inhibitor) to abolish phytochrome-dependent induction of *cab*. We found that treatment of SB-P cells with these inhibitors has no significant effect on *cab* expression (data not shown). Thus, they neither inhibit phytochrome activation of *cab* nor induce *cab* expression in continuous darkness.

The absence of apparent effects on *cab* expression could be due to the inability of these inhibitors to penetrate SB-P cells or to inhibit kinase activities present in this system. To address these possibilities, we established conditions to directly examine protein phosphorylation levels by pulse labeling SB-P cells with [^{32}P]orthophosphate. In addition to KN-62 and K-252a, we also tested the effects of bacterial toxins and W-7 on protein phosphorylation in SB-P cells. The results in Table 1 show that W-7, KN-62, and K-252a were all inhibitors of protein phosphorylation in SB-P cells. Pertussis toxin treatment also suppressed the level of general protein phosphorylation whereas cholera toxin showed less inhibitory effects. However, higher concentrations of cholera toxin also affected the level of ^{32}P incorporation. These observations show that the SB-P cells are likely to be permeable to the kinase inhibitors that we have tested.

Effects of Light and Toxins on cab Transcript Stability. To determine whether the stability of cab mRNA is affected by light and toxin treatments, we carried out a series of studies with the transcription elongation inhibitor cordycepin. This inhibitor has been shown to be effective in plant systems and several studies on the effects of light on mRNA stability have

Table 1. Effect of bacterial toxins and kinase inhibitors on the ^{32}P-labeling of SB-P cells

Addition	% labeling
None	100
Cholera toxin (1 μg/ml)	83
Cholera toxin (5 μg/ml)	38
Pertussis toxin (1 μg/ml)	11
Pertussis toxin (5 μg/ml)	2
W-7 (0.2 mM)	19
KN62 (20 μM)	46
K-252a (4 μM)	29

Pretreated SB-P cells (100 μl) were labeled with [^{32}P]orthophosphoric acid for 10 min. The relative incorporation of ^{32}P into the cells was determined by measuring the amount of acid-precipitable radioactivity per μg of protein extracted from the cells. The 100% labeling corresponds to 1817 ± 198 cpm/μg of protein.

been reported (21, 22). To verify that this inhibitor can efficiently shut off transcription in our system, we tested the ability of 0.2 mM cordycepin to prevent *hsp75* induction upon heat shock of SB-P cells. Fig. 3*A* demonstrates that indeed no detectable *hsp75* transcript can be induced by heat treatment after the addition of cordycepin. Preincubation was apparently not required since we heat-treated our cell cultures immediately after addition of this inhibitor. These results thus suggest that cordycepin is an efficient inhibitor of mRNA synthesis in SB-P cells and that its effects can be achieved rapidly. We then carried out a set of studies designed to measure the effects of light and bacterial toxins on cab mRNA decay rate. First, we compared the decay rate of cab transcripts in the dark with or without cordycepin. Fig. 3*B* shows that in the absence of cordycepin, decay of cab transcript was relatively slow, with a half-life of ≈10 h, when SB-P cells were transferred into the dark from light. When transcription was inhibited, the decay of cab transcript became significantly more rapid with a half-life of ≈2 h. These results suggest that transcription of *cab* was likely to continue for some time when untreated SB-P cells were transferred from the light into darkness. Interestingly, if the SB-P cells were kept in the light after addition of cordycepin, there was a short lag of ≈0.5 h before mRNA decay was observed. Thus, the presence of light appears to affect the stability of cab transcripts in SB-P cells. The apparent half-life of cab mRNA under this condition is ≈4 h. We note that the mRNA stability of some potato *rbcS* genes (21) and the oat *phyA* gene (22) is also increased slightly in the light as compared to darkness. In fact, with the oat *phyA* study, a similar lag phase in mRNA decay is observed in the light-treated plants. At present, the cause of these differences in mRNA stability is not known. However, since the magnitude of the difference in mRNA half-lives is not very large, it is unlikely that it plays a major role in the normal light-responsive behavior of these genes. We also examined the effects of pertussis toxin and cholera toxin on the decay of cab mRNA in darkness and in the presence of cordycepin. Similar to light, both toxins appeared to cause a slight delay of ≈0.5 h in the onset of cab mRNA decay. The kinetics of the decay, however, is very similar to that observed in the absence of toxin treatment. Since the stability of cab mRNA does not appear to be significantly affected, our results thus suggest that pertussis toxin and cholera toxin induce *cab* expression mainly through transcriptional activation.

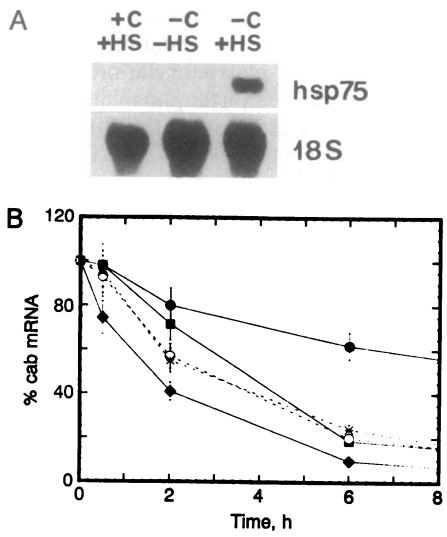

FIG. 3. (*A*) Effect of the inhibitor cordycepin on the heat induction of *hsp75*. A 50-ml soybean cell suspension was separated into 15-ml cultures in the presence (+C) or absence (−C) of the transcription inhibitor cordycepin 5′-triphosphate (0.2 mM). The cultures were transferred to a shaker at room temperature (−HS) or at 42°C (+HS) for 1 h. Northern blot analysis was then carried out to determine the level of *hsp75* expression. (*B*) Light, dark, and toxin effects on cab mRNA stability. Several SB-P cell suspensions were grown under continuous light for 2 weeks, mixed together, and then divided into five 60-ml cultures. At zero time, the cultures were treated as follows: ●, transferred to the dark; ◆, transferred to the dark after the addition of 0.2 mM cordycepin 5′-triphosphate; ■, remained in the light in the presence of cordycepin; ○, transferred to the dark with cholera toxin (1 μg/ml) and cordycepin; ×, transferred to the dark with pertussis toxin (1 μg/ml) and cordycepin. At the indicated time, 15-ml samples were taken from each culture for RNA isolation and quantitation of cab transcripts. Each point represents the mean ± SD of three experiments. The level of cab mRNA at time 0 is set as 100%.

DISCUSSION

G-Protein Involvement in Phytochrome-Dependent Gene Activation. The presence of G proteins in plants is now well established (8–14). However, the pathways in which these proteins are involved are still uncertain. Warpeha *et al.* (14) and Romero *et al.* (10) suggest that plant G proteins may be modulated by a blue-light receptor and phytochrome, respectively. Although Romero *et al.* (10) clearly suggested a functional link between G proteins and phytochrome-responsive gene expression in oat seedlings, the heterogeneity of the assay system makes future biochemical analyses of this process difficult. In addition, the concentration of cholera toxin needed for maximal induction of *cab* expression in oat seedlings was found to be in the range of 30–300 μg/ml. This relatively high concentration of toxin needed for *cab* activation is likely due to the inaccessibility of tissues to the toxin. In the present work, we have found that treatment of SB-P cells with two bacterial toxins can uncouple dependence of *cab* expression on phytochrome. After treatment with either cholera toxin or pertussis toxin, *cab* expression is activated and the level of this activity is no longer sensitive to further irradiation. This result is significant since it suggests that the events triggered by treatment with bacterial toxins are likely to be on the same pathway as those activated by phytochrome. The fact that the level of *cab* expression reached only ≈25% of the normal level suggests that the toxins may have activated some inhibitory pathways that can down-regulate *cab* expression. One likely candidate may be a desensitization pathway that normally acts to suppress *cab* expression under certain developmental or environmental conditions. The effects that we observed in the soybean cells may result from the simultaneous modulation of several distinct G proteins that are involved in different regulatory pathways. An equilibrium between positive and negative signals may thus explain the lower level of cab mRNA induction observed in the presence of the toxins. In any case, our results support previous observations on the effect of cholera toxin (10) and make the case for G-protein involvement in phytochrome-responsive gene expression quite compelling.

It is interesting to compare the kinetics of *cab* activation by bacterial toxins and phytochrome. Previous reports (15, 23), as well as this work, have shown that phytochrome activation of *cab* and *rbcS* shows relatively slow kinetics with a maximal level of mRNA accumulation reached within 6–10 h after onset of irradiation. In the present work, we found <5% of the light-inducible cab transcript 0.5 h after illumination. In contrast, the toxin-dependent activation of cab mRNA accumulation is relatively rapid, with maximal levels reached within 0.5–2 h. This result suggests that the signal transduction process that normally leads to *cab* expression can proceed quickly after the toxin-sensitive step. If the effects of the toxins are indeed the result of G-protein modification, our observations will then implicate the interaction between activated phytochrome and its primary signal transducer as the likely rate-limiting step for activation of *cab* transcription. In

this regard, we would like to point out that phytochrome is a soluble protein that is thought to be localized in the cytosol (24). Since heterotrimeric G proteins are known to be membrane associated, interaction between activated phytochrome and this signal transducer may play a significant role in determining the kinetics of gene regulation. This is unlike the other G-protein-coupled receptors that have been characterized to date. In those cases, diffusion may not be a limiting factor for the interaction between the membrane-bound receptors and the membrane-associated heterotrimeric G proteins.

Mechanism of *cab* Gene Activation by Bacterial Toxins. We should emphasize that our results do not directly demonstrate the involvement of G proteins. It is possible that cholera toxin and pertussis toxin may cause ADP-ribosylation of proteins in the SB-P cells that are distinct from G proteins. However, since these toxins have very different specificities and structures, the fact that they can both activate *cab* expression suggests that the target(s) of modification is likely to be Gα-related.

It is intriguing that cholera toxin and pertussis toxin have the same effects on *cab* expression in our studies. Similar effects were also observed in guard cells of fava beans where either cholera toxin or pertussis toxin caused inhibition of K^+ uptake and stomata opening (13). These results are reminiscent of that observed with mammalian adenylate cyclase where $G\alpha_s$ and $G\alpha_i$ are G proteins that up- and down-regulate this critical enzyme, respectively (5, 6). In this system, cholera toxin activates adenylate cyclase by activation of $G\alpha_s$ and pertussis toxin activates the same enzyme by inhibiting $G\alpha_i$. Our results suggest that phytochrome may activate gene expression via an analogous regulatory system. At present, we cannot distinguish whether phytochrome may activate a $G\alpha_s$-like protein or suppress a $G\alpha_i$-like protein. However, since genetic studies have suggested the presence of developmental regulators that normally repress expression of *cab* and *rbcS* in the dark (3, 4), a simple model may involve activation of a $G\alpha_s$ by phytochrome to overcome repression by a $G\alpha_i$ that is linked to a developmental stage- or dark-specific repressor. Studies with the photomorphogenic mutants may allow this hypothesis to be tested.

Toward a Mechanistic Model of the Signal Transduction Events Involved in Phytochrome-Dependent Gene Expression. To further establish the role of the toxin-sensitive step as an intermediate during light activation of *cab* expression, we tested the effects of W-7, a known inhibitor of *cab* activation by phytochrome (15). Indeed, we found that this reagent efficiently repressed the dark activation of *cab* by cholera and pertussis toxins. This observation is consistent with the interpretation that the bacterial toxins activate *cab* expression via the normal signal transduction pathway used by phytochrome. These data are summarized in a working model illustrated in Fig. 4. In this model, two distinct steps are proposed to mediate the effects of phytochrome on *cab* expression. The first involves a heterotrimeric G protein and the second involves a Ca^{2+}- or calmodulin-dependent enzyme that is W-7-sensitive. At present, we do not have any information on the identity of the latter step. One obvious possibility is the involvement of a Ca^{2+}/calmodulin-dependent kinase. In the present work, we have tried to correlate the effects of protein kinase inhibitors on protein phosphorylation with their effects on *cab* induction by phytochrome. Although we could demonstrate that the kinase inhibitors we tested had significant effects on protein phosphorylation in SB-P cells, no significant inhibition was observed for *cab* induction by phytochrome or the bacterial toxins. The lack of inhibition of *cab* activation in SB-P cells by kinase inhibitors thus suggests phosphorylation may not play a major role in this process. However, since none of our inhibitors completely abolishes protein phosphorylation in our system, the possibility remains that a small pool of inhibitor-resistant kinases may still be operative and is sufficient for the activation of *cab* transcription. Biochemical studies using this cell suspension will be necessary to shed more light on this part of the pathway.

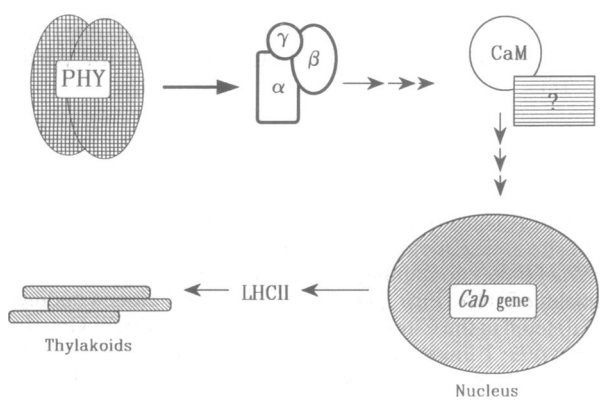

FIG. 4. Working model for phytochrome-regulated *cab* expression in soybean cell suspension. A heterotrimeric G-protein-dependent step is proposed to interact with activated phytochrome (PHY). The subsequent signal transduction events may involve a calmodulin (CaM)-dependent enzyme. At present, the identity of this protein is unknown. LHCII, light-harvesting chlorophyll a/b-binding protein of photosystem II.

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