# Photocontrol of Chloroplast Lipids in Fern Gametophytes

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*Dedicated to Prof. Dr. H. Schraudolf on the occasion of his 60th birthday*

Glycolipids, Linolenic Acid, Phytochrome, Blue Light Photoreceptor, Chloroplast Differentiation

In the gametophyte of the fern *Anemia phyllitidis* synthesis of linolenic acid esterified in monogalactosyldiglyceride requires light. By induction-reversion experiments it could be demonstrated that this light-dependent step is mediated by phytochrome. There is also evidence for phytochrome control of galactolipid and hexadecatrienoic acid synthesis. In continuous blue light the synthesis of linolenic acid is inhibited and linoleic acid accumulates. It is concluded that the blue light photoreceptor affects an inhibition of linoleic acid desaturase. In continuous blue light chloroplasts contain abundant multilayered thylakoids, the grana regions are not as distinct as in white light, and membranes appear less appressed. In continuous red light the membranes are reduced in number and contain less grana-like appressions. It is concluded that both photoreceptors are necessary for a coordinate synthesis and assembly of the individual components of the chloroplast membrane.

## Introduction

The membranes of higher-plant chloroplasts are characterized by specific lipids such as MGDG*, DGDG, SL, and PG. The two galactolipids which are the major components contain exceptionally high levels of the trienoic fatty acids linolenic acid (18:3) and hexadecatrienoic acid (16:3). An unusual fatty acid, trans-Δ3-hexadecenoic acid (t-16:1) esterified in PG is also a typical constituent of the chloroplast membrane [1].

It is generally known that chloroplast development in higher plants is most significantly regulated by light. Light intensity and light quality influence the relative amounts of chlorophylls, pigment-protein complexes, membrane proteins, and electron transport constituents. Also there is increasing evidence that light can affect the expression of both nuclear and chloroplastic genes [2]. Several different photoreceptors are involved in the regulation of these processes including phytochrome and one or more blue light photoreceptors.

Although lipids are the second most important constituent of the chloroplast membrane (ca. 15% of total weight), little attention has been paid to the question of whether light affects lipid metabolism. Generally, light stimulates the synthesis of chloroplast lipids, in particular the synthesis of linolenic acid [1]. In previous reports from this laboratory it was shown that in the fern *Anemia phyllitidis* the synthesis of linolenic acid is dependent on light [3, 4]. The aim of the present work was to determine the photoreceptors involved in this response. Fern gametophytes provide an excellent means of studying this question since they are composed of much more uniform tissue as compared to the leaves of higher plants. Furthermore, their development is well-known and is strongly influenced by light [5]. Results of this study show that phytochrome controls the induction of linolenic acid synthesis and the stimulation of galactolipid synthesis while blue light appears to be inhibitory to the desaturation of linoleic acid.

## Materials and Methods

### *Plant material*

Spores of *Anemia phyllitidis* (L.) Sw. and *Pteris vittata* L. were harvested from plants grown in the green house of the University of Ulm. Gametophytes were grown on a liquid salt medium as described previously [6] at 21 °C at different light regimes. Spores of *A. phyllitidis* were induced to germination

* *Abbreviations:* MGDG, monogalactosyldiacylglyceride; DGDG, digalactosyldiacylglyceride; PC, phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; SL, sulpholipid; 18:3-MGDG, linolenic acid esterified in MGDG; 16:3-MGDG, hexadecatrienoic acid esterified in MGDG.

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and growth in darkness with $5 \times 10^{-6}$ g/ml gibberellic acid [7] and with a 48 h red light irradiation for development in blue light. Spores of *P. vittata* were precultured on distilled water in white light as described previously [6] and were transferred to darkness after 7 days.

*Light sources*

White light was provided by white fluorescent lamps (Osram L65 W32, Warm White De Luxe), fluence rate 10 $Wm^{-2}$. Red light was obtained by the combination of two red plastic filters, Plexiglass type 501 (Röhm & Haas) with red fluorescent lamps (Philips TL 20W/15), fluence rate 1 $Wm^{-2}$. Far-red light was produced by Osram lamps Linestra 1604 filtered through Plexiglass type 501 and type 627, fluence rate 4.4 $Wm^{-2}$. The blue light source consisted of 8 Osram fluorescent lamps L20 W64 filtered through Plexiglass type 627, fluence rate 5.9 $Wm^{-2}$.

*Lipid analysis*

Lipids were extracted from gametophytes by homogenization with glass beads in a dismembrator (Braun-Melsungen) with chloroform/methanol, 2:1 v/v. They were purified according to Folch *et al.* [8]. Total lipids were fractionated into groups by column chromatography on a silicic acid column according to Kates [9]. The polar lipids were further fractionated on preparative silica gel plates impregnated with 0.15 M ammonium sulfate using acetone/benzene/water (91:30:8, by vol.). The various lipid classes were identified by co-chromatography of authentic compounds in different solvent systems and by specific reagents [9]. Fatty acid methyl esters were prepared from individual lipid classes by transmethylation with $BF_3$-methanol according to the procedure described by Van Wijngaarden [10]. Gas liquid chromatography was performed at 170 °C using a 180 cm × 2 mm glass column of 10% DEGS on chromosorb 100/120 mesh. Methyl pentadeconate was used as internal standard for quantification.

*Electron microscopy*

Gametophytes were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, postfixed for 2 h at 4 °C in 1% osmium tetroxid in water containing 0.1 M 3-amino-1,2,4,triazole to intensify membrane contrast [11]. The tissue was dehydrated in an ethanol series. Additional staining was achieved with uranyl acetate followed by lead citrate. Gametophytes were embedded in ERL (Serva), sections were viewed in an EM 109 (Zeiss).

## Results

*Light effects on polar lipids and their fatty acids*

A comparison of the major polar lipids of spores and gametophytes grown under different light qualities is shown in Table I. In spores and light-grown gametophytes the predominating polar lipids are the principal chloroplast lipids, *i.e.* MGDG, DGDG, and PG. In darkness the content of these lipids is low and the phospholipid PC predominates. The absolute amounts of the polar acyl lipids increase differentially in gametophytes grown for 21 days in white, red, and blue light, respectively. In contrast, in the absence of light the total amounts of these lipids decrease. The most significant effect of light appears on the major fatty acid of the photosynthetic membranes, *i.e.* linolenic acid esterified in MGDG (18:3-MGDG) (Table II). Spores contain only 2%

Table I. Polar lipids of spores and gametophytes of *Anemia phyllitidis* grown for 21 days under different light qualities (a, μg lipid $g^{-1}$ spores; b, %; means of 3 independent experiments).

| Lipid | Spores | | Gametophytes grown in white | | red | | blue | | darkness | |
|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | a | b | a | b | a | b | a | b |
| MGDG | 130 | 37 | 340 | 37 | 100 | 28 | 210 | 28 | 8 | 16 |
| DGDG | 60 | 18 | 220 | 24 | 100 | 29 | 170 | 23 | 3 | 6 |
| PG | 50 | 16 | 190 | 21 | 30 | 10 | 100 | 13 | 6 | 12 |
| PE | 40 | 13 | 80 | 9 | 60 | 17 | 120 | 16 | 10 | 19 |
| PC | 50 | 16 | 80 | 9 | 50 | 16 | 150 | 20 | 24 | 47 |
| Total | 330 | 100 | 910 | 100 | 340 | 100 | 750 | 100 | 51 | 100 |

Table II. Fatty acid composition of MGDG of spores and gametophytes of *Anemia phyllitidis* grown for 21 days under different light qualities (mole %, means of 3 independent experiments).

| Fatty acid | Spores | Gametophytes grown in | | | |
|---|---|---|---|---|---|
| | | white | red | blue | darkness |
| 16:0 | 19 | 5 | 8 | 6 | 22 |
| 16:1 | 1 | 2 | 3 | 2 | 0 |
| 16:3 | 0 | 1 | 4 | 0 | 0 |
| 18:0 | 3 | 1 | 0 | 1 | 17 |
| 18:1 | 27 | 6 | 7 | 18 | 9 |
| 18:2 | 47 | 25 | 19 | 70 | 39 |
| 18:3 | 2 | 53 | 56 | 2 | 2 |
| others | 1 | 7 | 2 | 1 | 11 |

of this fatty acid and it becomes the principal fatty acid in gametophytes grown in white or in red light. Surprisingly, in gametophytes grown in blue light or darkness, the proportion of this fatty acid is 2% as in spores. However, the absolute amounts are different. As compared to the spore, the amount of linolenic acid increases 69-fold in white light, 22-fold in red light, and 1.6-fold in blue light, but is only 1/16 in darkness. Similar but slightly lower values have been found for linolenic acid esterified in DGDG (data not given). Phospholipids contain only low amounts of linolenic acid and, in general, it has been found that light does not markedly affect the phospholipids of gametophytes (data not given, *cf.* [4]).

Synthesis of other chloroplast fatty acids was also found to be affected by light. Hexadecatrienoic acid, like linolenic acid, was synthesized only under white and red light conditions. The unusual fatty acid trans-hexadecenoic acid which is esterified in PG was found exclusively in white-light gametophytes and was 11% in 21 days old gametophytes.

*Effect of brief red light irradiations*

In order to determine whether the light dependent synthesis of linolenic acid is mediated by phytochrome, experiments with brief red light exposures were made. Dark-grown gametophytes which contain 2–5% linolenic acid were irradiated with a brief red light pulse. Fig. 1 shows that a 30 min red light irradiation results in a marked increase in the amount of 18:3-MGDG. The increase is from 5 to 7% immediately after the light treatment but becomes very prominent after 24 h. Therefore, in the following experiments gametophytes were analyzed 24 h or 48 h after irradiation. Fig. 2 shows that the stimulating effect of brief red light exposures can be fully reversed by brief far-red exposures given immediately after the red light. Fig. 2 also demonstrates the involvement of phytochrome in the stimulatory effect of light on the synthesis of both galactolipids but in this case the increase is smaller.

Phytochrome involvement in the control of linolenic acid synthesis can also be demonstrated in gametophytes of *P. vittata*. In a parallel experiment it was found that dark-grown gametophytes of *Pteris* contain 12% 18:3-MGDG. 48 h after a 30 min red light irradiation the amount had increased to 26% and this increase could be partially reverted by far-red light resulting in 20% 18:3-MGDG. Like *Anemia* also in *Pteris* a 30 min red light treatment was not sufficient to induce the synthesis of hexadecatrienoic acid.

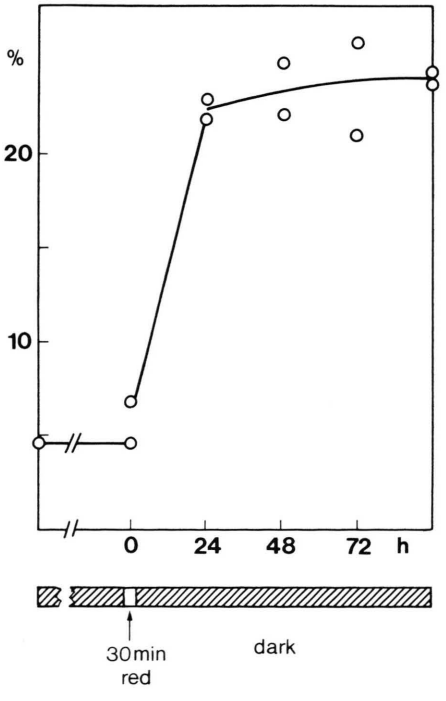


Fig. 1. Effect of red light on the proportion of linolenic acid in MGDG. Gametophytes of *A. phyllitidis* grown for 10 days in darkness were subjected to a single 30 min irradiation with red light and thereafter maintained in darkness for various times before being analyzed.

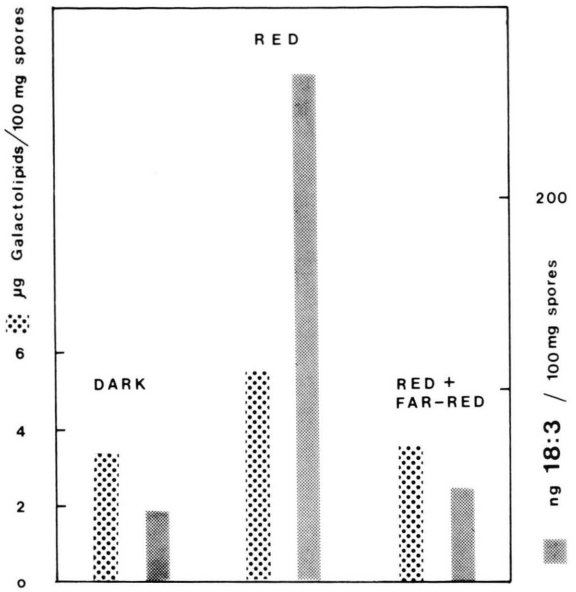


Fig. 2. Phytochrome involvement in the control of the synthesis of linolenic acid esterified in MGDG and of galactolipids. Dark-grown gametophytes of *A. phyllitidis* were irradiated either with 6 × (5 min red + 5 min dark) or with 6 × (5 min red + 5 min far-red). They were extracted 48 h after the light treatment, means of 4 independent experiments.

*Light effects on ultrastructure*

Electron micrographs from chloroplasts of light-grown gametophytes exhibit features which are characteristic of higher plant chloroplasts, *i.e.* the membranes are differentiated into stroma and grana thylakoids and plastoglobuli are present (Fig. 3a). In contrast to this is the structural organization of chloroplasts of gametophytes grown in blue light (Fig. 3b). In these organelles the stromal regions of the thylakoid system are extremely reduced while the grana regions are extended. Thus, the stroma is completely filled with multilayered membranes and a few starch grains. There are no distinct boundaries of the grana and the stacking appears less dense than in the grana stacks of plants from white light. Therefore it is difficult to decide whether the large grana regions are true grana or merely adhering membranes. In red light-grown gametophytes the chloroplasts contain large starch grains and the thylakoids appear to be reduced as compared to that of the white or blue-light chloroplasts. The thylakoids exhibit rare grana-like appressions (Fig. 3c).

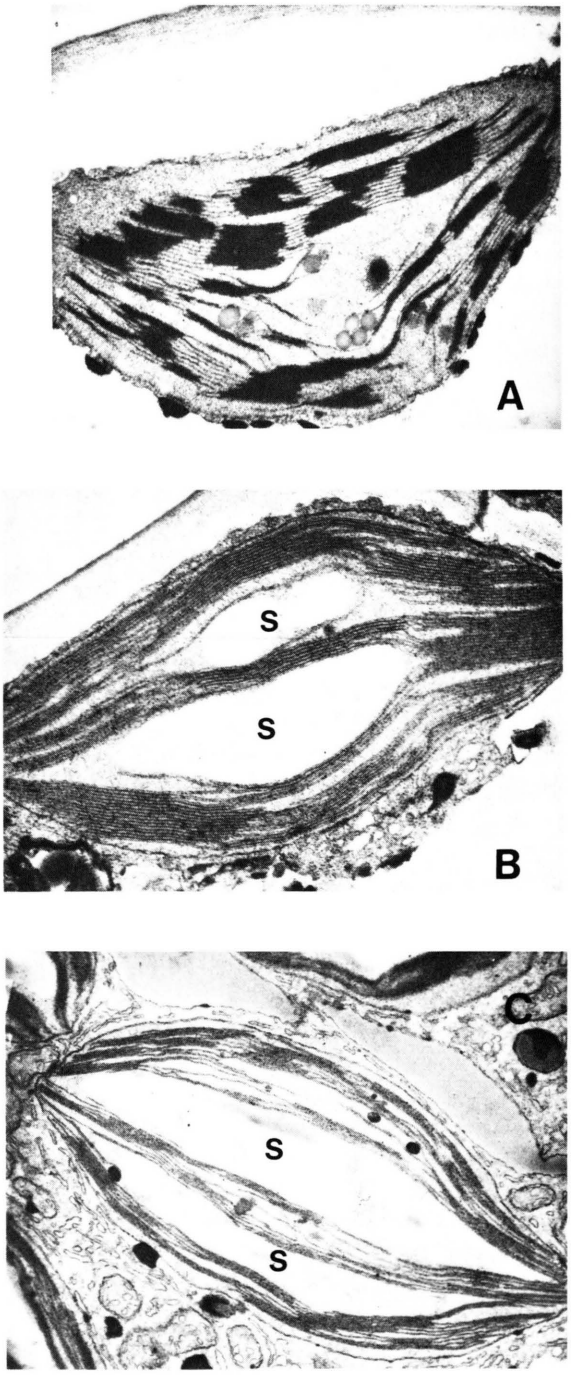


Fig. 3. Effect of continuous light of various qualities on chloroplast ultrastructure. 3a, white light, × 22000; 3b, blue light, × 15000; 3c, red light, × 25000; s, starch grain.

## Discussion

### *Red light effects*

The results presented in this study clearly show that in fern gametophytes the synthesis of linolenic acid and also of galactolipids is under the control of phytochrome. This could be demonstrated by induction-reversion experiments. In *A. phyllitidis* the induction effect of a brief red light irradiation can be completely reversed by a brief far-red light irradiation. The incomplete reversion in *P. vittata* gametophytes can be explained by the assumption that the inductive effect of 5 min red light may already be partially realized before being reversed by far-red light.

In this study the synthesis of 16:3-MGDG could not be induced by a 30 min red light treatment, only in continuous red light this fatty acid was present. Results from *Centranthus ruber* seedlings which respond to 10 min red light treatment (Gemmrich, unpublished results) indicate that in this species the synthesis of 16:3 is also under phytochrome control.

Phytochrome-mediated responses can generally be brought about by inducing or stimulating biosynthetic processes with red light [*cf.* 12]. From the finding that linolenic acid synthesis does not occur in darkness but upon exposure to red light, it is assumed that in *A. phyllitidis* phytochrome action on linolenic acid synthesis is inductive. The induction of linolenic acid synthesis by white light has been reported in several plants, for example in developing cotyledons of cucumber [13], and *Pharbitis nil* [14], and in barley leaves [15]. It seems likely that these inductive light effects are also mediated by phytochrome. The red light mediated increase in the amount of linolenic acid in *P. vittata* gametophytes (this report) and in pea leaves [16], the increase in galactolipids in *A. phyllitidis* gametophytes (this report) and in mustard seedlings [17] can be attributed to a modulation of the synthetic rate already occurring in darkness.

The results of this study are consistent with the hypothesis that phytochrome controls chloroplast development at different levels and through several independent initial actions [18], for example also through synthesis of linolenic acid, hexadecatrienoic acid, and of galactolipids.

### *Blue light effects*

The present study further establishes that a blue light photoreceptor is involved in the control of linolenic acid synthesis. From the extremely low increase of 18:3-MGDG together with the accumulation of its precursor linoleic acid in MGDG it is concluded that blue light inhibits linoleic acid desaturase. This phenomenon appears to be unique in ferns but it is not very surprising since it is well-known that the blue light photoreceptor controls the most important steps in fern development like spore germination (literature see [5, 19]), transition from one- to two-dimensional growth [5, 19], and antheridiogenesis [6]. Unlike our results, in angiosperms blue light seems not to inhibit linolenic acid synthesis. For example seedlings of maize [20], barley [21] and *Pharbitis nil* [14] are able to synthesize appreciable amounts of linolenic acid in blue light.

Our data give new insight in the relationship between lipid composition and function of the plastid. Preliminary results indicated that chloroplasts of blue light-grown gametophytes of *A. phyllitidis* are photosynthetic active and the results presented here clearly demonstrate that these chloroplasts are almost devoid of linolenic acid. From this it appears that the high level of linolenic acid which is up to 98% in angiosperm plants is not an absolute prerequisite for photosynthesis. Similar results have been obtained with *Arabidopsis* mutants with reduced levels of linolenic and hexadecatrienoic acid [22].

Another striking effect of blue light is on membrane arrangement. Recently, Tevini [21] observed an increase in multilayered thylakoids during greening of barley leaves in blue light. Consistently but more pronounced in this study, we find marked augmentation of thylakoids and concomitant stacking appears less dense, in particular within the grana regions. Quite different, in the radish seedling it was found that blue light, acting like high intensity white light, affects chloroplasts to contain fewer membranes with lower stacking degree and smaller grana when compared to those in red light [23]. The fact that fern gametophytes prefer shady habitats and are mostly destroyed in full sun light may account for this discrepancy.

In general the question arises whether the biochemical data may account for the ultrastructural modifications. Much recent work has shown that blue light as well as high intensity white light modifies the chlorophyll-protein complex, chlorophyll and carotenoid ratios, and the levels of constituents of the electron transport chain [24]. Blue light also enhances protein synthesis in isolated chloroplasts

from *Dryopteris filix-mas* [25] and, as shown in this report, inhibits synthesis of linolenic acid, hexadecatrienoic acid and trans-hexadecenoic acid. From these observations it becomes evident that all membrane components can be modified by blue light and similarly by red light and thus no conclusive data are available to explain the causal events leading to properly stacked membranes. Although the light-harvesting protein and trans-hexadecenoic acid (however *cf.* [26]) are useful candidates for the process of grana stacking [27] the question why membranes are stacked is still open [28]. Blue light grown gametophytes appear to be a suitable material for further studies.

In conclusion our results indicate that in fern gametophytes synthesis of constituent chloroplast lipids and fatty acids is under the control of both, phytochrome and a blue light photoreceptor. The modification of lipid composition and most probably also of protein composition may be the reason for alterated membrane assembly. The blue light photoreceptor seems to act as a counterpart to phytochrome such that the promotive effect of red light is cancelled by the inhibitory effect of blue light. In the case of plastidogenesis both photoreceptors together are responsible for a coordinate synthesis and assembly of the individual components of the photosynthetic membrane.

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