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Identification of possible signal transduction components mediating light induction of the *Gsa* gene for an early chlorophyll biosynthetic step in *Chlamydomonas reinhardtii*

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Abstract. Light-induced expression of the *Gsa* gene encoding the heme and chlorophyll biosynthetic enzyme glutamate 1-semialdehyde aminotransferase in *Chlamydomonas reinhardtii* was previously shown to involve Ca^{2+} and calmodulin (CaM) (C. lm et al. 1996, Plant Cell 8: 2245–2253). To further analyze the signal transduction pathway for light-induced *Gsa* expression, the effects of several pharmacological agents were examined. Treatment of light-dark synchronized cells with the heterotrimeric G-protein agonist Mas-7 caused partial induction of *Gsa* in the dark. The phospholipase C inhibitor U73122 inhibited light induction of *Gsa*. Exposure of cells to light caused a sustained 3-fold increase in cellular D-inositol 1,4,5-trisphosphate ($InsP_3$) concentration. KN-93, a specific inhibitor of Ca^{2+}/CaM-dependent protein kinase II, inhibited light induction of *Gsa*. In contrast, cyclosporin A, a specific inhibitor of the Ca^{2+}/CaM-dependent phosphoprotein phosphatase calcineurin, did not affect light induction of *Gsa*. These results, together with the earlier results, suggest the involvement of a canonical signal transduction pathway for light-regulated *Gsa* expression that involves a heterotrimeric G-protein activation, phospholipase C-catalyzed $InsP_3$ formation, $InsP_3$-dependent Ca^{2+} release, and activation of a downstream signaling pathway through a Ca^{2+}/CaM-dependent protein kinase.

Key words: *Chlamydomonas* (chlorophyll biosynthesis) – Chlorophyll biosynthesis – Light regulation (*Gsa* gene) – Signal transduction

Introduction

The ability of plants to adjust their development and metabolism in response to changes in environmental factors such as light, nutrients, and temperature is critical for their survival. Light is one of the most important dynamic environmental factors regulating photosynthesis, plant development, and the expression of genes. Studies of light-induced signal transduction have recently provided information about the components linking photoreceptors to gene expression. However, most of the available information is limited to phytochrome-mediated signal transduction, and our understanding of the signal transduction pathways for other light responses remains relatively poor.

For phytochrome responses, several signal transduction components have been identified by biochemical approaches (Millar et al. 1994). There is evidence that G proteins, Ca^{2+}, and calmodulin (CaM) are involved in phytochrome signaling cascades (Lam et al. 1989; Romero et al. 1991a,b; Neuhaus et al. 1993). Genetic screens have been used to identify several genes involved in light signal transduction (Quail 1995). While the specific biochemical functions of most of the proteins encoded by these genes are still unclear, the interaction of the proteins and their contribution to the phytochrome-mediated signaling have been studied in detail (Wei and Deng 1996).

For blue-light signaling in plants, the primary analytical tool has been genetics, particularly using *Arabidopsis thaliana* as a model system. Genetic screens for mutants with hypocotyls that fail to arrest their elongation in response to blue light have uncovered several mutants with altered blue-light responses. For example, the *hy4* gene has been shown to encode a blue-light receptor, CRY1 (Ahmad and Cashmore 1993). CRY1 is a soluble protein that is expressed at similar levels in dark- and light-grown *A. thaliana* seedlings (Lin et al. 1996). Mutants that are impaired in CRY1 have decreased sensitivity to blue light (Ahmad and Cashmore 1993), whereas transgenic plants overexpressing CRY1 have increased photosensitivity (Lin et al. 1996).

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Abbreviations: CaM = calmodulin; CsA = cyclosporin A; GSAT = glutamate 1-semialdehyde aminotransferase; $InsP_3$ = D-inositol 1,4,5-trisphosphate; PLC = phospholipase C

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A gene for a CRY1 homolog, CRY2 (Ahmad et al. 1998), and a gene for a protein (NPH1) that contains a serine/threonine kinase domain and other domains that may bind flavins (Huala et al. 1997) have also been cloned from *A. thaliana*. The *A. thaliana hy5* gene has been shown to encode a bZIP protein that interacts with light-responsive promoters (G-box) in light-induced genes (Chattopadhyay et al. 1998).

Biochemical and physiological approaches have been used to identify some intermediates of the blue-light signal transduction pathway in plants. A 40-kDa protein that appears to be the α subunit of a heterotrimeric G protein was identified in pea cell plasma membranes (Warpeha et al. 1991). The protein reacts with G-protein α-subunit antibody and binds GTP in a blue-light-dependent fashion. Using pharmacological approaches, it was shown that UV-B and UV-A/blue light-induced expression of the gene for chalcone synthase requires Ca^{2+} and reversible protein phosphorylation, and UV-B-induced chalcone synthase expression requires CaM (Christie and Jenkins 1996). Analysis of blue-light signal transduction pathways in plants is complicated by the fact that the responses of many genes to blue light are modulated, to varying degrees, by red/far red light phytochrome photoreception, and there are few examples of plant genes that are regulated solely by blue light (Hennig et al. 1999).

The unicellular green alga *Chlamydomonas reinhardtii* has been used as a model system for the study of plant-type photosynthesis and chloroplast structure and function. This organism is well suited for the study of blue light signal transduction because it apparently lacks phytochrome and phytochrome-based photoreversible responses (Bonenberger et al. 1994) but still has blue light-based and protochlorophyllide reductase-based photoresponses. One well characterized blue-light effect in this organism is the blue light requirement for sexual differentiation (Weissig and Beck 1991). A gene for a blue-light photoreceptor candidate that is similar to *A. thaliana* CRY1 and CRY2 has been described in *C. reinhardtii* (Small et al. 1995). From results obtained with pharmacological approaches, it was recently proposed that blue-light-induced gametic differentiation requires the activity of a "protein kinase C-like" kinase, a phosphoprotein phosphatase, adenosine 3′,5′-cyclic-monophosphate, and a protein tyrosine kinase (Pan et al. 1996).

We reported previously that two *C. reinhardtii* nuclear genes encoding enzymes for early steps of chlorophyll and heme biosynthesis are induced by light (Matters and Beale 1994; 1995a,b). These genes are *Gsa*, which encodes glutamate 1-semialdehyde aminotransferase (GSAT), and *Alad*, which encodes δ-aminolevulinic acid dehydratase. In cells that are synchronized in a 12-h-light and 12-h-dark regime, both genes are maximally expressed at 2 h into the light phase. Of the two genes, *Gsa* exhibits the greater influence of light on its expression, which has an absolute dependence on light. Light appears to be perceived by a blue-light photoreceptor (Matters and Beale 1995b; Herman et al. 1999). Light induction of *Gsa* requires a nitrogen source and acetate in the incubation medium, but external Ca^{2+} can partially replace the requirement for acetate (Im et al. 1996). Both Ca^{2+} and CaM are involved in both external Ca^{2+}-supported and acetate-supported light induction of *Gsa*.

We have tried to identify some additional components of the signal transduction chain involved in light-induced *Gsa* expression in *C. reinhardtii* using biochemical and pharmacological approaches. In this report, we describe evidence for the involvement of a heterotrimeric G protein, phospholipase C (PLC), D-inositol 1,4,5-trisphosphate ($InsP_3$), and a Ca^{2+}/CaM-dependent protein kinase in the signaling cascade.

Materials and methods

Culture conditions. Chlamydomonas reinhardtii wild-type strain CC124 was obtained from the *Chlamydomonas* culture collection (Duke University, Durham, N.C., USA) and was routinely maintained on Tris-acetate-phosphate (TAP) medium (Harris 1989) at 25 °C under light cycles (12 h of light and 12 h of dark). The light (52 μmol m^{-2} s^{-1}) was supplied by cool-white fluorescent tubes. Cultures were harvested in the mid-exponential growth phase (1×10^6 to 2×10^6 cells per mL) at the end of a dark phase by centrifugation.

Incubations. Agents were added from concentrated stock solutions. Stock solutions of Mas-7 (1 mM) were made up in H_2O. Stock solutions of KN-93 (30 mM), U73122 (1 mM), and U73343 (1 mM) were made up in dimethyl sulfoxide. Stock solutions of cyclosporin A (CsA) (25 mM) were made up in ethanol. In all experiments, all cultures including controls contained equal concentrations of the solvents used to make up the stock solutions.

For incubations containing added Ca^{2+}, harvested cells were washed twice with a solution consisting of 10 mM Pipes (pH 7.0) and 3 mM EGTA, and distributed into EGTA-treated flasks which contained 10 mM Pipes (pH 7.0), 1 mM EGTA, 7 mM NH_4Cl, and an amount of $CaCl_2$ calculated to give the desired free Ca^{2+} concentration at pH 7.0 in the presence of 1 mM EGTA, based on the equations of Blinks et al. (1982).

Acid-induced deflagellation was initiated by adding concentrated acetic acid to a culture of cells in TAP culture medium. After incubation for 10 s at pH 4.2, the culture was adjusted to pH 7.0 with 5 N KOH.

Determination of $InsP_3$ concentration. The $InsP_3$ concentration was determined by a radioreceptor assay using the commercially available kit (Amersham Life Science Inc., Arlington Heights, Ill., USA). Samples were prepared according to a protocol adapted from that described by Quarmby et al. (1992). Culture samples containing approximately 2×10^6 cells per mL were mixed with 1/20 volume of ice-cold 100% (w/v) trichloroacetic acid. The samples were then centrifuged at 4 °C for 10 min at 650*g*. Trichloroacetic acid was removed from the supernatant by extracting four times with five volumes of H_2O-saturated diethyl ether. The pH of the final aqueous phase was adjusted to 7.5 with 16% (w/v) sodium carbonate. The extracts were concentrated by vacuum centrifugation and analyzed for $InsP_3$ according to the protocol provided with the kit.

Isolation of RNA and gel blot analysis. Total RNA was isolated from 1×10^9 to 1×10^{10} cells that were resuspended in extraction buffer [50 mM Tris HCl (pH 8.0) 300 mM NaCl, 5 mM EDTA], treated with 40 μg per mL of proteinase K (Fisher Chemical Co., Pittsburgh, Pa., USA) for 20 min, and extracted with phenol-chloroform until the interface was clear. The aqueous layer was precipitated with ethanol, the pellet was resuspended in H_2O, and the RNA was precipitated overnight at 4 °C with an equal volume

of 4 M LiCl. The LiCl pellet was washed with ethanol, dried, and dissolved in H_2O.

The probe for GSAT mRNA was the full-length *Gsa* cDNA (Matters and Beale 1994). To quantitate relative RNA loading, blots were also probed with a cDNA for Cblp, a G-protein β-subunit-like polypeptide from *C. reinhardtii* (obtained from K.L. Kindle, Cornell University, Ithaca, N.Y., USA) that is known to be constitutively expressed in cells synchronized to light-and-dark regimes (Schloss 1990).

For gel blots, 20 μg of RNA was denatured with glyoxal, separated on a 1% (w/v) agarose gel, and blotted onto a nylon membrane (Nytran$^+$, Schleicher & Schuell). Blots were UV cross-linked and hybridized with nick-translated probes in a solution containing 50% (w/v) formamide, 5× SSPE (1× SSPE is 0.15 M NaCl, 10 mM sodium phosphate [pH 7.4], 1 mM EDTA), 2× Denhardt's reagent (1× Denhardt's reagent is 0.02% [w/v] Ficoll [Type 400, Pharmacia], 0.02% [w/v] polyvinylpyrrolidone, 0.02% [w/v] bovine serum albumin [Fraction V, Sigma]), and 50 μg per mL of sonicated salmon testes DNA at 45 °C. Final washes were done at 55 °C in 0.2× SSPE containing 0.1% (w/v) sodium dodecylsulfate. Autoradiographs were taken with Kodak X-OMAT AR film. For quantification, RNA gel blots were scanned with a Fujix BAS-1000 MacBAS bioimaging analyzer (Fuji Photo Film Co., Tokyo, Japan).

Results

The heterotrimeric G-protein activator Mas-7 induces Gsa *expression in the dark*. Because heterotrimeric G proteins are key upstream components of signal transduction pathways in many systems, we examined whether activation of G proteins in the dark induces expression of *Gsa*. Heterotrimeric G proteins are activated by mastoparan, which is a cationic amphipathic tetradecapeptide isolated from wasp venom. Mas-7 is a more potent analog of mastoparan. Incubation of cells for 2 h in the dark with Mas-7 induced partial expression of *Gsa* in a concentration-dependent manner (Fig. 1). In the light, Mas-7 partially inhibited *Gsa* expression. Possible explanations for the divergent effects of Mas-7 on *Gsa* expression in the light and dark are presented in the *Discussion*.

Chlamydomonas reinhardtii cells rapidly shed their flagella upon exposure to low pH. It has been shown that the activation of G proteins by mastoparan mimics the effect of acid shock in inducing deflagellation (Quarmby et al. 1992). Results with Mas-7 confirm this result. Exposure to 1 μM Mas-7, a concentration that induced *Gsa* expression in the dark, caused the cells to rapidly shed their flagella (data not shown). Approximately 20% of the cells partially regenerated their flagella after 30 min, and about 60% of the cells regenerated their flagella after 2 h, even in the continued presence of 1 μM Mas-7, although most of the cells did not resume active swimming within this time period. Because of the link between G proteins and acid-shock-induced deflagellation, we examined whether acid shock itself affects *Gsa* expression. Cells responded to acid shock by rapidly shedding their flagella. In contrast to the slow and incomplete recovery after Mas-7-induced flagellar shedding, over 50% of the cells regenerated their flagella within 30 min after acid shock, and over 90% of the cells had regenerated their flagella and were actively swimming within 90 min. Also in contrast to the effects of Mas-7, acid shock that triggered deflagellation did not induce *Gsa* expression in the dark, and it did not affect light induction of *Gsa*. These results suggest that even though heterotrimeric G proteins appear to be involved in the signal transduction pathways for both *Gsa* induction and flagellar shedding, the mechanism is not identical and the two stimuli can be differentiated by the cells to evoke different responses.

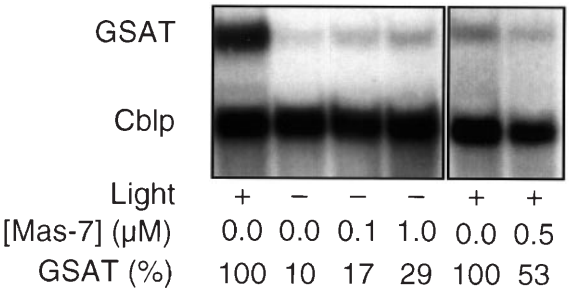

Fig. 1. Effects of Mas-7, a heterotrimeric G-protein activator, on *Gsa* induction. The indicated concentration of Mas-7 was added immediately before the onset of the light phase. All incubations (2 h) contained 10 mM Pipes (pH 7), 7 mM NH_3, 1 mM EGTA, 17.5 mM acetate, and sufficient Ca^{2+} to produce 10 μM free Ca^{2+}. Total RNA was extracted, electrophoresed on a 1% (w/v) agarose gel, blotted onto a nylon membrane, and hybridized with probes specific for GSAT mRNA and for constitutively expressed Cblp mRNA that served as a standard to control for potentially unequal gel loading. Data in the two boxes are derived from different experiments and the relative band densities cannot be directly compared because of differences in the specific radioactivities of the probes used in the two experiments. Below each lane are indications of the experimental treatments and the level of GSAT mRNA, which is expressed as a percentage of the level in the control sample, after normalization for the levels of Cblp mRNA in the samples

The PLC antagonist U73122 inhibits light-induced Gsa *expression*. Phospholipase-C-catalyzed hydrolysis of phosphatidyl-D-inositol 4,5-bisphosphate to $InsP_3$ and diacylglycerol is ubiquitous in signaling cascades in animal and plant systems (Munnik et al. 1998). The aminosteroid U73122 is a potent PLC antagonist that has been used to investigate the involvement of PLC in many signaling pathways (Pingret et al. 1998). U73343, a less active analog of U73122, has been used as a negative control for the nonspecific effects of U73122 on PLC. U73122 inhibited induction of *Gsa* expression by light at sub-micromolar concentrations, whereas the less active analog, U73343, inhibited *Gsa* expression only at concentrations greater than 5 μM (Fig. 2).

Light induces an increase in the cellular $InsP_3$ concentration. To further examine a possible role for PLC-dependent polyphosphoinositide hydrolysis as a signal transduction step in light induction of *Gsa*, we measured the effects of light on the cellular $InsP_3$ concentration. The $InsP_3$ concentration increased 3-fold at 3 s of light exposure and 7-fold at 12 s of light exposure (Fig. 3). The $InsP_3$ concentration then decreased to about the level at the 3-s point after 15 s, and this concentration was maintained for at least 30 min. The brief peak at 12 s was not seen in all experimental replicates, although it

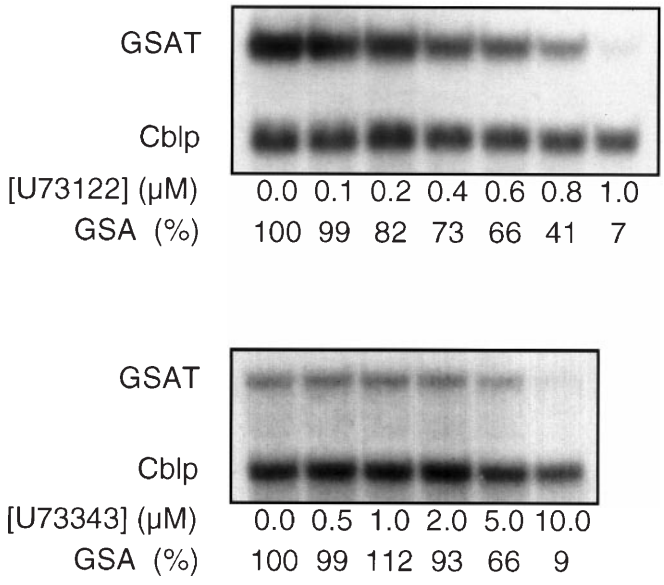

Fig. 2. Effects of the PLC inhibitors U73122 and U73343 on *Gsa* induction. The indicated concentration of U73122 (*top*) or U73343 (*bottom*) was added immediately before the onset of the light phase. All incubations (2 h) contained 10 mM Pipes (pH 7), 17.5 mM acetate, 7 mM NH_3, and 1 mM EGTA. RNA gel blots were produced, and the quantitative data are indicated, as described in the legend to Fig. 1

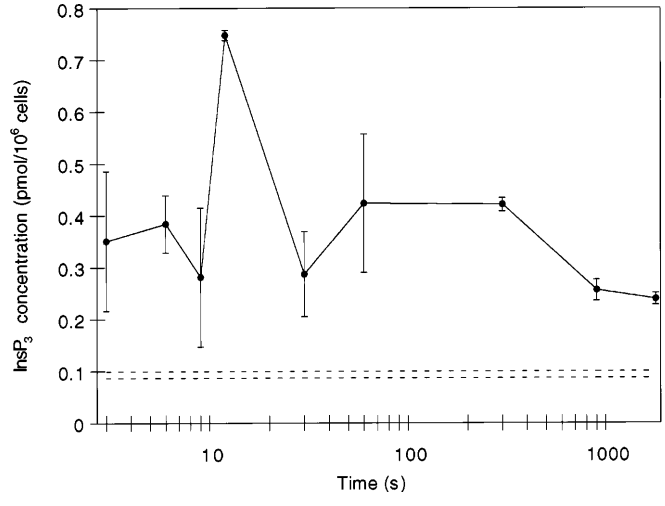

Fig. 3. Effect of light on cellular $InsP_3$ concentration. The amount of $InsP_3$ was quantified by a radio-receptor assay as described in *Materials and Methods*. Time from the beginning of light exposure is plotted on a logarithmic scale. *Solid circles* and error bars indicate the average and range of duplicate samples at the indicated times after the onset of exposure to light, respectively. The *dotted lines* indicate the $InsP_3$ concentration range in cells kept in the dark

may have occurred but was missed because of slight differences in the sampling times in the different experiments. However, the sustained 3-fold rise was seen in all experiments.

The Ca^{2+}/CaM-dependent protein kinase inhibitor KN-93 blocks Gsa *expression, but the Ca^{2+}/CaM-dependent phosphoprotein phosphatase inhibitor CsA does not.* Cal-

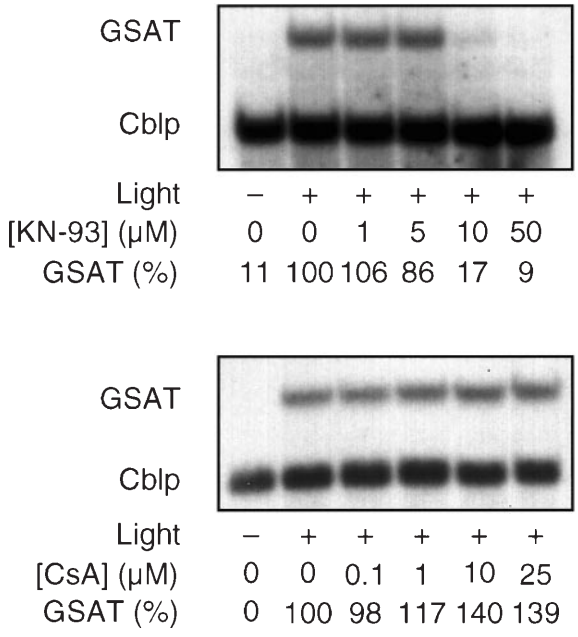

Fig. 4. Effects of KN-93 (*top*) and CsA (*bottom*) on *Gsa* induction. The indicated concentration of KN-93 or CsA was added immediately before the onset of the light phase. All incubations (2 h) contained 10 mM Pipes (pH 7), 7 mM NH_3, 17.5 mM acetate, and 1 mM EGTA. RNA gel blots were produced, and the quantitative data are indicated, as described in the legend to Fig. 1

modulin can potentially modulate the activity of several different kinds of CaM-binding proteins, including Ca^{2+}/CaM-dependent protein kinases such as CaM kinase II and phosphoprotein phosphatases such as calcineurin. It was previously reported that Ca^{2+}/CaM-dependent protein kinases are involved in a light-dependent responses in plant cells (Lu et al. 1996). We examined whether the signal for *Gsa* induction is transmitted from CaM via a Ca^{2+}/CaM-dependent protein kinase or a phosphoprotein phosphatase, using two specific inhibitors, KN-93 and CsA. Whereas KN-93 interferes with CaM binding to the CaM kinase II catalytic subunit (Sumi et al. 1991), CsA forms a complex with the Ca^{2+}/CaM-dependent protein kinase cyclophilin, and the complex binds to calcineurin to inhibit its activity (Cameron et al. 1995). Light-induced *Gsa* expression was completely abolished by 10 μM KN-93 (Fig. 4). In contrast, CsA did not affect *Gsa* expression at concentrations up to 25 μM. These results suggest that Ca^{2+}-activated CaM transmits the light signal for *Gsa* induction through a Ca^{2+}/CaM-activated protein kinase rather than through a Ca^{2+}/CaM-dependent phosphoprotein phosphatase such as calcineurin.

Discussion

Chlamydomonas reinhardtii has provided a useful experimental model for studying light-regulated gene expression by systems that do not involve phytochrome. Previously, we showed that two genes, *Gsa* and *Alad*,

which encode enzymes that catalyze early steps in chlorophyll biosynthesis, are induced by light in light-dark synchronized cells (Matters and Beale 1994; 1995a). These genes appear to respond specifically to light in the blue and green spectral regions, and not to red light (Matters and Beale 1995b). More recently, we have focused on one of these genes, *Gsa*, and showed that Ca^{2+} and CaM are involved in light-induced *Gsa* expression (Im et al. 1996). Other work has implicated a flavin-based photoreceptor for this light effect (Herman et al. 1999). We have continued to investigate the signal transduction pathway for light induction of *Gsa* expression using biochemical and pharmacological approaches. *C. reinhardtii* cells are very amenable to this approach and they have previously been shown to respond to a wide variety of pharmacological agents (Quarmby et al. 1992; Pan et al. 1996).

The ability of the heterotrimeric G-protein agonist Mas-7 to partially induce *Gsa* expression in the dark suggests that a heterotrimeric G protein is an early effector in the pathway. Mas-7 and its natural analog mastoparan are known to form amphipathic cationic α-helices which mimic stimulated membrane receptors and activate G proteins. Mastoparan has been used to show the involvement of G proteins in the signaling for rapid deflagellation induced by acid shock in *C. reinhardtii* (Quarmby et al. 1992). In that experiment, 30 s incubation with 5 μM mastoparan was sufficient to induce deflagellation without acid shock and to induce a rapid increase in the cellular $InsP_3$ concentration. Mastoparan has also been reported to increase PLC activity in carrot cell plasma membranes (Cho et al. 1995). In addition, G-protein agonists were capable of mimicking Nod factor activity by triggering *MtENOD12* transcription in differentiating root epidermis (Pingret et al. 1998). Because we observed only partial induction of *Gsa* with Mas-7, it is possible that the activation of the G protein by Mas-7 is not as high as by light or that other signaling molecule(s) may be involved that act synergistically in the early events of the signal transduction. However, another possible explanation for the relatively weak effect of Mas-7 on dark induction of *Gsa* that also provides an explanation for the inhibitory effect of Mas-7 on light induction of *Gsa*, is that G-protein agonists, which cause rapid flagellar shedding, may interfere with light signal transduction by removing signaling components that are located in the flagella. It was recently reported that photoreceptor-candidate flavin-binding proteins are concentrated in the flagella of *C. reinhardtii* (A. Dederichs, R. Hertel, and H. van den Ende, Eighth International Conference on the Cell and Molecular Biology of *Chlamydomonas*, June 2–7, 1998, Tahoe City, Calif., USA). If a G protein is a proximal downstream effector of the photoreceptor for *Gsa* induction, it is likely to be localized near the photoreceptor, perhaps in the flagella. It is possible, therefore, that after the initial activation by Mas-7, the signal transduction pathway for *Gsa* induction becomes interrupted by removal of effectors, including a G protein, as a result of flagellar shedding. The delayed and partially inhibited flagellar regeneration in the presence of Mas-7 may therefore interfere with the re-establishment of the light signal transduction pathway, which could account for both the relatively low level of dark induction and the partial inhibition of light induction of *Gsa* by Mas-7.

D-Inositol 1,4,5-trisphosphate is a ubiquitous second messenger that is generated from membrane phosphatidyl-D-inositol 4,5-bisphosphate by the action of PLC. The importance of phospholipid signaling in plants has recently been reviewed (Munnik et al. 1998). One effect of $InsP_3$ is to bind to receptors, triggering the opening of Ca^{2+} channels (Muir and Sanders 1997). It is known that $InsP_3$ causes the release of Ca^{2+} from internal stores across nonvacuolar membranes in plants (Muir and Sanders 1997) as well as *C. reinhardtii* (Kuin et al. 2000). Formation of $InsP_3$ was reported to be involved in the blue-light-induced leaflet movements of *Samanea saman*, where the induction of $InsP_3$ formation by blue light has been demonstrated (Kim et al. 1996). The cell-permeable aminosteroid U73122 has been reported to specifically inhibit PLC in both animal and plant systems (Pingret et al. 1998). The inhibition of *Gsa* expression by low concentrations of U73122, but only by much higher concentrations of the less active analog U73343, implicates PLC in transducing the signal for light induction of *Gsa*. The light-induced sustained 3-fold increase in intracellular $InsP_3$ concentration that we observed correlates with the need for a sustained light signal for *Gsa* induction that we previously reported (Matters and Beale 1995b), and provides additional support for the involvement of a PLC-catalyzed elevation of $InsP_3$ level as a link connecting upstream signaling events to downstream Ca^{2+} signaling.

Because the involvement of protein kinases and phosphatases in light signaling has been demonstrated in several plant and algal systems (Sheen 1993; Linden and Kreimer 1995), the involvement of a Ca^{2+}/CaM-dependent protein kinase and/or phosphoprotein phosphatase as downstream signal transduction components for light induction of *Gsa* was examined. The inhibitory effect of the CaM kinase II inhibitor KN-93 on light induction of *Gsa* in *C. reinhardtii*, and the absence of an effect of the calcineurin inhibitor CsA, suggests that a Ca^{2+}/CaM-dependent protein kinase such as CaM kinase II, rather than a Ca^{2+}/CaM-dependent phosphoprotein phosphatase such as calcineurin, transmits the signal from Ca^{2+}/CaM. It should be noted that although we were unable to find previous reports on the effects of CsA in *C. reinhardtii*, a cyclophilin gene has been cloned and characterized from *C. reinhardtii* (Somanchi et al. 1998), and CsA has been reported to elicit effects in intact plants (Deng et al. 1998) at concentrations below the maximum used in our experiment.

The conclusions we derive from experimental results reported here and in previous communications (Matters and Beale 1994, 1995b; Im et al. 1996; Herman et al. 1999) are summarized below. It should be emphasized that these results do not allow us to define the relative position of some signaling components, which are placed in the model on the basis of the consensus of conclusions derived from studies of other signaling

pathways. We propose that: (1) a heterotrimeric G protein is activated by a blue light photoreceptor that may contain a flavin chromophore; (2) the G-protein activation causes a stimulation of PLC activity; (3) the action of PLC causes an elevation in the cytoplasmic $InsP_3$ concentration; (4) the increased $InsP_3$ concentration causes an increase in the cytoplasmic Ca^{2+} concentration, by triggering the release of Ca^{2+} from intracellular stores and/or by increasing its influx from the medium; (5) Ca^{2+} combines with CaM; (6) the Ca^{2+}/CaM complex transmits the signal for *Gsa* induction through a Ca^{2+}/CaM-dependent protein kinase; (7) in contrast to other signaling pathways that are initiated by a brief signal (e.g. acid shock) that causes a spike in the concentrations of signal transduction components such as $InsP_3$ and Ca^{2+}, light induction of *Gsa* requires a sustained light signal that causes a sustained increase in the level of $InsP_3$ and Ca^{2+}. This model provides a useful basis to guide future biochemical and genetic studies of signal transduction pathways for blue light-regulated gene expression.

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