DISCUSSION

The role of calcium in physiological processes in animals was first recognized near the turn of the century. In plants too a number of physiological processes were reported to be regulated by calcium (Wynjones and Lunt, 1967), however, the mechanism of action was not well understood. The importance of Ca\(^{2+}\) as a regulatory ion, and its role as a second messenger became evident only after the discovery of Ca\(^{2+}\) binding protein, calmodulin (Cheung, 1970) in animal systems. The discovery of calmodulin in plants (Anderson and Cormier, 1978) and a few reports which followed on the calcium and calmodulin regulated enzymes and some physiological responses, gave some insight into the molecular mechanism of Ca\(^{2+}\) dependent regulation (Kretsinger, 1977). It is, however, not yet clear, if Ca\(^{2+}\) acts as a second messenger in plant systems. If that is proved a number of phenomenon involving signal transduction could be explained.

Many plant physiologists believed that Ca\(^{2+}\) could mediate light dependent processes as controlled by phytochrome. Based on the preliminary results obtained in different laboratories Roux et al. (1981) proposed a hypothesis, similar to that given for animal models (Fig. 34). However, in this the role of calcium as a second messenger in mediating light signals was stressed. They
PROPOSED MODEL FOR PHYTOCHROME ACTION
(After Roux et al., 1981)

FIG. 34
proposed that after perceiving the light signal, phytochrome could induce change in ion fluxes, especially in calcium and thereby resulting in an increased cytosolic \((\text{Ca}^{2+})_c\). This increased \((\text{Ca}^{2+})_c\) would lead to the activation of calmodulin to form a \(\text{Ca}^{2+}\text{CaM}\) complex. This complex would in turn act on various target enzymes and lead to a response.

The aim of the present study was, therefore, to study if calcium is involved as an intracellular second messenger in mediating physiological and developmental responses, especially in those regulated by light. The major results obtained in the present work on the light mediated calcium uptake, regulation of enzymes by \(\text{Ca}^{2+}\), calmodulin and light, and the involvement of \(\text{Ca}^{2+}\) and calmodulin in light mediated responses like cell proliferation, are discussed in the following pages.

**PHYTOCHROME REGULATION OF CALCIUM UPTAKE**

Although a few papers have appeared on the subject (Dieter and Marme, 1983; Dreyer and Weisenseel, 1979; Hale and Roux, 1980), it is not yet clear how phytochrome increases the calcium level inside the cell. The \((\text{Ca}^{2+})_c\) in the cytosol can be raised by increasing the influx of \(\text{Ca}^{2+}\) ions across the plasma membrane, or by the efflux of \(\text{Ca}^{2+}\) from the cell organelles. It could also be achieved by inhibiting the efflux of \(\text{Ca}^{2+}\) from cytosol.
Dreyer and Weisenseel (1979) first showed that in Mougeotia, a lower plant, red light induced an increase in the uptake of Ca$^{2+}$. This effect was reversed by a subsequent irradiation with far red light. In higher plants, there was some controversy regarding the direction of Ca$^{2+}$ movement as controlled by phytochrome. Hale and Roux (1980), using murexide dye, showed in oat protoplasts that there was initially a sudden influx of calcium ion upon irradiation with red light, and later the efflux occurred. This response was reversed by far-red light. However, Dieter and Marme (1983), found, in microsomes of zucchini, that Ca$^{2+}$ uptake was inhibited by irradiation with continuous far-red light suggesting that Pfr lowered the Ca$^{2+}$ efflux by affecting Ca$^{2+}$-ATPase and thus resulting in an increase in the free (Ca$^{2+}$)$_c$.

In the present investigation, uptake of $^{45}$Ca$^{2+}$ was studied to measure only the process of influx. For this, leaf discs were used and it was found, using red and far-red light, that Pfr increased the uptake of calcium in intact leaf discs. In intact system, however, since calcium could either enter the intracellular spaces or it could stick to the cell wall it was difficult to conclude if the influx resulted in an increase in (Ca$^{2+}$)$_c$ in the cytoplasm. The uptake studies were, therefore, carried out with isolated protoplasts. When protoplasts were irradiated with red light for 60-120 sec, there was a significant increase in Ca$^{2+}$ uptake. This increase was reversed by 5 min of...
far red light. This suggests that Pfr control of the direction of calcium flux could be from the extracellular media into the cytoplasm across the plasma membrane. This was found to be a very fast response and also the escape from reversibility was also very fast. A gap of one min between red and far-red light inhibited the reversal (Table 6). The uptake was found to increase for 2 min after which it started declining suggesting that with an increase in the calcium concentration in the cytoplasm, the calcium pump probably gets activated and starts effluxing excess Ca\(^{2+}\). This shows that the time of measurement of Ca\(^{2+}\) uptake is important to ascertain the exact role of Pfr. In our studies it is clear that initially Pfr increased the influx of Ca\(^{2+}\).

Attempts were made to study \(^{45}\)Ca\(^{2+}\) influx in protoplasts of wheat by Akerman et al. (1983) who could not observe any change by Pfr. The reason for the difference between their and our results could be the difference in the system used and also on the precautions that we have taken to record the results. We found that even if a slight exposure of light was perceived by the plants upto the stage of isolation of protoplasts and doing the uptake experiments, the red light effect could not be measured. Secondly, we observed variation in results obtained with plants raised from different seed stock. The increase by

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red light in such experiments varied from 40% to 120%. Another factor which affected the response was the use of nutrient medium compared to distilled water to germinate the seeds.

Although, it is clear that Pfr increases Ca^{2+} influx, the mechanism by which it does that is not well understood. It was found that the maximum amount of Ca^{2+} taken up in unirradiated and irradiated protoplasts was different. In red light irradiated protoplasts Ca^{2+} uptake was more even at low exogenous Ca^{2+} concentrations whereas in unirradiated protoplasts the Ca^{2+} level never reached the red level even after longer incubation time or even if incubated with higher concentration of Ca^{2+}. This gave indication that probably after light irradiation, there is some change caused in the membrane properties by Pfr. In Nitella, phytochrome has been shown to cause depolarization of the membrane potential (Weisenseel and Ruppert, 1977). If this happens in higher plants too, Pfr could result in opening of calcium channels. In plants the nature of such channels is not known but in animal system a number of reports are appearing on the characterization of Ca^{2+} channels (Nowycky et al., 1985). Studies on binding of Ca^{2+} channel blocker, verapamil, to plant membranes (Andrejauskas et al., 1985) suggest that Ca^{2+} channels might be present in plants. It is hoped that with the use of patch clamp technique with isolated plant protoplasts, this question would be resolved in near future.
Having found that Pfr increased calcium uptake in maize protoplasts, the next step was to check on the presence of calcium binding protein, calmodulin. This protein has been isolated from both lower and higher plants and has been shown to regulate a number of cellular processes in plants (Roux and Slocum, 1982; Hepler and Wayne, 1985). Calmodulin was purified from different parts of maize plants, seed, shoot and root, following the method of Biro et al. (1982). Some important properties of the protein were checked to confirm whether it is calmodulin or not. The protein got bound to CPZ Sepharose-4B column in a calcium dependent manner and could be eluted with EGTA (Jameison and Vanaman, 1979). It also could bind to octyl-sepharose column and the elution profile was similar to that obtained by Gopalkrishna and Anderson (1982). On SDS-PAGE a single band was observed after silver staining and had molecular weight of around 16,200. The protein also activated cAMP dependent phosphodiesterase enzyme (Cheung, 1970) and the stimulation of the enzyme activity was inhibited by anticalmodulin drugs and calcium antagonists.

In most of the studies on plant systems, calmodulin isolated from animal sources has been used for checking the activation of plant enzymes, like NAD kinase (Anderson and Cormier, 1978) and
Ca\textsuperscript{2+}ATPase (Dieter and Marme, 1980b). Roberts et al. (1984) when tested for the activation of animal myosin light chain kinase with spinach calmodulin, they found that the plant calmodulin could not activate the enzyme as much as the animal calmodulin could do. Even with an increase in the concentration of plant calmodulin, the activation was not to the same level as with animal calmodulin.

In the present study, calmodulin purified from seed, shoot and root tissue was tested for activation of phosphodiesterase and the percent stimulation was compared with calmodulin purified from goat testis. The activation achieved by seed and shoot calmodulin was less than that obtained by root calmodulin. In fact, the activation by root calmodulin was equivalent to testis calmodulin of goat. A similar trend was found when another animal enzyme, \textit{Ca}\textsuperscript{2+}Mg\textsuperscript{2+}ATPase, was tested for calmodulin activation. This enzyme has been used earlier for the assay of calmodulin (Hinds and Vincenzi 1982).

In most of the studies reported so far, it has been shown that calmodulin is a ubiquitous protein present in most of the eukaryotes and there is no difference between calmodulin purified from animal and plant sources (Marme, 1983). The reason for this differential activation in the present study is therefore not very clear, although in some reports earlier, plants calmodulin
showed less activation than animal calmodulin. In a report by Muto and Miyachi (1984) it is reported that in pea seedlings, the root had more calmodulin when expressed per mg protein basis. Allan and Trewavas (1985) also found that within the root segments, the root tips had 17 fold more active calmodulin when tested with plant NAD kinase, compared to the upper segments which had less active calmodulin. From these data and the present findings, there is some indication that calmodulin may differ in quantity and activity in different tissues and species. In fact, during seed germination, changes in calmodulin content has been reported (Cocucci, 1984) and also in Xenopus changes in mRNA of calmodulin have been shown during different stages of development (Dascal et al., 1986). Further work is required to understand the molecular basis of these differences.

REGULATION OF ENZYMES BY CALMODULIN AND PHYTOCHROME

From the foregoing discussion it is clear that light mediates calcium influx by mediating its effect through phytochrome and secondly that calmodulin is present in maize and it can on activation by Ca\(^{2+}\) activate the enzymes. For test systems, we had used animal enzymes, cAMP phosphodiesterase and RBC Ca\(^{2+}\).Mg\(^{2+}\).ATPase.
To check if any of the enzymes in maize is activated by Ca\(^{2+}\), CaM, and also regulated by Pfr we tested their effect on GDH and glyoxalase-I. The reason for checking GDH was that already in the laboratory work was progressing on the regulation of N\(_2\)-metabolizing enzymes by phytochrome and a report was published earlier that the enzyme is activated by Ca\(^{2+}\) (Takahashi and Furuhashi, 1980). However, there was no report on phytochrome regulation of this enzyme. The enzyme glyoxalase-I was found to be under phytochrome control (Pal, 1983) but there was no report on Ca\(^{2+}\) regulation of this enzyme. Also glyoxalase-I was reported earlier to have correlation with cell division and proliferation (Ramaswamy et al., 1984), a response that has been used in the present study to ascertain the role of calcium and calmodulin.

**Glutamate Dehydrogenase** - Since Pfr regulation of GDH was not reported earlier, the first step was to check whether GDH is under phytochrome control. Five day old maize seedlings when irradiated with 5 min of red light, showed 6 fold increase in the enzyme activity. The stimulation was reversed by 10 min of far-red light irradiation. This suggests that GDH is regulated by phytochrome by low energy response. This effect of phytochrome was also found to be age dependent and maximum stimulation was noticed in the 5 day old intact plants. The high energy response of Pfr was not tested for GDH activity.
When red treatment was given for 5 min and the plants kept in dark and extracted after different time interval, it was observed that the red light stimulation attains its maximum effect on GDH activity after 4h of dark period and then it stabilises off. When effect of Ca\(^{2+}\) was tested in vitro it showed maximum stimulation at 80 \(\mu\)M (Ca\(^{2+}\))\(_C\). Yamaya et al. (1984) studied the effect of Ca\(^{2+}\) on GDH activity from maize, and they found 100 \(\mu\)M (Ca\(^{2+}\))\(_C\) to be an optimum for maximum stimulation. Among Ca\(^{2+}\) dependent plant enzymes which are affected in vitro by only Ca\(^{2+}\) it is required in the millimolar range. For example, Muto and Beevers (1974) reported a stimulation of an alkaline lipase present in glyoxysomes by millimolar (Ca\(^{2+}\))\(_C\). Even purified ribulose 1,5-bisphosphate carboxylase purified from wheat was activated by (Ca\(^{2+}\))\(_C\) in the range of 5 to 15 mM. However, the enzymes which are regulated by calmodulin are sensitive to very low (Ca\(^{2+}\))\(_C\). Matsumoto et al. (1984) showed that ATPase activity in pea nuclei showed maximum stimulation at 50 \(\mu\)M (Ca\(^{2+}\))\(_C\).

To check, therefore, whether the Ca\(^{2+}\) effect was mediated through calmodulin or not, the partially purified enzyme was assayed with exogenous calmodulin. There was no further stimulation on addition of calmodulin. This could be due to the fact the in the extract the calmodulin could be already present. To test this calmodulin inhibitors, like TFP and compound 48/80,
were used in vitro (Gietzen et al., 1983). The enzyme activity was inhibited considerably which was reversed by the addition of exogenous calmodulin. This provided evidence for the possible involvement of calmodulin in activating GDH. To confirm this, GDH was purified from dark grown 5 day old coleoptiles according to Yamaya et al. (1984). After each purification step, the enzyme was assayed in the presence of a specific calmodulin inhibitor compound 48/80. With each step of purification the percent inhibition by 48/80 decreased (Table 13). After sephacryl-300 step the enzyme preparation was not totally pure and, therefore, it was passed through HPLC. With a pure enzyme preparation, compound 48/80 did not inhibit the activity. Even exogenous addition of calmodulin also did not have any effect. This showed that the factor responsible for 48/80 inhibition in sephacryl fraction was, therefore, separated by HPLC. Yamaya et al. (1984), also mentioned that they did not get any stimulation with calmodulin.

After HPLC there were two small peaks besides GDH peak (Fig. 21). One of the peak fraction when reconstituted with GDH showed an increase in GDH activity. To this reconstituted fraction when compound 48/80 was added the enzyme activity was inhibited and it showed stimulation with exogenous calmodulin. This suggests that calmodulin activates GDH only in the presence
of this protein factor, which needs further characterization. It, thus, appears that regulation of GDH by calmodulin could be via a protein factor which is responsible for its activity and calmodulin stimulation. Ranjeva et al. (1984) reported a situation somewhat similar to this in NAD - quinate oxidoreductase. They observed that a Ca<sup>2+</sup>-CaM dependent protein kinase regulated the activity of quinate oxidoreductase. Hence in this system also the enzyme was not directly regulated by calmodulin.

The enzyme GDH is thus regulated by Pfr Ca<sup>2+</sup> and calmodulin in presence of the protein factor. The question is how does phytochrome modulate the enzyme activity? Either it enhances the synthesis of active GDH, or it increases the Ca<sup>2+</sup> level which in turn could regulate the enzyme activity. More experiments will have to be done to prove either of these possibilities. However, in some experiments when the enzyme was extracted with EGTA the red light effect was more than if extracted without EGTA. This gives some indications that that stimulation of GDH in red light treated plants could not be due totally to direct activation of the enzymes by Ca<sup>2+</sup> but could be due to a higher level of the enzyme. After giving red light irradiation, a dark period of minimum 4 h was also necessary to get the red stimulation which indicates it to be a long term response. Further, after maximum stimulation of red light at 4 h, when the enzyme from dark grown plants was assayed with exogenous Ca<sup>2+</sup> in vitro, it showed 5-6
fold stimulation. However under similar conditions the stimulation by $\text{Ca}^{2+}$ was less in red light treated sets. This data indicates that in red light the level of the active enzyme is already high. This is partly reflected in the experiments where EGTA was added in vitro and it was observed that red light treated sets showed more inhibition than the dark enzyme. Also when TFP and compound 48/80 was used there was more inhibition in red light treated sets than in dark sets. However, it could not be ascertained whether phytochrome affects the protein factor or the calmodulin level which needs further study. It is, thus, clear from the above study, that phytochrome could mediate its responses via $\text{Ca}^{2+}$ and may be by calmodulin directly or indirectly leading to an increased enzyme level. Very few enzymes are known to be both phytochrome and calcium-calmodulin regulated like NAD kinase and $\text{Ca}^{2+}$ ATPase (Roux, 1984).

**Glyoxalase-I** - Glyoxalase-I has been shown to be present in plants (Ramaswamy et al., 1983) and it seems to be involved in the process of cell division and proliferation (Ramaswamy et al., 1984) by removing methyl glyoxal toxicity in the cells. There are reports that this enzyme is associated with microtubules (Gillespie, 1975). Cell division involves microtubule assembly and disassembly in the form of spindle which has been reported to be regulated by the $\text{Ca}^{2+}$ ions (Marcum et al., 1978). Infact role
of Ca$^{2+}$ and calmodulin in cell proliferation in animal systems has been emphasized (Means et al., 1982). In view of these reports, and also on preliminary data that it is controlled by Pfr in maize (Pal, 1983) attempts were made to check if Ca$^{2+}$ calmodulin regulate the activity of the enzyme.

In maize extracts prepared with EGTA in the extraction buffer, when TFP was added in vitro, glyoxalase activity was inhibited. This inhibition was reversed by the addition of exogenous calmodulin isolated from maize, and also by bovine brain calmodulin (Table 14).

Glyoxalase-I was studied in rapidly proliferating callus cells of Amaranthus, to assess the role of calmodulin in cell proliferation. This enzyme was inhibited by compound 48/80 and TFP in vitro which could be reversed by exogenous addition of calmodulin. In fact in Amaranthus even in vivo addition of TFP, blocked glyoxalase activity. These experiments suggest a possible role of calmodulin in regulating glyoxasase-I activity. This however, needs to be confirmed since the assays were done with partially purified enzyme preparations, in view of our experiments with GDH mentioned earlier where the reversal of the enzyme could be achieved in crude extract but not with purified enzyme. In Amaranthus glyoxalase - I activity was higher in
light grown callus compared to dark grown cultures suggesting a control of this enzyme by light.

ROLE OF CALMODULIN IN CELL PROLIFERATION

Since its discovery role of calmodulin has been established in a number of processes in animal systems (Means et al., 1982). In plants, there are some reports on the role of calcium and calmodulin in mediating cellular and physiological processes (Hepler and Wayne, 1985). However, no single process has been worked out in details. In the present study, an attempt was made to study a cellular response, cell proliferation, which was regulated by calcium and calmodulin as well as by light.

The role of Ca^{2+} in mitosis and cytokinesis has been studied to understand the process of cell proliferation (Hepler and Wolniak, 1983). In the present study, the callus cultures of *Amaranthus paniculatus* were used to understand the process of cell proliferation in some details. This system was taken because the callus clone used showed only extensive proliferation and it did not undergo any differentiation. Also these callus cultures did not show any greening.

To establish the role of Ca^{2+} and calmodulin in cell proliferation in *Amaranthus*, calmodulin inhibitors were used.
was observed that TFP blocked cell proliferation which suggests that calmodulin plays a role in cell growth as has also been shown by Elliott et al. (1983) in callus cultures of soybean. They used TFP in the range of 0.07 to 0.5 mM concentration. A similar conclusion of the involvement of calmodulin in bud formation in Torenia was recorded by Tanimoto and Harada (1986).

Since TFP could have other effects, it was desired to confirm that TFP used in the medium did block calmodulin in the callus cells. Calmodulin was assayed from callus grown on TFP and showed inhibition in its ability to activate cAMP dependent phosphodiesterase. Even in vitro it inhibited calmodulin activity.

The mechanism by which calmodulin regulates the process of cell proliferation is not clear. It has, however been shown to regulate the microtubule assembly and disassembly which in turn regulates cell division. Weisenberg (1972) demonstrated that Ca\(^{2+}\) can depolymerise microtubules in rat brain. Hepler and Wolniak (1983) showed how Ca\(^{2+}\) controls the assembly and disassembly of spindle microtubules and regulates both function and formation of mitotic apparatus.

In earlier studies from the laboratory it was reported that the enzyme glyoxalase-I plays some role in cell proliferation.
(Ramaswamy et al., 1983, 1984). It was, therefore, necessary to check the effect of TFP on the activity of glyoxalase-I. It was observed that the enzyme activity was inhibited by TFP and the inhibition was 75-80% of the control sets. This suggested that calmodulin could be regulating the activity of glyoxalase-I. Earlier some plant enzymes have been shown to be regulated by calmodulin in vitro. To check therefore the effect of TFP on in vitro activity of glyoxalase, the drug was added during assay of the partially purified enzyme. There was inhibition by TFP which was reversed by the addition of exogenous calmodulin. These data have been partially discussed earlier and to confirm the role of calmodulin in regulating glyoxalase-I activity, compound 48/80 was tested. It also inhibited the enzyme activity which was again reversed by the exogenous addition of calmodulin suggesting the possible involvement of calmodulin in regulating cell proliferation through one of the enzymes glyoxalase-I.

**IN INVOLVEMENT OF PHOSPHOINOSITIDES IN AGONIST MEDIATED RESPONSES**

The role of phosphoinositides cycle in animal systems in the transduction of message of the external stimulus has received great attention in recent years (Rasmussen and Barrett, 1984). The phosphoinositides especially PI bisphosphate on hydrolysis gives rise to IP$_3$ and diacylglycerol, the former results in an
increase in the cytosolic calcium concentration of the cell (Berridge, 1984). IP$_3$ releases internal calcium and diacylglycerol mediates its effect through protein kinase C (Hokin, 1985).

In plants there is no conclusive evidence that PI cycle is involved in agonist mediated responses. In the present study, we have got some indications on the involvement of PI cycle in the light dependent responses.

In $^{45}$Ca$^{2+}$ uptake experiments, it was found that protoplasts irradiated with red light for 1 min if left in dark for 1 to 3 min, uptake was enhanced as compared to protoplast which had been irradiated but not given the dark interruption period. This suggested that soon after perception of light stimulus, some processes are triggered in darkness, which in turn leads to enhanced influx of calcium.

Fain and Berridge (1979a,b) had shown that breakdown of PIP$_2$ by serotonin lead to an influx of Ca$^{2+}$ in blowfly salivary glands. Even Sadler et al. (1984) suggested an increase in Ca$^{2+}$ uptake as affected by serotonin. In view of this, question arises was, whether Pfr could result in breakdown of PIP$_2$ to affect Ca$^{2+}$ uptake. In order to test this, effect of serotonin was checked on the uptake of Ca$^{2+}$ in isolated protoplasts.
Experiments with labelled $\Ca^{2+}$ showed that serotonin mimicked the effect of red light. If both red light irradiation and serotonin were given together, there was no further stimulation (see Fig. 23). This suggested that Pfr could alter the PI cycle, the products of which in turn could affect the $\Ca^{2+}$ channels. Light mediated breakdown of PIP$_2$ has been shown in animal systems (Ghalayini and Anderson, 1984; Hayashi and Amakawa, 1985).

Talland and Wallace (1985) had shown that calmodulin antagonists, TFP and CPZ, also alter the PI cycle by elevating the level of phosphoinositides. In present experiments also, $^{45}\Ca^{2+}$ uptake was stimulated by TFP at 2.5 $\mu$M concentration. The effect was more pronounced in protoplasts, isolated from dark grown tissue. In red light irradiated protoplasts, the effect was not significant. This again suggests that Pfr could be bringing about its initial response by acting at the PI cycle level. Recently, Leli and Hauser (1986) have also claimed that TFP acts at the level of PI.

Another set of evidence for the involvement of PI cycle in cellular responses came with the use of lithium chloride, an inhibitor of inositol phosphatase, which limits the supply of inositol in the cell (Hallcher and Sherman, 1980). Effect of LiCl was checked on cell proliferation in Amaranthus. In this system, as mentioned earlier, the callus proliferation is
enhanced in the light grown cultures compared to dark grown cultures. It was found that LiCl inhibited calli growth and this inhibition was reversed by the exogenous addition of myo-inositol suggesting that PI cycle is required in sustaining cell proliferation in callus cultures. It has been indicated that phosphatidyl-inositol cycle might in some way be involved in the influx of Ca\textsuperscript{2+} in cytosol (Fain and Berridge, 1979b), a possibility not well worked out in plant system. However, in the callus cultures inhibited by LiCl, the effect of calcium ionophore A23187 was tested, which was found to reverse the LiCl effect. This implies that LiCl inhibition could be due to blockage of PI cycle resulting in low calcium concentration. The effect of LiCl, therefore, could be reversed by the addition of calcium ionophore. Calcium channel blockers also inhibited cell proliferation but up to 30 percent only, suggesting that may be internal cell calcium is enough to maintain the growth of the cells, or the blockers may be effective at higher concentrations, which were not tested in the present study. This inhibition was reversed by calcium ionophore-A23187 suggesting that calcium is an important ion for cell division. The exact mechanism by which phosphatidylinositol cycle and Ca\textsuperscript{2+} channels would be related is not clear at present and needs further work.

The mechanism by which the inhibition of phosphatidylinositol cycle affects cell proliferation is not
clear. From our earlier studies, we had some indication of the involvement of an enzyme glyoxalase-I in cell division and proliferation in callus cultures (Ramaswamy et al., 1983, 1984). The involvement of this enzyme was therefore looked into in the present system also. It was observed that the enzyme activity was significantly decreased with the addition of LiCl and this corresponded with the inhibition of callus growth. Again, the inhibition of glyoxalase-I was reversed by the exogenous addition of myoinositol suggesting that PI cycle does affect, in some way, the glyoxalase-I activity.

In above experiments, the precise role of LiCl, was not clear although it was found that the effect was not due to a salt effect, as NaCl had no effect. To check if LiCl did affect PI-cycle, levels of PI were measured in LiCl treated sets by labelling it with $^1^4$C-inositol. It was shown that LiCl inhibited 73% of PI level compared to the control sets.

Thus, the present data indicate involvement of PI cycle in light mediated response. Direct evidences would be required to ascertain the role of phosphatidylinositol species as second messenger in Pfr mediated responses.
OVERALL VIEW

Light regulates a large number of biochemical and physiological processes in plants. These effects are mediated through pigments like phytochrome, and cryptochrome. The mechanism by which these pigments modulate cellular functions and also the site of their action is not clear. There is no single model which can explain multiple responses triggered by light. It is possible that different processes are controlled by different pathways, however, a working hypothesis is proposed, based on the present findings and earlier works (Fig. 35). This hypothesis is intended to explain the light regulation of enzymes and also cell proliferation. The two processes, one at the biochemical and the other at the physiological level, has been studied in the present work.

Light perceived by phytochrome results in the conversion of Pr to Pfr. It is proposed that the initial action of Pfr is to modulate the phosphoinositide cycle, either acting via phospholipase C to help in the breakdown of PIP$_2$ to DG and IP$_3$ or probably through phosphokinases to increase the level of PIP$_2$ which in turn would be degraded. There is evidence that Pfr could itself act as a protein kinase (Lagarias et al., 1986). These processes would lead to an increase in cytosolic calcium by releasing endogenous pool of Ca$^{2+}$ or affecting in influx of Ca$^{2+}$ from outside. In the present study, the influx of Ca$^{2+}$ was
Fig. 35 PROPOSED MODEL FOR PHYTOCHROME REGULATION OF GENE EXPRESSION

Inactive and active form of protein is depicted as I and A respectively in the present model.
measured and was found to be affected by Pfr through PI cycle. There are other reports also which show that Pfr does regulate the influx of Ca\(^{2+}\) (Dreyer and Weisenseel, 1979; Hale and Roux, 1980) and also releases Ca\(^{2+}\) from endogenous pools, like from mitochondria (Roux et al., 1981). Recently, it has been shown that incubation of zucchini microsomes with IP\(_3\) does affect the release of calcium (Drobak and Ferguson, 1985). Involvement of phospholipid metabolism in signal transduction has also been suggested by Hartmann and Pfaffmann (1986).

Once calcium concentration increases in the cytoplasm it can initiate a few responses. At low concentration it can activate PKC in presence of DG. However, so far no effect of Pfr on PKC is reported although its presence has been shown in plant system (Schafer et al., 1985). The increase in Ca\(^{2+}\) ions could lead to activation of certain enzymes directly (Tomlinson and Turner, 1973) or bring about its affect through calmodulin, which has been reported in plants (Anderson and Cormier, 1978; Sane et al., 1984) and thus regulating a number of physiological processes (Hepler and Wayne, 1985). Even in the present study it was shown to regulate the process of cell proliferation. The activated calmodulin can control a series of responses. Either it activates the enzymes directly as shown in a few cases (Marme, 1983; Roux and Slocum, 1982), or it probably mediates its effect through other protein binding factors; like quinate oxido
reductase, through kinases (Ranjeva et al., 1983) or like GDH shown in the present study.

Although this hypothesis does explain the control of Pfr of those enzymes that are activated by light, it does not give an insight into the Pfr control of those enzymes which are de novo synthesized at the transcriptional level. In order to explain this it is suggested that active calmodulin could activate certain protein kinases, cytoplasmic or nuclear, which in turn would modulate the gene expression. It has been shown that some Ca^{2+}.CaM dependents protein kinases are present in the nuclei and are also regulated by Pfr (Datta et al., 1985). In our laboratory also, there is an evidence that Pfr does affect protein kinases (Doshi and Sopory, unpublished). And recently Otto and Schafer (1986) also suggested that Pfr could mediate its affect through protein kinases. In fact the process of new synthesis through calmodulin and kinases and their activation by Ca^{2+}.CaM could go on together and some enzymes might be controlled both ways as we think is probably the case with GDH.

The above hypothesis tries to explain how Pfr could be mediating its effect as it is known that it does require a transmitter (Mohr, 1983; Sharma and Sopory, 1984). This hypothesis needs further experimental support to stand the test of the time.