Plant Physiol. (1993) 102: 333–337

Update on Blue-Light Signal Transduction

Transduction of Blue-Light Signals[1]

Lon S. Kaufman*

Department of Biological Sciences, Laboratory for Molecular Biology, University of Illinois at Chicago, Chicago, Illinois 60680

BUT IS IT AN ELEPHANT?

In the old tale, several blind men are taken to an elephant. In turn, each is asked to examine and identify the mystery object. One blind man examines a leg and claims it to be a tree, another, examining the trunk, claims it to be a snake, and yet another, feeling a tusk, claims it to be a smooth rock. The pattern continues: each describes a component, none identifies the gestalt.

There are a wealth of blue-light responses in higher plants. It is clear that many are biochemically, physiologically, or genetically independent, and others are linked. The mechanism by which a photon of blue light is converted into a biochemical signal and transduced into a biological response is under investigation for several of these responses. In many cases, investigators have successfully defined one or more components of the signaling mechanism.

Taken together, the identified components have the potential to define an entire signal transduction mechanism; receptor, G-protein, diacylglycerol, inositol triphosphate, calcium, calmodulin, several kinases, ion channels, and so forth. However, direct demonstration of all the components has not been achieved in any one system. Indeed, in some cases specific carriers are known to be absent. Further, the locations of the respective carriers differ in accordance with the location of the specific response (Fig. 1). Perhaps the mechanisms responsible for transducing the blue-light signals are best represented by a herd of elephants.

Signal transduction paradigms are well established. However, recent data suggest that plants may have several novel signal carriers (Deng et al., 1992), and it is not certain that the paradigms will be transferable to plants. The majority of studies of signal carriers for blue-light responses in higher plants make use of standard biochemical tools: direct identification based on biochemical properties or antibody cross-reactivity, or indirect identification based on inhibitor/activator studies. These biochemical activities are correlated with the respective blue-light response by the photobiological activity (fluence response and/or time course), physical location, or involvement with the response as demonstrated by activator/inhibitor studies. The biochemical approach has recently been joined by a vigorous genetic approach.

There are four blue-light responses in higher plants currently receiving concentrated attention. Two occur in the leaf: blue-light-induced increase in stomatal aperture and blue-light-induced changes in gene expression; and two occur in the stem: blue-light-induced suppression of stem elongation and blue-light-induced curvature of the stem. These effects of blue light are observed in seedlings grown in continuous red light, in fully etiolated seedlings wherein far-red-light treatments follow the blue-light treatment, and/or in phytochrome-deficient mutants. Together, these suggest that the responses are due to excitation of a blue-light receptor and are not the result of phytochrome excitation. In each system, one or several signal carriers have been demonstrated.

STOMATAL APERTURE

Stomatal aperture increases in response to a single pulse of blue light. Upon irradiation, the plasma membrane becomes hyperpolarized, resulting in the opening of voltage-gated potassium channels. The inward flow of potassium initiates a series of metabolic events, guard cell swelling, and stomatal opening.

The blue-light-induced increase in stomatal aperture is inhibited by compounds such as phenylacetate and potassium iodide that, by quenching flavin-excited states (Hemmerich, 1967; Song et al., 1972), prevent energy transfer from excited flavins to nearby proteins, suggesting a flavin-based receptor but leaving open the possibility of a flavoprotein elsewhere within the signal transduction or response mechanisms (Vani and Raghavendra, 1989). Zeiger and co-workers have presented data suggesting that the carotenoid zeaxanthin might function as the receptor (Quinones et al., 1993).

There is not agreement on the source of the outward current leading to plasma membrane hyperpolarization. Immediately upon irradiation and continuing for several minutes thereafter, protons are extruded from guard-cell protoplasts (Shimazaki et al., 1986). This process does not require ATP. Approximately 30 s after irradiation, an ATP-dependent, transient, outward current is observed in guard-cell protoplasts (Assmann et al., 1985). The mechanism of proton extrusion and its relationship to the ATP-dependent outward current remain unclear. The delay between the two processes and the discordant ATP requirements would argue against a H^+-ATPase and have led to the proposal of a redox system (Raghavendra, 1990).

The amount of energy contained within the pulse of blue light is insufficient to result in the amount of proton extrusion or the observed current, indicating a signal transduction

[1] Supported in part by the National Science Foundation and the United States Department of Agriculture.

* Fax 1–312–413–2691.

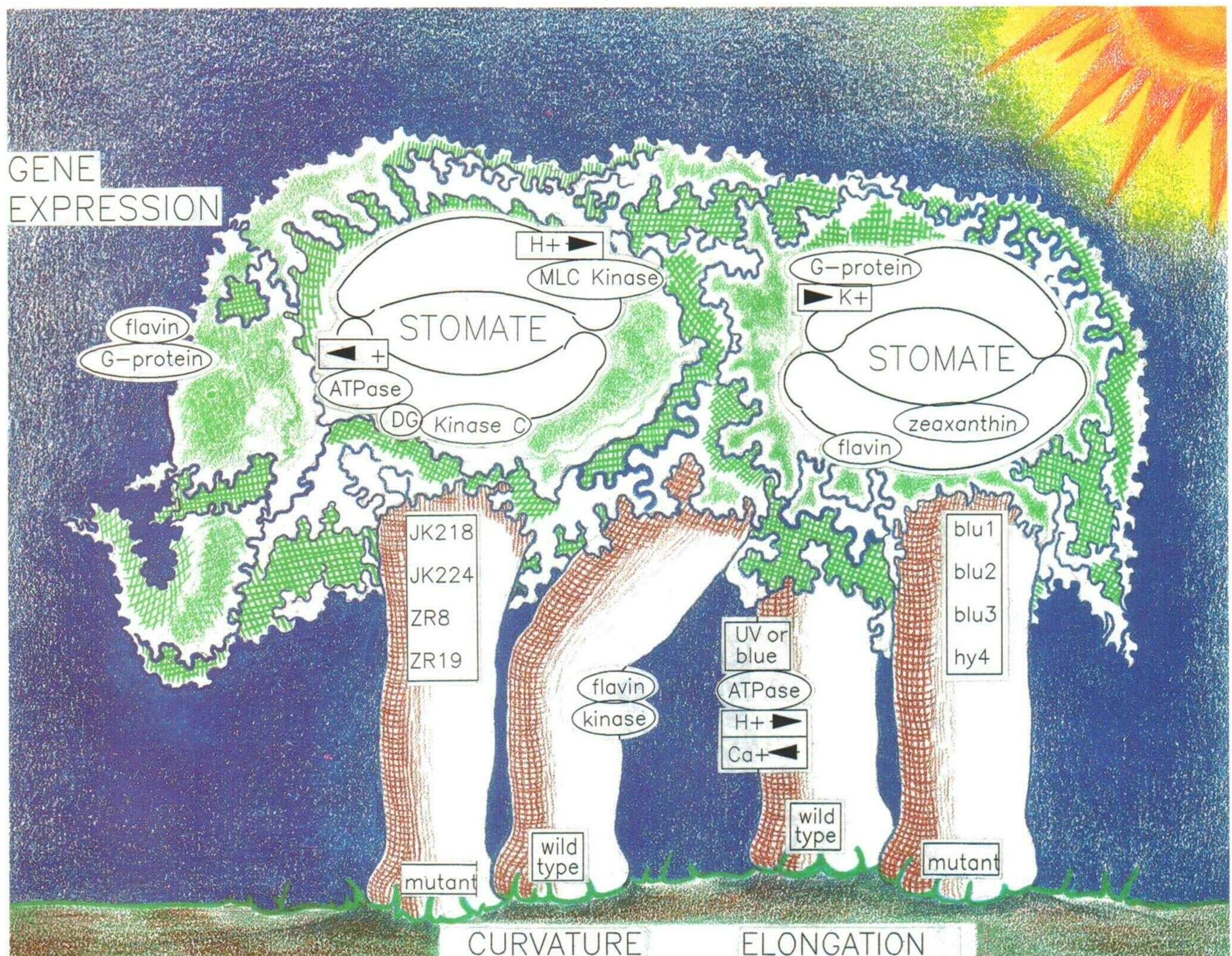

Figure 1. Representation of four blue-light regulated processes in higher plants: gene expression, stomatal aperture, stem curvature, and stem elongation. The representation depicts the mutants affecting stem curvature and elongation as well as the signal carriers proposed for each blue-light-regulated process.

mechanism. Both the proton extrusion and ATP-dependent current suggest a membrane-associated receptor and/or signal transduction mechanism.

Shimazaki and co-workers (Shimazaki et al., 1992), measuring the effects of inhibitors and antagonists on proton extrusion from guard-cell protoplasts of *Vicia faba*, suggest a role for calmodulin and a member of the calmodulin-dependent myosin light chain kinase group. They report a small effect from inhibitors of protein kinase C and no effect of inhibitors of cyclic nucleotide-dependent protein kinase or calmodulin-dependent protein kinase II.

On the basis of inhibitor/activator studies, Assmann and co-workers suggest that G-protein activation decreases the inward flow of potassium in guard-cell protoplasts from *V. faba* (Farley-Grenot and Assmann, 1991). The same group, using inhibitors, activators, and analogs, and measuring stomatal opening in *Commelina communis* and the ATP-dependent outward current in guard-cell protoplasts of *V. faba*, suggest roles for diacylglycerol and lipid-activated protein kinase C (Lee and Assmann, 1991). No role was identified for the cyclic nucleotide-dependent protein kinases.

GENE EXPRESSION

Blue light will affect the expression of several nuclear- and chloroplast-coded genes (e.g. Marrs and Kaufman, 1989, 1991; Warpeha and Kaufman, 1990; Wehmeyer et al., 1992). The response is certain to involve one or several transcription factors. The signaling mechanism responsible for the activation of these factors is unknown.

The *Cab* genes of pea respond immediately to a single pulse of blue light. The response occurs in the absence of cytoplasmic protein synthesis, indicating that the carriers are all present prior to the blue-light treatment (Marrs and Kaufman, 1989, 1991). Warpeha et al. (1991) have identified a blue-light-activated heterotrimeric GTP-binding regulatory pro-

tein associated with the plasma membrane of pea apical buds. The threshold fluence for blue-light excitation of the G-protein resembles that for blue-light-induced transcription of the *Cab* gene family in pea.

Plasma membranes derived from the apical buds of peas exhibit GTPase activity and GTP-γ-S binding when irradiated with blue light but not when irradiated with red light. The α-subunit was identified as a 40-kD polypeptide by several means, including cross-reactivity with polyclonal antibodies directed against transducin, blue-light-specific binding of a photoaffinity-labeling GTP analog, blue-light-specific ADP-ribosylation by cholera toxin, and blue-light-specific inhibition of ADP-ribosylation by pertussis toxin.

The receptor driving the G-protein activity is likely to be a flavoprotein (Warpeha et al., 1992). Compounds such as phenylacetate and potassium iodide, inhibiting transfer of excitation energy from flavins to nearby proteins, inhibit the ability of blue light to activate the G-protein. If the chromophore is a flavin, the concentration of potassium iodide necessary to inhibit the G-protein activation indicates that energy transfer would proceed through a triplet intermediate.

The link between this receptor and G-protein and the expression of specific *Cab* genes is correlative; the location and fluence-response characteristics are coincident. Although possible, there are no data to suggest the same G-protein functions in the guard cell.

STEM ELONGATION

The rate of stem elongation in dark- or red-light-grown seedlings slows in response to blue light. The response initiates within seconds of blue-light exposure, and probably results from a change in the cell wall's ability to relax and expand (Cosgrove, 1988). The slowed rate of growth will continue indefinitely if the light treatment is maintained. It is likely that the long-term response involves altered hormone flow within the stem. The relationship between the initial and long-term responses is unclear. This ignorance extends to the signal transduction mechanisms as well.

The most immediate effect of blue-light irradiation is a transient hyperpolarization of the plasma membrane of cucumber hypocotyl cells (Spalding and Cosgrove, 1992). The hyperpolarization, as large as 100 mV, precedes the cessation of stem elongation and is rectified within 2 to 3 min. The inhibitory effects of vanadate and KCN strongly suggest that depolarization is due to a plasma membrane H^+-ATPase. Repolarization appears to involve a calcium channel. No role was identified for potassium.

Membrane depolarization correlates with the suppression event both temporally and with respect to the threshold of the response. The relationship between the depolarization events in the cucumber hypocotyl and those in stomates is unknown. The magnitude of the depolarization in the hypocotyl is approximately twice that in stomates. As discussed above, the source of the proton extrusion in the stomate, ATPase, or redox system remains unclear.

Blue-light-induced suppression of hypocotyl elongation is eliminated in four mutants of *Arabidopsis thaliana*: *blu*1, *blu*2, *blu*3 (Liscum and Hangarter, 1991), and *hy*4 (Koorneef et al., 1980). The mutation in *blu*1 further separates the UV-A response from the blue-light response, leaving the potential for independent UV-A- and blue-light receptors and/or signal transduction mechanisms (Young et al., 1992).

It remains unclear if the signal transduction mechanism responsible for the immediate response is distinct from that responsible for the long-term response. Neither the initial kinetics of suppression nor the rapid depolarization have been reported for the *Arabidopsis* mutants.

STEM CURVATURE

The ultimate responsibility for phototropic curvature is certain to be altered hormone flow and, as a consequence, an altered growth pattern. The signal transduction mechanism responsible for phototropic curvature is probably distinct from the mechanism affecting suppression of stem elongation because the threshold fluences differ. Furthermore, a set of *Arabidopsis* mutants lacking, or altered in, phototropic curvature are distinct from the *blu* and *hy* mutants already described (Khurana and Poff, 1990). The individual mutants display only one of the phenotypes, whereas the double mutants display both phenotypes (Liscum et al., 1992).

The Briggs group, using plasma membranes purified from cells within the growing region of the stem, has identified a protein of approximately 120 kD (the actual molecular mass varies among different plant species) that undergoes a blue-light-induced phosphorylation (Reymond et al., 1992a; Short et al., 1992). The temporal, fluence, and spatial characteristics of the phosphorylation response correlate well with curvature (Short and Briggs, 1990). In addition, the *Arabidopsis* mutant JK224, which has a threshold fluence for curvature approximately 100-fold greater than wild-type *Arabidopsis*, is deficient in the quantity of this protein (Reymond et al., 1992b).

Triton- or CHAPS-solubilized preparations retain the blue-light-induced phosphorylation, suggesting a tight relationship among the receptor, kinase activity, and substrate (Short et al., 1993). Further, the 120-kD protein appears to contain a nucleotide binding site, suggesting that the 120-kD protein is autophosphorylated. It is possible that the 120-kD protein also functions as the receptor. Inhibitors of flavin excitation energy transfer prevent phosphorylation of the 120-kD protein, suggesting that the receptor or one of the signal carriers is a flavoprotein (Short et al., 1992).

The concentration of the flavin inhibitor potassium iodide necessary to prevent the phosphorylation is approximately 100-fold greater than that necessary to eliminate blue-light-driven G-protein activity in the plasma membranes of etiolated pea buds (see above), confirming that the two receptors, although both possibly containing flavins, are not identical. Further, the G-protein identified in the apical buds of peas is present in low concentration in the growing region of the pea stem (Warpeha et al., 1992) and the 120-kD protein was not identified in the apical bud of pea (Short and Briggs, 1990). Thus, these two chains are not only located in different regions of the stem but seem biochemically distinct.

HOW MANY BLUE ELEPHANTS ARE THERE?

The nature and number of blue-light signaling mechanisms has been discussed for decades and remains unclear. The

ignorance is exemplified by the frustration in attempting to define the receptor for any blue-light response. Action spectra indicate UV-A- and blue-light absorption. Inhibitor data suggest a flavin as the chromophore for several of the responses. This would account for the UV-A- and blue-light responses. Yet, the genetic separation of the UV-A and blue portions for suppression of stem elongation might argue for two chromophores, one responding to blue light and a separate chromophore responding to UV-A light. Galland and Senger (1988) have suggested that pterin might function in the UV-A response. Zeiger and co-workers (Quinones et al., 1993) have pointed to the possibility of the carotenoid zeaxanthin as a possible chromophore. The identification of heterotrimeric G-proteins suggests a seven transmembrane-segment receptor in the plasma membrane. However, it is not clear that all of the responses require G-protein activation.

The direct identification of a G-protein and the indirect evidence suggesting other recognized signal carriers such as calmodulin, calmodulin-activated myosin light chain kinase-like kinases, diacylglycerol, and protein kinase C suggest that the signal transduction paradigms established in other systems will be at least partially transferable to blue-light responses. The occurrence of common elements among the various responses, for example the hyperpolarization of the plasma membrane 30 s following blue-light irradiation of hypocotyl, wherein cessation of cell elongation occurs, and of guard cells, wherein swelling occurs, presents the possibility that some of the signal carriers are used in various blue-light responses or in different blue-light signal chains.

How many blue elephants are there? None yet.

ACKNOWLEDGMENTS

The author would like to thank those investigators who made works in press available prior to their publication and J. Gao, K. Folta, J. Marsh, J. Tilghman, and L. Wang for critical reading of the manuscript.

Received January 26, 1993; accepted March 7, 1993.
Copyright Clearance Center: 0032-0889/93/102/0333/05.

LITERATURE CITED

Assmann SN, Simonici L, Schroeder JI (1985) Blue light activates electrogenic ion pumping in guard cell protoplasts of *Vicia faba*. Nature **318:** 285–287

Cosgrove DJ (1988) Mechanisms of rapid suppression of cell expansion in cucumber hypocotyls after blue-light irradiation. Planta **176:** 109–116

Deng X-W, Matsuim M, Wei N, Wagner D, Chu AM, Feldmann KA, Quail PH (1992) COP1, an *Arabidopsis* photomorphogenic regulatory gene, encodes a novel protein with both a Zn-binding motif and a domain homologous to the B-subunit of trimeric G-proteins. Cell **71:** 791–801

Farley-Grenot K, Assmann AM (1991) Evidence for G-protein regulation of inward K^+ channel current in guard cells of fava bean. Plant Cell **3:** 1037–1044

Galland P, Senger H (1988) The role of pterins in the photoreception and metabolism in higher plants. Photochem Photobiol **48:** 811–820

Hemmerich P, Massey V, Weber G (1967) Photoinduced benzyl substitution of flavins by phenylacetate: a possible model for flavoprotein catalysis. Nature **213:** 728–730

Khurana JP, Poff KL (1990) Mutants of *Arabidopsis thaliana* with altered phototropism. Planta **178:** 400–406

Koornneef M, Rolff E, Spruit CJP (1980) Genetic control of light induced hypocotyl elongation in *Arabidopsis thaliana* (L.) Heynh. Z Pflanzenphysiol **100:** 147–160

Lee Y, Assmann SM (1991) Diacylglycerols induce both ion pumping in patch-clamped guard cell protoplasts and opening of intact stomata. Proc Natl Acad Sci USA **88:** 2127–2131

Liscum E, Hangarter RP (1991) *Arabidopsis* mutants lacking blue light-dependent inhibition of hypocotyl elongation. Plant Cell **3:** 685–694

Liscum E, Joung JC, Poff KL, Hangarter RP (1992) Genetic separation of phototropism and blue light inhibition of stem elongation. Plant Physiol **100:** 267–271

Marrs KA, Kaufman LS (1989) Blue light regulation of transcription for several nuclear genes in pea. Proc Natl Acad Sci USA **86:** 4492–4495

Marrs KA, Kaufman LS (1991) Rapid transcriptional regulation of the Cab and pEA207 gene families by blue light in the absence of cytoplasmic protein synthesis. Planta **183:** 327–333

Quinones MA, Lu Z, Zeiger E (1993) Zeaxanthin concentrations cosegregate with the magnitude of the blue light response of adaxial guard cells and leaf stomatal conductances in an F2 population of Pima cotton. (abstract No. 74). Plant Physiol **102:** S-15

Raghavendra AS (1990) Blue light effects on stomata are mediated by the guard cell plasma membrane redox system distinct from the proton translocating ATPase. Plant Cell Environ **13:** 105–110

Reymond P, Short TW, Briggs WR (1992a) Blue light activates a specific protein kinase in higher plants. Plant Physiol **100:** 655–661

Reymond P, Short TW, Briggs WR, Poff KL (1992b) Light induced phosphorylation of a membrane protein plays an early role in signal transduction for phototropism in *Arabidopsis thaliana*. Proc Natl Acad Sci USA **89:** 4718–4721

Shimazaki K, Iino M, Zeiger E (1986) Blue light dependent proton extrusion by guard cell protoplasts of *Vicia faba*. Nature **319:** 324–326

Shimazaki K, Kinoshita T, Nishimura M (1992) Involvement of calmodulin and calmodulin dependent myosin light chain kinase in blue light dependent H^+ pumping by guard cell protoplasts from *Vicia faba*. Plant Physiol **99:** 1416–1421

Short TW, Briggs WR (1990) Characterization of a rapid, blue light-mediated change in detectable phosphorylation of a pea plasma membrane protein from etiolated pea (*Pisum sativum*). Plant Physiol **92:** 179–185

Short TW, Ports M, Briggs WR (1992) A photoreceptor system regulating in vivo and in vitro phosphorylation of a pea plasma membrane protein. Photochem Photobiol **55:** 773–781

Short TW, Reymond P, Briggs WR (1993) A pea plasma membrane protein exhibiting blue light-induced phosphorylation retains photosensitivity following triton solubilization. Plant Physiol **101:** 647–655

Song PS, Moore TA, Kurtin WE (1972) Molecular luminescence studies of flavins. II Interaction involving the excited states. Z Naturforsch **27b:** 1011–1015

Spalding EP, Cosgrove DJ (1992) Mechanisms of blue light induced plasma membrane depolarization in etiolated cucumber hypocotyls. Planta **188:** 199–205

Vani T, Raghavendra AS (1989) Tetrazolium reduction by guard cells in abaxial epidermis of *Vicia faba*: blue light stimulation of a plasmalemma redox system. Plant Physiol **90:** 59–62

Warpeha KMF, Hamm HE, Rasenick MM, Kaufman LS (1991) A blue light activated GTP binding protein in the plasma membrane of etiolated pea. Proc Natl Acad Sci USA **88:** 8925–8929

Warpeha KMF, Kaufman LS (1990) Two distinct blue light responses regulate the levels of transcripts in pea. Planta **182:** 553–558

Warpeha KMF, Kaufman LS, Briggs WR (1992) A flavoprotein may

mediate the blue light activated binding of guanosine 5′-triphosphate to isolated plasma membranes of *Pisum sativum* L. Photochem Photobiol **55:** 595–603

Wehmeyer B, Cashmore AR, Schafer E (1990) Photocontrol of the expression of genes encoding chlorophyll *a/b* binding proteins and small subunit of ribulose-1,5-bisphosphate carboxylase in etiolated seedlings of *Lycopersicon esculentum* (L.) and *Nicotiana tabacum* (L.). Plant Physiol **93:** 990–997

Young JC, Liscum EL, Hangarter RP (1992) Spectral dependence of light stimulated hypocotyl elongation in photomorphogenic mutants of *Arabidopsis*: evidence for a UV-A photosensor. Planta **188:** 106–114