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YEARLY REVIEW

THE ROLE(S) OF CALCIUM IONS IN PHYTOCHROME ACTION

Introduction

Phytochrome, the red (R)*- and far-red light (FR)-absorbing morphogenetic photoreceptor, which occurs throughout the Plant Kingdom, was discovered by scientists at the US Department of Agriculture, Beltsville, MD. This year is the 40th anniversary of the prediction of the R/FR reversible pigment in plants and more than 30 years since its first spectroscopic detection. During the last four decades extensive progress has been made in understanding the molecular structure and function of phytochrome (for review see Furuya, 1987). *Phytochrome* genes have now been cloned for a few plant species (Quail *et al.*, 1987; Furuya, 1989; Sharrock and Quail, 1989). The photoregulation of genes, including the phytochrome gene itself, has been extensively studied (Quail *et al.*, 1987; Nagy *et al.*, 1988; Furuya, 1989; Tomizawa *et al.*, 1990). However, the molecular mechanism of phytochrome action is still obscure. Multiple response types could be an indication of different modes of action of the photoreceptor (Jordan *et al.*, 1986; Kronenberg and Kendrick, 1986; Schäfer *et al.*, 1986; Furuya, 1989). One attractive hypothesis is that calcium ions (Ca^{2+}) participate as a second messenger (Roux, 1984). During the last decade many papers have been published about the involvement of Ca^{2+} in the regulation of different phytochrome-regulated processes. Some of them were reviewed by Roux *et al.* in 1986. Since that time new and sometimes contradictory data have been published and this review is an update of knowledge concerning participation of Ca^{2+} in phytochrome action. The basic evidence for participation of Ca^{2+} in phytochrome action in different plant systems is reviewed and possible mechanisms of action of Ca^{2+} in plant photomorphogenesis are discussed.

Participation of Ca^{2+} in Phytochrome Action in Different Plant Systems

Biopotentials

The plasma membrane of some plants is electrically excitable. In a few lower-plant species light can trigger an "action potential" which appears to be an all-or-none response when the stimulus exceeds a certain threshold value (Trębacz, 1989 and references therein).

In isolated internodes of the green algae *Nitellopsis* sp., *Nitella opaca* AG. and *Nitella flexilis* (L.) AG., R (fluence 8.4 W m^{-2}) caused depolarization of their plasma membrane, which had a mean lag-time of 1.7 ± 0.3 s and reached a steady state within 1–2 min upon continuous irradiation (Weisenseel and Ruppert, 1977). Repolarization of the membrane occurred a few seconds after the light was turned off and was accelerated by FR applied immediately after the R pulse. The magnitude of R-triggered membrane depolarization in the algal cells was dependent upon the [Ca^{2+}] of the external medium. In Ca^{2+}-free medium the depolarization was only about 1 mV, whereas in the presence of 5 m*M* Ca^{2+} a mean depolarization of approximately 33 mV was recorded (Weisenseel and Ruppert, 1977). Red and FR change the mobility of the unicellular green alga *Mesotaenium caldariorum* in an electric field. The *Zeta*-potential, indicating the surface charge of the cells, was calculated from the velocity of the movement (Stenz and Weisenseel, 1986). After FR irradiation the electrophoretic mobility of the cells was faster and the *Zeta*-potential more negative than after R irradiation. There was no change in *Zeta*-potential after R and FR treatment in the presence of 1 m*M* ethyleneglycol-bis-(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA). The R effect on the *Zeta*-potential was also reduced by 10 μ*M* verapamil to the level of the FR control. From these data, Stenz and Weisenseel (1986) argued that phytochrome increases Ca^{2+} influx into the algal cells. In this way phytochrome

**Abbreviations*: AC, adenylate cyclase; Ca^{2+}, calcium ion; $[Ca^{2+}]_c$, cytoplasmic calcium ion; CaBP, calcium-binding physode; CPZ, chlorpromazine; DAG, 1,2-diacylglycerol; DB-cAMP, dibuthyryl cAMP; DHP, dihydroxypyridine; EDTA, ethylenediaminetetraacetic acid; EGTA, ethyleneglycol-bis-(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; FR, far-red light; GA, gibberellin; H_7, 1-(5-isoquinoline sulfonyl)-2-methylpiperazine; I_{Ca}, calcium channel current; IP_1, inositol 1-phosphate; IP_2, inositol 1,4-bisphosphate; IP_3, inositol 1,4,5-trisphosphate; OAG, 1-oleoyl-2-acetyl-sn-glycerol; PDE, phosphodiesterase; PEG, polyethylene glycol; Pfr, FR-absorbing form of phytochrome; PI, phosphatidylinositol; PIP_2, phosphatidylinositol 4,5-bisphosphate; PKA, protein kinase A; PKC, protein kinase C; PLA_2, phospholipase A_2; PLC, phospholipase C; PMA, phorbol 12-myristate 13-acetate; Pr, R-absorbing form of phytochrome; R, red light; TFP, trifluoperazine; TMB-8, 3,4,5-trimethoxy-benzoic acid 8-(diethylamino)-octyl ester; W_5, *N*-(6-amonohexyl)-1-naphthalenesulfonamide; W_7, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide.

may stimulate depolarization of the plasma membrane and/or secretion of less negatively or positively charged material to the surface of the cells.

Protoplast swelling

In 1983, Blakeley *et al.* published the first paper on phytochrome-controlled swelling of protoplasts, obtained from etiolated cereal leaves. Protoplasts, isolated from etiolated primary wheat (*Triticum aestivum* L.) leaves (Blakeley *et al.*, 1983; Bossen *et al.*, 1988), oat (*Avena sativa* L.) (Kim *et al.*, 1986; Chung *et al.*, 1988), maize (*Zea mays* L) (Zhou *et al.*, 1990) and barley (*Hordeum vulgare* L.) (U *et al.*, 1991), maintained at constant osmotic potential swelled upon R irradiation. The effect of R on protoplast swelling was photoreversible by subsequent FR, indicating the involvement of phytochrome (Blakeley *et al.*, 1983; Kim *et al.*, 1986; Bossen *et al.*, 1988; Zhou *et al.*, 1990; U *et al.*, 1991). Besides this effect *via* phytochrome, protoplasts also swelled in darkness after treatment with plant growth subtances: gibberellic acid (Blakeley *et al.*, 1983; Chung *et al.*, 1988; Bossen *et al.*, 1991; U *et al.*, 1991), indole-3-acetic acid, α-naphthaleneacetic acid, banzylaminopurine, abscisic acid (Bossen *et al.*, 1991), acetylcholine (Tretyn *et al.*, 1990a,d; Bossen *et al.*, 1991) and cAMP (Kim *et al.*, 1986; Chung *et al.*, 1988; Bossen *et al.*, 1990; U *et al.*, 1991). At 15°C protoplasts started to swell almost immediately after a R pulse and reached their maximum volume after 10 min in darkness at 15°C (Bossen *et al.*, 1988). Maize mesophyll protoplasts began to swell 5 min after R irradiation and increased by about 30% in comparison to the green safe light control (Zhou *et al.*, 1990). However, at 4°C the process only reached completion after 12 h incubation (Chung *et al.*, 1988).

In some cases, R-induced protoplast swelling appeared to be stimulated in the presence of K^+ (Blakeley *et al.*, 1983) or in an isosmotic modified Murashige–Skoog's nutrient solution (Kim *et al.*, 1986; Chung *et al.*, 1988). Bossen *et al.* (1988) found that phytochrome-controlled swelling of etiolated wheat protoplasts had an absolute prerequisite for Ca^{2+} in the surrounding medium and that the presence or absence of K^+ had no influence on the response. Neither Mg^{2+} nor Ba^{2+} replaced this requirement for Ca^{2+}. Furthermore, protoplast swelling was stimulated in darkness in the presence of Ca^{2+} and the calcium ionophore A 23187 (calimycin) (Bossen *et al.*, 1988; U *et al.*, 1991). However, phytochrom-stimulated, Ca^{2+}-dependent swelling of protoplasts failed in a medium containing the chelator EGTA (Bossen *et al.*, 1988; U *et al.*, 1991) or calcium-channel blockers verapamil and La^{3+} (Bossen *et al.*, 1988). These results strongly suggest that R causes the opening of calcium channels in the plasma membrane. This suggestion has been recently confirmed by Tretyn *et al.* (1990c), who found that nifedipine, a very specific calcium-channel blocker, completely prevents the R-induced, Ca^{2+}-dependent swelling of wheat protoplasts. However, nifedipine is only active when it is added to the medium before a R pulse (Tretyn *et al.*, 1990c). Moreover, as with A 23187, protoplasts swell in darkness in a medium containing Ca^{2+} and the agonist of the "L-type" animal calcium channels, Bay K-8644 (Tretyn *et al.*, 1990c). Bossen *et al.* (1988) and subsequently Tretyn *et al.* (1990c) proposed that a R-induced swelling of protoplasts is stimulated by an increase in cytoplasmic $[Ca^{2+}]$ ($[Ca^{2+}]_c$). Recently, U *et al.* (1991) have shown that R increases $^{45}Ca^{2+}$ uptake by etiolated barley mesophyll protoplasts and FR overcomes the effect of R, suppressing uptake of this ion. A R pulse increases and FR pulse decreases $[Ca^{2+}]_c$ in etiolated oat protoplasts (Chae *et al.*, 1990). The phytochrome-controlled transduction chain leading to protoplast swelling has been studied by Bossen *et al.* (1988, 1990, 1991) and U *et al.* (1991). A GTP-binding protein (G-protein), phosphatydylinositides, adenosine 3′:5′-cyclic monophosphate (cAMP) and the calcium-binding protein, calmodulin, could all take part in phytochrome-induced protoplasts swelling. The non-hydrolyzable GTP analogue, guanosine-5′-O-(3-thiotriphosphate) (GTP-γ-S) added to electroporated protoplasts stimulated their swelling in darkness or after a control FR irradiation. After a recovery period this compound was only active when Ca^{2+} was added to the external medium. Introduction of GDP-β-S by electroporation into protoplasts before a R pulse inhibited the phytochrome-stimulated swelling response (Bossen *et al.*, 1990).

The phospholipase C (PLC) inhibitor, neomycin (10 μ*M*), as well as lithium chloride (10 μM), an inhibitor of inositol-1-phosphatase, prevented R and GTP-γ-S-induced swelling of wheat protoplasts, while having no effect on the FR-irradiated control. The precursor of the phosphoinositide pathway, *myo*-inositol, had no effect on protoplast volume after R or FR treatment. However, this compound at 1 m*M* nullifed the inhibitory effect of Li^+ on the R-induced swelling response (Bossen *et al.*, 1990). The participation of calmodulin in protoplast swelling is unclear. A very specific antagonist of this regulatory protein, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W_7), stimulated an increase in protoplast volume both in darkness or after control FR irradiation and failed to prevent the R-induced response. A non-active analogue of this compound, *N*-(6-aminohexyl)-1-naphthalenesulfonamide (W_5), used at the same concentration as W_7 (10 μ*M*) failed to affect R or FR irradiated protoplasts (Bossen *et al.*, 1990). These observations were explained on the basis of an indirect effect on the $[Ca^{2+}]_c$ *via* a calmodulin-activated plasma membrane ATPase being inactivated (Bossen *et al.*, 1990). Effectiveness of the calmodulin inhibitor

(chlorpromazine [CPZ]) in blocking the R-induced, Ca^{2+}-dependent swelling response could be due to a non-specificity mechanism (Tretyn *et al.*, 1990a).

The effect of cAMP and the participation of cAMP-dependent protein kinase C (PKC) in the regulation of phytochrome-controlled protoplast swelling have been described (Kim *et al.*, 1986; Chung *et al.*, 1988; Bossen *et al.*, 1990; U *et al.*, 1991). The membrane-permeable analogue of cAMP, dibuthyryl-cAMP (DB-cAMP) (Kim *et al.*, 1986; Chung *et al.*, 1988; Bossen *et al.*, 1990), prostacycline, an activator of adenylate cyclase (AC) (Kim *et al.*, 1986), and phorbol 12-myristate 13-acetate (PMA), an activator of PKC, stimulate swelling of oat or wheat mesophyll protoplasts in darkness or after control FR irradiation. However, R or PMA-induced increase in protoplast volume was completely prevented by the PKC inhibitor, 1-(5-isoquinoline sulfonyl)-2-methylpiperazine) (H_7) (Bossen *et al.*, 1990). The stimulation of protoplast swelling by DB-cAMP in darkness was not inhibited by neomycin and Li^+ (Bossen *et al.*, 1990).

Recently, Zhou *et al.* (1990) have studied the biological significance of phytochrome-mediated swelling of etiolated leaf protoplasts. They compared the effect of phytochrome on the swelling of protoplasts isolated from *Pisum sativum* L. (pea) leaves, maize coleoptiles and both etiolated and R pre-irradiated maize leaves. Red light failed to induce swelling of pea leaf and maize coleoptile protoplasts. Furthermore, R failed to stimulate swelling of protoplasts isolated from R-pretreated unrolled maize leaves. However, R stimulated swelling of protoplasts isolated from etiolated maize leaves (Zhou *et al.*, 1990). These authors suggest that phytochrome-stimulated swelling of protoplasts is correlated with the leaf unrolling response and not leaf expansion. Both swelling of etiolated wheat protoplasts and unrolling of the etiolated leaf material from which protoplasts were isolated are under phytochrome control and only occur if Ca^{2+} is present in the medium (Tretyn and Kendrick, 1990; for details see below).

Mougeotia *chloroplast reorientation*

Considerable indirect evidence has accumulated on the role of Ca^{2+} in the sensory transduction pathway from the photoreceptors (phytochrome and blue-light photoreceptor) (Haupt, 1987; Schönbohm, 1987; Walczak *et al.*, 1984) to the terminal response (actin-based chloroplast reorientation) in the green alga *Mougeotia* (Wagner and Klein, 1981; Grolig and Wagner, 1988; Wagner and Grolig, 1991). Furthermore, calmodulin or a closely related homologue has been isolated from *Mougeotia* and its involvement in the signalling process has been proposed (Wagner *et al.*, 1984). However, pharmacological studies in different laboratories using calmodulin inhibitors (trifluoperazine [TFP], W_7 and others) have led to conflicting interpretations with respect to the requirement of calmodulin in this process (Serlin and Roux, 1984; Wagner *et al.*, 1984; Schönbohm *et al.*, 1990b). Schönbohm *et al.* (1990b) observed that TFP at 10 μM, applied over extended time periods, had no effect on the final chloroplast response, while higher TFP concentrations merely resulted in considerable side effects. Hence, they concluded no specific TFP effect and therefore no involvement of calmodulin in the chloroplast movement of *Mougeotia*. However, Wagner *et al.* (1984) showed that the application of 10 μM TFP, 15 min before the induction of movement in *Mougeotia*, noticeably delayed the beginning of reorientation without effect on the percentage of finally responding cells. This result indicated that TFP (possibly *via* calmodulin) influences the kinetics of chloroplast movement in *Mougeotia*. The difference noted by Schönbohm's group is apparently a consequence of the different kinetic parameter they utilized.

Two hypotheses have been put forward on the role of phytochrome in the chloroplast reorientation of *Mougeotia*: (1) The phytochrome gradient is translated directly into a calcium gradient, to steer chloroplast positioning (Haupt and Weisenseel, 1976; Serlin and Roux, 1984). The vectorial mechanism could be envisaged as a FR-absorbing form of phytochrome (Pfr)-mediated opening of calcium channels in the plasma membrane (vectorial calcium effect). The suggestion made earlier, that phytochrome itself is the plasma membrane calcium channel (Hendricks and Borthwick, 1967) appears unlikely in light of recent evidence indicating that phytochrome in *Mesotaenium* and *Mougeotia* is at best only a peripheral membrane protein (Kidd and Lagarias, 1990; Hanstein *et al.*, 1991). (2) Calcium is uniformly distributed in the cytoplasm and its sole function is to establish cytoskeletal competence for the performance of chloroplast movement (scalar calcium effect). The vectorial mechanism can be envisaged as a Pfr-mediated regulation of anchorage sites to actin on the *Mougeotia* plasma membrane (vectorial anchorage effect) (Wagner and Klein, 1981; Grolig and Wagner, 1988).

The first hypothesis originally appeared to be supported by results obtained by two different approaches: observation of R/FR reversible $^{45}Ca^{2+}$ influx into the cell from the external medium by micro-autoradiography (Dreyer and Weisenseel, 1979); the action of phytochrome could be mimicked by local application of calcium ionophore A 23187 (Serlin and Roux, 1984). Recently, this hypothesis has been challenged by the following results: application of various calcium-entry blockers (Schönbohm *et al.*, 1990a) or incubating the cells in calcium-depleted media (Schönbohm *et al.*, 1990a; Russ *et al.*, 1991) failed to influence the final chloroplast position with respect to light. Furthermore, patch-clamp data on *Mougeotia* protoplasts

(cell-attached configuration), do not support phytochrome-regulation of ion-channel activity compatible with the rapid physiological response (Lew *et al.*, 1990a,b). Only after a considerable delay of 2–5 min can a R-activated potassium channel be observed. This response is too slow to establish an ion gradient affecting chloroplast reorientation, because by the time it occurs the chloroplast has already started to reorient (Haupt and Trump, 1975; Walczak *et al.*, 1984). Finally the calcium antagonist, 3,4,5-trimethoxy-benzoic acid 8-(diethylamino)-octyl ester (TMB-8), fails to prevent chloroplast movement (Lew *et al.*, 1990b). Evidence is therefore mounting against the involvement of phytochrome-controlled calcium channels in the reorientation of the *Mougeotia* chloroplast. Evidence is growing in favour of the second hypothesis. Calcium-binding vesicles, evidently identical to physodes (Tretyn *et al.*, 1991a), have been discovered within the *Mougeotia* cytoplasm, but most abundantly in the area where the chloroplast is in contact with the cortical cytoplasm (Wagner and Rossbacher, 1980; Grolig and Wagner, 1987). Recent investigations demonstrate the capability of these globules to exchange calcium for osmium, potassium or neutral red, much like an ion-exchanger (Russ *et al.*, 1988; Grolig and Wagner, 1989; Tretyn *et al.*, 1991a). Since the *Mougeotia* physodes, like those in brown algae (Ragan, 1976) lack a membrane, they may have this function *in vivo* (Tretyn *et al.*, 1991a).

Measurements of $[Ca^{2+}]_c$ in *M. scalaris* with the fluorescent calcium indicator-dye indo-1 showed that pulsed irradiation with 365 nm light caused a rapid increase in $[Ca^{2+}]_c$ (within less than 1 s) independent of the external $[Ca^{2+}]$ (Russ *et al.*, 1991). These observations suggest that calcium is released from intracellular stores, such as the calcium-binding physodes (Wagner *et al.*, 1992). Light of 365 nm was at least twice as effective in raising $[Ca^{2+}]_c$ as 450 nm light, whereas the effect of R was undetectable. The experimental procedure used included pulses of 365 nm light to excite indo-1 to enable measurement. Hence, Russ *et al.* (1991) argued that any small R-induced $[Ca^{2+}]$ changes would have been masked by the strong blue-light effect on calcium release during indo-1 excitation. This is also supported by the finding that fluorescence of chlorotetracycline-strained calcium-binding physodes (CaBP) decreased in a FR-reversible manner after irradiation with R (Wagner *et al.*, 1987). Therefore the slow R-induced activation of a calcium-dependent potassium channel (Lew *et al.*, 1990a,b) could be a consequence of a minor R-mediated calcium release from the CaBP, which leaves the microtubules relatively unaffected (see below; Grolig, 1992).

How is the observed light-induced rise in $[Ca^{2+}]_c$ related to the chloroplast movement? In addition to the phenomenon of chloroplast reorientation with respect to the direction of light, the velocity of the movement has also been reported to depend on the light conditions (Haupt, 1982). In accordance with Schönbohm (1987), Russ *et al.* (1991) observed that blue light-mediated movement is faster than that induced by R, and UV-A-mediated movement is the fastest of all. This behaviour matches perfectly with the progressive rise of $[Ca^{2+}]_c$ observed at shorter wavelengths. This conclusion is further strengthened by the observation that both the calcium-channel agonist Bay K-8644 and the calcium ionophore A 23187 increased the velocity of low irradiance chloroplast movement under low irradiance conditions (Russ *et al.*, 1991).

The prominent rise of $[Ca^{2+}]_c$ after treatment with 365 or 450 nm light is reflected by changes in the mechanical properties of the cytoplasm (Weisenseel, 1968; Schönbohm and Hellwig, 1979). Schönbohm (1987) found an immediate decrease of chloroplast anchorage in high irradiance blue light, in contrast to a slow increase of chloroplast anchorage in R. Similarly, Weisenseel (1968) has shown that *Mougeotia* cells kept at low temperature retain the capacity to carry out reorientational movement in blue light, but not in R. Finally, Kraml *et al.* (1988) reported a considerable increase of the velocity of phytochrome-mediated chloroplast movement, if cells of the closely related alga *Mesotaenium* were irradiated with blue light in addition to R. All these light-mediated changes in cytoplasmic viscosity could be taken as an indication for the existence of a cytoskeletal network which resists chloroplast repositioning under R, low $[Ca^{2+}]_c$ conditions, but "loosens up" under blue light, high $[Ca^{2+}]_c$ conditions.

Microtubules in various systems are known to depolymerize at high $[Ca^{2+}]_c$ levels (Zhang *et al.*, 1990). In *Mougeotia* interphase cells, the microtubules are located just beneath and in close contact to the plasma membrane (Foos, 1970; Galway and Hardham, 1986). It is not known to what extent this feature interacts with the observed $[Ca^{2+}]_c$ changes *in vivo*, but in "ghosts" of *Mougeotia* protoplasts membrane-bound microtubules are remarkably stable and depolymerize at high $[Ca^{2+}]$ only after pretreatment with Triton X-100 (Kakimoto and Shibaoka, 1986). The microtubule-depolymerizing agents colchicine, nocodazole and vinblastine all accelerate R-mediated chloroplast movement by a factor of 2, while taxol, a microtubule-polymerizing agent, retards movement (Serlin and Ferrell, 1989).

The data on the involvement of calmodulin discussed above led Russ *et al.* (1991) to suggest that a physically- or chemically-induced release of Ca^{2+} from the calcium-binding "vesicles"/physodes, may cause a local calmodulin-mediated (Wagner *et al.*, 1984; Gratzer and Baines, 1987) microtubule depolymerization and this in turn could lead to a decrease in viscosity in the cortical region of *Mougeotia* cells (Grolig, 1992; Wagner *et al.*, 1992). One ecological function for the faster chloroplast reori-

entation under UV-A and blue light could be the necessity to prevent damage of the photosynthesis apparatus as a result of sudden intense irradiation (Nultsch *et al.*, 1981).

Summarizing, the hypothesis that the role of calcium in the light-induced chloroplast reorientation (Wagner and Klein, 1981; Grolig and Wagner, 1988), typically occurring in *Mougeotia* and *Mesotaenium*, is not part of the signalling process has been strengthened by recent research. Probably, the main function of calcium is to establish cytoskeletal competence in the amazingly thin cell cortex of 0.2–0.3 μm enabling rapid response when a signal is dispatched by an incipient beam of light (Wagner *et al.*, 1992). Except for calmodulin, the cytoskeletal components and regulatory factors that are involved in establishing competence remain unknown. However, microtubules are good candidates to play a role since their polymerization/depolymerization behaviour to modifications in $[Ca^{2+}]_c$ is compatible with the observed changes in cytoplasmic viscosity accompanying chloroplast reorientation.

Germination of fern spores and Spirodela *turions*

Germination of many unicellular non-green (*Adiantum capillus-veneris* L., *Dryopteris paleacea* SW.) and green (*Onclea sensibilis* L.) fern spores and multicellular *Spirodela polyrhyza* L. Schleiden turions is stimulated by light.

Low fluence R stimulates germination of *Onclea sensibilis* spores and FR applied immediately after R reverses the effect (Wayne and Hepler, 1984). Red-light stimulation of fern-spore germination has only been observed in the presence of Ca^{2+}: 3 μ*M* of this cation stimulates 50% germination of R-irradiated spores. Phytochrome-stimulated germination of *Onoclea* species occurs even if Ca^{2+} is added to the medium 8 h after R irradiation. A longer interval betwen the R pulse and application of Ca^{2+} results in a rapid decline in germination. The calcium ionophore, A 23187, also stimulates spore germination of *Onoclea* in darkness in the presence of Ca^{2+}. The calcium-channel blockers La^{3+} and Co^{2+}, and calmodulin inhibitors TFP and CPZ all inhibit the R-induced, Ca^{2+}-dependent spore germination of *Onoclea* (Wayne and Hepler, 1984).

By means of atomic-absorption spectroscopy, Wayne and Hepler (1985) have shown that about 5 min after the onset of R irradiation there is an increase in $[Ca^{2+}]_c$ in hydrated *Onoclea* spores. They also showed that La^{3+} (300 μ*M*) added to the medium before a R pulse completely prevents Ca^{2+} uptake.

Iino *et al.* (1989) studied the effect of R on the cell cycle progression and DNA content of germinated non-green *Adiantum capillus-veneris* spores. The first step in germination of G_0-stopped (G_0 = part of the interphase of the cell cycle) spores is stimulation of DNA synthesis during the S phase of the cell cycle and subsequent cell division. Spores remain quiescent when hydrated in darkness in a solution containing high (1 m*M*) or low (32 n*M*) $[Ca^{2+}]$. Moreover, a gradual decrease in DNA content was observed, especially in spores incubated in a low $[Ca^{2+}]$ medium. A R pulse stimulated DNA increase in spores incubated in a high $[Ca^{2+}]$ medium. Spores were most sensitive to a R pulse (10 min, fluence 3×10^3 J m^{-2}) after 2 days of dark hydration in a medium containing 1 m*M* $CaCl_2$. An increase in DNA ranging from 60 to 100% was observed 50–70 h after the R pulse. The highest increase in DNA content was found when Ca^{2+} was added to the medium immediately after the R pulse. However, R resulted in an increase in the DNA content even if the 1 m*M* Ca^{2+} was delayed by 15 h. Longer delays result in the R-inductive effect being progressively lost (Iino *et al.*, 1989). A FR pulse (10 min, fluence 10^3 J m^{-2}) after R almost completely prevented the DNA increase, even if it was applied 10 h after the R pulse. After this time FR was less effective (Iino *et al.*, 1989).

The effect of phytochrome and Ca^{2+} on germination of *Dryopteris paleacea* spores has been extensively studied by Scheuerlein and colleagues (Dürr and Scheuerlein, 1990; Scheuerlein *et al.*, 1988, 1989, 1991). In non-green *Dryopteris* spores the first visible evidence of germination is the appearance of chlorophyll synthesis. Using an epifluorescence microscope or a spectroscopic determination of extracted chlorophyll it is possible to determine the first step of spore germination 2 days after light treatment (Scheuerlein *et al.*, 1988). Both *in vivo* and *in vitro* determined chlorophyll synthesis occurs only if Ca^{2+} is present in the surrounding medium (Scheuerlein *et al.*, 1988). The half-maximal response was observed in a medium containing 3 μ*M* free Ca^{2+} and the optimum was reached around 100 μ*M*. Red light-stimulated germination of *Dryopteris* occurred if Ca^{2+} was added during the first 30 h after the light treatment. After longer periods Ca^{2+} was less effective in stimulating germination and 48 h after a R pulse no significant response was observed, compared to the Ca^{2+}-free control (Dürr and Scheuerlein, 1990). During this period (from 30 to 50 h after R treatment) Ca^{2+} must be present in the medium for at least 9 h for maximal response (Dürr and Scheuerlein, 1990). A second R pulse applied 24 or 42 h after the first pulse increased the response to Ca^{2+}, as well as shifting sensitivity to this cation during prolonged dark intervals (Dürr and Scheuerlein, 1990). Polyethylene glycol (PEG), which is thought to inhibit the action of Pfr (Psaras and Haupt, 1989), also delayed the Ca^{2+} requirement by approx. 30 h. Red light-stimulated germination of *Dryopteris* spores can be completely reversed by a subsequent FR treatment given up to 10 h after a R pulse. Spores irradiated with R reached 50% escape from reversi-

bility after 22 h incubation (Dürr and Scheuerlein, 1990). Red light-induced Ca^{2+}-dependent *Dryopteris* germination was significantly reduced by addition of 10 μ*M* or higher La^{3+} to the medium; germination was completely blocked at 10 m*M* La^{3+} (Scheuerlein *et al.*, 1989).

Dormant turions of *Spirodela polyrhyza* after a cold after-ripening period can be stimulated to germinate by activation of phytochrome (Appenroth *et al.*, 1989). Red light, followed by a few days of darkness, results in germination of *Spirodela* turions, whereas FR has no effect (Appenroth and Augsten, 1990; Appenroth *et al.*, 1990). Far-red light fully reverses the R effect if given during the first day after R irradiation, after which its effectiveness gradually decreases. Red light-induced turion germination occurs only when Ca^{2+} is present in the medium. The half-maximal response for Ca^{2+} was observed at 16 ± 2 μ*M* (Appenroth *et al.*, 1990). The presence of Ca^{2+} before or during the first 12 h after R irradiation had no effect on turion germination. The highest sensitivity of *Spirodela* to Ca^{2+} was observed on the second day after R, after which there was a sharp decrease in response (Appenroth and Augsten, 1990).

Treatment of etiolated *Spirodela* with the calcium *ionophore* A 23187 (1 μ*M*) for 24 h stimulated germination of about 25% of turions in the presence of 1 m*M* $Ca(NO_3)_2$. Both shorter or longer treatment of turions incubated in a medium containing Ca^{2+} and A 23187 had no effect on their germination (Appenroth *et al.*, 1990). Inhibition of Ca^{2+} uptake produced by calcium-influx blockers: La^{3+}, Co^{2+} and Mn^{2+} influence turion germination to different degrees. Whereas La^{3+} and Co^{2+} nullify the R-inductive effect, Mn^{2+} was much less effective. Lanthanium ions were virtually ineffective if added to the medium containing Ca^{2+} during the first 24 h after a R pulse. Verapamil, another calcium-channel blocker, at concentrations higher than 100 μ*M* decreases the R-induced, Ca^{2+}-dependent response (Appenroth *et al.*, 1990). However, calmodulin inhibitors TFP and CPZ were more inhibitory at concentrations one order of magnitude lower than verapamil (Appenroth *et al.*, 1990).

Seed germination

Phytochrome controls germination of seeds of many plant species. Light can promote or inhibit germination and such seeds are referred to as positively and negatively photosensitive seeds, respectively (Cone and Kendrick, 1986).

Presence of Ca^{2+} (1 m*M*) or EGTA (1 m*M*) in the incubation medium had no effect on germination of negatively photosensitive seeds of *Phacelia tanacetifolia* Benth. cv. Blue Clai in darkness. However, in the presence of A 23187 (10 μ*M*) and EGTA a 50% reduction of germination was observed, whereas a calmodulin antagonist, calmidazolium (10 μ*M*) nearly completely stopped this process (Cocucci and Negrini, 1991), indicating that activation of calmodulin is a requirement for germination. In addition, Ca^{2+} or EGTA in the presence or absence of A 23187 failed to affect photoinhibition of germination of *P. tanacetifolia* (Cocucci and Negrini, 1991).

In the case of many species gibberellins (GA) can overcome the requirement of seeds for light (Hilhorst, 1990, and reference therein). For example, germination of *Sisymbrium officinale* (L.) Scop. is stimulated by R or GA in darkness. It was concluded that Pfr increases the rate of GA synthesis and/or the sensitivity of seeds to GA (Hilhorst, 1990). Similar interaction between Pfr and GA has been also observed during germination of the fern spores *Lygodium japonicum* (Kagawa and Sugai, 1991). In both cases the authors concluded that Ca^{2+} may be a second messenger in phytochrome-controlled germination. Participation of Ca^{2+} in GA-stimulated synthesis and secretion of some hydrolases (occur during the early stages of germination) has been demonstrated with aleurone cells of barley seeds (caryopses) (Bush and Jones, 1988 and references therein).

The oat seedling as a model system

Aerial tissues of etiolated oat seedlings are one of the richest sources of phytochrome, from which it has been isolated and biochemically characterized (Vierstra and Quail, 1986). The structure and regulation of phytochrome genes in this species are well documented (Quail *et al.*, 1987), but its photomorphogenesis, although often studied, is less well understood.

Phytochrome controls membrane permeability to Ca^{2+} and its subcellular distribution in etiolated oat cells. Hale and Roux (1980), using the chromometallic dye murexide, found that R induces an efflux of Ca^{2+} from etiolated oat protoplasts to the surrounding medium. Far-red light reversed the R effect resulting in a decrease in external $[Ca^{2+}]$. Furthermore, R and FR had no effect on membrane permeability to Ca^{2+} in protoplasts isolated from green (light-grown) oat-leaf tissues.

Tretyn (1987) studied the effect of phytochrome on $^{45}Ca^{2+}$ accumulation by etiolated oat coleoptile sections and found that R irradiation stimulates its uptake. Far-red light not only nullified the effect of R, but decreased the rate of $^{45}Ca^{2+}$ accumulation compared to the dark control. Red-light stimulation of Ca^{2+} uptake by sections of etiolated oat coleoptiles was decreased by about 43 and 52% by the calcium channel blockers verapamil (100 μ*M*) and La^{3+} (100 μ*M*), respectively (Tretyn, 1987).

The effect of phytochrome on free $[Ca^{2+}]_c$ in oat cells was studied by Chae and co-workers (1990) using the acetoxymethyl esterified form of the Ca^{2+} fluorescent-indicator dye, quin-2 (quin-2/AM). In

non-irradiated protoplasts isolated from etiolated oat seedlings the $[Ca^{2+}]_c$ was 30 n*M*. A 5-min R pulse caused an increase of $[Ca^{2+}]_c$ to 193 n*M*. Subsequent irradiation with FR almost completely reversed the R effect decreasing $[Ca^{2+}]_c$ to 48 n*M* (Chae *et al.*, 1990). Furthermore, a FR pulse given in the presence of EGTA in the surrounding medium decreased $[Ca^{2+}]_c$ to 3 n*M*.

Red-light irradiation affects the sterol induced increase in $[Ca^{2+}]_c$ in oat protoplasts (Yeo *et al.*, 1990). Incoporation of cholesterol in protoplast membranes caused a 58% increase in free $[Ca^{2+}]_c$. The $[Ca^{2+}]_c$ was further increased to 130% when the cholesterol-incorporated protoplasts were irradiated with a R pulse. Sterols such as sitosterol or stigmasterol failed to affect these phytochrome induced changes in $[Ca^{2+}]_c$, but a constant increase in free $[Ca^{2+}]_c$ was observed in protoplasts treated by these compounds and irradiated with R (Yeo *et al.*, 1990).

Red and FR change intracellular Ca^{2+} localization in etiolated oat coleoptile cells. Using a potassium antimonate technique and x-ray microanalysis, Tretyn *et al.* (1991b) found that Ca^{2+} is mainly associated with the outer and inner surface of the plasma membrane of etiolated oat coleoptiles. After exposure of seedlings to R, precipitates of calcium antimonate were additionally observed in cisternae of the endoplasmic reticulum. Oat coleoptile cells exposed to R followed immediately by FR had precipitates visible on the outside of the plasma membrane, within the cell walls and in the vacuoles (Tretyn *et al.*, 1991b).

Red-light irradiation increased the phosphorylation of 15 different proteins in etiolated oat protoplasts in the presence of Ca^{2+} (1 m*M*). When Ca^{2+} was removed from the medium containing protoplasts (with EGTA), the intensity of phosphorylation of these proteins was drastically reduced. Phosphorylation of 2 of the 15 proteins studied was dependent on the wavelength of irradiation (Park and Chae, 1989). Furthermore, the phosphorylation intensity of these 2 proteins (27 and 32 kDa) was especially high after 2–3 min irradiation when the influx of Ca^{2+} into the protoplasts was not blocked. However, FR suppressed the phosphorylation of these polypeptides (Park and Chae, 1989). Photoreversible, Ca^{2+}-dependent *in vivo* phosphorylation of the 27 and 32 kDa proteins from etiolated oat protoplasts appears to be performed by PKC (Park and Chae, 1990). When the PKC inhibitor, H_7, or the inositol phospholipid metabolic blocker, Li^+, were present in the medium containing the protoplasts the phosphorylation of these proteins was substantially reduced. However, the PKC activator, OAG (1-oleoyl-2-acetyl-sn-glycerol) and the tumour promoting phorbol ester, PMA, both increased the phosphorylation of these proteins (Park and Chae, 1990).

Leaf unrolling

In cereal seedlings R induces unrolling of etiolated primary leaf sections. The R-stimulated unrolling of 7-day-old barley (Viner *et al.*, 1988, 1991) or 8-day-old wheat (Tretyn and Kendrick, 1990) etiolated leaf sections is inhibited by their preincubation in 1 m*M* EGTA. Application of 1 m*M* Ca^{2+} during a 30 min treatment period after R irradiation overcomes the EGTA inhibitory effect on leaf unrolling (Tretyn and Kendrick, 1990). Addition of 5 m*M* Ca^{2+} to the EGTA containing medium between 0.5 and 1 h after R partially reduces its inhibitory effect, but when added 2 h after the light treatment was completely ineffective (Viner *et al.*, 1988, 1991). Sensitivity of barley leaf tissues to Ca^{2+} decreased simultaneously with the Pfr level. However, as little as 19% of Pfr formed after a R pulse induced a full leaf-unrolling response (Viner *et al.*, 1988).

The R-induced, Ca^{2+}-dependent increase in apparent leaf width of etiolated wheat was completely nullified by a 10 min pretreatment of tissues in the calcium-channel blocker nifedipine (1 μ*M*). The same concentration of the calcium-channel agonist, Bay K-8644, stimulated unrolling of leaf sections in darkness or after a control FR pulse in medium containing 1 m*M* $CaCl_2$ (Tretyn and Kendrick, 1990).

Leaf movement

Leaf movements of *Mimosa pudica* L., *Albizzia julibrissin* Durraz., *A. lophantha* Benth., *Cassia fasciculata* Michx., *Samanea saman* (Jacq.) Merrill and other plant species are induced by transferring leaves from light to darkness (scotonasty) or from darkness to light (photonasty) (Satter and Galston, 1981, and references therein; Roblin *et al.*, 1989). Phytochrome and the blue light-absorbing photoreceptor appear to be involved in this process. Dark-induced closure of leaflets is under phytochrome control, whereas blue and FR-absorbing pigments control the light-induced opening (Roblin *et al.*, 1989). Inhibition of dark-induced leaflet closure occurs with the addition of EGTA; TMB-8, an intracellular calcium antagonist (Roblin *et al.*, 1990); verapamil; $LaCl_3$; nifedipine (Roblin *et al.*, 1989), the first two of which also reduce light-induced opening of *Cassia* leaflets (Roblin *et al.*, 1990). However, calcium-channel blockers had no effect on movements by light-on signals (Roblin *et al.*, 1989). Compounds such as A 23187 (Roblin and Fleurat-Lessard, 1984), W_7 and CPZ (calmodulin antagonists) (Roblin *et al.*, 1988), inhibited scotonastic, but stimulated photonastic leaflet movements (Roblin *et al.*, 1990). The calcium-channel agonist Bay K-8644 had no effect on light-induced leaflet opening, but significantly increased the rate of dark-induced leaflet closure (Roblin *et al.*, 1989, 1990).

Phytochrome controls nyctinastic movement of *A. lophantha* leaflets (Moysset and Simon, 1989). Depending on the external concentration applied A 23187 or Ca^{2+}, can induce an increase in nyctinastic closure of leaflets. In adequate concentrations they counteract FR-induced leaflet closure (Moysset and Simon, 1989). The intracellular calcium antagonist, TMB-8, inhibits the effect of R, but had no effect on the FR-induced response (Moysset and Simon, 1989).

Flower induction

Halaban and Hillman (1970) found that flowering of *Lemna perpusilla* Torr. grown under an inductive photoperiod on a full nutrient solution with sucrose could be inhibited by transfer of plants to water at particular times during the dark period. The highest degree of inhibition of the process was observed about 1–2 h after the time of maximal sensitivity of *Lemna* to light treatments that influence flowering. Addition of either $Ca(NO_3)_2$ or K_2HPO_4 partially prevents the effect, whereas NH_4NO_3 or $MgSO_4$ increases the degree of inhibition (Halaban and Hillman, 1970).

Recently, Friedman *et al.* (1989) have presented data concerning participation of Ca^{2+} in the phytochrome-controlled flower induction of *Pharbitis nil* CHOIS. This short-day plant can be induced to flower by a single photoinductive dark period. Induction of flowering occurs after pretreatment with 20 m*M* EGTA given 8 h before the dark period. Application of 30 m*M* $CaCl_2$, 30 min after EGTA application completely reverses the inhibitory action of the chelator. Furthermore, plants sprayed with A 23187 at different times from the beginning until 4 h of the subinductive dark period (11 h 45 min) produced more flowers per plant than non-treated controls. However, treatment with $LaCl_3$ (5 m*M*), W_7 (150 μ*M*) and CPZ (200 μ*M*) all reduced the flowering response (Friedman *et al.*, 1989). Tretyn *et al.* (1990b) failed to confirm these results using EGTA, calcium-channel blockers (La^3, nifedipine and verapamil) and the calcium-channel agonist (Bay K-8644). The calmodulin inhibitor CPZ and the inositol phospholipid metabolic inhibitor Li^+ also had no effect.

Interaction Between Phytochrome and Ca^{2+}

In order to characterize the relationship between phytochrome and Ca^{2+}, the Ca^{2+}-sensitive phase after R irradiation has been studied for several responses. Results of these experiments are summarized in Table 1.

Table 1. Investigation of the Ca^{2+}-dependent phase for different phytochrome responses. Different plant systems were irradiated with a red-light pulse in Ca^{2+}-free medium and then Ca^{2+} was supplied at different times after the irradiation. The escape time is the time after which responsiveness to Ca^{2+} addition is lost

Response	Escape time	Reference
Wheat protoplast swelling	< 10 min	Bossen *et al.* (1988)
Barley leaf unrolling	1–2 h	Viner *et al.* (1988)
Spirodela turion germination	> 12 h	Appenroth and Augsten (1990)
Fern spore germination		
Onclea sensibilis	< 8 h	Wayne and Hepler (1984)
Dryopteris paleacea	≈ 15 h	Dürr and Scheuerlein (1990)
Adiantum capillus-veneris	15–20 h	Iino *et al.* (1989)

Bossen *et al.* (1988) showed that to obtain a full swelling response of etiolated wheat protoplasts, Ca^{2+} had to be supplied to the medium within the first 10 min after a 1 min saturating R pulse. In the case of etiolated barley leaf sections, maximum unrolling occurred if Ca^{2+} was supplied to the bathing solution up to 2 h after a R inductive pulse (Viner *et al.*, 1988). Experiments which were performed with *Spirodela* turions showed that Ca^{2+} only stimulates germination when applied during the Pfr-requiring phase (Appenroth and Augsten, 1990). The most Ca^{2+}-sensitive period was found to be between 24 and 48 h after a R pulse. Independent of the delay between a R pulse and a FR pulse to remove Pfr, only a negligible response to Ca^{2+} addition was observed (Appenroth and Augsten, 1990). Interaction between phytochrome and Ca^{2+} observed during swelling of wheat protoplasts (Bossen *et al.*, 1988), unrolling of barley leaf sections (Viner *et al.*, 1988), and germination of *Spirodela* turions (Appenroth and Augsten, 1990) differed from that recorded for R-induced fern spore germination (Wayne and Hepler, 1984; Iino *et al.*, 1989; Scheuerlein *et al.*, 1989). Wayne and Hepler (1984) studied the relationship between phytochrome photoconversion and Ca^{2+} transport. Red light-stimulated germination of *Onoclea* occurs even if Ca^{2+} was added to the medium up to 8 h after irradiation. In the absence of Ca^{2+}, FR partially reversed the inductive effect of R, but only when given up to 5 min after R. The ability of FR to reverse the inductive R effect was not observed when Ca^{2+} was present at the time of R irradiation (Wayne and Hepler, 1984). Phytochrome phototransformation clearly does not require Ca^{2+}. The first cell division of *Adiantum* spores is induced by R even in the absence of Ca^{2+}. A R-induced DNA increase in the spores was unaffected even if a saturating concentration of Ca^{2+} was added 15 h after R (Iino *et al.*, 1989). Much longer retention of the capacity to respond to Ca^{2+} after phytochrome photoconversion was observed by Scheuerlein *et al.* (1989) for R-induced germination of fern spores

Table 2. The effect of red-light *via* phytochrome on Ca^{2+} influx/efflux into/from different plant cells or on the intracellular free $[Ca^{2+}]$ ($[Ca^{2+}]_0$) measured by different analytical methods

Method of measurement	Plant material	Effect	Reference
Murexide—spectrophotometry	*Mougeotia* protoplasts	Efflux	Roux (1984)
	Vallisneria protoplasts	Efflux	Takagi and Nagai (1988)
	Oat protoplasts	Efflux	Hale and Roux (1980)
	Wheat protoplasts	Efflux	Bossen (1990)
Autoradiography	*Mougeotia* cells	Influx	Dreyer and Weisenseel (1979)
Atomic absorption spectroscopy	*Onoclea* spores	Influx	Wayne and Hepler (1985)
$^{45}Ca^{2+}$ uptake studies	Maize protoplasts	Influx	Das and Sopory (1985)
	Oat coleoptile sections	Influx	Tretyn (1987)
	Mesotaenium protoplasts	Influx	Berkelman and Lagarias (1990)
	Barley protoplasts	Influx	U *et al.* (1991)
Quin-2—fluorimetry	Oat protoplasts	Increase $[Ca^{2+}]_c$	Chae *et al.* (1990)
Fura-2—fluorimetry	*Dryopteris* spores	Decrease $[Ca^{2+}]_c$	Scheuerlein *et al.* (1991)

of *Dryopteris paleacea.* In this case there was no germination induction when Ca^{2+} was added to the medium as late as 40 h after R. Moreover, Ca^{2+} addition was fully effective 24 h after R treatment, when the spores had escaped from FR reversibility (Scheuerlein *et al.*, 1989).

Phytochrome control of Ca^{2+} fluxes

Phytochrome controls membrane permeability to different ions, including Ca^{2+} (Kendrick and Bossen, 1987). Results concerning the influence of R on Ca^{2+} fluxes are contradictory (Table 2). Dreyer and Weisenseel (1979) showed by means of autoradiography that R stimulates $^{45}Ca^{2+}$ accumulation into the cells of the green alga *Mougeotia*. A few years later Wayne and Hepler (1985) using atomic absorption spectroscopy discovered that R stimulates an increase in the total $[Ca^{2+}]_c$ of *Onoclea* spores. Both authors showed that subsequent exposure to FR nullifies R-induced Ca^{2+} accumulation by both *Mougeotia* and *Onoclea* cells. Stimulation of $^{45}Ca^{2+}$ uptake by R has been confirmed by a number of authors for etiolated maize (Das and Sopory, 1985), barley (U *et al.*, 1991) protoplasts, *Mesotaenium* protoplasts (Berkelman and Lagarias, 1990), and etiolated oat coleoptile section cells (Tretyn, 1987). In all these plant systems FR, applied after a R pulse, significantly reduces the R stimulation of $^{45}Ca^{2+}$ uptake.

Results which contradict the above cases have been obtained on the basis of studies using the Ca^{2+}-sensitive dye, murexide. Hale and Roux (1980), Roux (1984), Takagi and Nagai (1988) and Bossen (1990) have all shown that R stimulates an efflux and FR results in influx of Ca^{2+} into the cells of etiolated protoplasts of oat, *Mougeotia* sp., *Vallisneria gigantea* Graebner and wheat (Table 2).

There are a limited number of studies on phytochrome control of $[Ca^{2+}]_c$. Using an acetoxymethyl ester of the Ca^{2+} fluorescent indicator dye, *quin-2* (quin-2/AM) Chae *et al.* (1990) have found that 5 min R irradiation of etiolated oat protoplasts results in an approximately 5-fold increase in $[Ca^{2+}]_c$, while a subsequent irradiation with 5 min FR leads to a significant reduction in $[Ca^{2+}]_c$. However, Scheuerlein *et al.* (1991) utilizing a different fluorescent calcium indicator dye, fura-2, discovered that in germinated spores of *Dryopteris*, R leads to a decrease in $[Ca^{2+}]_c$ in comparison to non-irradiated cells. Since these measurements were performed 20 h after R treatment it is difficult to say if observed changes in $[Ca^{2+}]_c$ in individual *Dryopteris* spores represent primary or secondary effects.

The question as to whether R and FR directly influence intracellular free Ca^{2+} is still unanswered. However, it is clear that phytochrome can change membrane permeability to Ca^{2+} ions. In our opinion, results of experiments performed with murexide are rather difficult to interpret. Murexide does not penetrate plasma membrane and the results only show net changes of Ca^{2+} in the surrounding medium. From studies with Ca^{2+} fluorescent-indicator dyes it is known that the presence of chelating agents of this ion in the medium decrease $[Ca_{2+}]_c$ of cells maintained in darkness (Scheuerlein *et al.*, 1991) or irradiated with R (Chae *et al.*, 1990; Scheuerlein *et al.*, 1991). Presence of membrane impermeable chelators of murexide in the surrounding medium can lead to a non-physiological efflux of the Ca^{2+} from the cytoplasm to the external medium. Red light-stimulated increase of membrane permeability to Ca^{2+} may increase its leakage from the cytoplasm to the medium.

In etiolated plant cells $[Ca^{2+}]_c$ is in the same concentration range as in non-stimulated animal cells (Chae *et al.*, 1990; Scheuerlein *et al.*, 1991)

and increases significantly after R irradiation (Chae *et al.*, 1990). In animal cells different stimuli change $[Ca^{2+}]_c$ *via* an increase or decrease of membrane permeability for extracellular Ca^{2+}. Membrane fluxes of this ion are regulated by two well defined elements: calcium channels (Tsien and Tsien, 1990) and calcium pumps (Schatzman, 1989). There is some evidence pointing to the existence of similar elements in the membranes of plant cells. The presence and molecular properties of different plant ion channels including those for Ca^{2+} have been reviewed recently by Sanders and Slyman (1989) and Tester (1990). Influx of Ca^{2+} into many plant cells is modified by different compounds which inhibit or stimulate, in a manner consistent with the operation of particular types of calcium channels similar to those in animals (Tester, 1990). Some of these agents can influence Ca^{2+}-dependent photomorphogenetic responses. Wayne and Hepler (1985) showed that La^{3+} inhibits the R-induced increase in intracellular calcium in the spores of *Onoclea sensibilis*. Stenz and Weisenseel (1986) found that the R-induced reduction of the *Zeta*-potential of the green alga *Mesotaenium* disappeared in media with a low $[Ca^{2+}]$ or after adding the calcium-channel blocker, verapamil. Both La^{3+} and verapamil decrease the rate of $^{45}Ca^{2+}$ uptake by cells of oat etiolated coleoptile sections (Tretyn, 1987). Takagi and Nagai (1988) have reported that three different calcium-channel blockers, La^{3+}, nifedipine, and verapamil, result in the cessation of phytochrome-controlled cytoplasmic streaming in *Vallisneria gigantea* Graebner protoplasts. Calcium-dependent streaming is induced only when phytochrome exists in the Pfr form (Takagi *et al.*, 1990). All three of the above-mentioned calcium-channel blockers inhibited phytochrome-mediated movements of *Cassia fasciculata* leaflets (Roblin *et al.*, 1989, 1990). Lanthanium ions inhibit the phytochrome-controlled germination of *Onoclea sensibilis* (Wayne and Hepler, 1984), *Dryopteris paleacea* fern spores (Scheuerlein *et al.*, 1989) and *Spirodela polyrha* turions (Appenroth *et al.*, 1990).

Both Ca^{2+}-dependent, phytochrome-stimulated swelling of etiolated wheat protoplasts (Bossen *et al.*, 1988; Tretyn *et al.*, 1990c) and unrolling of etiolated wheat leaves (Tretyn and Kendrick, 1990) is nullified by verapamil, La^{3+}, and nifedipine, a DHP derivative. Nifedipine was only active when it was added to the medium containing protoplasts before a R pulse (Tretyn *et al.*, 1990c). In contrast, another DHP derivative, Bay K-8644, well known as an antagonist of "L-type" calcium channels (Tsien and Tsien, 1990), stimulates wheat protoplast swelling (Tretyn *et al.*, 1990c) and leaf unrolling (Tretyn and Kendrick, 1990) after control FR irradiation. Furthermore, Bay K-8644 promotes phytochrome-mediated *Cassia fasciulata* leaf movement (Roblin *et al.*, 1989, 1990) and increases the velocity of the R-mediated *Mougeotia scalaris* Hassel chloroplast rotation (Russ *et al.*, 1991). Most authors agree that phytochrome regulates transmembrane Ca^{2+} fluxes (Hepler and Wayne, 1985; Moysset and Simon, 1989; Roux *et al.*, 1986; Stenz and Weisenseel, 1986; Takagi and Nagai, 1988; Takagi *et al.*, 1990; U *et al.*, 1991; Wayne and Hepler, 1984, 1985; Weisenseel and Ruppert, 1977) *via* regulation of calcium-channel activity (Bosen, 1990; Bossen *et al.*, 1988; Roblin *et al.*, 1989; Tretyn, 1987; Tretyn and Kendrick, 1990; Tretyn *et al.*, 1990a). Tretyn *et al.* (1990c) have proposed that phytochrome controls the activity of DHP-sensitive "L-type" calcium channels.

Unlike calcium channels there is direct evidence concerning the effect of phytochrome on calcium pumps (Ca^{2+}-translocating ATPase). Calcium pumps have been identified and biochemically characterized from tissues of many plant species (Rasi-Caldogno *et al.*, 1989; see Briskin, 1990, for review). Dieter and Marmé (1981, 1983) demonstrated that activity of the microsomal Ca^{2+} pump is stimulated by calmodulin. However, when vesicles were prepared from FR-irradiated corn seedlings the pump was no longer under the control of calmodulin (Dieter and Marmé, 1981, 1983).

In some plant systems FR irradiation decreases the rate of $^{45}Ca^{2+}$ uptake (Tretyn, 1987; U *et al.*, 1991) and $[Ca^{2+}]_c$ (Chae *et al.*, 1990) compared to dark controls. However, the mechanism of action of the physiologically inactive R-absorbing form of phytochrome (Pr) on these processes remains unclear.

Sources of Ca^{2+}

There are two different Ca^{2+} sources available for utilization by light-stimulated cells: from the extracellular sources and intracellular stores. The concentrations in soil solutions or in "fresh" water are not very high, values of 1 μM Ca^{2+} or less. These ranges of $[Ca^{2+}]$ are sufficient, but not optimal for phytochrome-stimulation of germination of fern spores: *Onoclea* (Wayne and Hepler, 1984), *Dryopteris* (Scheuerlein *et al.*, 1989), *Adiantum* (Iino *et al.*, 1989) or *Spirodela* turions (Appenroth and Augsten, 1990). During plant development Ca^{2+} may accumulate inside particular cells or tissues. For example, in *Onoclea* spores, large amounts of Ca^{2+} have been found within the cell wall (Wayne and Hepler, 1985). Only after depletion of the cell wall-localized Ca^{2+} by EGTA is it possible to demonstrate that phytochrome-controlled fern-spore germination is a Ca^{2+}-dependent process (Wayne and Hepler, 1984; Scheuerlein *et al.*, 1989). Moreover, adequate amounts of the calcium chelator can inhibit other R-stimulated processes: leaf unrolling (Viner *et al.*, 1988; Tretyn and Kendrick, 1990), leaf movement (Roblin *et al.*, 1990) and photoperiodic flower induction (Friedman *et al.*, 1989; Tretyn *et al.*, 1990b). On

these grounds we believe that in many plant systems phytochrome controls movement of Ca^{2+} from the apoplast to the symplast. Experiments on protoplast swelling show that even after removing the cell wall they must be very carefully washed with EGTA solution before being used in an experiment (Bossen *et al.*, 1988; Tretyn *et al.*, 1990b). Cytochemical studies performed on etiolated oat coleoptiles have shown that most of the antimonate precipitable Ca^{2+} is present both in the proximity of the extracellular and intracellular side of the plasma membrane (Tretyn *et al.*, 1991b). In animal cells special protein, so-called "annexin", can take part in binding of Ca^{2+} to the phospholiped components of the plasma membrane cytoplasmic surface. Recently, Clark *et al.* (1991) have found annexin-like protein in pea plumules which has properties similar to that found in animal cells. It appears that plasma membrane-bound calcium moves into the cytosol of the oat coleoptile cells after R irradiation (Tretyn *et al.*, 1991b). After washing the cells with ethylenediaminetetraacetic acid (EDTA) or EGTA it is possible to remove Ca^{2+} bounded to the cell wall and extracellular side of the plasma membrane (Wayne and Hepler, 1985).

Some Ca^{2+}-dependent, light-regulated processes, such as *Mougeotia* chloroplast re-orientation, have a very low $[Ca^{2+}]$ requirement in the surrounding medium. However, special osmiophilic globules are observed inside the cells of this green alga (Grolig and Wagner, 1989). These CaBP are devoid of any membrane-like structures and accumulate large amounts of calcium (Tretyn *et al.*, 1991). A similar function of *Mougeotia* CaBP is shown by the "tannin" vacuoles observed in cortical parenchyma cells of motor organs localized at the leaflet base of *Mimosa* (Toryiama and Jaffe, 1972). The way in which light initiates release of Ca^{2+} from such internal stores is difficult to envisage. Roblin *et al.* (1989) have proposed that R stimulates Ca^{2+} influx from the apoplast into the cells, while blue light can release the Ca^{2+} from its intracellular stores.

The Mechanism of Action of Ca^{2+} in Photomorphogenesis

Light is one of the most important environmental stimuli for bacteria, fungi, animals and plants. Thus nearly all these organisms, during their evolution, have developed mechanisms by which they are able to detect both quantitative and qualitative aspects of the light environment, indicating the direction of irradiation. Moreover, some bacteria and both lower and higher plants utilize light as an energy source in photosynthesis.

The best characterized photoreceptor in animals is rhodopsin (Fung, 1985; Rayer *et al.*, 1990); it has also been found in some bacteria (bacteriorhodopsin) and the green alga *Chlamydomonas* (for review see Wagner and Marwan, 1991). Rhodopsin is a membrane component of rods and cones of the eye retina in vertebrates and in visual cells of invertebrates (Rayer *et al.*, 1990). In the case of *Halobacterium* and *Chlamydomonas* this pigment is also an integrated membrane component (Wagner and Marwan, 1991). Rhodopsin consists of a polypeptide (opsin) to which a carotenoid-derivative chromophore (retinal) is covalently linked (Rayer *et al.*, 1990). The primary structure of opsin in different species has many characteristics in common with all rhodopsins and other membrane receptors. The proteins possess seven transmembrane segments interconnected by loops on both side of the membrane. First and second cytoplasmic loops are the target for a G-protein in animals (Fung, 1985) called trandsducin (G_t). Moreover, in the C-terminal part of the polypeptide of both invertebrates and vertebrates rhodopsin has serine and threonine residues which undergo light-induced phosphorylation (Rayer *et al.*, 1990). After absorption of one photon by rhodopsin, isomerization of the retinal chromophore from 11-*cis* to the all-*trans* configuration is observed (Rayer *et al.*, 1990). In the case of *Halobacterium*, light stimulates isomerization of all-*trans* retinal to the 13-*cis* form of the chromophore (Wagner and Marwan, 1991). Re-isomerization of the chromophore group of rhodopsin is accompanied by conformational changes of the protein (Fung, 1985). In invertebrates the light activated form of rhodopsin (*meta*-rhodopsin) is thermally stable at physiological temperatures and is re-isomerized by light to the physiologically inactive form (Rayer *et al.*, 1990).

Some biochemical and physicochemical properties of rhodopsin are very similar to those of phytochrome. Both pigments consists of protein and chromophore components. Absorption of light by the chromophores of rhodopsin and phytochrome leads to their isomerization (in the case of phytochrome it is 15-*cis*/*trans* isomerization, Rüdiger, 1987). Similar to the retinal rhodopsin chromophore in invertebrates, *cis*/*trans* and *trans*/*cis* isomerization of the phytochrome chromophore by light are accompanied by conformational changes of the protein resulting in the physiologically active Pfr form being converted to the inactive (Pr) form, and *vice versa*. It has been shown by means of monoclonal antibodies that conformational differences between Pr and Pfr extend over the whole length of the molecule (Schneider-Poetsch *et al.*, 1989). However, only certain regions of the phytochrome polypeptide chain undergo photoconversion-induced conformational changes and these are probably involved in phytochrome's mode of action: segments corresponding to 4–10 kDa, 74, 83–84 and 100 kDa from the N-terminus (Quail *et al.*, 1987). Recently, Schneider-Poetsch and coworkers (Schneider-Poetsch and Braun, 1991; Schneider-Poetsch *et al.*, 1991) analysed published phytochrome sequence data and made a comparison with bacterial sensor

proteins responsive to environmental stimuli. They found 23–26% homology for a 240 amino acid segment near to the C-terminus. Both in phytochrome and bacterial sensor proteins this domain undergoes conformational changes which are caused by stimulus of the N-terminal region. By analogy to bacterial sensor protein these workers postulate that phytochrome, after light-stimulated conformational change of the C-terminus, transduces this signal by interactions with additional (not identified) proteins.

Phytochrome and rhodopsin have one important difference. Whereas rhodopsin is a typical integral membrane protein, there is no such evidence for this in the case of phytochrome in plants. Immunocytochemical studies have shown that phytochrome is a cytoplasmic protein. Moreover, analysis of phytochrome polypeptides shows no extended hydrophobic sequences typical of membrane proteins (for review see Jordan *et al.*, 1986; Vierstra and Quail, 1986). This difference between rhodopsin and phytochrome probably reflects the former's single mode of action within the membrane, while phytochrome might interact with several different components within the cell. In fact, in animal (Rayer *et al.*, 1990) and *Chlamydomonas* cells (Harz and Hegemann, 1991) rhodopsin regulates membrane conductance. In contrast to phytochrome, in animals rhodopsin does not take part in the regulation of photomorphogenesis. Similar to phytochrome, bacterial sensor proteins also lack hydrophobic transmembrane regions in the N-terminal region of the polypeptide chain (Schneider-Poetsch *et al.*, 1991).

Signal Transduction Chains

Light absorption by phytochrome is transduced by an unknown cascade which leads to changes in membrane conductance, growth and development. Photoconversion of phytochrome and ultimate photomorphogenetic responses appear to be connected by internal messenger(s). Calcium ions have been proposed as playing this role (Roux *et al.*, 1986) as discussed above. This section reviews data concerning participation of second messengers in phytochrome action.

There are only a limited number of studies about the signal transduction chain involved in phytochrome action. At the present state of knowledge it seems that the process of light transduction which occurs in *Halobacterium*, rod cells of vertebrates and visual cells of invertebrates and in the green alga *Chlamydomonas*, is similar to that observed in many plants (Table 3). The transduction of light stimuli which takes place in invertebrates especially is very similar to that observed during phytochrome action. In invertebrates this involves light activation of a G_t, the target of which is PLC, a rhodopsin kinase and finally Ca^{2+} as the second messenger (Fung, 1985; Rayer *et al.*, 1990). All these elements have also been recognized in plant cells and they probably function in the same way as in animals.

GTP-binding proteins

The G-proteins in animals are a heterotrimer consisting of three distinct polypeptides α ($\approx$40 kDa), β ($\approx$36 kDa) and γ (8 kDa). The α subunit binds GTP and is the activator of target proteins, whereas β and γ subunits form a complex which modulates the activity of the α subunit (Stryer and Bourne, 1986; Gilman, 1987). Furthermore, the α subunit of a G_p can be specifically activated through ADP-ribosylation by bacterial toxins (Stryer and Bourne, 1986; Gilman, 1987).

The presence of a protein which binds [^{35}S]GTP-γ-S has been found in many plant systems (Hasunuma *et al.*, 1987; Blum *et al.*, 1988; Drøbak *et al.*, 1988; Korolkov *et al.*, 1990; Romero *et al.*, 1991b). These proteins were ADP-ribosylated in the presence of cholera toxin (Romero *et al.*, 1991a) and they possess GTPase activity (Korolkov *et al.*, 1990) and were recognized by antibodies raised against a highly conserved peptide of most G_p α-subunits (Blum *et al.*, 1988; Korolkov *et al.*, 1990; Romero *et al.*, 1991b). Furthermore, [^{35}]GTP-γ-S-binding proteins have been isolated from membrane fractions (Hasunuma *et al.*, 1987; Blum *et al.*, 1988; Drøbak *et al.*, 1988; Korolkov *et al.*, 1990). Despite the fact that G-proteins isolated from plants may differ in molecular mass they have similar biochemical and molecular properties to G_p isolated from animal cells (Stryer and Bourne, 1986; Gilman, 1987).

There is limited evidence that G-protein activation is involved in phytochrome action. Bossen and co-workers (1990) have shown that the R-induced, Ca^{2+}-dependent, swelling of etiolated wheat mesophyll protoplasts is nullified by an inhibitor of G-proteins, GDP-β-S. However, an activator of G-proteins, GTP-γ-S, induced swelling of the protoplasts to the same extent as after R, both in darkness and after a control FR irradiation as long as essential Ca^{2+} was present (Bossen *et al.*, 1990).

Hasunuma *et al.* (1987) were the first to report binding of [^{35}S]GTP-γ-S to proteins in extracts of *Lemna paucicostata* 441 containing membrane components. They found that R and FR inhibited GTP-binding to these proteins and proposed that phytochrome (Pfr) may function in the same way as the photoexcited state of rhodopsin in animals. Subsequently, Korolkov *et al.* (1990) published data confirming the presence of a G-protein in plants: the eyespot of *Chlamydomonas reinhardtii*. This protein can take part in light induced and rhodopsin-dependent locomotion of the alga (Korolkov *et al.*, 1990).

Extensive studies of the possible involvement of G-proteins in the phytochrome transduction chain have been performed by Romero and colleagues

Table 3. Comparison of bacterial, animal and plant photoreceptors. (Data from: Fung, 1985; Rayer *et al.*, 1990; Brederoo *et al.*, 1991; Wagner and Marwan, 1991)

	Photoreceptor		Transduction chain			
Taxonomic group	Type	Location	Ca^{2+}	G	IP_3	Location
Bacteria						
Halobacterium	Rhodopsin	Membrane	−	+	ND*	Within the membrane
Animals						
Invertebrate	Rhodopsin	Membrane	+	+	+	Within the membrane
Vertebrate	Rhodopsin	Membrane	+	+	+	In the cytosol
Plants						
Chlamydomonas	Rhodopsin	Membrane	+	+	+	In the cytosol
Other plants	Phytochrome	Cytosol	+	+	+	In the cytosol

*ND = not determined; G = GTP-binding protein; IP_3 = inositol 1,4,5-trisphosphate.

(Romero *et al.*, 1991a,b). They found that compared to FR or the dark control, R increases binding of [^{35}S]GTP-γ-S to a protein fraction isolated from pre-irradiated etiolated oat seedlings. Moreover, *in vitro* binding of GTP-γ-S to G-protein(s) from the oat extract was inactivated by the Pr form of exogenously added purified phytochrome. However, the binding capacity remained fully active when the Pfr form was added to the medium (Romero *et al.*, 1991b). Romero *et al.* (1991a) have also studied the effect of cholera toxin on the chlorophyll *a*/*b*-binding protein *Cab* and phytochrome *Phy* genes. Expression of both these genes is phytochrome-regulated, the former positively and the latter negatively. After 2-h incubation of etiolated oat seedlings in darkness in different doses of cholera toxin, Romero *et al.* (1991a) found that the toxin regulates the expression of *Cab* and *Phy* genes: *Cab* up-, and *Phy* down-regulated.

From studies with animal cells it is well known that activation of G_p leads to an exchange of GDP for GTP bound to their α-subunit. The GTP-bound to the α subunit affects the activity of other effector molecules, that generate intracellular signals. Adenylate cyclase, cGMP phosphodiesterase (cGMP PDE), possibly PLC and phospholipase A_2 (PLA_2) and more than one type of ionic channel have been shown to be direct targets for G proteins (Brown and Brinbaumer, 1990). A single G protein may have several different membrane targets and can be linked to about 70 different receptors, at least 8 different K^+, 1 Na^+ and 2 different Ca^{2+} channels (Brown and Brinbaumer, 1990). In different animal cells they directly regulate voltage and DHP-sensitive Ca^{2+} channels (Brown and Brinbaumer, 1990; Schultz *et al.*, 1990; Trautwein and Hescheler, 1990). The presence of similar channels in etiolated wheat mesophyll protoplasts has been postulated by Tretyn *et al.* (1990c). Furthermore, Bossen *et al.* (1990) have obtained results consistent with the existence of a G-protein in the protoplasts. We believe that, at least in etiolated wheat protoplasts, membrane Ca^{2+} transport can be controlled by a G_p-type system which may be directly bound to a DHP-sensitive L-type channel.

The phosphoinositide cascade

During hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) by membrane-bound PLC, two intracellular signal molecules, *viz.* 1,2-diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3) are generated, which activate PKC, whereas IP_3 mobilizes Ca^{2+} from intracellular stores (Berridge, 1987). The activity of a plasma membrane located PLC can be modulated *via* a PKC-controlled phosphorylation, whereas IP_3 receptors are associated with the endoplasmic reticulum by phosphorylation by a cAMP-dependent protein kinase A (PKA) (Bansal and Majerus, 1990). The breakdown of PIP_2 to DAG and IP_3 appears to be regulated by a G-protein (Stryer and Bourne, 1986; Berridge, 1987).

Phosphoinositides have been isolated from different plant systems (for review see Boss, 1989; Hartmann and Pfaffmann, 1990; Bonner *et al.*, 1991). Furthermore, PLC has been isolated from wheat (Melin *et al.*, 1987) and *Pharbitis nil* (Bonner *et al.*, 1991) membranes. In both cases the enzyme was activated by a low [Ca^{2+}], wheres G_p activators failed to affect PLC activity (Melin *et al.*, 1987; Bonner *et al.*, 1991). In *Samanea* leaflets, rapid light-induced changes have been shown in the concentration of inositol 1-phosphate (IP_1) and phospholipids (Morse *et al.*, 1987, 1990). The very convenient system, moss protonemata, has been used for a study on phytochrome-controlled signal transduction in lower plants (for review see Hartmann and Pfaffmann, 1990). Phototropic response of *Ceratodon purpureus* (Hedw.) Brid. protonemata is regulated *via* phytochrome. A 5 min pulse of R increased the activity of PLC in dark-grown cells, whereas FR after R reversed the effect to the level of the dark control. Furthermore, R increased both the rate of PIP_2 degradation and the levels of inosi-

tol 1,4-bisphosphate (IP_2) and IP_3. Lithium ions (inhibitor of inositol-1-phosphatase) decreased the level of phosphatidylinositol (PI), and increased the endogenous levels of IP_1, IP_2 and IP_3 in R-preirradiated protonemal tissues (Hartmann and Pfaffmann, 1989). Recently, Bossen *et al.* (1990) found that Li^+ and neomycin, an inhibitor of PLC, inhibit both R- and GTP-γ-S induced swelling of protoplasts while having no effect on the FR-irradiated phytochrome-controlled phototropic response of *Ceratodon purpureus* (Hartmann and Pfaffman, 1989). In the latter case, Li^+ (10^{-3} *M*) was only active if it was present during or after R irradiation (Hartmann and Pfaffmann, 1989). Lithium ions also inhibited another phytochrome-regulated process: R-induced chloroplast reorientation in *Mougeotia scalaris* (Wagner *et al.*, 1989).

Phytochrome and proteins phosphorylation

Since the discovery of cAMP-dependent PKA in 1968, reversible phosphorylation of proteins has been shown to be the major mechanism for the regulation of protein function in animal cells (for review see Kikkawa and Nishizuka, 1986). As in animals, reversible protein phosphorylation also plays a regulatory role in the transduction of many different extracellular signals in plants (for review see Ranjeva and Boudet, 1987; Poovaiah *et al.*, 1987; Blowers and Trewavas, 1989), including light (for review see Singh and Song, 1990).

A calcium-dependent protein kinase has been found in soluble as well as membrane fractions of many plant systems (Anderson, 1989; Blowers and Trewavas, 1989). Datta *et al.* (1985) have shown the R, Ca^{2+}-dependent phosphorylation of three different proteins: 47, 64 and 77 kDa. Far-red irradiation, calcium chelators and calmodulin inhibitors nullify these phosphorylations in isolated pea nuclei. From the same material a polyamine-stimulated protein kinase was found and purified to 90% homogeneity (Li and Roux, 1991). To date nothing is known about phytochrome regulation of this enzyme. Phytochrome-controlled dephosphorylation of two proteins (29 and 30 kDa) and phosphorylation of another (30 kDa) in etiolated oat coleoptile has been described (Otto and Schäfer, 1988), as well as phosphorylation of two proteins (27 and 32 kDa) in etiolated oat protoplasts (Park and Chae, 1989). Phosphorylation of the 27 and 32 kDa proteins from oat protoplasts was substantially reduced both by the PKC inhibitor (H_7) and Li^+, whereas the activators of PKC, OAG and PMA, both enhanced phosphorylation of these proteins. From their *in vivo* studies Park and Chae (1990) proposed that phytochrome action in oat protoplasts is associated with a PKC-dependent protein phosphorylation. Participation of PKC in regulation phytochrome-controlled processes has also been postulated by Bossen *et al.* (1990) and Haas *et al.* (1991). Bossen *et al.* (1990) have shown that H_7 inhibits the R-induced swelling of etiolated wheat protoplasts. However, PMA induced swelling of the protoplasts after control FR irradiation (Bossen *et al.*, 1990). Staurosporine, an inhibitor of PKC significantly decreases phytochrome-mediated, Ca^{2+}-dependent germination of *Dryopteris filix-mas* L. spores (Haas *et al.*, 1991). The presence of a protein kinase activity associated with the phytochrome molecule itself has been a matter of much discussion. Wong *et al.* (1986, 1989) have shown that purified phytochrome preparations possess protein kinase activity. However, Kim *et al.* (1989) and Grimm *et al.* (1989) have shown that the phytochrome-associated protein kinase activity is due to a protein which co-purifies with phytochrome. Despite this discrepancy, the fact that phytochrome co-purifies with a protein kinase may suggest that these proteins have affinity with each other. The phytochrome molecule itself could be a substrate for protein kinases. Wong *et al.* (1986) found that mammalian PKA phosphorylates both Pr and Pfr, whereas PKC intensively phosphorylates the Pr form. Wong *et al.* (1990) and McMichael and Lagarias (1990) have demonstrated that an *in vitro* polycation-stimulated protein kinase phosphorylates the serine rich N-terminus of purified oat phytochrome.

Using isolated nuclei from etiolated oat seedlings Romero *et al.* (1991a) have studied the mechanism of phytochrome-regulated protein phosphorylation. By means of Western blot analysis and antibodies against animal G-protein at least two G-proteins (24 and 75 kDa) were discovered in the etiolated oat seeding nuclei. Cholera toxin, which stimulates ADP-ribosylation of a G-protein, enhances phosphorylation of two proteins (10 and 54 kDa) and inhibits phosphorylation of two others (60 and 75 kDa). The phosphorylation of 60 and 75 kDa proteins was also stimulated by R. Addition of GDP-γ-S to the nuclei preparations stimulated phosphorylation of a 60-kDa protein, however, the phosphorylation of three other proteins were inhibited. The increase in GDP-γ-S-induced phosphorylation of the 60-kDa protein was reduced by FR irradiation of oat nuclei (Romero *et al.*, 1991a). Participation of plant nuclear protein phosphorylation in light-regulated gene expression has been postulated (Datta and Cashmore, 1989). At least in the oat system this process could be regulated by a G-protein signal transduction cascade (Romero *et al.*, 1991b).

Calmodulin–calcium binding protein

The universal role of calmodulin, in Ca^{2+}-dependent, phytochrome-controlled processes was pointed out by Roux *et al.* (1986). Since that time, many new results concerning participation of calmodulin in phytochrome action have been published.

Calmodulin has been found in all plants investi-

gated so far. It is low-molecular mass (16.7 kDa) acidic, calcium-binding protein (for review see Allan and Hepler, 1989). This highly structurally and functionally conserved protein throughout the plant and animal kingdom is activated by calcium. Binding of Ca^{2+} by the protein induces its conformational change. Activated calmodulin can bind to many target proteins and modulates their activity (for review see Allan and Hepler, 1989).

Calmodulin has been found in *Mougeotia* (Wagner *et al.*, 1984) and oat (Biro *et al.*, 1984), two model plant systems for studying the mechanism of phytochrome action. There are many results concerning the effect of calmodulin antagonists on photomorphogenesis in plants. The calmodulin antagonists TFP (Wagner *et al.*, 1984, 1987) and W_7 (Serlin and Roux, 1984) inhibit R-induced chloroplast reorientation in the cells of the green alga *Mougeotia*. The calmodulin inhibitors CPZ and TFP inhibited Ca^{2+}-dependent, phytochrome-controlled germination of spores of *Onoclea sensibilis* (Wayne and Hepler, 1984) and germination of *Spirodela polyrhiza* turions (Appenroth *et al.*, 1990). Chlorpromazine and W_7 modified phytochrome-regulated movement of *Cassia fasciculata* leaflet (Roblin *et al.*, 1988, 1990) and reduced the flowering response in the photoperiodic induced *Pharbitis nil* seedlings (Friedman *et al.*, 1989). Recently, Lam *et al.* (1989) published data suggesting involvement of calmodulin in the regulation of light-induced expression of *Cab* genes.

The Alternative Pathway: Adenylate Cyclase and Cyclic-AMP

Hormonal regulation of intracellular cAMP levels represents one of the major mechanisms of hormonal action in eukaryotic cells. The level of cAMP is regulated through modulation of AC. The activity of this enzyme is under the control of a G-protein (Northup, 1985). Adenylate cyclase is activated by G_s (S = stimulatory for AC) and inhibited by G_i (i = inhibitory for AC) (Gilman, 1987). Both classes of G-proteins differ from each other in the structure of the α-subunit (Northup, 1985; Gilman, 1986). Besides cAMP and AC, cyclic nucleotide phosphodiesterase (cAMP PDE) and PKA, which catalyze the transfer of the γ-phosphoryl group of ATP to the seryl or threonyl residues of its protein substrate, are elements of the AMP system.

Since the review by Amrhein (1977) there is no up-to-date review of the metabolism and the mode of action of cAMP in plants. However, Song and coworkers (Kim *et al.*, 1986; Chung *et al.*, 1988; U *et al.*, 1991) have shown that cAMP can mimic R in stimulating swelling of etiolated oat (Kim *et al.*, 1986; Chung *et al.*, 1988) and barley protoplasts (U *et al.*, 1991). A similar effect of cAMP on the swelling of etiolated wheat protoplast has been described by Bossen *et al.* (1990). Red light and the calcium ionophore A 23187 not only result in protoplast swelling but both factors also increase protoplast cAMP level, whereas EGTA had no effect on cAMP (U *et al.*, 1991).

Recently, Hahn and Song (1991) have shown that in etiolated oat seedlings R suppresses and stimulates the AC and GTPase activity, respectively. Far-red light reverses the R effect suggesting participation of phytochrome in the regulation of the activity of both enzymes in oats. Furthermore, they found that cholera toxin (by catalysis of ADP-ribosylation of a G-protein) completely blocked GTPase activity, having only a small effect on AC. These authors suggest that in etiolated oat seedlings phytochrome controls a G_s of G-protein.

The mechanism of action of cAMP in phytochrome-regulated signal transduction is unclear. U *et al.* (1991) have suggested that in etiolated barley protoplasts photoactivation of phytochrome by R may lead to an increase in $[Ca^{2+}]_c$, and subsequently to an increase in cAMP level. As mentioned above, protoplast swelling appears to be controlled by a G_p-type system (Bossen *et al.*, 1990). In animal cells a G-protein may directly or indirectly, *via* AC, control the activity of DHP-sensitive Ca^{2+} channels (Trautwein and Hescheler, 1990). In the latter case, AC controls the activity of Ca^{2+} channels *via* cAMP-PKA. It has been shown that PKA phosphorylation of L-type channels leads to an increase in calcium channel current (I_{ca}), whereas dephosphorylation of this protein decreases I_{ca}. Furthermore, ATP-γ-S leads to a maximal increase of I_{ca} (Trautwein and Hescheler, 1990).

It is unknown whether the same mechanism of calcium channel activity *via* phosphorylation/dephosphorylation cycles occurs in plant cells. However, phosphorylation/dephosphorylation of different proteins have been observed during regulation of some processes in plants (for review see Ranjeva and Boudet, 1987).

Concluding Remarks

Table 4 summarizes phytochrome-mediated and Ca^{2+}-dependent processes in plants. These responses can be divided into two groups: direct (transmembrane and *Zeta*-potential, leaf movement), and indirect (associated with changes of membrane permeability to Ca^{2+} and/or increase in $[Ca^{2+}]_c$, enzymes activity, cytoskeleton modification). Furthermore some of these processes appear to be operated through a calcium-dependent modulation of the responses (Ca^{2+} is not necessary in the extracellular medium), whereas others are unidirectional and occur only if Ca^{2+} is present in the surrounding environment. The first group of responses are observed mainly in lower plants (*e.g.* chloroplast movement in algae; protonemata phototropism in mosses), while the second group is more typical of morphogenetic responses observed during

Table 4. The participation of Ca^{2+} in different phytochrome-controlled phenomena

Level of control	Plant species	Phytochrome-regulated process	Effect of Ca^{2+}	References
Membrane properties and permeability	*Nitella* sp.	Membrane potential	Depolarization	Weisenseel and Ruppert (1977)
	Mesotaenium cadariorum	*Zeta*-potential	Decreasing	Stanz and Weisenseel (1986)
	Phaseolus aureus	Root-tip adhesion	Stimulation	Tanada (1968)
Membrane permeability, osmotic pressure, and cytoskeleton activity	*Triticum aestivum*	Protoplast swelling	Stimulation	Bossen *et al.* (1988)
	Cassia fasciculata	Leaf movement	Modulation	Roblin *et al.* (1990)
	Albizzia lophantha			Moysset and Simon (1989)
	Hordeum vulgare	Leaf unrolling	Stimulation	Viner *et al.* (1988)
	Triticum aestivum			Tretyn and Kendrick (1990)
	Mougeotia scalaris	Chloroplast movement	Stimulation	Grolig and Wagner (1988)
Enzyme and metabolic activity	*Avena sativa*	Acetylcholinesterase	Inhibition	Kim *et al.* (1990)
	Lygodium japonicum	Gibberellin synthesis	Stimulation	Kagawa and Sugai (1991)
		NAD kinase	Activation	Dieter and Marmé (1980)
	Zea mays	Mitochondrial ATPase	Inhibition	Serlin *et al.* (1984)
	Spinacia oleracea	Peroxidase secretion	Stimulation	Sticher *et al.* (1991)
Gene expression, DNA and protein synthesis	*Onoclea sensibilis*	Spore germination	Stimulation	Wayne and Hepler (1984)
	Dryopteris paleacea			Dürr and Scheuerlein (1990)
	Adiantum capillus-veneris	DNA synthesis	Stimulation	Iino *et al.* (1989)
	Spirodela polyrhiza	Turion germination	Stimulation	Appenroth and Augsten (1990)
	Pharbitis nil	Flower induction	Participation	Friedman *et al.* (1989)

cell division (*e.g.* fern spores) and differentiation of higher plants (*e.g.* leaf unrolling). In some cases, such as in phytochrome-controlled flower induction or regulation of seed germination, participation of Ca^{2+} in regulation of these processes seems to be very complicated. Collectively published data strongly suggests that there is not simply one type of Ca^{2+} action in photomorphogenesis. The mode of action of Ca^{2+} in phytochrome-controlled processes appears to be determined by state of differentiation (cell competence). However, even in one cell alternative phytochrome-regulated pathways (calcium-dependent and independent) are likely to exist.

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