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REVIEW

Protochlorophyllide reduction – what is new in 2005 ?

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Abstract

Because the transformation of protochlorophyllide (Pchlide) to chlorophyllide (Chlide) is an irradiation-dependent process, it is at the heart of the photosynthetic membrane biogenesis, turnover, and adaptation to changes of the environment. I review here the new data published during the year 2004 on Pchlide reduction to Chlide.

Additional key words: irradiation; temperature.

Introduction

The development and maintenance of life on earth predominantly depends on photosynthesis. In this frame, radiation quality and quantity is one of the most important environmental factors, which affect physiology, biochemistry, growth, and development of photosynthetic organisms. To this end the understanding of several processes such as plant greening, *i.e*. the mechanisms leading to a functional photosynthetic apparatus, and response to changing irradiation constitute major issues, which are mostly studied in etiolated angiosperm leaves.

Gymnosperms are able to green in the dark while angiosperms can only green in the light. The search for the enzymes catalyzing protochlorophyllide (Pchlide) reduction in the dark and under irradiation revealed two different types of enzymes to which I will refer to as a light-independent Pchlide reductase (DPOR) and as a light-dependent Pchlide reductase (LPOR). The search for homologous genes in other groups of photosynthetic organisms lead to the conclusion that cyanobacterium, fern, moss, algae, and gymnosperm genomes contain the genes encoding both LPOR and DPOR, whereas angiosperm genomes contain only the genes encoding LPOR. The reduction of Pchlide to chlorophyllide (Chlide) is catalyzed by LPOR. Up to three structurally related but differentially regulated isoenzymes of LPOR have been found in several organisms (reviewed by Schoefs and Franck 2003, Masuda and Takamiya 2004). In *Arabidopsis thaliana lporA* and *lporB* genes, but not *lporC*, are strongly expressed early in seedling development. By contrast, the expression of *lporB* and *lporC*, but not *lporA*, is observed in older seedlings and adult plants (reviewed by Schoefs and Franck 2003, Masuda and Takamiya 2004). The *lpor* genes are encoded in the nucleus and the proteins are synthesized on free cytosolic polysomes that are not attached to the plastid outer envelope. In most papers cited in this contribution the type of LPOR isoenzymes was not studied.

In the dark, LPOR enzymes are unable to process their substrates. LPOR is therefore of particular interest because actually it is only one of the two enzymes identified so far in nature, in which the enzymatic activities are switched on by the absorption of visible photons (reviewed by Schoefs and Franck 2003). Thus under irradiation LPOR catalyzes the reduction of photoactive Pchlide to Chlide, a reaction, which initiates two 'Pchlide-Chlide' cycles (reviewed by Schoefs 2001). Each cycle consists in a succession of intermediates leading to the formation of chlorophyll (Chl) molecules and to the regeneration of the photoactive Pchlide pool (Schoefs 2001) (Fig. 1). Schoefs (2001) has suggested that it is the non-photoactive to photoactive Pchlide ratio (R), which directs the product of photoreduction into either cycle.

Several reviews dealing with Pchlide to Chl transformation appeared recently (Chl biosynthesis: Schoefs and Bertrand 2005; Chl catabolism: Eckhardt and Grimm

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Abbreviations: δ-ALA – δ-aminolevulinic acid; Car – carotenoid; Chl – chlorophyll; Chlide – chlorophyllide; DPOR – light-independent protochlorophyllide reductase; LPOR – light-dependent protochlorophyllide oxidoreductase; Pchlide – protochlorophyllide; PLB – prolamellar body; PS – photosystem; WT – wild type.

2004, Hoertensteiner and Matile 2004; plastid ultrastructure: Staehelin 2004; Pchlide-Chlide cycle: Schoefs 2001; Pchlide photoreduction: Schoefs and Franck 2003, Masuda and Takamiya 2004; Pchlide reduction: Fujita and Bauer 2003; tetrapyrrole signalling: Vasileuskaya *et al.* 2004). Consequently, in this contribution only the new data published during the year 2004 are summarized. The first part of this contribution deals with the different types of Pchlide found in non-irradiated tissues. In the second part new data about the 'Pchlide-Chlide' cycles are summarized, and in a third part new aspects dealing with regulation of Chl production are reviewed.

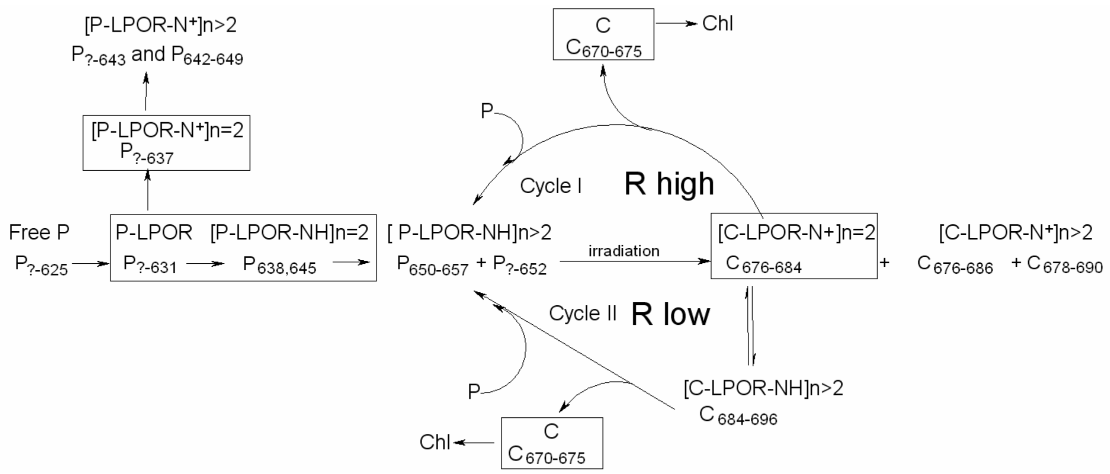

Fig. 1. The 'protochlorophyllide-chlorophyllide' cycles in angiosperms. The frames indicate the Pchlide and Chlide forms found *in vitro* when protochlorophyllide photoreduction was assayed using over-expressed *Synechocystis* LPOR. C – chlorophyllide, Chl – chlorophyll, n – number of ternary complexes in the aggregate, N^+ – $NADP^+$, NH – NADPH, P – protochlorophyllide, R – non-photoactive to photoactive Pchlide ratio.

The Pchlide status in dark-grown plant tissues

In the absence of radiation no Chl is produced and the leaves accumulate Pchlide together with carotenoids (Cars), the later being the most abundant pigments. Consequently the dark-grown leaves appear yellow. Accumulation of Pchlide in the dark is known as etiolation and the plant material is referred to as etiolated. The time required to reach the etiolated stage from germination is species-dependent (*e.g.* *Phaseolus vulgaris*: 10–15 d; *Triticum aestivum*: 6–7 d; *Arabidopsis thaliana*: 3–4 d). During etiolation Pchlide molecules accumulate under two different states denoted as non-photoactive Pchlide and photoactive Pchlide. By definition, photoactive Pchlide is a Pchlide, which is transformed to Chlide during a short irradiation (some ms). Biochemical studies have shown that photoactive Pchlide is an aggregate containing at least two ternary complexes, each of them being composed by one molecule of enzyme loading one molecule of Pchlide and one molecule of NADPH in the active site (reviewed by Schoefs and Franck 2003). Mathematical analyses of both the spectrum of non-photoactive and photoactive Pchlide emission bands have revealed that in etiolated tissues both pools are spectrally heterogeneous (reviewed by Schoefs and Franck 2003) (Table 1). Most of the studies performed on Pchlide and Pchlide-LPOR complexes have been done on plants having open leaves or cotyledons, and almost no data are available on other types of vegetables such as those having stacked leaves like cabbage or salad. The status of Pchlide has been investigated in 'non-standard' plant materials such as dark-forced shoots of grapevine (*Vitis vinifera* L.) (Böddi *et al.* 2004) and layers of white cabbage leaves (*Brassica oleracea* var. *capitata*) (Solymosi *et al.* 2004b).

Dark forcing is a well-known method used in grapevine propagation. In this original system, cuttings of grapevine collected for propagation are forced in the dark up to 100 d. The first dark-forced shoots appear after 2 weeks and reach a length of 20 cm after 6 weeks (Fig. 2). Simiarly to leaves (*e.g.* Schoefs *et al.* 1998) grapevine dark-forced tissues accumulate limited amounts of Pchlide and Cars during the etiolation process and appear yellow (Fig. 2). The 77 K fluorescence spectra of dark-forced leaves and petioles of grapevine were similar to those found with other dark-forced species (Skribanek *et al.* 2000) or with leaves with proplastids (Schoefs and Franck 1993), *i.e.* the non-photoactive to photoactive ratio is high (Table 2).

Solymosi *et al.* (2004b) measured the radiation transmission through layers of white cabbage (*Brassica oleracea* var. *capitata*) leaves. Irradiance decreased to 1 % under the fourth leaf layer, and under ten leaf layers the sensor of the *Li-Cor* photometer did not detect any radiation. Pigment analyses showed Chl only in the outermost leaf layer and Pchl(ide) only in the innermost leaf layer. The comparison of the 77 K fluorescence spectra recorded from the outermost leaf layer towards the heart of the cabbage head showed that photosystem 2 (PS2) emission band disappeared first, followed by the photosystem 1 (PS1) one. In the 8th leaf, the bands of both non-photoactive and photoactive Pchlide appeared. Both fluorescence intensities increased towards the centre of the head, that of non-photoactive Pchlide being always the strongest. Therefore R was always high (Table 2) and the 77 K fluorescence spectrum of the innermost leaf was similar to those obtained for leaves with proplastids (Schoefs and Franck 1993, Schoefs *et al.* 1994). Gaussian de-convolutions of non-irradiated and flashed innermost leaves revealed the presence of several bands of non-photoactive and photoactive Pchlide already described for leaves (Table 1).

When photoactive Pchlide accumulates, a particular membrane network, exhibiting a para-crystalline structure—the prolamellar body—is formed in the plastids (etioplasts) (reviewed in Staehelin 2004). Immunocytolocalization showed that most of the photoactive Pchlide is located in this structure (Ryberg and Dehesh 1986). Although pigment analyses of photoactive Pchlide isolated in the native state have indicated the presence of Cars (Ouazzani Chahdi *et al.* 1998), the role(s) of Cars in non-irradiated leaves remain(s) unclear because they are connected neither to Pchlide molecules (Ignatov *et al.* 1993) nor to newly formed Chlide molecules (Franck and Mathis 1980). The possibility that Cars and Pchlide interfere with the prolamellar body (PLB) organization was investigated in the presence of metabolic inhibitors. Moro *et al.* (2004) changed the Car composition using two different Car inhibitors and Savchenko *et al.* (2004) studied the consequence(s) of inhibition of tetrapyrrole synthesis by levulinic acid.

Fig. 2. Photograph of a dark-forced shoot of grapevine (*Vitis vinifera* L.). (Courtesy of B. Böddi.)

Table 1. Comparison of the spectral heterogeneity of the non-photoactive and photoactive protochlorophyllide pools in etiolated bean leaves (Schoefs *et al.* 2000), dark-forced grapevine tissues (Böddi *et al.* 2004), in the innermost cabbage leaf (Solymosi *et al.* 2004b), PLB incubated with heavy metals (Mysliwa-Kurdziel *et al.* 2004, Solymosi *et al.* 2004a), and *in vitro* with LPOR from cyanobacteria (Heyes *et al.* 2002). ND: non-determined.

Spectral form	Photoactivity	Dark forced grapevine tissues		Innermost cabbage leaf	Wheat PLB incubated with metals			*In vitro* with LPOR from cyanobacteria
		leaves	petioles		Cd	Cr	Fe, Hg	
$P_{?\text{-}625}$	-	+	+	ND	ND	ND	ND	ND
$P_{?\text{-}631}$	-	+	+	+	ND	ND	ND	+
$P_{?\text{-}637}$	-	+	+	+	ND	ND	+	+ (?)
$P_{?\text{-}643}$	-	+(?)	+(?)	+	ND	ND	ND	ND
$P_{642\text{-}649}$	-	ND	ND	+	ND	+	+	-
$P_{638\text{-}645}$	+	+(?)	+(?)	+	+	ND	+	+
$P_{?\text{-}652}$	+	ND	ND	+	ND	ND	ND	
$P_{650\text{-}657}$	+	+	+	ND	+	+	+	
$P_{?\text{-}667}$	-	+	+	+	ND	+	ND	

In the presence of norflurazon or amitrole the accumulation of colourless Car precursors phytoene and phytofluene or the accumulation of lycopene and upstream precursors is triggered, respectively (Moro *et al.* 2004). The structure of PLB was severely disturbed in amitrole-treated plants, but not in norflurazon-treated plants. Because amitrole also modifies the lipid composition of membranes (Di Baccio *et al.* 2002), it is not certain that the PLB disorganization in amitrole-treated plants is due to the accumulation of Car precursors. The type of disorganization observed is reminiscent of those observed in the *ccr2* mutant of *Arabidopsis* (deficiency in Car isomerase), which also accumulates linear precursors (Park *et al.* 2002). Altogether the results suggest that the accumulation of linear Cars such as lycopene may interfere with the building of PLB. If the change in the organization of PLB in amitrole-treated plants is obvious, no cytological quantitation was performed in this study in order to assess that the organization of PLBs in norflurazon-treated plants is really not modified. Despite the fact that amitrole treatment does not affect the LPOR content of the membranes, a decrease in the amounts of Pchlide and Cars was clearly observed together with the alteration in PLB organization. This suggests that the amount of LPOR is not strictly regulated by the amount of Pchlide (see below). All these modifications resulted in the dramatic increase of R in the amitrole-treated plants (Moro *et al.* 2004) (Table 2).

Table 2. Correlation between the value of R and the type of 'Pchlide-Chlide' cycle used to produce the newly formed Chlide molecules in different plant material, tissues, and growth conditions.

Material and condition	Value of R	Pchlide-Chlide cycle
Leaves with proplastids	High	I
Leaves with etioplasts	Low	II
Leaves with etioplasts + norflurazon	Low	II
Leaves with etioplasts + amitrole	High	I but incomplete
Leaves from dark-forced grapevine	High	ND
Petioles from dark-forced grapevine	High	ND
Most-inner leaf of white cabbage	High	I
Red-radiation-grown	High	I
Etioplasts from bundle sheath cells	High	I
Leaves treated with levulinic acid	High	ND

In the second study (Savchenko *et al.* 2004) the consequence of an inhibition of tetrapyrrole synthesis by levulinic acid, an inhibitor of the δ-aminolevulinic acid dehydratase (reviewed by Bertrand and Schoefs 2005), on the etiolation process in barley leaves was described. The inhibition of Pchlide synthesis had pleiotropic effects (Savchenko *et al.* 2004): (*1*) delay in leaf growth, (*2*) reduction of the Car content, (*3*) reduction of the number of plastids containing a PLB, (*4*) increase of R (see below and Table 2), despite of an unchanged amount of LPOR enzymes. Such an effect, which was also observed by Moro *et al.* (2004) (see above), is contradictory with the biochemical studies showing that LPOR import is driven by active synthesis of Pchlide (reviewed by Masuda and Takamiya 2004). Recently, conflicting results appeared in the literature. Reinbothe *et al.* (1995) were the first to describe a Pchlide-dependent mode of import of LPOR in isolated plastids, but the results could not be repeated (Aronsson *et al.* 2000, Dahlin *et al.* 2003) and this mode of import was considered as an artefact (Aronsson *et al.* 2003). Kim and Apel (2004) reinvestigated the difficult question of LPORA import in plastids of intact seedlings. Their results demonstrate that the import of LPORA into plastids of cotyledons is substrate-dependent, while the import into plastids of leaves is substrate independent.

The effects of levulinic acid on lipid-LPOR interactions and PLB-membrane microviscosity were also studied using fluorescence probes pyrene and diphenylhexatriene, respectively (Savchenko *et al.* 2004). In the presence of pyrene, pyrene excitation did not result in a fluorescence of Pchlide in levulinic acid-treated leaves and pyrene eximerization increased. Because a Pchlide fluorescence signal and a decrease in pyrene eximerization were observed in protein-free artificial micelles containing free Pchlide (Denev *et al.* 2000), Savchenko *et al.* (2004) concluded that non-photoactive Pchlide cannot be a free-pigment but should be composed of pigments somehow associated with protein(s). Although a detailed study of the spectral composition of the non-photoactive Pchlide was not performed in this study, the conclusion of this work constitutes an additional argument for the model of formation of photoactive Pchlide proposed by Schoefs and collaborators (Schoefs 2001, Schoefs and Franck 2003, Bertrand and Schoefs 2005). In this model, two main spectral forms of non-photoactive Pchlide, *i.e.* $P_{?\text{-}631}$ and $P_{?\text{-}637}$ (Schoefs *et al.* 2000a) are actually pigment-LPOR complexes (Fig. 1). This does not rule out the possibility for Pchlide to be bound to other proteins, which remain to be identified. As far as the interaction between LPOR and lipids is concerned, the comparison of the energy transfer from pyrene to LPOR in levulinic acid-treated and control plants indicates that the microenvironment of the protein was slightly modified by the treatment (Savchenko *et al.* 2004). In order to determine whether the absence of photoactive Pchlide modifies the fluidity of the plastid inner membranes, the microviscosity of the membrane was probed using diphenylhexatriene. The results indicate that the absence of the large aggregates of photoactive Pchlide and of Cars modifies the membrane fluidity (Savchenko *et al.* 2004).

There is increasing evidence that the increment of heavy metals in the environment constitutes a major problem (reviewed by Delfino 2004, Poirier and Bertrand 2005). The photosynthetic apparatus is one of the numerous target sites of heavy metals identified so far (*e.g.* Bertrand *et al.* 2001, reviewed by Mysliwa-Kurdziel *et al.* 2002, Poirier and Bertrand 2005). Heavy metals may also interact with the Chl biosynthetic pathway (reviewed by Mysliwa-Kurdziel and Strzalka 2002). New data dealing with the impact of heavy metals on the Pchlide-Chlide cycle have been published by Mysliwa-Kurdziel and Strzalka (2004) and Solymosi *et al.* (2004a). These authors showed that the dark-incubation of isolated PLB with chrome [Cr(IV)], iron [Fe(III)], cadmium [Cd(II)], or mercury [Hg(II)] triggers a decrease of the amount of photoactive Pchlide and an increase of the amount of non-photoactive Pchlide much larger than when the PLBs are incubated with water (Table 1). Thus these metals are able to destabilize the large aggregates of photoactive Pchlide. The higher the content of either metal, the faster is the decrease. Using iron and mercury [Hg(II)], a large pigment degradation was also observed (Mysliwa-Kurdziel and Strzalka 2004, Solymosi *et al.* 2004a). When the photoreduction was assayed in heavy-metal-incubated PLBs using a single ‘saturating’ flash (the flash was saturating when tested with non-treated samples), the fluorescence at 657 nm did not disappear completely suggesting that the pool of photoactive Pchlide could not be entirely transformed to Chlide by a single saturating flash. This demonstrates that the tested heavy metals are able to interact through several ways with the Pchlide-Chlide cycle. Some of these ways have been identified (Mysliwa-Kurdziel and Strzalka 2004):

(*1*) Interactions with the LPOR active site: Cd interacts with SH groups and thus can interfere with those localized in the LPOR active site (reviewed by Schoefs and Franck 2003).

(*2*) Modification of the redox potential: Cr(VI) is a powerful oxidant (Cr^{6+}/Cr^{3+} = 1.44 V), which can trigger oxidation of NADPH to $NADP^+$ ($NADPH/NADP^+$ = –0.32 V). The replacement of NADPH by $NADP^+$ inside most probably occurs in Cr-incubated samples because an emission band at approximately 649 nm, typical for LPOR-Pchlide-$NADP^+$ ternary complexes (reviewed by Schoefs and Franck 2003), is clearly seen after photoreduction (Table 1). In addition, the dark-incubation with Cr(VI) triggered the increase of the emission at approximately 667 nm, which has been associated with the formation of very large aggregates of non-photoactive Pchlide (reviewed in Schoefs and Franck 2003, Bertrand and Schoefs 2005) (Table 1). Another effect of the change in the oxidation state of NADPH can be the reduction of the size of the photoactive aggregates with formation of dimers ($P_{638-645}$).

(*3*) Change in the environment allowing pigment degradation: A strong decrease of pigment content, together with an increase of protopheophorbide formation, *i.e.* release of Mg from the Pchlide, has been recorded after the incubation of isolated PLB with Fe and Hg. As the release of the Mg ion at the centre of Chl constitutes one of the first steps of the Chl degradation pathway (reviewed by Bertrand and Schoefs 1999), one can hypothesize that Fe and Hg made the PLB’s-pigment more available for degradation.

The Pchlide-Chlide cycles

The first step of the Pchlide-Chlide cycles consists in the absorption of photons by Pchlide molecules and its reduction to Chlide by LPOR enzymes (Fig. 1). The comparison of the ‘Pchlide-Chlide’ cycle in leaves at different development stages lead to the conclusion that two ‘Pchlide-Chlide’ cycles run together after the irradiation (Fig. 1). The proportion of Chlide molecules entering each cycle depends on the plastid developmental stage and seems to be correlated to the proportion of non-photoactive Pchlide present in the leaf before the photoreduction (Table 2). Consequently, when the non-photoactive to photoactive Pchlide ratio is high—the factor R defined by Schoefs (2001)—as in embryonic leaves, most of the Chlide molecules produced during the irradiation follow cycle I (Fig. 1). Cycle I can be divided in two phases: (*1*) release of the Chlide molecules without dislocation of the large aggregates, and (*2*) reconstitution of photoactive Pchlide through binding of non-photoactive Pchlide molecules by LPOR enzymes (Schoefs *et al.* 2000b). When R is low, as in etiolated leaves, most of the Chlide molecules (85–88 %) produced during the irradiation follow cycle II (Fig. 1). Cycle II can be divided into three parts (Fig. 1): (*1*) replacement of $NADP^+$ by NADPH; (*2*) dislocation of the large aggregates containing Chlide molecules, and (*3*) reconstitution of photoactive Pchlide through binding of non-photoactive Pchlide molecules by LPOR enzymes. Regardless the cycle involved in Chlide formation, the newly formed Chlide molecules are esterified (Fig. 1). Actually the transformation of Chlide to Chl is a four step process, which requires esterification in itself (formation of Chlide geranylgeraniol) followed by three successive hydrogenations (transformation of Chlide geranylgeraniol to Chlide phytol, *i.e.* regular Chl). When the kinetic of Chlide esterification is established in irradiated barley leaves, it presents two phases: a fast phase completed within 1 min after photoreduction, and a slow phase with a plateau 20 min after photoreduction (Rassadina *et al.* 2004). The Chlide molecules esterified during the fast phase correspond to those formed through cycle I (Bertrand and Schoefs 2000, Domanskii and Rüdiger 2001). This fast esterification would be possible because LPOR and Chl synthase, preloaded with tetraphenyl diphosphates, would form complexes (Schmid *et al.* 2002). The kinetic of the rapid phase was not influenced by exogenous hormones (cytokinin, abscisic acid), inhibitors of protein synthesis (cycloheximide or chloramphenicol), or the Chl esterification inhibitor (NaF), but the ratio between the intermediates was changed: the GG hydrogenation was inhibited (Rassadina *et al.* 2004). In contrast, the application of an exogenous cytokinin such as kinetin increased the speed of the slow phase, whereas NaF and cycloheximide decreased it. The effect of NaF was already described by Wiktorsson *et al.* (1996) and was explained by the inhibition of LPOR de-phosphorylation, which precludes PLB disaggregation and dislocation of the large aggregates containing the newly formed Chlide molecules. The effect of cycloheximide seems to indicate that the amount of Chl synthase is low in etiolated plants and the expression of the corresponding gene is regulated by irradiation (Rassadina *et al.* 2004). Abscisic acid and chloramphenicol do not have an effect.

As already mentioned, Chlide molecules to be esterified should have left the LPOR active site. Consequently, the LPOR enzyme can bind new molecules of Pchlide and in the presence of NADPH photoactive Pchlide regeneration occurs (reviewed in Schoefs 2001). This step is designed as the photoactive Pchlide regeneration. Because the transformation of Chlide to Chl in cycles I and II proceeds at different speeds (see above), the regeneration of photoactive Pchlide kinetics also has two phases (Schoefs *et al.* 2000b, Rassadina *et al.* 2004).

The effects of inhibitors of Car biosynthesis on the formation of photoactive Pchlide have been described in the first part of this review. When norflurazon did not influence the value of R and the amitrole treatment triggered a dramatic increase of R, which became high, consequently most of the newly formed Chlide molecules were engaged in cycle I instead in cycle II. In addition in amitrole-treated samples the Pchlide-Chlide cycle could not be completed because photoactive Pchlide did not regenerate (Moro *et al.* 2004).

The leaves of plants performing C_4 photosynthesis have two populations of chloroplasts. The first one is localized in the bundle sheath tissues and typically the chloroplasts are devoid of grana. The second population is localized in the mesophyll and typical chloroplasts have grana. When compared, the chloroplasts from these tissues also differ in many points such as polypeptide composition and primary photosynthetic and CO_2 fixation processes (Edwards and Walker 1983). Do the differences between these two types of chloroplasts appear during the etiolation process or do they result from a differentiation process occurring during chloroplast maturation? It is difficult to find in literature an answer to this interesting question. For instance PLBs from bundle sheath cells have a reduced size and more prothylakoids compared to those from the mesophyll cells (Rüffer-Turner *et al.* 1984). Marchand *et al.* (2004) dissected the formation of Chl molecules in bundle sheath cells and evaluated the consequences of their findings in term of PS2 biogenesis. As expected from the ultrastructural data (Rüffer-Turner *et al.* 1984), a high value of R (2.32) was found in the etioplasts from bundle sheath cells, while the value of R for intact leaves was low (0.38) (Marchand *et al.* 2004) (Table 2). Consequently, Chl molecules were mostly formed through the 'Pchlide-Chlide' cycle I in isolated bundle sheath plastids. When studied in the intact leaves, the 'Pchlide-Chlide' cycle producing Chlide molecules was usually of type II. This result clearly illustrates that heterogeneity in the mechanisms producing Chl molecules can exist inside the same tissue.

Franck *et al.* (1997) showed that the formation of a particular spectral form of Chlide of the 'Pchlide-Chlide' cycle II—$C_{684\text{-}696}$ (Fig. 1)—is an obligatory step on the pathway leading to PS2 assembly. As this intermediate is only formed during cycle II (reviewed in Schoefs 2001), PS2 formation in bundle sheath plastids is very low (Marchand *et al.* 2004). Besides the scientific conclusions of this study, we should keep in mind that the deductions made on the basis of studies on intact leaves are a kind of mean of all the processes taking place inside these leaves and be aware that at the tissue level heterogeneity does exist. I think that the complete understanding of the greening process should further include other factors than molecules and also their localization inside the tissues.

A crucial question, which remains largely unsolved, is how the data on the Pchlide-Chlide cycles obtained with higher plants can be extrapolated to lower organisms, including non-flowering land plants, green, brown, and red algae, and cyanobacteria. The *in vitro* studies performed recently by Heyes *et al.* (2002, 2003) using over-expressed *lpor* gene from cyanobacterium enlightened this problem. Using LPOR from *Synechocystis* they showed that the Pchlide-Chlide cycle is composed by one light-driven reaction followed by two reactions that do not require irradiation (Heyes *et al.* 2002, 2003). The starting point of the cycle is the formation of photoactive Pchlide, probably $P_{638\text{-}644}$, suggesting that in the absence of a complex lipid environment, such as that of PLB, the formation of larger aggregates does not occur. *In vitro* R is always high. Consequently, it is not surprising that under irradiation the Chlide form probably corresponds to $C_{670\text{-}675}$ (Fig. 1). A detailed analysis of the spectral changes indicates one intermediate absorbing at 681 nm and emitting at 684 nm (Heyes *et al.* 2003). This confirms my deduction about the products of the different photoactive Pchlide spectral forms of dark-grown higher plants (Schoefs 2000, Schoefs and Bertrand 2005) (Fig. 1). Using LPOR enzymes from the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1, Heyes and Hunter (2004) got evidence for the formation of an additional intermediate absorbing at 685 nm and emitting at 689 nm. Reconstitution experiments showed that this spectral form corresponds to LPOR-Chlide-NADPH complexes (Heyes and Hunter 2004).

Chl synthesis during greening and in green plants

The production of Chl molecules is not restricted to the period during which the first photons are absorbed by the leaf. Actually, the production of Chl molecules is only very active during greening. This is shown by the fact that at the beginning of barley leaf greening, the capacity to accumulate Pchlide in the dark and the chlorophyllase activity are high. In contrast, during senescence all these parameters are weak (Pshibytko *et al.* 2004). Pchlide photoreduction is catalyzed by different LPOR isoenzymes—LPORA-LPORC—at the beginning of greening and in green tissues (see above). Although the spectral properties of the analysis of non-photoactive and photoactive Pchlide in dark-grown plants over-expressing *lporA* or *lporB* genes are similar to those found in etiolated tissues (Franck *et al.* 2000), no detailed analysis of the non-photoactive and photoactive Pchlide present in greening leaves have been reported before the work by Amirjani and Sundqvist (2004). After 1 h of irradiation, small amounts of $P_{?-625}$ and $P_{?-631}$ were observed (Table 3). The presence of an accompanying band of photoactive Pchlide, as observed by Schoefs and Franck (1991) and Schoefs *et al.* (1992), is probably dependent on the irradiance used for greening. After an extended period of irradiation no Pchlide was detected (Amirjani and Sundqvist 2004). When the samples were placed back in dark for 4 h, several spectral forms of non-photoactive ($P_{?-631}$) and photoactive ($P_{648-653}$, $P_{650-657}$) Pchlides accumulated *de novo* (Fig. 1), but the ratio between the spectral forms was dependent on the preceding irradiation time. During the period of darkness, PLB reforms and structural observations indicate that the pattern of the membrane might be different from that found in non-irradiated tissues (Henningsen 1970). In this study the type of LPOR of isoenzymes was not determined, but LPORC distribution has been studied in leaves of *Beta vulgaris* (Tripathy *et al.* 2004).

Table 3. Comparison of the spectral heterogeneity of the non-photoactive and photoactive protochlorophyllide pools observed in etiolated bean leaves (Schoefs *et al.* 2000), during greening (Amirjani and Sundqvist 2004), and in red-radiation-grown wheat (Sood *et al.* 2004). ND: non-determined, XD: X h in the dark, XL: X h under irradiation.

Spectral form	Photo-activity	During greening			Red-radiation-grown wheat leaves + 6D	
		1L	3L	1L+4D	Root-shoot transition zone uncovered	covered
$P_{?-625}$	-	+	-	ND	ND	ND
$P_{?-631}$	-	+	-	+	+	+
$P_{?-637}$	-	ND	-	ND	+ if incubated with δ-ALA	ND
$P_{?-643}$	-	ND	-	ND	ND	ND
$P_{642-649}$	-	ND	-	ND	ND	ND
$P_{638-645}$	+	-	-	ND	ND	ND
$P_{?-652}$	+	-	-	+	+	ND
$P_{650-657}$	+	-	-	+	ND	+
$P_{?-667}$	-	ND	-	ND	ND	ND

Sood *et al.* (2004) studied the effect of irradiation of the root-shoot transition zone on the accumulation of Pchlide in leaves in red-radiation-grown wheat (500 µmol m^{-2} s^{-1}, wavelength = 670±26 nm). The leaves from plants with an irradiated root-shoot transition zone differed from those which zone was not irradiated, by the absence of the spectral bands corresponding to photosystems in fluorescence spectra. In either sample no fluorescence from Pchlide was found during the irradiation but the amount of Pchlide increased when the samples were replaced to darkness for 6 h. In the former type of samples (with an irradiated root-shoot transition zone), both non-photoactive Pchlide $P_{?-631}$ and photoactive Pchlide $P_{?-653}$ were accumulated. The value of R was high and under an additional flash Chlide was formed through the cycle I (Tables 1 and 3). After the flash some fluorescence remained at 650 nm, reflecting the presence of $P_{642-649}$. When the samples were incubated with δ-aminolevulinic acid (δ-ALA), the universal precursor of tetrapyrroles (reviewed by Bertrand and Schoefs 2005), non-photoactive Pchlide emitting fluorescence at 634 nm accumulated. This result confirms earlier results obtained with etiolated material treated in similar conditions (Sundqvist 1973). In the latter type of plants (with a non-irradiated root-shoot transition zone) the incubation with δ-ALA in the dark results mostly in the accumulation of non-photoactive Pchlide. The emission band was broad and a flash transformed the few photoactive Pchlide formed in these conditions (Sood *et al.* 2004).

So far the synthesis of Chlide *a* molecules was only considered in this review. Besides Chl *a*, the photosynthetic apparatus of green algae and higher plants as well as some Prochlorophytes contain also Chl *b*. The biosynthetic pathway leading to the formation of Chl *b* has been recently elucidated. Actually, part of the Chlide *a* pool is transformed to Chlide *b* (*e.g.* Schoefs *et al.* 1998) through the catalytic action of Chlide *a* oxygenase (CAO) and then esterified (reviewed in Bertrand and

Schoefs 2005). The rate of Chl accumulation not only changed during greening but also under environmental stresses, such as heat-shock or water deficit (*e.g.* Tewari and Tripathy 1998, Zayed and Zeid 1998). In order to increase our understanding of the impacts of stresses on Chl accumulation at different stages of greening, Pshibytko *et al.* (2004) measured the amount of Chls ($a+b$) in the first leaf of barley grown at 22–20 °C for 4, 7, or 11 d under a photoperiod of 16 h and then submitted the leaf to a heat stress (40 °C, 3 h) or to water deficit (immersion of the roots into 3 % *PEG 6000*, 45 h). Compared to the control plants, the heat stress somewhat reduced (by 10 %) the total Chl content at every developmental stage investigated. Water deficit had a different impact with regard to the greening stage. In the 4-d-old leaves the Chl amount was higher (17 %) in stressed leaves than in the control ones. No change was observed in the 7-d-old leaves, whereas in the 11-d-old leaves the Chl amount was reduced. In order to determine whether the changes in Chl amount induced by the stress were due to a block in the Chl synthesis or due to an increase of the Chl catabolism, the rate of Pchlide accumulation in the dark (3 h) and the activity of chlorophyllase—the enzyme, which catalyzes the first step of Chl catabolism (reviewed by Bertrand and Schoefs 1999, Hoertensteiner and Matile 2004)—were measured. In wild type (WT) plants the chlorophyllase activity decreased almost linearly along greening. Both heat stress and water deficit had similar consequences on the activity of chlorophyllase (Pshibytko *et al.* 2004). In young seedlings the activity was strongly reduced (by more than 50 %), not affected in 7-d-old seedlings, and strongly increased in old plants (by more than 50 %). The changes in chlorophyllase activity can be explained in term of enzyme inactivation and by a change in the photosynthetic membrane arrangement. In young seedlings the heat treatment mostly affects the enzyme activity without modifying the membrane organization (Pshibytko *et al.* 2003). Consequently, a drop in the chlorophyllase activity was monitored when compared to the control leaves. Considering that the enzyme inactivation is similar at every developmental stage, the fact that the modification in the membrane arrangement may lead to a larger amount of substrate available for chlorophyllase, therefore the activity of the enzyme increases. Altogether the result indicates that under these stresses the chlorophyllase activity does not change significantly after the 4th day of development (Fig. 3). The curve describing the capacity to accumulate Pchlide in the dark during greening of non-stressed plants has two phases: (*1*) a large and fast decrease, which occurs during the first 6 d of greening, and (*2*) a slow decrease, which reaches a minimal value in 11-d-old plants (Pshibytko *et al.* 2004). Such a decrease is expected as the demand in Chl molecules decreases when the plastids reach the mature stage. Regardless the greening stage, the water deficit had no impact on Pchlide accumulation, whereas the heat stress decreased this capacity (Pshibytko *et al.* 2004). The older the leaves, the stronger the decrease was (Pshibytko *et al.* 2004). Again this effect can be explained by the disturbance of photosynthetic membranes by heat stress (Pshibytko *et al.* 2003). Actually, the formation of the large aggregates of photoactive Pchlide requires the structuration of lipids as shown by the formation of the PLB. As the heat stress influences the lipid organization only at a late developmental stage, it is not surprising that the impairment of Pchlide accumulation in the dark was larger in 11-d-old seedlings than in the 4-d-old ones.

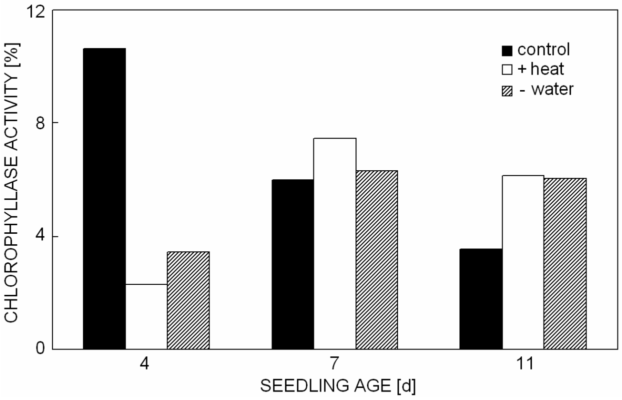

Fig. 3. Temperature and water-deficit stresses on the chlorophyllase activity at different developmental stages of the first leaf of barley (4-, 7-, or 11-d-old). The plants were grown at 22–20 °C under a photoperiod of 16 h and then submitted to a heat stress (40 °C, 3 h) or to water deficit (immersion of the roots into 3 % *PEG 6000*, 45 h) (redrawn from Pshibytko *et al.* 2004).

Regulation of Chl synthesis

Isolated tetrapyrroles can easily transfer the energy of absorbed photons to oxygen, leading to the formation of activated oxygen species, which in turn can damage the surrounding molecules (Ricchelli 1995). In non-irradiated leaves and at the beginning of the greening process Pchlide and the newly formed Chlide molecules are not energetically connected to Cars, which are able to quench both triplet Chl and activated oxygen species. Thus neither Pchlide nor Chlide are protected against damage by radiant energy (*e.g.* Hirschberg and Chamovitz 1994; reviewed by Schoefs and Franck 2003) and the accumulation of free Pchlide and Chlide can result in a lethal photo-oxidative damage as soon as the plant is irradiated (*e.g.* op den Camp *et al.* 2003). Consequently, the cell production of tetrapyrrole molecules should be strongly regulated in order to prevent the accumulation of non-protected pigments. How this regulation proceeds is still unclear, but some members of the regulation network have already been identified (reviewed by Vasileuskaya *et al.* 2004, Schoefs and Bertrand 2005). Several aspects such as irradiance, endogenous signalling, or extra-nuclear mutations dealing with the regulation of tetrapyrrole synthesis have been investigated in cyanobacteria, ferns, and angiosperms.

Regulation by radiant energy: sensing and signalling

Numerous photosynthetic organisms are capable of acclimation to changes to irradiance and radiation quality. Among prokaryotes the group of cyanobacteria, which performs the complementary chromatic adaptation, is the best-studied in this respect. Complementary chromatic adaptation was originally used to describe a photo-reversible change in the colour phenotype that is dependent on the colour of radiation in which the cells are grown (Kehnoe and Grossman 1994). The adaptation consists of a shift in the ratio of the two phycobiliproteins, phycocyanin and phycoerythrin. In green radiation phycoerythrin accumulates, whereas in red radiation phycocyanin accumulates. Many studies on this topic have been performed since its first description by Gaidukov (1903). In order to identify new genes regulated by radiation colour, Stowe-Evans *et al.* (2004) performed a genomic DNA micro-array analysis in *Fremyella diplosiphon*. Among the numerous genes for which the expression was up-regulated by green radiation, one finds *chlL* and *chlN*, two genes encoding polypeptides of DPOR.

The control of tetrapyrrole synthesis was also investigated in cyanobacterium species, which do not perform chromatic adaptation such as *Synechocystis* sp. PCC 6803. Regulation of the expression of the genes (*chlL*, *chlN*, and *chlB*) encoding the polypeptides composing DPOR are expressed in the dark and repressed under nitrogen-fixing conditions (reviewed in Fujita and Bauer 2003), “white light” (Hihara *et al.* 2001, Huang *et al.* 2002), ultraviolet radiation (Huang *et al.* 2002), iron deficiency (Singh *et al.* 2003), and change in the redox state of photosynthetic electron transport chain (Hihara *et al.* 2003). Other studies have suggested that the small Cab-like proteins ScpB-ScpE are involved in the regulation of tetrapyrrole biosynthesis in *Synechocystis* sp. PCC 6803. This hypothesis was based on the fact that in single *scpB* or *scpE* deletion mutants Pchlide contents decreased under irradiation-activated heterotrophic growth (15 min “white light” *per* 24 h), whereas upon transfer to continuous irradiation the mutants had a slower recovery of the Chl content than the control cells (Xu *et al.* 2002). Because of the high similarity in the primary sequences of the ScpB-ScpE proteins it was hypothesized that they have a complementary regulatory function in Chl biosynthesis and therefore they may functionally compensate for each other to some degree. In order to test this hypothesis, Xu *et al.* (2004) compared the effects of multiple *scp* gene mutations on pigment biosynthesis in different background strains. When the number of deleted *scp* gene(s) increased, a progressive decrease of the Chl content was observed, especially in the PS1-less and PS1-less/PS2-less strains that have already a very low Chl content. A detailed pigment analysis revealed that in these last two strains, Chlide molecules but not Pchlide ones were progressively accumulated, when the number of deleted *scp* genes increased. Taking into account that in PS1-less/*chlL* (*chlL* is a gene encoding one of the polypeptides composing DPOR) strains grown in the presence of δ-ALA different intermediates accumulated depending on the *scp* gene mutated (*scpE*: Mg-protoporphyrin IX and Mg-protoporphyrin IX monomethyl ester; *scpB*: Pchlide) (Xu *et al.* 2002), the strains missing multiple *scp* genes were unable to compete with WT strains when co-cultivated under irradiation, because the mutants accumulated tetrapyrrole intermediates (Xu *et al.* 2004). Thus Scp proteins probably play major and complex roles in the regulation of Chl production in cyanobacteria.

Eukaryotic photosynthetic cells can also react to changes in the radiation colour and/or irradiance. These changes such as de-etiolation are mediated by photoreceptors such as phytochromes and cryptochromes (reviewed by Casal 2000, Neff *et al.* 2000). The former photoreceptor type actually belongs to a family containing up to 5 photoreceptors denoted as PhyA-PhyE [tobacco: PhyA-PhyB—Adam *et al.* 1997; *Arabidopsis*: PhyA-PhyE—Clark *et al.* 1994]. Each type of phytochrome monitors the incident red and far-red radiation reaching the plant by switching reversibly between two conformers upon sequential absorption of red and far-red radiation. Within the PhyA type of phytochrome there exist two spectroscopically and photochemically distinguishable subpopulations denoted PhyA’ and PhyA”. The former is the major irradiation-labile and longer-wavelength species dominating in tissue growth, whereas the latter is the minor relatively irradiation-stable and shorter-wavelength species, more evenly distributed in plants (reviewed in Sineshchekov 2004). The activated phytochrome transduces subsequently the perceived information to the target(s). Among the numerous loci involved in the phytochrome signalling pathways, one finds the phytochrome-interacting factors (PIF) that bind directly to the photoreceptor (reviewed by Quail 2002). PIF proteins are members of the bHLH family of transcriptional factors, which modulate target gene expression. Recently, a new PIF protein, designated as PIF1 [*Arabidopsis* Genome Initiative (AGI) locus number At2g20180], also a member of the bHLH family was discovered (Ni *et al.* 1998) and tested for a possible implication in the regulation of Chl biosynthesis (Huq *et al.* 2004). Mutants over-expressing PIF1 show some perturbations in seedling growth, but neither these nor the T5-DNA-insertion mutants with altered expressions present normal photo-responsiveness of germination. This suggests that PIF1 is not necessary for normal seedling de-etiolation. However, a marked bleaching phenotype was observed in *pif1* mutants (Fig. 4). Pigment analysis revealed that *pif1* mutants accumulate more Pchlide than wild type. This phenotype is qualitatively reminiscent of *flu* mutants, which also accumulate Pchlide (reviewed by Vasileuskaya *et al.* 2004, Schoefs and Bertrand 2005). Therefore the bleaching of *pif1* and *flu* mutants seems to have the same origin: a photo-oxidative stress triggered by the transfer of photon energy absorbed by the excess of Pchlide (and may be other tetrapyrrole intermediates) to oxygen. However, direct targets of *flu* and *pif1* appear to be different (Huq *et al.* 2004). In order to determine the action of PIF1 in the regulation circuit controlling Chl biosynthesis, Huq *et al.* (2004) compared the Chl accumulation and bleaching in wild type (WT) and in *pif1* and *phyA* or *phyB* mutants grown in the dark for increasing time periods before a period of greening. Comparison of bleaching rate in the WT and *pif1* showed that the longer the etiolation the stronger the bleaching phenotype. This suggests that PIF1 acts as a repressor of the transformation of Pchlide precursors into Pchlide. Therefore when the expression of the gene *pif* is impaired, non-photoactive Pchlide is especially accumulated (Fig. 4). On the other hand, when Chl accumulation curves obtained with the different mutant lines are compared, Chl accumulation is faster in *pif1* mutants and slower in both *phy* mutants than in WT (Fig. 5). Altogether these findings suggest that phytochrome A (PhyA) and/or phytochrome B (PhyB) can repress PIF1 activity under irradiation. Interactions between PIF1 and PhyA or PhyB were indeed demonstrated, the strongest affinity being measured with PhyB. PIF1 is localized in the nucleus. Its bHLH domain allows it to bind to the G-box DNA sequence motif (CACGTG) (Huq *et al.* 2004), which has been identified in many promoters of light-regulated genes (Martinez-Garcia *et al.* 2000, Huq and Quail 2002), but DNA-PIF1 complexes were not capable to interact with PhyA or PhyB. However, physical interactions between PhyA or PhyB and PIF1 negatively modulate PIF1, the transcriptional activity.

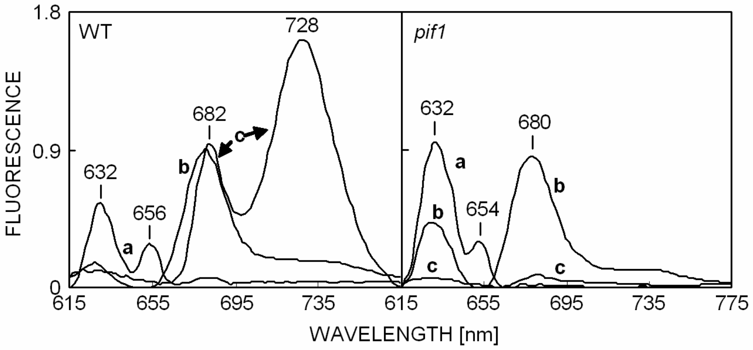

Fig. 4. 77 K fluorescence spectra of wild type (WT) and *pif* mutant recorded (a) before the irradiation and after (b) 10 min or (c) 24 h of irradiation (data redrawn from Huq *et al.* 2004).

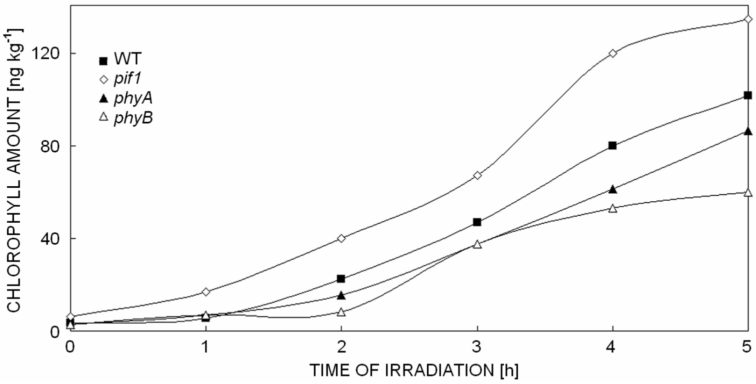

Fig. 5. Perturbation of the chlorophyll (Chl) accumulation capacity in *pif1, phyA*, or *phyB* mutants of *Arabidopsis thaliana*. 2-d-old or 3-d-old dark-grown seedlings were transferred to continuous "white light" for different time periods and the Chl amount was measured after extraction in *N',N'*-dimethylformamide (data redrawn from Huq *et al.* 2004.)

Another target of PhyA seems to be the process leading to the formation of the photoactive Pchlide aggregates. Two studies dealt with this particular step of the biogenesis of the photosynthetic apparatus: Antipova *et al.* (2004) investigated the control of LPOR accumulation in tobacco by PhyA comparing the LPOR amount in WT and *phyA* anti-sense transgenic mutant of tobacco. When grown in far-red (FR) radiation (λ≥680 nm), the mutant seedlings contained more than three times more Chl molecules than WT, which was able to accumulate only a very small amount of Chl due to the irradiation conditions (see Sineshchekov *et al.* 2004). Dot-blot analysis of LPOR showed that under FR, but not in darkness, the anti-sense mutant contained more LPOR than the WT (Antipova *et al.* 2004) confirming that PhyA regulates the *lpor* gene expression. Sineshchekov *et al.* (2004) studied another aspect of photoactive Pchlide formation, the relative accumulation of non-photoactive and photoactive Pchlide. They showed that when grown in constant FR ($\lambda \geq 720$ nm), the content in non-photoactive and photoactive Pchlide in upper stems of tobacco and pea increased dramatically, while no Chl molecules accumulated. The presence or absence of Chl molecules in FR-irradiated tissues seems to depend primarily on the quality of the filters used for irradiation (see Antipova *et al.* 2004). Similar changes in the non-photoactive and photoactive Pchlide contents, but of lower amplitude, were also observed in pea leaves and tobacco cotyledons. These results agree with those obtained by Antipova *et al.* (2004) (see above), but contrast with those obtained with upper stems of tomato and *Arabidopsis* cotyledons because in these species FR did not induce any change in the non-photoactive to photoactive Pchlide ratio. Sineshchekov *et al.* (2004) concluded that the effect of FR on biogenesis of photoactive Pchlide is not only dependent on the species but also on the organ. The meaning of this differential regulation remains unclear and the discrepancies between the results presented in Figs. 1 and 2 are of no help for the understanding. The comparison of the FR effects on the change in the R value in tobacco transgenic lines over-expressing full-length PhyA or N-terminally truncated (Δ7-69 oat PhyA), which belongs to the PhyA” (Sineshchekov *et al.* 1999), leads to the conclusion that PhyA’ is responsible for the mediation (Sineshchekov *et al.* 2004). The effect would be inhibited by PhyA”.

The size of the light-harvesting antenna is often measured by the Chl *a*/*b* ratio and that size can change according to environmental changes. For instance, when the irradiance decreases, the Chl *a/b* ratio decreases (*e.g.* Walters and Horton 1994). How the modifications in the value of Chl *a/b* are realized and controlled, were the main questions of Harper *et al.* (2004). These authors found that the modifications in Chl *a/b* correlate with changes in *CAO* mRNA and CAO protein contents. For example, when the plants were moved from moderate irradiance (230 µmol m^{-2} s^{-1}) to shade irradiance (45 µmol m^{-2} s^{-1}) Chl *a/b* decreased after one day and the *CAO* mRNA contents increased at the same time, while the CAO protein contents did not increase until one day later. Similar results were obtained with the *cch1* mutant, which is a leaky Chl mutant with a pronounced yellow-green phenotype under moderate irradiance. This phenotype results from a mutation in the *chlH* gene encoding the H subunit of Mg-chelatase (Mochizuki *et al.* 2001). In the mutant some protoporphyrin IX molecules are accumulated. When the *cch1* mutant, previously grown in low irradiance (95 µmol m^{-2} s^{-1}), is transferred to moderate irradiance a large increase of Chl *a/b* was monitored, while the *CAO* mRNA and CAO protein contents decreased. The amount of Chl molecules also decreased in the mutant but not in the WT. The decrease in the total Chl amount in the *cch1* mutant can be due to photo-bleaching triggered by the protoporphyrin IX accumulated, or due to an inhibition of δ-ALA synthesis by the protoporphyrin IX accumulated (reviewed by Bertrand and Schoefs 2005). To test these hypotheses, the protoporphyrin IX amount was measured before and after the transfer to moderate irradiance of *cch1* and WT plants. In both plant types a slight increase in the pigment content was observed after the transfer. After two days of growth at moderate irradiance, the capacity to transform protoporphyrin IX into Pchlide was tested by shifting the plants to the dark. In both plant lines Pchlide accumulated, indicating that the protoporphyrin IX was normally transformed to Pchlide. Altogether these results suggest that the Chl *b* synthesis is not merely regulated by Chlide *a* availability, but that adjustment to Chl availability can take place at CAO protein abundance. The findings also suggest that changes in *CAO* mRNA and protein contents as a response to changes in irradiance are part of a long-term acclimating mechanism.

Impact of extranuclear mutations on Pchlide synthesis

Chloroplast biogenesis requires the cooperation of nuclear and chloroplast genomes (Taylor 1989). According to the endosymbiotic theory this cooperation is a consequence of the transfer of many genes from the original chloroplast to the actual nuclear genome (Martin *et al.* 2002). Usatov *et al.* (2004) studied the ultrastructure and the pigment content in plastids from the variegated mutant line *var10* of sunflower (Beletskii *et al.* 1969). The plants from *var10* lines exhibit leaves with alternating white and green areas, which therefore constitute a very clear phenotype. The study of the plastid ultrastructure at the border between white and green areas of leaves from the *var10* mutant showed that the content in plastid is heterogeneous with chloroplasts and plastids without thylakoids (Usatov *et al.* 2004). In contrast, cells from the white areas only contained plastids deprived of thylakoids but with vacuoles. Accordingly, the pigment analyses of samples from white and green leaf areas showed large differences. Green areas had contents of Chls and Cars similar to the WT leaves, while in the white zone the content was strongly reduced (Chls: 0.2–3.0 %; Cars: 8–10 %). As expected, the pigment deficiency was strongly affected by the growth conditions, *i.e.* irradiance and/or temperature (Table 4). These measurements indicated that pigment deficiency in the *var10* line is not a stable trait. In order to determine whether leaf bleaching is solely due to the Car deficiency or to a deregulation of Chl precursor synthesis in the *var10* line, Usatov *et al.* (2004) compared the capacity to

Table 4. Effects of irradiance [W m^{-2}] and temperature [°C] on the amounts of photosynthetic pigments [% of wild type, WT] in the variegated mutant line *var10* of sunflower.

Irradiance	Temperature	Chlorophylls	Carotenoids
0.04	20-22	100.0	100
0.04	28-30	17.0	73
11.00	20-22	0.1–0.2	8–10

synthesize δ-ALA and Pchlide of WT and *var10* mutant plants. The results demonstrate that the mutant tissue comprises all the necessary enzymes and cofactors to produce Chl precursors in the dark, but the amount of some of them such as Chl synthase—the enzyme, which catalyzes the esterification of Chlide—can be lower than in the WT (Vezitskii *et al.* 1999). Because only Chl but not Chlide is able to bind the polypeptides of the photosynthetic apparatus, the cells with a low amount of Chl synthase will not be able to transform the newly formed Chlide molecules to Chl ones. Therefore these cells accumulate non-protected tetrapyrroles, which can easily be photo-oxidized.

References

Adam, E., Kozma-Bognar, L., Schafer, E., Nagy, F.: Tobacco phytochrome: genes, structure and expression. – Plant Cell Environ. **20**: 678-684, 1997.

Amirjani, M.R., Sundqvist, C.: Regeneration of protochlorophyllide in green and greening leaves of plants with varying proportions of protochlorophyllide forms in darkness. – Physiol. Plant. **121**: 377-390, 2004.

Antipova, T.V., Gapeeva, T.A., Volotovsky, I.D.: Photoregulation of protochlorophyllide oxidoreductase and rubisco large subunit accumulation in phytochrome A-deficient transgenic tobacco plants. – Russ. J. Plant Physiol. **51**: 152-155, 2004.

Aronsson, H., Sohrt, K., Soll, J.: NADPH:protochlorophyllide oxidoreductase uses the general import route into chloroplasts. – J. biol. Chem. **381**: 1263-1267, 2000.

Aronsson, H., Sundqvist, C., Dahlin, C.: POR hits the road: import and assembly of a plastid protein. – Plant mol. Biol. **51**: 1-9, 2003.

Beletskii, Y.D., Razoriteleva, E.K., Zhdanov, Y.A.: [Cytoplasmic mutations of sunflower induced by *N*-nitrosomethylurea.] – Dokl. Akad. Nauk SSSR **186**: 1425-1426, 1969. [In Russ.]

Bertrand, M., Schoefs, B.: Photosynthetic pigment metabolism in plants during stress. – In: Pessarakli, M. (ed.): Handbook of Plant and Crop Stress. 2nd Ed. Pp. 527-543. Marcel Dekker, New York – Basel 1999.

Bertrand, M., Schoefs, B.: The formation of chlorophyll from chlorophyllide in leaves containing proplastids is a four-step process. – FEBS Lett. **486**: 243-246, 2000.

Bertrand, M., Schoefs, B.: Protochlorophyllide photoreduction – a review. – In: Pessarakli, M. (ed.): Handbook of Photosynthesis. 2nd Ed. Pp. 97-107. Taylor & Francis, Boca Raton – London – New York – Singapore 2005.

Bertrand, M., Schoefs, B., Šiffel, P., Roháček, K., Molnar, I.: Cadmium inhibits epoxidation of diatoxanthin to diadinoxanthin in the xanthophyll cycle of the marine diatom *Phaeodactylum tricornutum*. – FEBS Lett. **508**: 153-156, 2001.

Böddi, B., Bóka, K., Sundqvist, C.: Tissue specific protochlorophyll(ide) forms in dark-forced shoots of grapevine (*Vitis vinifera* L.). – Photosynth. Res. **82**: 141-150, 2004.

Casal, J.J.: Phytochromes, cryptochromes, phototropin: photoreceptor interactions in plants. – Photochem. Photobiol. **71**: 1-11, 2000.

Clark, T., Mathews, S., Sharrock, R.A.: The phytochrome apoprotein family in *Arabidopsis* is encoded by five genes: the sequences and expression of *phyD* and *phyE*. – Plant mol. Biol. **25**: 413-427, 1994.

Dahlin, C., Aronsson, H., Almkvist, J., Sundqvist, C.: Protochlorophyllide-independent import of two NADPH:protochlorophyllide oxidoreductase proteins (LPORA and PORB) from barley into isolated plastids. – Physiol. Plant. **109**: 289-303, 2003.

Delfino, J.J.: Revisiting heavy metals in the environment: using wetlands for their removal. – Ecol. Model. **178**: 35-38, 2004.

Denev, I.D., Stupak, A., Klyuchareva, E.A., Savchenko, G.E.: Spectral and kinetic characteristics of pyrene fluorescence in the model systems and membranes of etioplasts. – Zh. prikl. Spektroskop. **67**: 743-747, 2000.

Di Baccio, D., Quartacci, M.F., Dalla Vecchia, F., La Rocca, N., Rascio, N., Navari-Izzo, F.: Bleaching herbicide effects on plastids of dark-grown plants. Lipid composition of etioplasts in amitrole and norflurazon-treated barley leaves. – J. exp. Bot. **53**: 1857-1865, 2002.

Domanskii, V., Rüdiger, W.: On the nature of the two pathways in chlorophyll formation from protochlorophyllide. – Photosynth. Res. **68**: 131-139, 2001.

Eckhardt, U., Grimm, B., Hortensteiner, S.: Recent advances in chlorophyll biosynthesis and breakdown in higher plants. – Plant mol. Biol. **56**: 1-14, 2004.

Edwards, G.E., Walker, D.A.: C3, C4: Mechanisms, and Cellular and Environmental Regulation of Photosynthesis. – Blackwell Scientific, Oxford 1983.

Franck, F., Eullaffroy, P., Popovic, R.: Formation of long-wavelength chlorophyllide (Chlide 695) is required for the assembly of Photosystem II in etiolated barley leaves. – Photosynth. Res. **51**: 107-118, 1997.

Franck, F., Mathis, P.: A short-lived intermediate in the photoenzymatic reduction of protochlorophyll(ide) into chlorophyll(ide) at a physiological temperature. – Photochem. Photobiol. **32**: 799-803, 1980.

Franck, F., Sperling, U., Frick, G., Pochert, B., Cleve, B., Apel, K., Armstrong, G.A.: Regulation of etioplast pigment-pigment complexes, inner membrane architecture, and protochlorophyllide *a* chemical heterogeneity by light-dependent NADPH:protochlorophyllide oxidoreductase A and B. – Plant Physiol. **124**: 1678-1696, 2000.

Fujita, Y., Bauer, C.E.: The light-independent protochlorophyllide reductase: a nitrogenase-like enzyme catalyzing a key reaction for greening in the dark. – In: Kasdish, K.M., Smith, K.M., Guilard, R. (ed.): The Porphyrin Handbook. Chlorophylls and Bilins: Biosynthesis, Synthesis, and Degradation. Pp. 109-156. Elsevier Science, New York 2003.

Gaidukov, N.: Weitere Untersuchungen über den Einfluss farbigen Lichtes auf die Farbung der Oscillarien. – Ber. deutsch. bot. Gesell. **21**: 484-492, 1903.

Harper, A.L., Gesjen, S.E. von, Linford, A.S., Peterson, M.P., Faitcloth, R.S., Thissen, M.M., Brusslan, J.A.: Chlorophyllide *a* oxygenase mRNA and protein levels correlate with the chlorophyll *a/b* ratio in *Arabidopsis thaliana*. – Photosynth. Res. **79**: 149-159, 2004.

Henningsen, K.W.: Macromolecular physiology of plastids. VI. Changes in membrane structure associated with shifts in the absorption maxima of the chlorophyllous pigments. – J. Cell Sci. **7**: 587-621, 1970.

Heyes, D.J., Hunter, C.N.: Identification and characterization of the product release steps within the catalytic cycle of protochlorophyllide oxidoreductase. – Biochemistry **43**: 8265-8271, 2004.

Heyes, D.J., Ruban, A.V., Hunter, C.N.: Protochlorophyllide oxidoreductase: spectroscopic characterization of the dark reactions. – Biochemistry **42**: 523-528, 2003.

Heyes, D.J., Ruban, A.V., Wilks, H.M., Hunter, C.N.: Enzymology below 200 K: the kinetics and thermodynamics of the photochemistry catalyzed by protochlorophyllide oxidoreductase. – Proc. nat. Acad. Sci. USA **99**: 11145-11150, 2002.

Hihara, Y., Kamei, A., Kanehisa, M., Kaplan, A., Ikeuchi, M.: DNA microarray analysis of cyanobacterial gene expression during acclimation to high light. – Plant Cell **13**: 793-806, 2001.

Hihara, Y., Sonoike, K., Kanehisa, M., Ikeuchi, M.: DNA microarray analysis of redox-responsive genes in the genome of the cyanobacterium *Synechocystis* sp. strain PCC 6803. – J. Bacteriol. **185**: 1719-1725, 2003.

Hirschberg, J., Chamovitz, D.: Carotenoids in cyanobacteria. – In: Bryant, D.A. (ed.): The Molecular Biology of Cyanobacteria. Pp. 559-579. Kluwer Academic Publ., Dordrecht – Boston – Lancaster 1994.

Hoertensteiner, S., Matile, P.: How leaves turn yellow: catabolism of chlorophyll. – In: Nooden, L.D. (ed.): Plant Cell Death Processes. Pp. 182-202. Elsevier, San Diego 2004.

Huang, L., McCluskey, M.P., Ni, H., LaRossa, R.A.: Global gene expression profiles of the cyanobacterium *Synechocystis* sp. strain PCC 6803 in response to irradiation with UV-B and white light. – J. Bacteriol. **184**: 6845-6858, 2002.

Huq, E., Al-Sady, B., Hudson, M., Kim, C., Apel, K., Quail, P.H.: PHYTOCHROME-INTERACTING FACTOR 1 is a critical bHLH regulator of chlorophyll biosynthesis. – Science **305**: 1937-1941, 2004.

Huq, E., Quail, P.H.: PIF4, a phytochrome-interacting bHLH factor, functions as a negative regulator of phytochrome B signaling in *Arabidopsis***.** – EMBO J. **21**: 2441-2450, 2002.

Ignatov, N.V., Belyaeva, O.B., Litvin, F.F.: Possible role of flavin components of protochlorophyll(ide)-protein complexes in primary processes of protochlorophyllide photoreduction in etiolated plant leaves. – Photosynthetica **29**: 235-241, 1993.

Kehnoe, D.M., Grossman, A.R.: Complementary chromatic adaptation: photoperception to gene regulation. – Semin. Cell Dev. Biol. **5**: 303-313, 1994.

Kim, C., Apel, C.: Substrate-dependent and organ-specific chloroplast protein import in plants. – Plant Cell **16**: 88-98, 2004.

Marchand, M., Dewez, D., Franck, F., Popovic, R.: Proto-chlorophyllide phototransformation in the bundle sheath cells of *Zea mays*. – J. Photochem. Photobiol. B **75**: 73-80, 2004.

Martin, W., Rujan, T., Richly, E., Hansen, A., Cornelsen, S., Lins, T., Leister, D., Stoebe, B., Hasegawa, M., Penny, D.: Evolutionary analysis of *Arabidopsis*, cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus. – Proc. nat. Acad. Sci. USA **99**: 12246-12251, 2002.

Martinez-Garcia, J., Huq, E., Quail, P.H.: Direct targeting of light signals to a promoter element-bound transcription factor. – Science **288**: 859-863, 2000.

Masuda, T., Takamiya, K.: Novel insights into the enzymology, regulation and physiological functions of light-dependent protochlorophyllide oxidoreductase in angiosperms. – Photosynth. Res. **81**: 1-29, 2004.

Mochizuki, N., Brusslan, J.A., Larkin, R., Nakatani, A., Chory, J.: *Arabidopsis* genome uncoupled 5 (*gun5*) mutant reveals the involvement of Mg-chelatase H subunit in plastid-to-nucleus signal transduction. – Proc. nat. Acad. Sci. USA **98**: 2053-2058, 2001.

Moro, I., Dalla Vecchia, F., La Rocca, N., Navari-Izzo, F., Quartacci, M.F., Di Baccio, D., Rüdiger, W., Rascio, N.: Impaired carotenogenesis can affect organization and functionality of etioplast membranes. – Physiol. Plant. **122**: 123-132, 2004.

Mysliwa-Kurdziel, B., Prasad, M.N.V., Strzalka, K.: Heavy metal influence on the light phase of photosynthesis. – In: Prasad, M.N.V., Strzalka, K. (ed.): Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants. Pp. 229-255. Kluwer Academic Publ., Dordrecht 2002.

Mysliwa-Kurdziel, B., Strzalka, K.: Influence of metals on biosynthesis of photosynthetic pigments. – In: Prasad, M.N.V., Strzalka, K. (ed.): Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants. Pp. 201-227. Kluwer Academic Publ., Dordrecht 2002.

Mysliwa-Kurdziel, B., Strzalka, K.: Influence of Cd(II), Cr(VI) and Fe(III) on early steps of deetiolation process in wheat: fluorescence spectral changes of protochlorophyllide and newly formed chlorophyllide. – Agr. Ecosyst. Environ. **106**: 199-207, 2004.

Neff, M.M., Fankhauser, C., Chory, J.: Light as an indicator of time and place. – Genes Dev. **14**: 257-271, 2000.

Ni, M., Tepperman, J.M., Quail, P.H.: PIF3, a phytochrome-interacting factor necessary for normal photoinduced signal transduction, is a novel basic helix-loop-helix protein. – Cell **95**: 657-667, 1998.

op den Camp, R.G.L., Przybyla, D., Ochsenbein, C., Laloi, C., Kim, C., Danon, A., Wagner, D., Hideg, E., Göbel, C., Feussner, I., Nater, M., Apel, K.: Rapid induction of distinct stress responses after the release of singlet oxygen in *Arabidopsis*. – Plant Cell **15**: 2320-2332, 2003.

Ouazzani Chahdi, M.A., Schoefs, B., Franck, F.: Isolation and characterization of photoactive complexes of NADPH:protochlorophyllide oxidoreductase from wheat. – Planta **206**: 673-380, 1998.

Park, H., Kreunen, S.S., Cuttriss, A.J., Della Penna, D., Pogson, B.J.: Identification of the carotenoid isomerase provides insight into carotenoid biosynthesis, prolamellar body formation and photomorphogenesis. – Plant Cell **14**: 321-332, 2002.

Pshibytko, N.L., Kalitukho, L.N., Zhavoronkova, N.B., Kabashikova, L.F.: The effect of heat shock and water deficit on the state of chloroplast photosynthetic membranes of different age leaves of *Hordeum vulgare* L. – Biol. Membr. **20**: 121-127, 2003.

Pshibytko, N.L., Kalitukho, L.N., Zhavoronkova, N.B., Kabashikova, L.F.: The pool of chlorophyllous pigments in barley seedlings of different ages under heat shock and water deficit. – Russ. J. Plant Physiol. **51**: 15-20, 2004.

Quail, P.H.: Phytochrome photosensory signalling network. – Nature Rev. mol. cell. Biol. **3**: 85-93, 2002.

Rassadina, V., Domanskii, V., Averina, N.G., Schoch, S., Rüdiger, W.: Correlation betwen chlorophyllide esterification, Shibata shift and regeneration of protochlorophyllide650 in flash irradiated etiolated barley leaves. – Physiol. Plant. **121**: 556-567, 2004.

Reinbothe, S., Runge, S., Reinbothe, C., van Cleve, B., Apel, K.: Substrate-depednent transport of the NADPH:protochlorophyllide oxidoreductase into isolated plastids. – Plant Cell **7**: 161-172, 1995.

Ricchelli, F.: Photophysical properties of porphyrins in biological membranes. – J. Photochem. Photobiol. B **29**: 109-118, 1995.

Rüffer-Turner, M.E., Bradbeer, J.W., Stocking, C.R., Montes, G., Wara-Aswapati, O.: Chloroplast development in *Zea mays*. – Israel J. Bot. **33**: 133-147, 1984.

Ryberg, M., Dehesh, K.: Localization of NADPH-protochlorophyllide oxidoreductase in dark-grown wheat (*Triticum aestivum*) by immuno-electron microscopy before and after transformation of the prolamellar bodies. – Physiol. Plant. **66**: 616-624, 1986.

Savchenko, G.E., Klyuchareva, E.A., Abramchik, L.M.: Formation of pigment apparatus in etiolated barley leaves under the influence of levulinic acid. – Russ. J. Plant Physiol. **51**: 316-325, 2004.

Schmid, H.C., Rassadina, V., Oster, U., Schoch, S., Rüdiger, W.: Pre-loading of chlorophyll synthase with tetraphenyl diphosphate is an obligatory step in chlorophyll biosynthesis. – J. biol. Chem. **383**: 1769-1776, 2002.

Schoefs, B.: Photoreduction of Protochlorophyllide *a* to Chlorophyllide *a* During the Biogenesis of the Photosynthetic Apparatus in Higher Plants. – Dissertation 2000.

Schoefs, B.: The protochlorophyllide-chlorophyllide cycle. – Photosynth. Res. **70**: 257-271, 2001.

Schoefs, B., Bertrand, M.: Protochlorophyllide photoreduction – a review. – In: Pessarakli, M. (ed.): Handbook of Photosynthesis. 2nd Ed. Pp. 97-107. Taylor & Francis, Boca Raton – London – New York – Singapore 2005.

Schoefs, B., Bertrand, M., Franck, F.: Plant greening: Biogenesis of photosynthetic apparatus in bean leaves irradiated shortly after the germination. – Photosynthetica **27**: 497-504, 1992.

Schoefs, B., Bertrand, M., Franck, F.: Spectroscopic properties of protochlorophyllide analyzed *in situ* in the course of etiolation and in etiolated leaves. – Photochem. Photobiol. **72**: 85-93, 2000a.

Schoefs, B., Bertrand, M., Funk, C.: Photoactive protochlorophyllide regeneration in cotyledons and leaves from higher plants. – Photochem. Photobiol. **72**: 660-668, 2000b.

Schoefs, B., Bertrand, M., Lemoine, Y.: Changes in the photosynthetic pigments in bean leaves during the first photoperiod of greening and the subsequent dark phase. Comparison between old (10-d-old) leaves and young (2-d-old) leaves. – Photosynth. Res. **57**: 203-213, 1998.

Schoefs, B., Franck, F.: Photosystem II assembly in 2-day-old bean leaves during the first 16 hrs. of greening. – Compt. rend. Acad. Sci. Paris Sér. III **313**: 441-445, 1991.

Schoefs, B., Franck, F.: Photoreduction of protochlorophyllide to chlorophyllide in 2-d-old dark-grown bean (*Phaseolus vulgaris* cv. Commodore) leaves. Comparison with 10-d-old dark-grown (etiolated) leaves. – J. exp. Bot. **44**: 1053-1057, 1993.

Schoefs, B., Franck, F.: Chlorophyll biosynthesis: Mechanisms and evolution. – Photochem. Photobiol. **78**: 543-557, 2003.

Schoefs, B., Garnir, H.-P., Bertrand, M.: Comparison of the photoreduction of protochlorophyllide to chlorophyllide in leaves and cotyledons from dark-grown bean as a function of age. – Photosynth. Res. **41**: 405-417, 1994.

Sineshchekov, V.A.: Phytochrome A: functional diversity and polymorphism. – J. Photochem. Photobiol. Sci. **3**: 608-611, 2004.

Sineshchekov, V.A., Belyaeva, O.B., Sudnitsin, A.: Up-regulation by phytochrome A of the active protochlorophyllide, Pchlide^{655}, biosynthesis in dicots under far-red light. – J. Photochem. Photobiol. B **74**: 47-54, 2004.

Sineshchekov, V.A., Clough, R.C., Jordan-Beebe, E.T., Vierstra, R.D.: Fluorescence analysis of oat *phyA* deletion muatnts expressed in tobacco suggests that the N-termianl domain determines the photochemical and spectroscopic distinctions betwen PhyA’ and PhyA”. – Photochem. Photobiol. **69**: 728-732, 1999.

Singh, A.K., McIntyre, L.M., Sherman, L.A.: Microarray analysis of the genome-wide response to iron deficiency and iron reconstitution in the cyanobacterium *Synechocystis* sp. PCC 6803. – Plant Physiol. **132**: 1825-1839, 2003.

Skribanek, A., Apatini, D., Inaoka, M., Böddi, B.: Protochlorophyllide and chlorophyll forms in dark-grown stems and chlorophyll forms in dark-grown stems and stem-related organs. – J. Photochem. Photobiol. B **55**: 172-177, 2000.

Solymosi, K., Lenti, K., Mysliwa-Kurdziel, B., Fidy, J., Strzalka, K., Böddi, B.: Depending on concentration Hg^{2+} reacts with different components of the NADPH:protochlorophyllide oxidoreductase macrodomains. – Plant Biol. **6**: 358-363, 2004a.

Solymosi, K., Martinez, K., Kristol, Z., Sundqvist, C., Böddi, B.: Plastid differentiation and chlorophyll biosynthesis in different leaf layers of white cabbage (*Brassica oleracea* cv. *capitata*). – Physiol. Plant. **121**: 520-529, 2004b.

Sood, S., Tyagi, A.K., Tripathy, B.C.: Inhibition of Photosystem I and Photosystem II in wheat seedlings with their root-shoot transition zones exposed to red light. – Photosynth. Res. **81**: 31-40, 2004.

Staehelin, L.A.: Chloroplast structure: from chlorophyll granules to supra-molecular architechture of thylakoid membranes. – Photosynth. Res. **76**: 185-196, 2004.

Stowe-Evans, E.L., Ford, J., Kehoe, D.M.: Genomic DNA microarray analysis: identification of new genes regulated by light color in the cyanobacterium *Fremyella diplosiphon*. – J. Bacteriol. **186**: 4338-4349, 2004.

Sundqvist, C.: The relationship between chlorophyllide accumulation, the amount of protochlorophyllide$_{636}$ and protochlorophyllide$_{650}$ in dark grown wheat leaves treated with δ-aminolevulinic acid. – Physiol. Plant. **28**: 464-470, 1973.

Taylor, W.C.: Regulatory interactions between nuclear and plastid genomes. – Annu. Rev. Plant Physiol. Plant mol. Biol. **40**: 211-233, 1989.

Tewari, A.K., Tripathy, B.C.: Temperature-stress-induced impairment of chlorophyll biosynthesis reaction in cucumber and wheat. – Plant Physiol. **117**: 851-858, 1998.

Tripathy, B.C., Mohapatra, A., Pattanayak, G.K.: Subplastidic distribution of chlorophyll biosynthetic intermediates and characterization of protochlorophyllide oxidoreductase C. – ACS Symp. Ser. **887**: 107-128, 2004.

Usatov, A.V., Rassadina, V.V., Averina, N.G., Lezhneva, L.A., Dudko, Y.S., Mashkina, E.V., Prikhozhenko, E.Y., Kolokolova, N.S.: Structural and functional characteristics of the mutant plastids from extranuclear variegated forms of sunflower. – Russ. J. Plant Physiol. **51**: 156-163, 2004.

Vasileuskaya, Z., Oster, U., Beck, C.F.: Involvement of tetrapyrroles in inter-organellar signaling in plants and algae. – Photosynth. Res. **82**: 289-299, 2004.

Vezitskii, A.Y., Lezhneva, L.A., Shcherbakov, R.A., Rassadina, V.V., Averina, N.G.: [Activity of chlorophyll synthase and chlorophyll *b* reductase in the chlorophyll-deficient plastome mutants of sunflower.] – Fiziol. Rast. (Moscow) **46**: 580-585, 1999. [In Russ.]

Walters, R.G., Horton, P.: Acclimation of *Arabidopsis thaliana* to the light environment: Changes in composition of the photosynthetic apparatus. – Planta **195**: 248-256, 1994.

Wiktorsson, B., Ryberg, M., Sundqvist, C.: Aggregation of NADPH:protochlorophyllide oxidoreductase is favoured by protein phosphorylation. – Plant Physiol. Biochem. **34**: 23-34, 1996.

Xu, H., Vavilin, D., Funk, C., Vermaas, W.: Small Cab-like proteins regulating tetrapyrrole biosynthesis in the cyanobacterium *Synechocystis* sp. PCC 6803. – Plant mol. Biol. **49**: 149-160, 2002.

Xu, H., Vavilin, D., Funk, C., Vermaas, W.: Multiple deletions of small Cab-like proteins in the cyanobacterium *Synechocystis* sp. PCC 6803. Consequences for pigment biosynthesis and accumulation. – J. biol. Chem. **279**: 28971-27979, 2004.

Zayed, M.A., Zeid, I.M.: Effect of water and salt stresses on growth, chlorophyll, mineral ions and organic solutes contents, and enzymes activity in mung bean seedlings. – Biol. Plant. **40**: 351-356, 1998.