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MINI-REVIEW

The Role of Plasma Membrane Redox Activity in Light Effects in Plants

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Abstract

Stimulations by light of electron transport at the plasma membrane make it possible that redox activity is involved in light-induced signal transduction chains. This is especially true in cases where component(s) of the chain are also located at the plasma membrane. Photosynthetic reactions stimulate trans-plasma membrane redox activity of mesophyll cells. Activity is measured as a reduction of the nonpermeating redox probe, ferricyanide. The stimulation is due to production of a cytosolic electron donor from a substance(s) transported from the chloroplast. It is unknown whether the stimulation of redox activity is a requirement for other photosynthetically stimulated processes at the plasma membrane, but a reduced intermediate may regulate proton excretion by guard cells. Blue light induces an absorbance change (LIAC) at the plasma membrane whose difference spectrum resembles certain *b*-type cytochromes. This transport of electrons may be due to absorption of light by a flavoprotein. The LIAC has been implicated as an early step in certain blue light-mediated morphogenic events. Unrelated to photosynthesis, blue light also stimulates electron transport at the plasma membrane to ferricyanide. The relationship between LIAC and transmembrane electron flow has not yet been determined, but blue light-regulated proton excretion and/or growth may depend on this electron flow. No conclusions can be drawn regarding any role for phytochrome because of a paucity of information concerning the effects of red light on redox activity at the plasma membrane.

Key Words: Signal transduction; photosynthesis; blue light; plasma membrane redox; light-induced absorbance change; flavin; cytochrome *b*; sphingoid bases.

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Introduction

The components of a redox system exists at the plasma membrane, and energy associated with the flow of e^- between these carriers may elicit morphogenic responses in plants and animals (Bienfait and Luttge, 1988; Crane and Barr, 1989). Because absorption of light by various pigments often results in e^- transport (Schmidt, 1984a), it is possible that the plasma membrane-localized redox activity participates in signal transduction chains initiated by irradiation of different spectral qualities. The purpose of the following short review is to examine some of the more recent evidence in plants relating to this possibility.

Photosynthetically Active Radiation

Effects of Photosynthesis on Redox Activity

Besides providing chemical energy in the chloroplast, photosynthesis is indirectly responsible for regulating certain cell processes, some of which occur at the plasmalemma. It is known, for example, that photosynthesizing cells excrete H^+ more rapidly than cells in the dark (Felle and Bertl, 1986). This results in a hyperpolarization of the membrane potential (e.g., Ullrich-Eberius *et al.*, 1983), which, together with the steeper H^+ gradient, increases the protonmotive force, thereby providing energy for fluxes of important components of the cell. A role for plasma membrane redox activity in these processes is possible, since light also stimulates the transport of e^- across the plasma membrane of green cells from a cytosolic donor; this is measured as a reduction of nitroblue tetrazolium (Elzenga and Prins, 1987) or of exogenous ferricyanide (Ivankina *et al.*, 1984; Lass *et al.*, 1986; Dharmawardhane *et al.*, 1987; Neufeld and Bown, 1987). There appears to be no effect of light on ferricyanide reduction by mesophyll cells when exogenous NADH is the e^- donor (Dharmawardhane *et al.*, 1987).

An involvement of photosynthesis in transplasmalemma e^- transport is indicated by two lines of evidence. First, DCMU, a specific inhibitor of noncyclic photosynthetic e^- transport, eliminates the light-induced stimulation of redox activity at the plasmalemma without affecting dark controls (Table I; Dharmawardhane *et al.*, 1987; Novak *et al.*, 1988). It was also noted that light has no effect on transmembrane redox activity of bleached, photosynthetically incompetent leaves from plants grown on the carotenoid synthesis inhibitor, norflurozon (Table I; Rubinstein *et al.*, 1989). Secondly, a broad-band action spectrum for the stimulation of ferricyanide reduction by green mesophyll cells is similar to that for photosynthetic O_2 evolution

Table I. Effect of White Light and Stearylamine on Transplasma Membrane Electron Transport by Photosynthetically Competent or Incompetent Oat Leaf Segments[a]

Treatment		Ferricyanide reduction (% dark control)[b]		
		Green	Green + DCMU[c]	White[d]
−SA	Dark	100	100	100
	Light	176	108	75
+SA[e]	Dark	108	68	113
	Light	748	312	356

[a] Data from Rubinstein *et al.* (1989).
[b] Average rate for the three controls was 2.5 μmol · g fr wt^{-1} · h^{-1} as determined by a colorimetric assay using bathophenanthroline disulfonic acid.
[c] DCMU added 15 min prior to assay; concentration = 75 μM.
[d] White segments were from leaves of 8- to 9-day-old plants grown in 0.1 mM norflurozon.
[e] Stearylamine (SA) used as an analog of sphingosine (see Dharmawardhane *et al.*, 1989). Concentration = 1 mM added 15 min before assay.

(Dharmawardhane *et al.*, 1987). The question then arises as to how a process restricted to the chloroplast can influence an event at the plasmalemma.

To answer this question, it must be understood that photosynthesis stimulates the dark rate of e^- transport across the plasmalemma, rather than inducing or activating a separate redox system. Evidence for this is that apparent K_m's (Dharmawardhane *et al.*, 1987; Neufeld and Bown, 1987), pH profiles (Dharmawardhane *et al.*, 1987), and saturation curves for divalent cations (Ivankina *et al.*, 1984; Dharmawardhane *et al.*, 1987) are similar for both light and dark activities. Only the apparent V_{max} is stimulated by light (Dharmawardhane *et al.*, 1987).

Other relevant data concern the presence and nature of a stimulatory factor(s) which appears to be responsible for the photosynthetic effect on the rate of transplasma membrane e^- flow. There is a 4-s lag before a stimulation of redox activity is observed, followed by a 15-min period before the optimum rate is attained (Rubinstein *et al.*, 1989). The decay of the light rate in the dark has a half-time of less than 3 min (Dharmawardhane *et al.*, 1987). These data point to the appearance in the cytosol of a relatively stable chemical product of photosynthesis rather than a short-lived photochemical intermediate.

The chemical product does not appear to be photosynthetic O_2 which might activate mitochondrial processes, since the stimulation of redox activity by light is unchanged when respiration is inhibited (Dharmawardhane *et al.*, 1987). NADPH and ATP from the chloroplast are eliminated as stimulatory factors, since neither are known to translocate readily to the cytosol (Heber, 1974). Furthermore, ATP levels in the cytosol do not increase in the light

(Goller *et al.*, 1982). Triose phosphate has been implicated, however, since it can be exported from the chloroplast via the phosphate translocator when the triosephosphate/P_i ratio is low (Heber, 1974). Cytosolic triosephosphate can then be metabolized by reverse glycolysis and the pentose phosphate pathway to produce NADPH, an e^- donor for transplasmalemma redox activity (Sijmons *et al.*, 1984; Qiu *et al.*, 1985). Both triosephosphate and reduced pyridine nucleotides are known to increase in the cytosol when mesophyll cells are exposed to light (Hampp *et al.*, 1985).

Evidence against triosephosphate as the photosynthetic product that is indirectly responsible for the increased rate of transplasmalemma e^- transport was presented by Elzenga and Prins (1989). They showed that light-induced ferricyanide reduction is retarded when the CO_2 level is raised by adding $KHCO_3$ to *Elodea* cells. It was argued that the rate of e^- transport at the plasma membrane should be stimulated under these conditions, since triosephosphate levels in the chloroplast, and hence in the cytosol, would increase. Therefore, malate translocated from the plastid was suggested to be the chemical intermediate whose subsequent oxidation in the cytosol generated reduced pyridine nucleotide (Elzenga and Prins, 1989).

It is known, however, that as triosephosphate accumulates in the chloroplast, a higher triosephosphate/P_i ratio may result, which would activate the enzyme, ADP glucose pyrophosphorylase, and favor starch formation (Hall and Rao, 1987). Operation of this pathway would lead to less, rather than more, triosephosphate available for translocation to the cytosol and could be responsible for the observed inhibition of redox activity. To help settle this issue, data are needed for amounts of cytosolic triosephosphate under high and low bicarbonate levels. In any case, there seems to be agreement that the supply of NAD(P)H is limiting in the dark and that photosynthesis serves indirectly to increase the level of the e^- donor. This is achieved by producing a translocatable compound in the chloroplast whose conversion in the cytosol leads to the production of NAD(P)H.

Relationship of Redox Activity to Events Regulated by Photosynthesis

An important question to answer is whether redox activity is an intermediate between photosynthetic processes and the subsequent changes in membrane potential, H^+ excretion, and fluxes of other solutes. Even though redox activity as detected by ferricyanide reduction is also stimulated by light, the possibility remains that events such as solute fluxes, which occur at the plasma membrane, are regulated separately from transmembrane e^- transport. The fact that added ferricyanide induces H^+ excretion similar to that of light (Rubinstein and Stern, 1990) is suggestive of an important role for cell surface redox activity, but, contrary to the effects of light, the oxidant

causes a membrane depolarization (Lass *et al.*, 1986; Novak *et al.*, 1988; Marrè *et al.*, 1988), an inhibition of ion uptake, and a stimulation of ion efflux (Lass *et al.*, 1986; Rubinstein and Stern, 1986; Marrè *et al.*, 1988). In other words, e^- flow in the presence of the nonphysiological redox probe may be a cause of the acidification being measured, independent of light effects (Rubinstein and Stern, 1990).

It is still possible, however, that an accelerated transport of e^- at the plasmalemma to its natural acceptor, either O_2 or some other substance, is an intermediate event leading to H^+ excretion in the light. Serrano *et al.* (1988) detected an ATP-dependent outward current from the guard cell plasma membrane using the patch clamp technique in the whole cell configuration. The current is inhibited by vanadate and probably represents H^+ excretion via the H^+-ATPase. The current flow is stimulated by red light after a lag of a few seconds and is inhibited by DCMU, indicating that photosynthesis is involved. But the inhibition by DCMU is observed even when ATP is added to the cytosolic surface of the membrane. The authors conclude that a reduced photosynthetic product may be limiting for H^+ excretion (Serrano *et al.*, 1988); the product may stimulate e^- transport at the plasma membrane, which then activates H^+ excretion. A system of this sort occurs in the chloroplast, where light-regulated H^+-ATPase activity is in part controlled by redox reactions (Shahak, 1985).

Further evidence for a relationship between light, e^- transport, and H^+ fluxes was presented by Elzenga *et al.* (1989). Using plasma membrane-enriched vesicles, they showed that ATPase activity, presumably representing the H^+ pump, was inhibited by NEM, oxidized glutathione, and NAD^+. The NAD^+ inhibition was alleviated by increasing concentrations of NADH. The authors suggest that light-induced H^+ excretion via the ATPase may be regulated by redox activity.

Blue Light

Effect of Blue Light on Redox Activity

Redox activity in the form of a blue light-induced absorbance change (LIAC) has been detected *in vivo* by light-minus-dark difference spectroscopy in organisms as diverse as the fungi *Trichoderma* (Fig. 1A), *Phycomyces* and *Neurospora* (Gressel and Rau, 1983), the slime mold *Dictyostelium* (Poff and Butler, 1974), and in wheat and maize celeoptiles (Senger and Briggs, 1981). The photoresponse has also been observed in plasma membrane-enriched fractions from *Neurospora* (Brain *et al.*, 1977a; Gressel and Rau, 1983), *Acetabularia* (Caubergs *et al.*, 1984), maize coleoptiles (Brain *et al.*, 1977a;

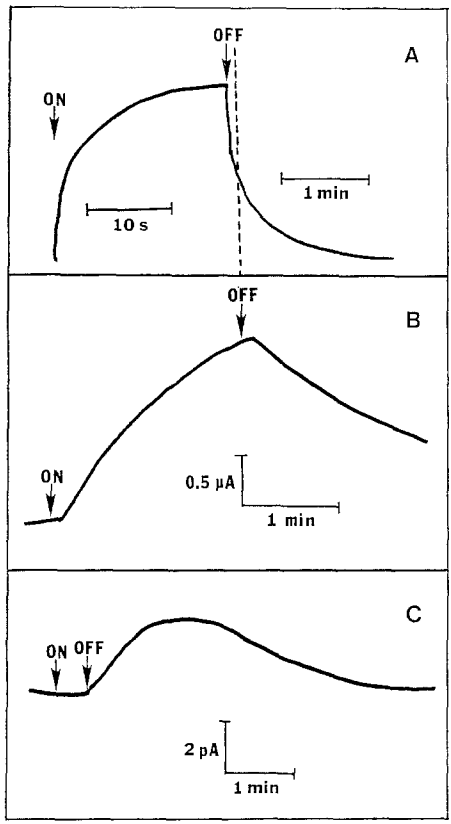

Fig. 1. Effects of blue light on LIAC, transplasma membrane electron transport, and outward current. A: Kinetics of blue light-induced absorbance (LIAC) of *Trichoderma* mycelia. Broad band blue light at 560 W m^{-2} was given for 20 s. The ordinate represents relative absorbance measured by a beam alternating between 440 and 460 nm. Dashed line indicates half-time for decay of the LIAC. Data from Horowitz *et al.* (1986). B: Kinetics of blue light-induced transplasma membrane electron transport. Oat leaf segments were pretreated with 1 mM stearylamine (a sphingoid base analog) for 1 h before irradiation with 35 μmol m^{-2} s^{-1} broad band blue light. Redox activity measured electrochemically by the method of Chalmers *et al.* (1984) as the current generated during the reoxidation of ferricyanide. Data of Jurgens, Rubinstein, and Stern (unpublished). C: Kinetics of whole-cell outward current (presumably H^+ excretion) determined by the patch clamp method. Cell membrane potential was clamped at -50 mV. A 30-s pulse of 50 μmol m^{-2} s^{-1} blue light was superimposed on 500 μmol m^{-2} s^{-1} background red light. Data are for guard cell protoplasts of *Vicia faba* from Assmann *et al.* (1985).

Goldsmith *et al.*, 1980; Widell and Larsson, 1984), cauliflower inflorescences, barley and oat shoots, and wheat and oat shoots (Widell and Larsson, 1984). For the *in vitro* system, the only effective e^- donor appears to be EDTA and the absorbance change is enhanced under anaerobic conditions maintained by the addition of glucose and glucose oxidase (Goldsmith *et al.*, 1980).

The LIAC is most likely the result of flavin-mediated photoreduction of a *b*-type cytochrome, since action spectra for LIAC's compare favorably with flavin absorption spectra (Munoz and Butler, 1975; DeFabo, 1980; Widell and Larsson, 1984). A *b*-type cytochrome is implicated because of the characteristics of the difference spectrum; also, a cytochrome *b* mutant in *Neurospora* shows an altered LIAC (Brain *et al.*, 1977b). Both noncovalently bound flavin (0.2–2.5 nmol mg protein^{-1}) and *b*-type cytochrome (0.1–0.5 nmol mg protein^{-1}) are usually found in purified plasma membrane preparations (Møller and Crane, 1990).

Amplifying data of previous reports based on less pure membrane preparations, Asard *et al.* (1989) and Askerlund *et al.* (1989) examined the cytochrome composition of plasma membranes purified by aqueous two-phase partitioning from cauliflower inflorescences, zucchini, and bean hypocotyls, spinach, sugar beet and barley leaves, and barley roots. The *b*-type cytochromes were spectrally characterized and their midpoint redox potentials determined. Generally, two to three *b*-type cytochromes were identified whose concentrations in the plasma membranes were species dependent. The major component (60 to 70% of the total) was a high-potential cyt *b* 557–561, with a midpoint potential of +110 to +160 mV, which was reduced 80 to 100% by ascorbate. Other evidence suggests that this high-potential *b*-type cytochrome is involved in the flavin-mediated blue light response (Widell *et al.*, 1983; Kjellbom and Larsson, 1984; Caubergs *et al.*, 1988).

In an artificial membrane system, flavins conjugated with C18-hydrocarbons were bound to vesicles prepared from natural or synthetic phospholipids (Schmidt, 1984b). With cytochrome *c* in the lumen acting as an e^- acceptor and EDTA in the outside medium as e^- donor, the vesicles transferred redox equivalents across the membrane to cytochrome *c* on exposure to blue light under both aerobic and anaerobic conditions. This demonstrates the potential of flavins in natural membranes to mediate e^- flow in blue light.

Redox activity at the plasma membrane can also be determined by measuring the reduction of ferricyanide in the medium bathing cells or tissues. This relatively simple method showed that photosynthesis can stimulate transplasma membrane e^- transport (see previous section), but the procedure also can be used to characterize blue light effects. Dharmawardhane *et al.* (1989) have shown that sphingoid bases (SB) and certain analogs stimulate e^- flow across the plasma membrane of oat mesophyll cells up to fourfold in the light. Curiously, the same SB concentrations which stimulate in the light inhibit or have no effect on ferricyanide reduction in the dark. Light-induced redox activity in the presence of SB is halved when leaves are pretreated with the photosynthetic inhibitor DCMU, or when photobleached leaves are used instead, but a twofold stimulation over dark controls remains

(Table I; Dharmawardhane *et al.*, 1989). A broad band action spectrum for the response in photosynthetically incompetent leaves shows that the activity is restricted to the blue region (Dharmawardhane *et al.*, 1989; Rubinstein *et al.*, 1989).

Since SB inhibit protein kinase activity associated with transplasma membrane e^- transport in neutrophils (Hannum and Bell, 1989), the inhibition or ineffectiveness of SB on e^- transport in the dark and the SB-induced activation in blue light are most easily explained by assuming that two redox protein complexes (RP_1 and RP_2) exist at the plasma membrane and that each is affected in an opposite manner by phosphorylation (Dharmawardhane *et al.*, 1989). Analogous to the transplasma membrane NADPH oxidase of neutrophils (Lambeth, 1988), it is proposed that RP_1 must be phosphorylated to be maximally active; RP_1 operates in the dark and is accelerated by products of photosynthesis. RP_2, on the other hand, may have to be dephosphorylated before it is activated, as is the case for rhodopsin (Kuhn and Wilden, 1987) and some plant redox enzymes (Budde and Chollet, 1988); RP_2, then, would be the redox complex activated by blue light.

Evidence for two redox systems is that (a) when SB are present, blue and only blue light is active in photosynthetically incompetent leaves; (b) the stimulation of redox activity by light in the presence of SB is maintained for at least 20 min after they are washed out, while the dark inhibition is no longer seen under these conditions; (c) a 15-min preincubation in SB is needed before stimulations by light are observed, but in the dark, SB inhibit in less than 5 min (Dharmawardhane *et al.*, 1989); (d) after preincubation in SB, a lag period of 0.5 sec or less is observed before light stimulates redox activity, but 4 sec must elapse before an increase by light is observed without SB (Rubinstein *et al.*, 1989).

Even though more than one blue light photoreceptor may exist (e.g., Iino, 1988b; Palit *et al.*, 1989), it would be useful to determine if the LIAC is related or identical to the SB-induced blue light stimulation of ferricyanide reduction. LIAC measures changes in a *b*-type cytochrome while ferricyanide probably oxidizes a flavin directly, but both activities occur at the plasma membrane. Furthermore, e^- transport to ferricyanide is stimulated by blue light in SB-treated leaves bleached by norfluorzon (Rubinstein *et al.*, 1989); this makes it possible that a flavin is the pigment being activated under these conditions. As mentioned above, flavins have also been implicated in the LIAC. The kinetics of cytochrome *b* reduction during LIAC and transplasma membrane e^- transport to ferricyanide are compared in Fig. 1A and 1B.

Further investigations into similarities between these two processes may now be conducted since recent results show that ferricyanide-stimulated redox activity also can be seen *in vitro* with membrane preparations identical to those used for LIAC (Hassidim *et al.*, 1987). Thus, one can now ask if SB

also stimulate LIAC, if e^- flow to ferricyanide *in vitro* is stimulated by blue light, and if action spectra and other photochemical parameters are similar for the two processes. Another meaningful approach would be to identify mutants depleted in one type of redox activity and ask if the other type is also missing.

Relationship of Redox Activity to Events Regulated by Blue Light

Regardless of whether the LIAC detected in plasma membrane preparations is identical to redox activity measured *in vivo*, it is important to consider whether these redox processes are part of the signal transduction chain of blue light (see reviews by Senger and Briggs, 1981; Briggs and Iino, 1983; Widell, 1987), a chain which may consist in part of phosphorylation reactions (Gallagher *et al.*, 1988; Short and Briggs, 1990). Although no specific blue light response has been shown to depend absolutely on LIAC, in *Neurospora* the action spectrum for the photoinhibition of the circadian rhythm of conidiation was shown to be similar to the action spectrum for the photoreduction of cytochrome *b* (Munoz and Butler, 1975). Furthermore, methylene blue, which sensitizes LIAC *in vitro*, similarly sensitizes the rhythmic conidiation response (Feldman, 1989).

The finding that a photosporulation mutant in *Trichoderma* exhibits an altered LIAC (Horowitz *et al.*, 1986) also points to a physiological importance for this reaction. In contrast, the LIAC was not changed in a phototropism mutant of *Phycomyces* (Lipson and Presti, 1977). What is more, known inhibitors of flavin photochemistry and blue-light responses (azide, iodide, and phenylacetic acid) had little effect on the blue-photoinduced primordial formation in *Coprinus* (Durand, 1985).

A physiological role for LIAC in plants has been implied because the action spectrum for LIAC is similar to that seen for induction of phototropism in oats (Widell *et al.*, 1983). Furthermore, Leong and Briggs (1982) found that both the LIAC of microsomal preparations and phototropism (but not growth or gravitropism) were enhanced by the diphenyl ether herbicide, acifluorfen. The increase of LIAC was shown to be due to retardation of dark reoxidation of a *b*-type cytochrome. It was suggested that the herbicide acts at the blue photoreceptor end of the stimulus-response chain associated with phototropism (Leong and Briggs, 1982).

Further work with the herbicide was done on *Trichoderma* (Gaba and Gressel, 1987). While the effect of acifluorfen on LIAC was not studied, blue light-induced sporulation was increased by the herbicide. However, here its effect appeared to be on dark reactions unrelated to the photoreceptor, since acifluorfen also enhanced sporulation when applied 30 min after light exposure; LIAC in *Trichoderma* was shown to decay within a minute after

photoinduction (Fig. 1A; Horowitz *et al.*, 1986). Considering the reports cited above, it would appear that the physiological relevance of LIAC in fungi and plants must still be resolved.

Another approach to investigate the physiological relevance of blue light-stimulated redox activity (both LIAC and e^- transport to ferricyanide) would be to study light effects on processes located at the plasma membrane which are part of the signal transduction chain. One such process is the excretion of H^+ controlled by the H^+-pumping ATPase. For example, in guard cell protoplasts, H^+ excretion is stimulated by blue light (Shimizaki *et al.*, 1986). Assmann *et al.* (1985) have shown in a patch clamp study that less than 1 min after a 30-s pulse of blue light, an ATP-dependent hyperpolarization of the membrane potential results, presumably due to H^+ export (Fig. 1C). This leads to activation of voltage-regulated K^+ channels in the plasma membrane, an influx of K^+, and the uptake of H_2O, which results in the opening of the stomatal pore.

An unanswered question is whether redox activity in the guard cell plasma membrane is an intermediate between blue light and H^+ excretion. The transport of e^- to ferricyanide has been detected in guard cells and epidermal strips, and ferricyanide additions can change rates of $^{86}Rb^+$ uptake, an ion analogous to K^+ (Roth-Bejerano *et al.*, 1988). Furthermore, blue light stimulates the rate of ferricyanide (Roth-Bejerano and Itai, 1990) or tetrazolium reduction (Vani and Raghavendra, 1989). In the latter case, the blue, and to some degree the red, light stimulation was increased by 3 μM riboflavin or FMN. However, the assay method is not quantitative and reduced tetrazolium could only be observed at the plasma membrane after addition of exogenous NADH. It is unlikely that significant pools of pyridine nucleotides normally exist outside the plasma membrane.

In order to implicate redox activity at the plasma membrane as an intermediate reaction between blue light and H^+ pumping in guard cells, it is necessary to obtain precise data showing that an accelerated rate of e^- transport occurs during the 30-s or so lag before H^+ excretion (Fig. 1C; Assmann *et al.*, 1985). In mesophyll cells, a lag of only 4 s (or less in the presence of SB) occurs between light exposure and detection of ferricyanide reduction (Rubinstein *et al.*, 1989), so if similar kinetics hold with guard cells, redox activity could play a causal role. One way to demonstrate such a relationship would be to inhibit specifically e^- transport at the plasma membrane, perhaps with a particular drug or an antibody to the redox complex, and ask if blue light effects are still observed.

The arguments regarding the relationship of redox activity at the plasma membrane with stomatal opening could also be invoked for light-induced leaf expansion and leaf movements. Both processes are triggered, at least in part, by blue light, and H^+ excretion is a component of the signal transduction

chain (Lee and Satter, 1989; VanVolkenburgh *et al.*, 1990). As mentioned above, redox activity is present at the plasma membrane of mesophyll cells, but no data exist concerning its presence in pulvini cells, the motor tissue for leaf movements.

Blue light also controls elongation of certain dicots. Pea epicotyl growth was inhibited by 40% 2–3 min after a 30-s blue pulse ($80\,\mu mol\,m^{-2}\,s^{-1}$) (Laskowski and Briggs, 1989), and Cosgrove (1981) has shown that $11\,W\,m^{-2}$ blue light inhibited hypocotyl growth of intact *Sinapis* and *Cucumis* plants within 30 s, a rate much faster than the appearance of phototropism or the inhibitory effects of red light on growth (Cosgrove, 1985). Blue light appears to affect the wall yielding coefficient, not the yield threshold or mechanical extensibility (Cosgrove, 1988). Frozen–thawed hypocotyls under tension show an extension or "creep" during long-term studies (Cosgrove, 1989). The creep appears to be controlled enzymatically, which in turn could be regulated by sulfhydryl reagents. If thiol reduction is limiting for growth, one might ask if a blue light-activated redox system at the plasma membrane may directly or indirectly affect SH groups of the wall enzyme(s) responsible for creep.

Segments of *Cucumis* hypocotyls are also inhibited by blue light ($100\,\mu mol\,m^{-2}\,s^{-1}$) with somewhat slower kinetics than intact plants (Shinkle and Jones, 1988). The blue light inhibition is prevented by ascorbate and intensified by malate. Since the extracted cell wall peroxidase activity is also inhibited by ascorbate, the enzyme is implicated in the blue light effect. It is hypothesized that peroxidase action leads to a stiffening of the wall by cross-linking certain wall components (Shinkle and Jones, 1988). While Cosgrove (1988) did not detect a wall stiffening by blue light, it is still possible that the inhibition of hypocotyl growth involves a peroxidase-mediated step using H_2O_2 produced by transplasma membrane e^- transport.

Even though the requirement for wall acidification as a regulatory factor for growth of dicot stems is controversial, it is possible that the rate of H^+ excretion becomes limiting during blue light irradiances. A membrane depolarization is seen immediately after exposure of cucumber hypocotyls to blue light (Spalding and Cosgrove, 1989), and this may be due in part to an inhibition of the H^+ pump. A related observation may be the inhibition by blue light of the tonoplast H^+-pumping ATPase (Krauss *et al.*, 1987). It was shown that blue light irradiation of vacuolar membranes inhibited intravesicular acidification, but only in the presence of added flavins, ATP, and O_2. Other evidence suggests that SH groups regulate H^+ pumping (Hager and Lanz, 1989) and that H_2O_2 mediates the photoinactivation (Krauss *et al.*, 1987). Recently, it has been proposed that transplasma membrane e^- transport can generate H_2O_2 (Apostol *et al.*, 1989). Thus, one might consider a signal transduction chain in which blue light absorption by an endogenous flavin leads to reduction of O_2 to H_2O_2. The H_2O_2 may then

oxidize essential SH groups on the H^+-ATPase or affect other processes required for elongation. While SH groups have been identified at the ATP-binding site of plasma membrane ATPases (Serrano, 1988), it must be pointed out that the pump measured by Krauss *et al.* (1987) was the tonoplast type and that high irradiances were used (58.5 $W m^{-2}$).

Red Light

Red light effects unrelated to photosynthesis are usually mediated by phytochrome. Like blue light, the signal transduction chain of this photoreversible pigment appears to involve phosphorylation events (Otto and Schafer, 1988; Wong *et al.*, 1989). Although the pigment itself may not be a kinase, a small percentage of the protein is phosphorylated when extracted (Grimm *et al.*, 1989). Red light can also interact with blue light effects on processes such as stomatal opening (Assmann, 1988), phototropism (Steinitz *et al.*, 1988; Iino, 1988a), and growth (Warpeha and Kaufman, 1990).

Events at the plasma membrane, including membrane potential and H^+ transport are, affected by red light within seconds of irradiation (Pratt, 1989). So, if the H^+ pump is in part regulated by redox activity (see discussion above), a role for e^- transport at the plasma membrane can be inferred. But there are no published reports that red light activates the transport of e^- at the plasma membrane in the absence of photosynthesis. However, we have preliminary results indicating that under certain conditions, red light irradiation of photosynthetically incompetent leaves stimulates transplasma membrane redox activity; it remains to be seen if phytochrome is involved in this response.

Summary

A scheme implicating plasma membrane redox activity in certain effects of light is presented in Fig. 2. We hypothesize that photosynthetically active radiation supplements the cytosolic level of reducing power [i.e., NAD(P)H]. The NAD(P)H may be oxidized by one or more redox protein complexes which are composed of flavin and a *b*-type cytochrome. Two complexes, RP_1 and RP_2, are proposed based on results using SB as discussed above. The complex designated RP_2 may contain the components responsible for LIAC and is activated by blue light. Either complex may regulate certain proteins in the membrane whose activity is controlled by sulfhydryl reduction.

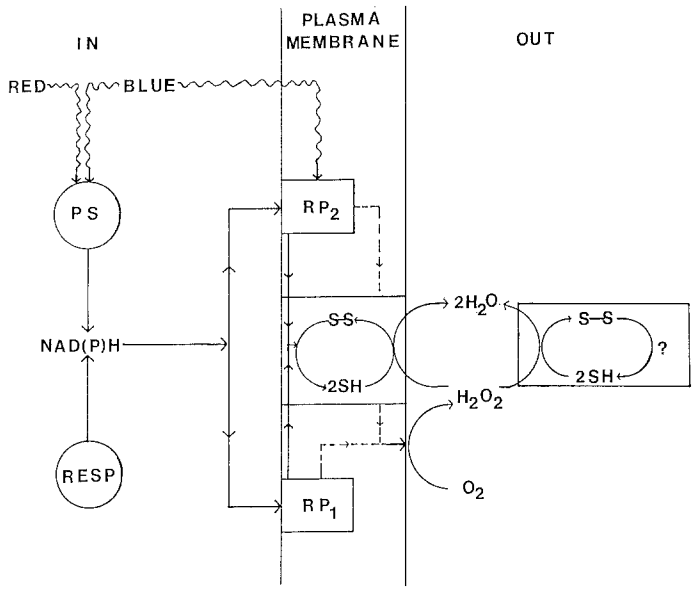

Fig. 2. A speculative model to explain the activation and effects of redox reactions associated with the plant plasma membrane. The two redox protein complexes (RP_1 and RP_2) are reduced by NAD(P)H supplied by respiration (RESP) or photosynthesis (PS). Red and blue light may affect redox activity indirectly via PS, or blue light may act directly on RP_2. Solid lines represent e^- transport within the membrane. Dashed lines represent e^- transport to O_2. SH–SS conversion would regulate membrane-bound or wall-bound enzymes. For further details, refer to Summary.

The redox complexes may also donate e^- to O_2 to produce O_2^- which is dismutated to H_2O_2. The H_2O_2 oxidizes available SH groups in the membrane or in the cell wall.

If the H^+-ATPase can be regulated by sulfydryl groups and if reduction of disulfide groups is maintained by redox activity at the plasma membrane, then a direct or indirect activation of a redox complex by light could account for stimulations of H^+ excretion and the multitude of important events dependent on this process. Likewise, if H_2O_2 plays a role in oxidation of sulfydryl groups both in the plasma membrane and cell wall and if redox activity leads to H_2O_2 production, then activation of a redox complex by light could have a negative effect on processes at the cell surface. The direction taken by e^- flow from the redox complexes (i.e., either to activate protein by reduction of disulfide groups or inactivate protein by production of H_2O_2) could depend on the presence of blue light, the availability of cytosolic reducing power, and any number of as yet unknown developmental or environmental signals.

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