Adaptation of Chloroplast-Ultrastructure and of Chlorophyll-Protein Levels to High-Light and Low-Light Growth Conditions*

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In saturating light radish seedlings grown in high-light growth conditions (90 W · m^{-2}) possess a much higher photosynthetic capacity on a chlorophyll and leaf area basis than the low-light grown plants (10 W · m^{-2}). The higher CO$_2$-fixation rate of HL-plants is due to the presence of HL-chloroplasts which possess a different ultrastructure and also different levels of the individual chlorophyll-carotenoid-proteins than the LL-chloroplasts of LL-seedlings.

1. *Ultrastructure:* The high-light adapted chloroplasts are characterized by fewer photosynthetic membranes per chloroplast section, by low grana stacks (only few thylakoids per granum), a lower stacking degree of thylakoids, a higher proportion of non-appressed membranes (stroma thylakoids + end grana membranes) and a high starch content. The LL-chloroplasts possess no starch, their grana stacks are higher (up to 17 thylakoids per granum) and also significantly broader than that of HL-chloroplasts.

2. *Chlorophyll-proteins:* The photosynthetic apparatus of HL-chloroplasts contains a larger proportion of chlorophyll *a*-proteins of photosystem I (CPIa + CPI) and of photosystem II (CPa, the presumable reaction center of PS II) than the LL-chloroplasts which possess a higher proportion of light-harvesting chlorophyll *a*/*b*-proteins (LHCP$_1$, LHCP$_2$, LHCP$_3$, LHCP$_y$). The higher levels of LHCPs in LL-plants are associated with a higher ground fluorescence fo and maximum fluorescence fp of the *in vivo* chlorophyll.

3. *Chlorophyll and carotenoid ratios:* The chloroplasts of HL-plants possess a higher proportion of chlorophyll *a* and *β*-carotene (higher values for the ratios chlorophyll *a*/*b* and lower values for *a*/*c* and *x*/*c*) which reflect the increased level of the chlorophyll *a*/*β*-carotene-proteins CPIa, CPI and CPa. The higher level of light-harvesting chlorophyll *a*/*b*-xanthophyll-proteins (LHCPs) in LL-plants is also indicated by an increased content of xanthophylls and chlorophyll *b* as seen from lower *a*/*b* and higher *x*/*c* and *a*/*c* ratios.

4. The results indicate that plants possess the capacity for an ontogenetic adaptation of their photosynthetic apparatus to the incident light intensity. The HL-chloroplasts of HL-plants which contain less antenna chlorophyll, are adapted for a more efficient photosynthetic quantum conversion at light saturation than the LL-chloroplasts with high grana stacks. The correlation between higher levels of light-harvesting chlorophyll *a*/*b*-proteins (LHCPs) and a higher stacking degree of thylakoids, and the involvement of LHCPs in stacking is discussed.

Introduction

Growth, development and morphology of plants at high light quanta fluence rates (HL-plants) are quite different from those of plants grown at low light-intensity (LL-plants) demonstrating the ability of plants and of chloroplasts to respond with a distinctive growth-response to the incident light intensity. This capacity for light adaptation is documentated by specific changes in the morphology, physiology, biochemistry and structure of leaves and of chloroplasts [1–6].

The photosynthetic apparatus of sun leaves and leaves from HL-plants (sun-type or HL-chloroplasts) is adapted for high rates of photosynthetic light quanta conversion [2–6]. This is associated with a different chemical composition of thylakoids, as seen by higher values for the ratio chlorophyll *a*/*b*

Abbreviations: *a*/*b*, ratio chlorophyll *a*/*b*; *a*/*c*, weight ratio chlorophyll *a* to *β*-carotene, CPI and CPIa, the two P700 containing chlorophyll *a* *β*-carotene-proteins of photosystem I; CPa, chlorophyll *a* *β*-carotene-protein of photosystem II, *c*/*x*, ratio *β*-carotene/xanthophylls; fo, ground fluorescence of the *in vivo* chlorophyll fluorescence; fp, maximum level of the *in vivo* chlorophyll fluorescence; FP, free pigments; HL, high-light growth condition; LHCPs, sum of the 4 light-harvesting-chlorophyll *a*/*b*-proteins LHCP$_1$, LHCP$_2$, LHCP$_3$ and LHCP$_y$; LL, low light growth condition; PAGE, polyacrylamide-gel electrophoresis; PS I and PS II photosynthetic photosystem I and II; SDS, sodium dodecylsulphate; Tris, tris (hydroxymethyl)-aminomethan; v/v, volume per volume; w/v, weight per volume; x/c, weight ratio xanthophylls to *β*-carotene.

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[1–4, 7] lower values for the ratio xanthophylls to β-carotene (x/c) [1, 2, 8, 9] and a higher proportion of electron carriers, *e.g.* plastoquinone [1, 8, 9], phylloquinone [1, 8] and cytochromes [3, 4], and a higher level of RuBP-carboxylase on a chlorophyll basis [4, 10]. The ultrastructure of sun-type and HL-chloroplasts is characterized by smaller amounts of lamellar material, narrow and low grana stacks [2, 3] and large starch grains [1, 12]. In contrast, the shade-type and LL-chloroplasts not only exhibit high grana stacks [2, 3, 11] but also broader grana and a higher stacking degree of thylakoids [2, 11, 12].

Carotenoids and chlorophylls are quantitatively bound to the photochemically active thylakoids in form of several pigment-proteins which can be isolated by polyacrylamide gel electrophoresis of SDS solubilized chloroplasts [13–16]. There are 4 light-harvesting chlorophyll *a*/*b*-proteins $LHCP_1$, $LHCP_2$, $LHCP_3$ and $LHCP_Y$ which are characterized by low chlorophyll *a*/*b* values (1.1–1.3) and high values for the ratio chlorophyll *a*/β-carotene [16–17]. CPI and CPIa are the photochemically active, P700-containing chlorophyll *a*/β-carotene proteins of photosystem I [15–18]. $LHCP_1$ and $LHCP_2$ are regarded as dimers or oligomers of the presumable monomer $LHCP_2$ and CPIa as a possible oligomer of CPI [15, 19]. The minor component CPa, a chlorophyll *a*/β-carotene protein [16, 17] seems to represent the reaction center of photosystem II [15, 18, 20].

Since the individual pigment-proteins of chloroplasts possess a distinct pigment composition [17], the different chlorophyll and carotenoid composition of sun-type and HL-chloroplasts (changed ratios a/b and x/c, when compared to LL-chloroplasts) may be caused by changes in the ratio of the light-harvesting chlorophyll *a*/*b*-proteins (LHCPs) to the chlorophyll *a*-proteins CPI, CPIa, CPa. In shade-type and LL-chloroplasts with their larger chlorophyll antenna and larger photosynthetic unit size one can expect a higher proportion of LHCPs. This may also be the reason for the higher stacking degree and grana width of LL-chloroplasts. To test this we studied chloroplast ultrastructure, pigment content and pigment-protein levels in HL and LL-radish plants.

Materials and Methods

Radish seedlings (*Raphanus sativus* L. "Saxa Treib") were grown on peat in the dark for 3 d (25 °C, 65% relative humidity) and then illuminated with continuous white light (Osram HQIE lamps 400 W; 8 cm running water filter) of high (20 klux $= 90\ W \cdot m^{-2}$) or low quanta fluence rates (1 klux $= 10\ W \cdot m^{-2}$).

The photosynthetic pigments were extracted in acetone and light-petrol. Chlorophylls were determined in diethylether [21]. The levels of carotenoids were measured after separation by thin layer chromatography [1] or by high performance liquid chromatography [17].

The photosynthetic CO_2-fixation rates were measured in an open system with an infrared gas analyzer (URAS 2T Hartmann and Braun) at 24 °C using an Osram HQIE lamp 1000 W as light source with a running water layer as IR filter. The chlorophyll fluorescence induction curve was obtained at room temperature from 20 min darkened radish cotyledons using a special apparatus consisting of a Helium-Neon laser (4 mW; 632.8 nm as excitation light). The emitted fluorescence was separated from the excitation light by a Schott filter (RG 665) and registered via a photomultiplier (Type 7256 RCA).

Leaf segments were fixed in buffered 5% glutardialdehyde (pH 7.4), postfixed in buffered 2% OsO_4 (pH 7.4) and poststained with uranylacetate and lead citrate as previously described [11]. The stacking degree, the ratio of appressed to non-appressed membranes and the thylakoid frequency per chloroplast section (total thylakoid length in µm per 10 µm² median chloroplast section) were determined by measuring the length of stacked (appressed) and non-stacked membranes (stroma-thylakoids + end grana membranes) with a kilometer tracer.

The isolation of chloroplasts was performed in a sorbit-containing phosphate buffer [16]. For the solubilization of chloroplasts two different digestion buffer systems were applied. The buffer A solution consisted of Tris-HCl-buffer (50 mM; pH 6.8) containing 1% mercaptoethanol (v/v), 10% glycerol (v/v) and 0.5% SDS (w/v). The final solution contained about 0.15 µM chlorophyll per ml (weight ratio chlorophyll/SDS = 1:40). Buffer B, a modified Anderson buffer [22], consisted of a Tris-HCl-buffer (300 mM; pH 8.8), 13% glycerol (v/v) and 0.5% SDS (w/v).

Since the amounts of free pigments found after PAGE directly depend on the digestion time, the latter was kept very low. To achieve this, the chloroplast preparations incubated in the SDS-solubilization-buffer, stirred for 2 min and then centrifuged for 3 min at 10000 × *g* to remove small amounts of undissolved membrane material (if present) and particularly to remove considerable amounts of starch in the HL-chloroplast preparations. In the case of LL-chloroplasts (no starch) this centrifugation step could be omitted, stirring was then performed for 5 min. The thus solubilized chloroplast lamellae were immediately transferred to freshly prepared polyacrylamide gel cylinders.

Disk electrophoresis and gel preparations were performed in the cold room at 4 °C [16], following the method of Laemmli [23]. The relative amounts of the individual chlorophyll-protein bands were determined by scanning at 663 nm (RFT scanning densitometer). This wavelength was found to be best suited to simultaneously register in a comparative way both chlorophylls *a* and *b*. At wavelengths above 663 nm the proportion of protein-bound chlorophyll *a* would be overestimated and beyond 663 nm the amounts of chlorophyll *b*.

Results

Radish seedlings grown at high quanta fluence rates possess a higher photosynthetic CO_2-fixation capacity than plants which greened under low-light growth conditions. The higher photosynthesis rates are seen on a chlorophyll and cotyledon area basis (Fig. 1). These enhanced CO_2-fixation rates of the HL-plants are associated with higher rates of dark respiration.

The cotyledons of the HL-plants contained about the same chlorophyll content (ca. 100−120 µg per pair of cotyledons) and also a similar leaf area (ca. 3.3−3.6 cm² per pair of cotyledons). Thus on a 1 cm² leaf area basis HL and LL-cotyledons exhibited about the same chlorophyll content (HL 31 µg; LL 29 µg *a* + *b*). The mean values for the ratio chlorophyll *a*/*b* from several cultivations were higher in HL (3.2−3.7) than in LL-cotyledons (2.7−3.0). The higher chlorophyll *a*/*b* ratios are coupled to lower values for the ratio xanthophylls to β-carotene x/c and chlorophyll *a* to β-carotene a/c (Table III). These altered pigment ratios indicate that HL-plants possess another chloroplast-type in their cotyledons (sun-type or HL-chloroplasts) than the LL-plants which contain shade-type or LL-chloroplasts.

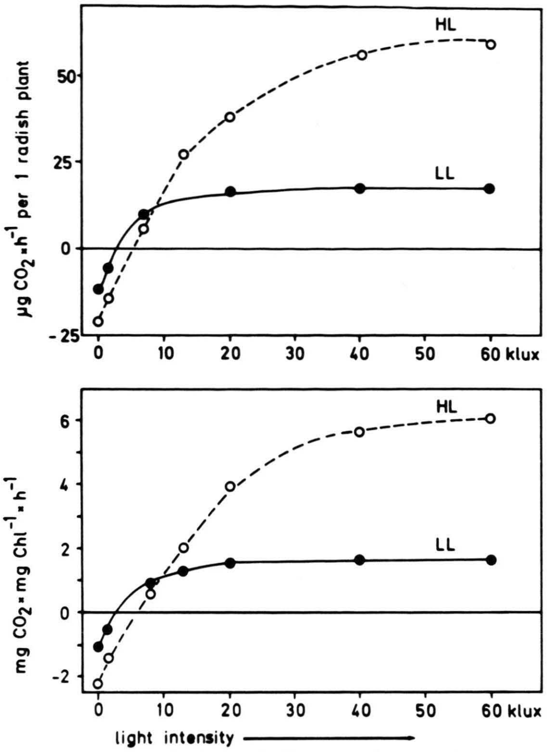

Fig. 1. Differences in photosynthetic CO_2-assimilation (mg $CO_2 \cdot h^{-1}$) of 7 d old HL and LL-radish plants (cotyledon stage) on a plant and on a chlorophyll basis. After 3 d dark growth the seedlings greened at either high (HL: 20 klux) or low light quanta fluence rates (LL: 1 klux).

Chloroplast ultrastructure: HL-chloroplasts not only differ in their chlorophyll-carotenoid-composition from LL-chloroplasts but also in their ultrastructure (Fig. 2). They contain fewer photosynthetic membranes (lower total thylakoid length) per chloroplast section, their grana stacks are narrower and contain fewer thylakoids, they exhibit a high starch content, more and larger plastoglobuli, a higher proportion of non-appressed membranes (= stroma thylakoids + end grana membranes) and a correspondingly lower ratio for appressed to non-appressed membranes (Table I).

The LL-chloroplasts, in turn, are characterized by higher and broader grana stacks, a higher stacking degree and a higher ratio of appressed to non-appressed membranes; however, they do not contain starch (Table I).

Chlorophyll-carotenoid-proteins: The differences in pigment composition, ultrastructure and thylakoid

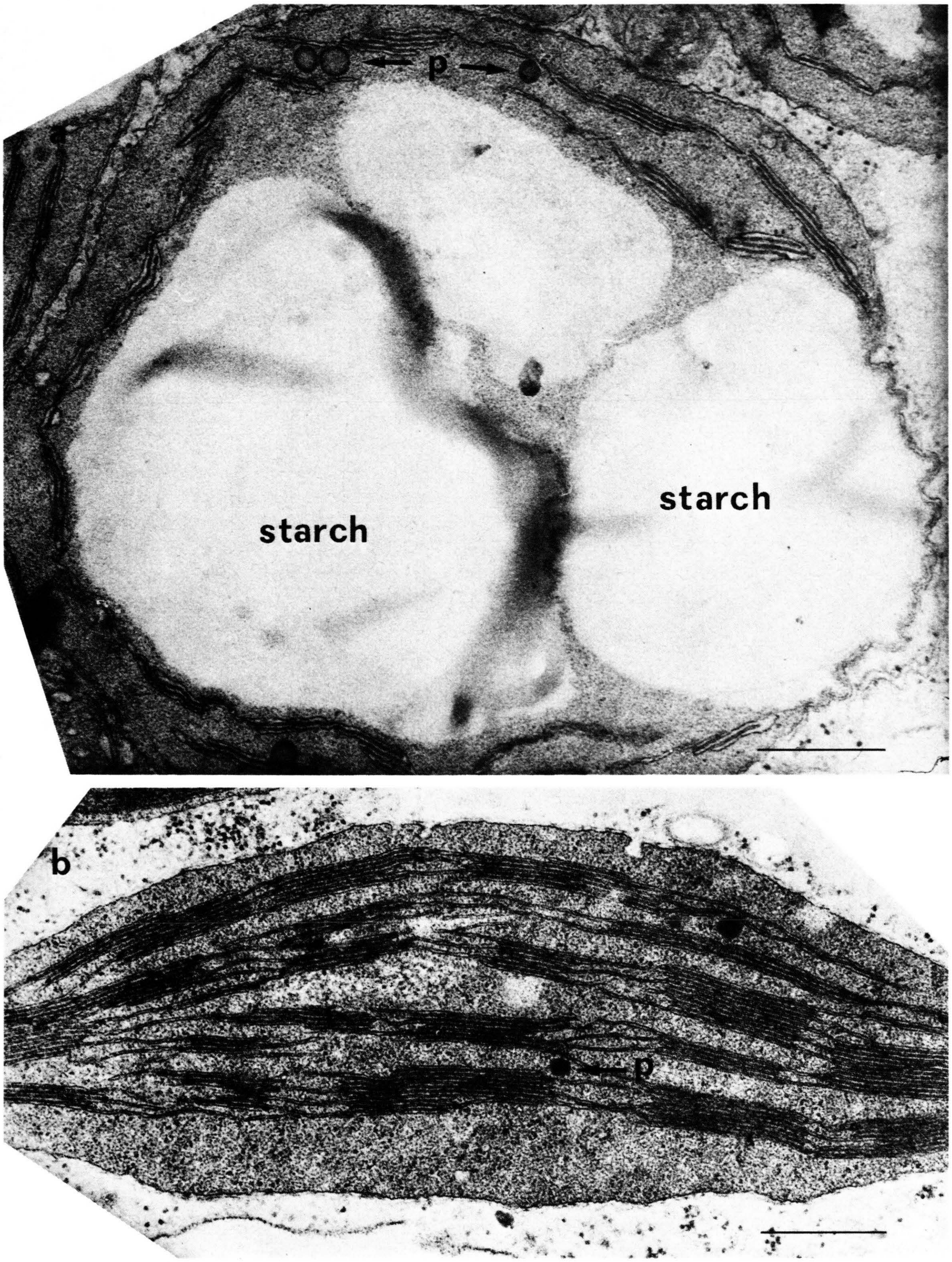

Fig. 2. Ultrastructure of chloroplasts from green cotyledons of 8 d old radish plants (3 dark growth + 5 d illumination). a) High-light (sun-type) chloroplast from plants greened at 20 klux (90 W · m^{-2}) and b) low-light (shade-type) chloroplast from plants greened at 1 klux (10 W · m^{-2}). (5% glutardialdehyd + 2% OsO_4; *p* = plastoglobuli; bar = 0.5 μm).

Table I. Differences in the ultrastructure of chloroplasts from 6 and 8 d old radish seedlings greened for 3 and 5 d under high-light and low-light growth conditions. The differences between HL and LL-chloroplasts are significant; error probability beyond 0.1% ($P < 0.001$).

Parameter	Age of plants	HL-chloroplasts $90\ W \cdot m^{-2}$	LL-chloroplasts $10\ W \cdot m^{-2}$
stacking degree of thylakoids (%) [a]	6 d	55 ± 5	62 ± 3
	8 d	55 ± 4	64 ± 3
ratio of appressed to non-appressed membranes [a]	6 d	1.2	1.6
	8 d	1.2	1.8
average grana width (µm)	6 d	0.28	0.34
	8 d	0.26	0.37
average number (and range) of thylakoids per granum [b]	6 d	3.0 (2 – 10)	3.9 (2 – 17)
	8 d	2.7 (2 – 6)	4.5 (2 – 17)
total length of thylakoids (in µm) per 10 µm² area of chloroplast section [c]	6 d	117 ± 27	197 ± 28
	8 d	88 ± 18	220 ± 32
per cent starch content per chloroplast section (% starch area of chloroplast area) [a]	6 d	34.1	0
	8 d	49.9	0
number of plastoglobuli per 100 chloroplast sections	6 d	749	303
	8 d	639	185

[a] Based on 40 median chloroplast sections.

[b] Based on 1200 (HL) and 1600 grana stacks (LL) of 40 chloroplasts.

[c] Based on 20 median chloroplast sections; the thylakoid length was determined per stroma matrix section; in the case of HL-chloroplasts the chloroplast section occupied by starch was deducted.

arrangement between HL and LL-chloroplasts are due to differences in the levels of the various chlorophyll-carotenoid-proteins of the photosynthetic apparatus. Seven chlorophyll-carotenoid-proteins with different mobility and pigment composition can be separated by polyacrylamide gel-electrophoresis of isolated, SDS-solubized HL and LL-chloroplasts. There are four light-harvesting chlorophyll *a*/*b* xanthophyll-proteins ($LHCP_1$, $LHCP_2$, $LHCP_3$, $LHCP_y$) which are characterized by low a/b ratios (1 – 1.3) and a high level of lutein and neoxanthin [17]. $LHCP_3$ is regarded as the monomer, and the other LHCPs as possible dimers and oligomers. CPa is a chlorophyll *a*/*β*-carotene protein thought to be the reaction center of photosystem II [18] which exhibits high amounts of *β*-carotene (~ 1 mol per 2 mol of chlorophyll *a*) [16, 17]. There are two more chlorophyll a-proteins, the P700-containing CPI and CPIa of photosystem I [18], which also contain a high level of *β*-carotene and only very low amounts of xanthophylls (lutein, violaxanthin and neoxanthin) [17].

The relative proportions of total chlorophyll found in the various chlorophyll-carotenoid-proteins depend on the composition and pH of the buffer system used in the solubilization of the isolated chloroplasts. Buffer A (pH 6.4) results in a larger fraction of $LHCP_3$ and $LHCP_1$ and little $LHCP_2$ and $LHCP_y$, whereas in buffer system B (pH 8.8) the levels of $LHCP_3$ and $LHCP_1$ are decreased with a simultaneous increase in the amounts of $LHCP_2$ (Fig. 3; Table II). The buffer system also affects the level of the chlorophyll *a*/*β*-carotene-proteins of photosystem I. Buffer A yields a higher proportion

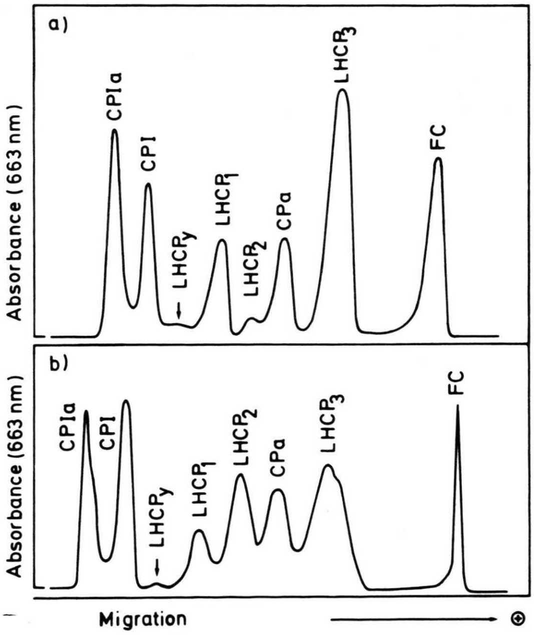

Fig. 3. Densitometer scans of the seven different chlorophyll-carotenoid-proteins from radish chloroplasts separated by SDS-PAGE using buffer A (a) and buffer B (b) for digestive solubilization. The disintegration pattern depends on the composition of the digestion buffer. Buffer A yields a higher proportion of CPIa and $LHCP_3$ and buffer B a higher percentage of CPI and $LHCP_2$. FP = free chlorophyll.

Table II. Percentage distribution of chlorophyll $a + b$ in the different chlorophyll-proteins of HL and LL-chloroplasts from cotyledons of 7 d old Raphanus seedlings (3 d dark growth + 4 d continuous illumination at high or low quanta fluence rates). The separation of chlorophyll-proteins was performed by SDS-PAGE using two different buffer systems. In both cases, HL-chloroplasts contain more chlorophyll in the chlorophyll *a*-proteins of PS I (CPIa + CPI) and PS II (CPa) than the LL-chloroplasts which possess more LHCPs. Mean values of 6 – 10 determinations from 3 cultivations with 4 runs per each preparation.

	Buffer A		Buffer B	
	HL	LL	HL	LL
a) *photosystem I (PS I)-proteins*				
CPIa	16.7	14	12	9
CPI	12.0	10.2	17	16
sum of PS I *	**28.7**	24.2	**29**	25
b) *photosystem II (PS II)-proteins*				
CPa *	**11.0**	9.0	**13.2**	10.4
LHCPs *	44.0	**50.7**	47.8	**55.7**
sum of PS II *	55.0	59.7	61.0	66.1
c) *free chlorophyll*				
	16.3	16.1	10.0	8.9
d) *% chlorophylls in LHCPs*				
LHCP_y	2.1	2.7	2.6	3.7
LHCP_1	11.0	13.4	7.2	7.8
LHCP_2	3.1	3.3	11.3	11.9
LHCP_3	27.8	31.3	26.7	32.3
e) *pigment-protein ratios*				
PS II/PS I *	1.9	2.5	2.1	2.6
PS I/CPa	2.6	2.7	2.2	2.4

* The differences between HL and LL-chloroplasts are significant ($P < 0.005$).

of CPIa than CPI, whereas buffer B produces a slightly higher proportion of CPI. The level of the chlorophyll *a*/*β*-carotene-protein CPa is somewhat higher in the buffer system B. An advantage of buffer A is a better separation of the chlorophyll-proteins which gives purer fractions for the chemical analysis. The level of the free chlorophylls (FP) in buffer A is, however, higher (16%) as in the buffer system B (9 – 10% free chlorophyll).

We have compared the absorption spectra of the different chlorophyll-carotenoid-proteins and found no significant differences between them whether using solubilization buffer A or B. This is shown for CPIa in Fig. 4. All LHCPs showed the typical maxima at 672 nm (chlorophyll a) and 653 nm (chlorophyll b) and also maxima at 471 and 439 nm, as previously described [16]. CPIa and CPI show identical spectra ($\lambda_{\max}$ at 677.5 to 678 nm); the red absorption maximum of CPa is at 672 nm [16]. All three chlorophyll *a*/*β*-carotene-proteins (CPIa, CPI, CPa) are lacking the characteristic chlorophyll *b* absorption band at 653 nm. The good agreement in the absorption spectra of the corresponding chlorophyll-proteins obtained with buffer system A and B shows that there are no principal differences in the desintegration pattern by applying the two different buffer systems. It depends, however on the particular buffer system whether more monomeric (*e.g.* LHCP_3) or a higher proportion of dimeric or oligomeric forms (*e.g.* LHCP_2) are obtained. The hypothesis that the observed LHCPs are different aggregation forms of LHCP_3 is further supported by a rather similar chlorophyll-xanthophyll composition of all four LHCPs [17].

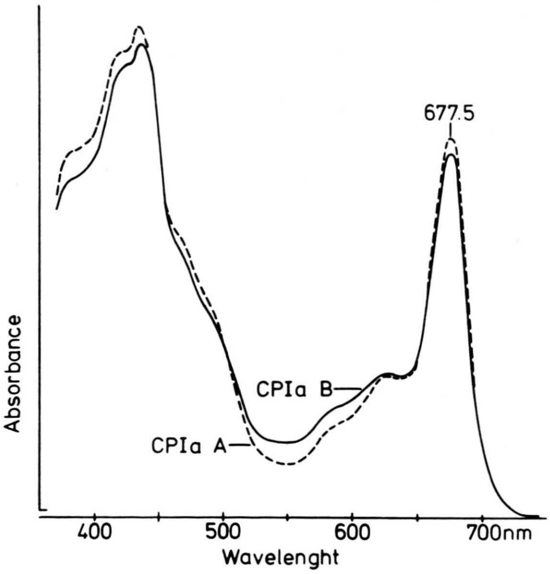

Fig. 4. Comparison of the absorption spectra of the chlorophyll *a*-protein CPIa isolated from radish chloroplasts by SDS-PAGE after applying solubilization buffer A (CPIa A) or buffer B (CPIa B).

Light-induced changes in chlorophyll-protein levels: SDS-PAGE of SDS-solubilized HL-chloroplasts and LL-chloroplasts of radish cotyledons reveal for both chloroplast types all seven previously described chlorophyll-proteins [16]. There are, however, significant differences in the relative amounts of the individual pigment-proteins as seen from the percent distribution of total chlorophyll in the pigment fractions (Table II). Depending on the buffer system HL-chloroplasts possess 28 to 29% of chlorophyll in CPIa + CPI and 44 to 47,8% in the LHCPs. LL-chloroplasts, in turn, exhibit lower amounts of CPIa + CPI (24–25%) and higher levels of the LHCPs (50.7 to 55.7%).

The ratio of total pigment-proteins from photosystem II (LHCPs + CPa) to that of photosystem I (CPIa + CPI) ranges in HL-chloroplasts between 1.9 and 2.1 for the buffer system A and B respectively; in LL-chloroplasts the corresponding values are 2.5 and 2.6. These ratios show that at LL-conditions a higher proportion of chlorophyll is packed into protein components of photosystem II. The slight decrease of the CPa level in LL-chloroplasts is more than compensated by the increase of the LHCPs. As compared to HL-chloroplasts the percentage of chlorophyll in the LHCPs of LL-chloroplasts is increased by 6.7 to 7.9% (Table II) and the increase in the stacking degree also lies in the same range (7–9%, Table I). This suggests that the LHCPs participate in thylakoid stacking.

The ratio of the two photochemically active photosystem I components CPIa + CPI to CPa, the presumable reaction center of photosystem II, is not significantly different (Table II), indicating that the decrease of CPIa + CPI in LL-chloroplasts is paralleled by an almost equivalent decrease in the level of CPa.

Chlorophyll fluorescence: The differences in pigment-protein levels between HL and LL-radish leaves are also reflected in the fluorescence induction signal obtained by illumination of 20 min darkened plants (Kautsky effect [24]). HL-leaves exhibit a low ground fluorescence fo and also a low maximum fluorescence fp (Fig. 5). The level of fo slightly decreases from the 4th of the 7th day of cotyledon greening in the HL-plant, parallel to a slow decrease in the level of chlorophyll $a + b$. It is important to note that cotyledon and plant development, including chlorophyll accumulation and also chlorophyll destruction proceed much faster at HL than at LL-growth conditions. The LL-cotyledons which possess a more stable chlorophyll content show a higher value for fo which does not change during development. The fp level of the LL-cotyledon is slightly decreased on the 7th day and is reached somewhat earlier.

From the relative amounts of fo and fp one can calculate the photosynthetic unit size [25]; a high ground fluorescence fo is found when many antenna chlorophylls are present (large photosynthetic unit size). The higher fo level of LL-cotyledons thus indicates a greater proportion of light-harvesting chlorophyll a/b-proteins which further supports the data in Table II. More antenna chlorophylls and a larger size of the photosynthetic units are also found in shade leaves of the beech which possess higher fo and fp levels than the sun leaves [2, 6].

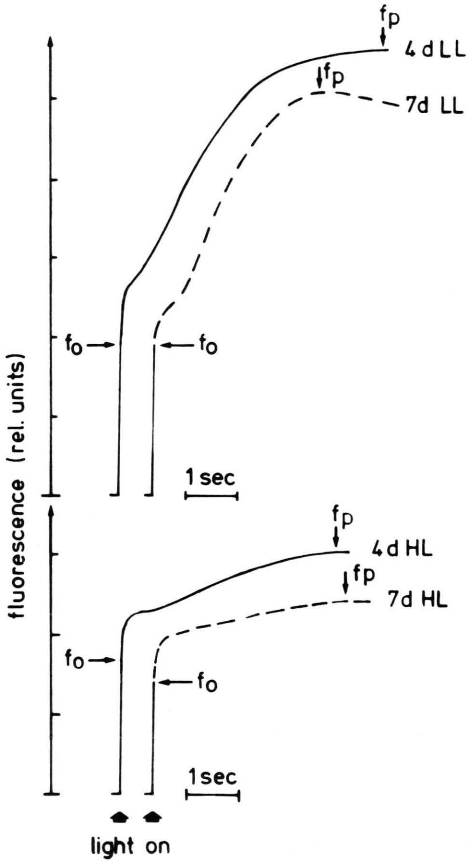

Fig. 5. Chlorophyll fluorescence induction curves (Kautsky effect) of HL and LL-radish cotyledons after 4 and 7 d of greening at high (HL) or low light quanta fluence rates (LL). fo = ground fluorescence, fp = maximum fluorescence.

Prenylpigment ratios as indicators: The different ratios of chlorophyll a/b and of xanthophylls to β-carotene (x/c) in sun and shade leaves of trees had already been recognized by Willstätter and Stoll 1913 [7] and Seybold and Egle 1937 [26]. The same differences are also found between leaves from HL and LL plants [1, 2, 8]. Radish cotyledons of HL-seedlings also exhibit higher a/b and lower x/c ratios (Table III) indicating a higher proportion of chlorophyll a and among the carotenoids a higher percentage of β-carotene than in LL-leaves. The

Table III. Prenylpigment ratios in the cotyledons of 7 d old HL and LL-radish plants and in different chlorophyll-carotenoid proteins isolated from SDS-solubilized Raphanus chloroplasts. Mean values from several determinations [17]. The values given in parantheses are the ratios found in very pure preparations (a, b = chlorophyll *a* and *b*; c = β-carotene; x = sum of xanthophylls).

	a/b	a/c	c/x	x/c
HL-plant	3.7	14	~ 0.30	3.4
LL-plant	2.8	20	~ 0.23	4.3
chlorophyll a-proteins:				
CPIa + CPI	9 (21)	10	1.2 – 1.8	0.6 – 0.8
CPa	3 (8)	8 (4)	1.5 – 3.0	0.3 – 0.7
light-harvesting proteins:				
LHCPs	1.1 – 1.3	60 – 180	0.01 – 0.02	50 – 100

different chlorophyll *a* and β-carotene composition of HL and LL-chloroplasts is also evident in the ratios of chlorophyll a/c and c/x which are different for HL and LL-chloroplasts. The reason for these differences in the prenylpigment ratios between the leaves of HL and LL-plants are the different levels of the individual chlorophyll-proteins (Table II) which possess destinct chlorophyll-carotenoid compositions.

The chlorophyll *a*-proteins CPIa + CPI isolated by a single SDS-PAGE step and also the purer CPa fraction exhibit high *a/b* and c/x ratios and low values for the ratios a/c and x/c (Table III). In contrast, the LHCPs are characterized by low a/b (1.1 – 1.3) and c/x ratios (0.01 – 0.02) and high values for a/c (60 – 180) and x/c (50 – 100). The consequence of the higher levels of LHCPs in the LL-plants is a decreased ratio of *a/b* and a higher value of x/c (Table III). The increased percentage of chlorophyll *a*/β-carotene-proteins (CPIa, CPI and CPa) at HL-growth conditions is correspondingly documented by higher a/b and lower x/c and a/c ratios. The pigment ratios are thus good indicators for the relative pigment-protein composition of the photosynthetic apparatus. This modification of the pigment ratios by changes in the levels of chlorophyll-carotenoid-proteins is summarized in Fig. 6.

Discussion

It has been shown in this investigation that the ultrastructure of chloroplasts and the level of the individual chlorophyll-carotenoid-protein is regulated by the light intensity applied during growth and development. The formation of HL-chloroplasts with a higher proportion of photosystem I pigment proteins (CPIa + CPI) at high light quanta fluence rates and of LL-chloroplasts with higher levels of LHCPs at low light intensity shows the good onto-

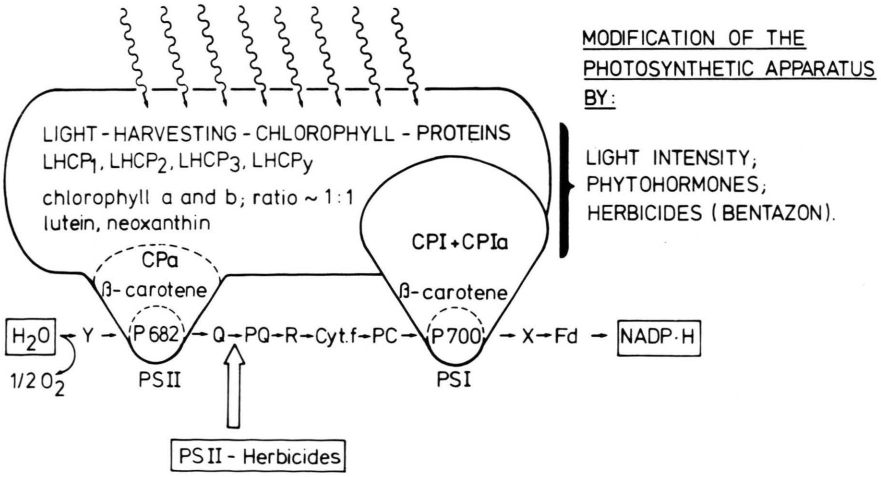

Fig. 6. A model of the organization of the 7 chlorophyll-carotenoid-proteins in a functional photosynthetic apparatus with indication of the photosynthetic electron transport chain. The predominant localization of chlorophyll b, lutein and neoxanthin in the light-harvesting chlorophyll *a/b*-proteins LHCPs and of β-carotene (with chlorophyll *a*) in CPI + CPIa and in CPa is indicated. The relative amounts of LHCPs can be modified by light intensity as shown here, by bentazon application (shade-type adaptation) [58] and under the influence of phytohormones, *e.g.* kinetin [4, 58]. PSI, PSII = photosystem I and photosystem II.

genetic adaptation response of the photosynthetic apparatus to the incident light.

On a chlorophyll basis the HL-chloroplasts of HL-leaves possess at light saturation a capacity for photosynthetic quantum conversion 4 times higher than the chloroplasts of LL-radish leaves. As a result they accumulate large starch grains; the rate of assimilate formation is apparently higher than the rate of assimilate export out of the leaf. Higher CO_2-fixation rates are also found in sun and HL-leaves of other plants, when compared to shade and LL-leaves [2, 3, 4, 6].

The higher capacity for photochemical quantum conversion of absorbed light in the HL-cotyledons is also documented by lower levels for the ground and maximum chlorophyll fluorescence (fo; fp). In contrast, photosynthesis of LL-cotyledons and LL-chloroplasts, with high and broad grana stacks and a higher level of LHCPs is light saturated at a much earlier stage than that of HL-leaves. In LL-leaves emphasis is placed on the formation of large pigment antennae (more LHCPs and a larger photosynthetic unit size) in order to increase light absorption and to overcome the light shortage. This is a physiologically reasonable adaptation-response of the photosynthetic apparatus at low quanta fluence rates. At light saturation this LL-adapted photosynthetic apparatus absorbs more light in its large antenna than it can photochemically transform; a large proportion of the absorbed energy will be dissipated by fluorescence, which is seen in the higher fluorescence induction curves.

The chloroplasts of HL-leaves, which do not experience light shortage, are adapted to high rates of light quanta conversion which is also documented by the higher level of the photochemically active, P-700 containing pigment-proteins CPI and CPIa of photosystem I and of CPa, the presumable reaction center of photosystem II. Their higher light-saturation point and lower chlorophyll fluorescence are certainly not only caused by the higher rates of quantum conversion but also by the lower level of LHCPs and a lower absorption of incident light quanta (smaller photosynthetic unit) than in LL-leaves.

From the fact that HL-chloroplasts possess less thylakoids per chloroplast (as seen from lower values of total thylakoid length per chloroplast section, Table I) and that the chlorophylls are quantitatively bound to thylakoids, one has to conclude that HL-chloroplasts possess less chlorophyll than LL-chloroplasts. This has been shown for the sun-chloroplasts of sun leaves from the beech which possess about 20 to 50% less chlorophyll per chloroplast than shade chloroplasts [6]. Fagus sun leaves, however, possess more chloroplasts per mesophyll cell (palisade paremchyma), which compensates for the lower thylakoid and grana content of the sun chloroplasts [6]. Since the HL and LL-radish cotyledons contain about the same chlorophyll content per leaf and leaf area, a higher number of chloroplasts per cell and leaf area must be assumed for the HL-cotyledons as compared to LL-radish cotyledons. The higher photosynthetic capacity of the HL-cotyledons is accordingly the combined action of a high-light adapted chloroplast type (the HL-chloroplast) and an increased number of chloroplasts per mesophyll cell.

The higher CO_2-fixation capacity of HL-leaves and HL-chloroplasts is not only associated with a higher number of reaction centers with reference to total chlorophyll but also with more photosynthetic electron transport chains, as seen from higher levels of the electron carriers plastoquinone [1, 9], phylloquinone K_1 [1, 8] and cytochromes [4]. It has been shown that the coupling factor CF_1 is located only on exposed thylakoid regions (stroma thylakoids and end grana membranes) [27–29]; that is in the non-appressed membrane regions. The chloroplasts of HL-leaves with their low grana stacks and their lower value for the ratio of appressed to non-appressed membranes (for radish see Table I) contain more CF_1 per chlorophyll than LL-chloroplasts [27–29], indicating also a higher photophosphorylation capacity. Chloroplasts isolated from HL-Sinapis plants in fact exhibit higher phosphorylation rates than chloroplasts from LL-plants [30].

CPa probably consisting of a single 43 KD peptide [31] contains β-carotene [17] and possibly also the naphthoquinone phylloquinone K_1 [32], both of which are potential quenchers of excitation energy. This chlorophyll a/β-carotene-protein can be resolved into two components [33]; one of these may represent the P 680-containing reaction center of PS II and the other the inner antenna surrounding it [34]. Some CPa fractions isolated from radish chloroplasts possess low values for the ratio chlorophyll *a*/*b* (2.5–3.5), while other purer preparations exhibit higher a/b values (5.5 to 8) and contain a higher proportion of β-carotene (lower a/c ratio) which supports this suggestion. This purer CPa may represent the basic reaction center core of PS II to which different amounts of LHCPs can be added, as shown in Fig. 6 and Fig. 7.

The increased stacking degree of thylakoids in the LL-radish chloroplasts is paralleled by an increased level of the light-harvesting chlorophyll-proteins (LHCPs). A higher percentage of LHCPs (57%) was also detected in the shade plant *Malaxis monophyllos* when compared with the sun plant spinach (LHCPs 43%); in contrast, the levels of the photosystem I CPI complexes were much lower in *Malaxis* (14%) than in spinach (27%) [35]. A lower percentage of chlorophyll in the pigment-proteins CPI + CPIa and a higher proportion of LHCPs was also found in the shade plants *Alocasia* and *Helmholtzia,* while the levels of CPa of the shade plants were similar to those of the sun plants spinach, pea and barley [36]. This raises the question as to whether the LHCPs are involved in the stacking of thylakoids. There are several observations which support this assumption.

By freeze-fracturing of spinach chloroplast thylakoids several faces with subthylakoid particles are detected, indicating the assymmetry of the photosynthetic membrane. The EF face of fully greened plants gives a particle-size distribution histogram with 2 maxima at 10.5 and 16.4 nm [37]. When plants are greened in intermittent light, the particles are smaller, and chlorophyll *b* and the LHCP are lacking. After transfer of the plants to continous light chlorophyll *b* and LHCP are formed, stacking occurs and the EF particle-size distribution shows the two maxima correctly. On this basis it is postulated that LHCPs bind in varying amounts to a basic PS II core particle (CPa?) to form the EF particles with the characteristic size distribution [37]. This view is further supported by the observation that the larger EF particles are missing in a chlorophyll *b*-deficient *Chlamydomonas* mutant [38] and are of reduced size in a partially chlorophyll-deficient soybean mutant with a low LHCP level [39] and in a barley mutant that lacks chlorophyll *b* and LHCP [40]. From these data it is assumed that LHCPs are a component of the large EF face particles.

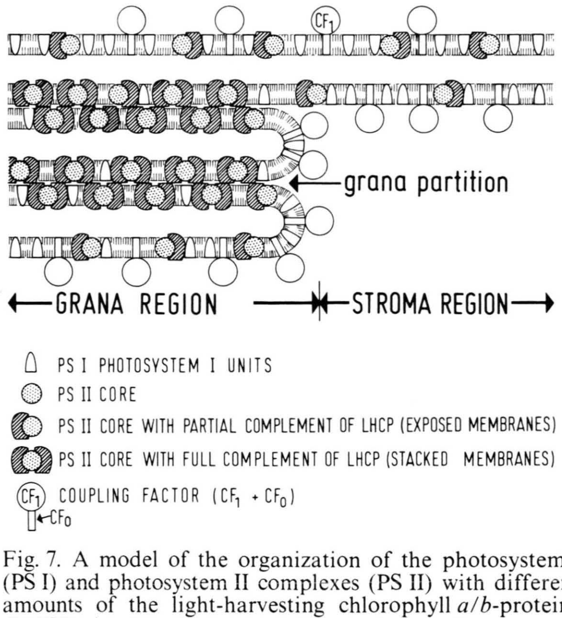

Fig. 7. A model of the organization of the photosystem I (PS I) and photosystem II complexes (PS II) with different amounts of the light-harvesting chlorophyll *a*/*b*-proteins (LHCP) in the photosystem II complexes of grana and stroma thylakoids. (Based on Staehelin *et al.* [60], modified and revised according to new results [43, 51–53].)

Unstacking of thylakoids has been achieved by applying low-salt buffer [41], while restacking can be induced by a low concentration of divalent cations (2 mM $MgCl_2$) or high levels of monovalent cations ($>$ 100 mM NaCl) [42, 43]. In the presence of cations isolated LHCPs can also form aggregates [44]. When the LHCPs of two adjacent thylakoids are responsible for stacking, then part of the LHCP or its peptide must be located at the outer surface of the thylakoid [45], while the major part of the LHCP as a hydrophobic polypeptide, is certainly embedded in the photosynthetic bilayer membrane. One end, however, seems to protrude slightly from the outer surface. When this protruding peptide is removed by trypsin treatment (cleavage of 20 amino acids) [46], the ability of thylakoids to form grana is lost [47, 48]. The Mg^{2+}-ion induced aggregation of LHCP-containing liposome-vesicles is also lost after trypsin-treatment of the LHCPs [49]. Further support that only the polypeptide part of LHCP is responsible for stacking comes from a barley mutant which lacks pigmented LHCP and chlorophyll *b* but forms thylakoid stacks, this is possible because one major polypeptide of the light-harvesting complex is present [50].

More recently it was shown that the stacked membrane regions of chloroplasts contained predominantly photosystem II (incl. LHCPs) and only few photosystem I units [51, 52]. Studies on the distribution of photosystem II activity between grana and stroma lamellae showed that 75 to 80% of all photosystem II centers are localized in regions of stacked membranes (grana partitions), a distribution that was similarly found for the intra-membranous particles of the EF face [53]. The fact that the EF particles of stroma lamellae are smaller and that the photosystem II activity of stroma-lamellae requires higher light intensities for maximum rates than that of grana-lamellae indicate that the photosystem II units of stroma thylakoids possess a smaller chlorophyll antenna [45]. From the freeze-fracture work it is evident that the photochemically active core complex of PS II (CPa?) is surrounded in the stroma lamellae by fewer aggregates of the LHCPs than in the stacked membrane regions of the grana partitions [37, 45].

This uneven distribution of photosystem I units and of photosystem II units (with different amounts of LHCPs) as well as the importance of LHCPs as a prerequisite for stacking is shown in the model of the grana and stroma thylakoid organization (Fig. 7). The role of monovalent or bivalent cations (*e.g.* Mg^{2+}) in stacking seems to be the induction of an LHCP association with PS II-cores and the formation of cross-links between the LHCPs of adjacent grana membranes [48, 53]. Adhesion between the LHCP particles of two membranes seems to be mediated by hydrophobic interactions and the cations are possibly needed to neutralize surface charges on the particles [43].

A final remark concerns the question as to how the high-light adaptation response is realized. The formation of the apoprotein of LHCP, which is coded in nuclear genes and synthesized in the cytoplasm [54, 55] is induced by active phytochrome [56] and the accumulation of the pigment-loaded LHCP is photocontrolled by protochlorophyllide [57]. It can be assumed that phytochrome is in its active form under the low and high quanta fluence rates of the white light applied here. Since the HL-chloroplast modification can be simulated with blue light, while red light yields a shade-type adaptation response [58, 59], it is concluded that a blue light receptor (cryptochrome?), which measures light quantity, may be responsible for the HL-induced modification of the photosynthetic apparatus into a HL-adapted chloroplast. The LL-chloroplast with a higher amount of LHCPs formed at low light intensity and in red light seems to be the result of the phytochrome-induced regular chloroplast development program, which can then be modified in response to the incident light intensity.

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