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REVIEW

Biosynthesis, accumulation and emission of carotenoids, α-tocopherol, plastoquinone, and isoprene in leaves under high photosynthetic irradiance

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Abstract The localization of isoprenoid lipids in chloroplasts, the accumulation of particular isoprenoids under high irradiance conditions, and channelling of photosynthetically fixed carbon into plastidic thylakoid isoprenoids, volatile isoprenoids, and cytosolic sterols are reviewed. During leaf and chloroplast development in spring plastidic isoprenoid biosynthesis provides primarily thylakoid carotenoids, the phytyl side-chain of chlorophylls and the electron carriers phylloquinone K1, α-tocoquinone and α-tocopherol, as well as the nona-prenyl side-chain of plastoquinone-9. Under high irradiance, plants develop sun leaves and high light (HL) leaves with sun-type chloroplasts that possess, besides higher photosynthetic CO_2 assimilation rates, different quantitative levels of pigments and prenylquinones as compared to shade leaves and low light (LL) leaves. After completion of chloroplast thylakoid synthesis plastidic isoprenoid biosynthesis continues at high irradiance conditions, constantly accumulating α-tocopherol (α-T) and the reduced form of plastoquinone-9 (PQ-9H_2) deposited in the steadily enlarging osmiophilic plastoglobuli, the lipid reservoir of the chloroplast stroma. In sun leaves of beech (*Fagus*) and in 3-year-old sunlit *Ficus* leaves the level of α-T and PQ-9 can exceed that of chlorophyll b. Most plants respond to HL conditions (sun leaves, leaves suddenly lit by the sun) with a 1.4–2-fold increase of xanthophyll cycle carotenoids (violaxanthin, zeaxanthin, neoxanthin), an enhanced operation of the xanthophyll cycle and an increase of β-carotene levels. This is documented by significantly lower values for the weight ratio chlorophylls to carotenoids (range: 3.6–4.6) as compared to shade and LL leaves (range: 4.8–7.0). Many plant leaves emit under HL and high temperature conditions at high rates the volatile compounds isoprene (broadleaf trees) or methylbutenol (American ponderosa pines), both of which are formed via the plastidic 1-deoxy-D-xylulose-phosphate/2-C-methylerythritol 5-phosphate (DOXP/MEP) pathway. Other plants by contrast, accumulate particular mono- and diterpenes. Under adequate photosynthetic conditions the chloroplastidic DOXP/MEP isoprenoid pathway essentially contributes, with its C_5 isoprenoid precusors, to cytosolic sterol biosynthesis. The possible cross-talk between the two cellular isoprenoid pathways, the acetate/MVA and the DOXP/MEP pathways, that preferentially proceeds in a plastid-to-cytosol direction, is shortly discussed.

Keywords Andy A. Benson · β-Carotene · Chlorophylls · Chloroplast adaptation · Carbon flow into isoprenoids · Chloroplast envelope · Cross-talk between the two isoprenoid pathways · DOXP/MEP pathway · Fosmidomycin · Isoprenoid biosynthesis · MEP pathway · Methylbutenol · Mevinolin · Phylloquinone K1 · Plastoglobuli · Sterol formation · Sun chloroplasts · Zeaxanthin

Abbreviations

$a + b$	Total chlorophylls
a/b	Ratio of chlorophyll a to b
Chl	Chlorophyll
$(a + b)/(x + c)$	Weight ratio of chlorophylls to carotenoids
A	Antheraxanthin
c	Carotenes

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DMAPP	Dimethylallyldiphosphate
DOXP/MEP pathway	Plastidic 1-deoxy-D-xylulose-4-phosphate/2-C-methylerythritol 5-phosphate pathway
GAP	Glyceraldehyde-3-phosphate
IPP	Isopentenyl diphosphate
MBO	2-methyl-3-buten-2-ol
MEP	2-C-methylerythritol 5-phosphate
MVA	Mevalonic acid
PPFD	Photosynthetic photon flux density
V	Violaxanthin
$x + c$	Total carotenoids
x	Xanthophylls
Z	Zeaxanthin

Carbon assimilation and biosynthetic capacities of chloroplasts

The photosynthetic light and associated electron transport reactions of chloroplasts that are driven by two photosystems, produce ATP and NADPH used in the CO_2 fixation and assimilation reactions (Calvin–Benson cycle) yielding sugar phosphates, starch, fatty acids, lipids and isoprenoids. The "path of carbon in photosynthesis," including cyclic regeneration of the carbon dioxide acceptor molecule, was discovered in Berkeley using $^{14}CO_2$ (Bassham et al. 1954; Benson et al. 1952; Calvin and Bassham 1962). The essential contributions of Andy A. Benson in Melvin Calvin's photosynthesis laboratory have been described in personal, historical perspectives of that research (Benson 2002a, b; see also Walker 2007). A.A. Benson also identified glycerol and sugars confined to lipids as early ^{14}C-labeled photosynthetic products, such as galactolipids (Benson et al. 1958), which then led to the detection of phosphatidyl glycerol in chloroplasts (Benson and Maruo 1958; Benson and Strickland 1960) as well as the plant sulfolipid (Benson et al. 1959a; Benson and Miyano 1961, 1962; Benson 1963) and the recognition of chloroplast lipids as carbohydrate reservoirs (Benson et al. 1959b; Ferrari and Benson 1961; as reviewed in Benson 1971). The essential roles of phosphatidylglycerol and sulfolipid in photosynthesis established in early and recent research have now been summarized (Benning 2007; Wada and Murata 2007).

The biosynthetic capacities of chloroplasts are not only restricted to photosynthetic CO_2 assimilation, ATP and NADPH formation, but also comprise other activities. Thus, chloroplasts and all other plastid forms are the sole site for *de novo* fatty acid biosynthesis in the plant cell (Stumpf 1984) as was later confirmed with chloroplast herbicides (Golz et al. 1994). In addition, the different types of chloroplast isoprenoid lipids, such as carotenoids as pure prenyllipids and the isoprenoid side-chains of chlorophylls, α-tocopherol, phylloquinone K1 and plastoquinone-9 are formed via the plastidic 1-deoxy-D-xylulose 4-phosphate/2-C-methylerythritol 5-phosphate (DOXP/MEP) pathway of isopentenyl diphosphate (IPP) and isoprenoid biosynthesis (Lichtenthaler et al. 1997a, b, 1999; Schwender et al. 1996). Moreover, chloroplasts possess the complete enzymatic machinery to synthesize the porphyrin ring for chlorophyll and cytochrome biosynthesis (Beale 1999; von Wettstein et al. 1995). For this reason, various chloroplast compounds, other than sugar phosphates—such as the fatty acids in glycerolipids, carotenoids, the prenyl side-chains of Chls and plastidic prenylquinones as well as the porphyrin ring moiety of Chls—are quickly labeled when $^{14}CO_2$ is applied to leaves and chloroplasts under photosynthetic conditions.

Chloroplast glycero lipids and isoprenoid lipids

Chloroplasts are surrounded by two biomembranes, i.e., the inner and outer envelope. The two photosynthetic light reactions and associated electron transport reactions proceed in the photochemically active thylakoids of the plastid stroma, whereby one has to differentiate exposed stromal thylakoids and appressed granal thylakoids (Meier and Lichtenthaler 1981). Like other cellular biomembranes the envelope and thylakoid biomembranes consist of a glycerolipid bilayer with specific integral and functional proteins. In contrast to cytosolic cellular biomembranes, thylakoids possess, in addition to phospholipids, sugar-containing glycolipids—i.e., chloroplast-specific mono- and di-galactolipids and the sulfolipid. These carbohydrate-containing glycerolipids (galacto- and sulfolipids), which have been termed glycolipids, were described very early as components of green leaves and chloroplasts by Benson's group (Benson et al. 1959a, b). J.F.G.M. Wintermans, a former post-doc of A.A. Benson at Pennsylvania State University, determined, after his return to Europe, for the first time the quantitative levels of phospholipids and glycolipids with respect to chlorophyll in isolated spinach chloroplasts (Wintermans 1960). Later it was shown that, in addition to phospho- and glyco-lipids, thylakoids also possess the lipophilic photosynthetic pigments, Chls and carotenoids, together with the lipophilic prenylquinones (plastoquinone-9, phylloquinone K1, α-tocoquinone) as electron carriers and, in addition, the lipid antioxidant α-tocopherol (Lichtenthaler and Calvin 1964). By combining the glycerolipid data of Wintermans (1960) with the pigment and isoprenoid lipid results of

Lichtenthaler and Calvin (1964), the first time a table was established with the level of all thylakoid lipids of spinach chloroplasts, based on one gram atom manganese (Lichtenthaler and Park 1963). It turned out that Chls comprise between 21% and 24%, carotenoids 4–5%, and the three prenylquinones together with α-tocopherol 3–5% of the total thylakoid lipids, whereas phospholipids make up 11%, galactolipids almost 50%, and sulfolipid 5–6% of the thylakoid lipids. Thus, isoprenoid pigments and prenylquinones taken together make up about 33% and diacylglycerol lipids the remaining two-thirds of the thylakoid lipids. The specific arrangement of pigments and glycerolipids in the photosynthetic biomembrane was not known in the beginning 1960s but under considerable discussion.[1] Today it is clear that the photosynthetic pigments are bound to several chlorophyll-carotenoid proteins (Green and Durnford 1996, Lichtenthaler et al. 1982a, Thornber 1975) which are functionally integrated in the thylakoid biomembrane together with the reaction centers of photosystem I and II (Nelson and Yocum 2006).

[1] At that time I was a post-doc with Melvin Calvin and, after the thylakoid lipid table had been published (Lichtenthaler and Park 1963), Andy Benson became quite interested in the functional organization of membrane lipids and contacted me in Berkeley. During my subsequent visit to his laboratory in La Jolla/San Diego in the summer of 1963, I had on basis of the thylakoid lipid table an extensive discussion with Andy Benson on the arrangement of phospho- and glycolipids in photosynthetic biomembranes. From the dimensions of the double lines seen for thylakoid membranes in electron microscopic observations at high magnification and the length of the fatty acid chains in the glycerolipid molecules, the two of us agreed that a bilayer was spatially possible and that the thylakoid membrane most probably consisted of a bilayer of glycerolipids in which various functional proteins were embedded and attached. We also discussed in a very stimulating approach how chlorophylls with their porphyrin ring and phytyl side-chain were oriented in such a lipid bilayer and how plastidic prenylquinones with their phytyl or nona-prenyl side-chain might be embedded, together with the carotenoids, as C40-isoprenoids to give a possible thylakoid lipid bilayer structure. The thylakoid lipid table and various aspects of our discussion subsequently became essential parts of Andy Benson's review paper, "Plant Lipid Membranes" (Benson 1964), in which he showed various possibilities for the arrangement of glycerolipids in biomembranes and pointed out the kind of information and research needed to make progress in our understanding of the functional organization of plant and thylakoid membrane lipids. This topic was further advanced by Weier and Benson (1967). In this context, one has to consider that our present knowledge that all cellular membranes are composed of a basic lipid bilayer structure was not known at that time. Likewise, in the early 1960s, it was not known that photosynthetic pigments are bound to particular chlorophyll–carotenoid–protein complexes, such as the light-harvesting Chl *a*/*b* complexes, LHCPs (or LHCII), photosystem II (CPa) and photosystem I pigment–protein complexes (CPI and CPIa) (Bennett 1983; Lichtenthaler et al. 1982a; Thornber 1975). Further, that the pigments are not packed together with glycerolipids in the thylakoid bilayer. (H. K. Lichtenthaler, March 2007).

Localization of isoprenoids in chloroplasts

In chloroplasts there exist three sites for the localization of isoprenoid pigments and prenylquinones, (i) thylakoids, (ii) chloroplast envelope and (iii) osmiophilic plastoglobuli of the plastid stroma.

Thylakoids contain the chlorophylls, several carotenoids and the prenylquinones plastoquinone-9, phylloquinone K1, and α-tocoquinone, as well as low amounts of the lipophilic antioxidant, α-tocopherol (Lichtenthaler and Calvin 1964). The photosynthetic pigments are usually bound to several special chlorophyll–carotenoid–protein complexes (Bennet 1983; Lichtenthaler et al. 1982a, b; Thornber 1975). Carotenoids comprise as regular components β-carotene (and small amounts of α-carotene), lutein, neoxanthin and the three xanthophyll cycle carotenoids zeaxanthin (Z), violaxanthin (V) and antheraxanthin (A). The latter are interchangeable by the light-triggered xanthophyll cycle that is active particularly at high light conditions and de-epoxidizes V via A to Z (Demmig-Adams and Adams 1996; Schindler et al. 1994; Schindler and Lichtenthaler 1996). This localization of chloroplast isoprenoid lipids is shown in Fig. 1.

Envelopes consist of the outer and inner biomembranes and represent a yellow biomembrane system containing primarily the carotenoids violaxanthin and lutein, as first isolated in Andy Benson's laboratory in 1973 (Douce et al. 1973). A second paper from the same laboratory confirmed this unique carotenoid composition and showed that envelopes isolated from chloroplasts of dark spinach leaves had a 3.5 times higher level of violaxanthin than lutein (+zeaxanthin), whereas the xanthophyll ratio was only 0.75 in envelopes isolated from illuminated leaves (Jeffrey et al. 1974). This finding indicated that the level of V, the major carotenoid of the xanthophyll cycle, can apparently change in the envelope, dependent on its reduction to Z in the light-driven xanthophyll cycle. Later Lichtenthaler, Douce and coworkers in a close cooperation could show that

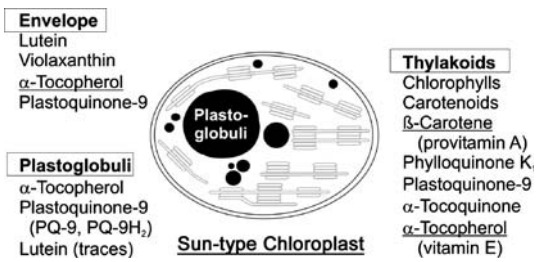

Fig. 1 Scheme of a sun chloroplast with indication of the three sites of plastidic isoprenoid localization: (1) the chlorophyll-free envelope, (2) the photochemically active thylakoids with chlorophylls, and (3) the chlorophyll-free osmiophilic plastoglobuli of the plastid stroma. The two plastidic vitamins essential for human nutrition, β-carotene (provitamin A) and α-tocopherol (vitamin E), are underlined

chloroplast envelopes also contain α-tocopherol and the prenylquinone plastoquinone-9 together with small amounts of phylloquinone K1 and α-tocoquinone (Lichtenthaler et al. 1981a). They further demonstrated that the relative levels of these isoprenoids (prenylquinones as well as carotenoids) were quite different from those of thylakoids. The latter exhibit a weight ratio of plastoquinone-9 to α-tocopherol of 4.0, and envelopes, with α-tocopherol as their major component, a ratio of only 0.4. Moreover, in isolated spinach envelopes the three major carotenoids were violaxanthin (41%), lutein (28%) and Z (12%), with a β-carotene level of only 10% and A and neoxanthin at less than 5% each (Lichtenthaler et al. 1981a). By contrast, spinach thylakoids (isolated from darkened spinach harvested in the early morning) contain primarily β-carotene (33%), lutein (40%) and violaxanthin (16%) and as minor components neoxanthin (7%) and Z + A (almost 4%). Carotenoids have also been found in envelopes of non-green plastids, together with mono- and di-galactolipids, phosphatidylcholin and the phosphate translocator (Alban et al. 1988). Today it is well established that the envelope is the site of synthesis of various thylakoid compounds, such as galactolipids, sulfolipids, and certain phospholipids as well as the site for the final steps of carotenoid and prenylquinone biosynthesis (Joyard et al. 1998). The envelope has also multiple functions in the import and export of compounds into and from chloroplasts (Heber and Heldt 1981).

Plastoglobuli are regular globular plastid structures that primarily represent a reservoir for excess amounts of plastoquinone-9 (predominantly the reduced form plastohydroquinone-9 or PQ-9H_2), α-tocopherol and possibly other excess plant lipids (Lichtenthaler 1968; Lichtenthaler and Sprey 1966) that cannot be stored in the thylakoids. The osmiophilic plastoglobuli of chloroplasts do not contain chlorophylls or phylloquinone K1, and they only contain trace amounts of carotenoids. By contrast, plastoglobuli of carotenoid-rich chromoplasts possess carotenoids and secondary carotenoids. In plastoglobuli of the thylakoid-free barley etioplasts we also found carotenoids (lutein and violaxanthin), in addition to plastoquinone-9 and α-tocopherol. Young chloroplasts of freshly developed leaves exhibit few and rather small plastoglobuli (diameter range: 0.07–0.2 μm). With increasing age of chloroplasts and leaves, the number and size of plastoglobuli increases, particularly in sun-exposed leaves and leaves that regularly receive enough light to perform photosynthesis at good rates. Concerning size and frequency of plastoglobuli, there exist two strategies in plants: (1) in spinach and other herbaceous plants the number of plastoglobuli in older leaves increases to several hundred per chloroplast at a rather small diameter of 0.1–0.2 μm (Lichtenthaler 1969a), and (2) in older sun-exposed leaves of beech and oak, however, fewer but considerably larger plastoglobuli (diameter size: 0.4–2.0 μm) are formed (Lichtenthaler 1968, 1971b). Also, in several-year-old *Ficus* leaves few, but larger, plastoglobuli (diameter 0.3–3.0 μm) accumulate (Lichtenthaler and Weinert 1970). Besides plastoquinol-9 and α-tocopherol, plastoglobuli tend to contain some galactolipids as found e.g., in isolated plastoglobuli of *Ficus* and *Tilia*, but only traces of phospholipids. The question remains open, whether plastoglobuli store neutral lipids, such as triglycerides. Although distinct amounts of triacylglycerols were described for isolated beech plastoglobuli (Tevini and Steinmüller 1985), this is not certain for beech, because the isolated chloroplast and plastoglobuli fractions of the leaves are contaminated by large cytosolic lipid droplets that contain neutral triglycerides. As a matter of fact, all leaf cells of beech contain one of these large cytosolic lipid bodies (about the size of chloroplasts) that, due to the high tannin content of beech leaf cells, co-sediment with chloroplasts.

Differences in ultrastructure and pigment composition of sun and shade chloroplasts

Sun and shade leaves of trees as well as leaves of high-light (HL) and low-light (LL) plants differ considerably in their quantitative composition of photosynthetic pigments, electron carriers, chloroplast ultrastructure, and photosynthetic rates (Anderson et al. 1995; Boardman 1977; Givnish 1988; Lichtenthaler et al. 1981b; Meier and Lichtenthaler 1981; Wild et al. 1986). Leaves that develop under high irradiance (sun leaves and HL-leaves) possess sun-type chloroplasts that are adapted to much higher rates of photosynthetic CO_2 assimilation on a leaf area basis and on a chlorophyll basis in comparison to shade leaves or leaves from LL-plants (reviewed by Lichtenthaler and Babani 2004). Sun leaves exhibit a higher Chl *a* + *b* content per leaf area unit, higher values for the ratio Chl *a*/*b*, a much lower level of light-harvesting Chl *a*/*b* proteins (LHCII), and, as a consequence, a lower stacking degree of thylakoids (lower amounts of appressed membranes) than shade leaves and LL-plants with their low-irradiance shade-type chloroplasts (Lichtenthaler et al. 1981b, 1982b, 1984). The ultrastructure of sun-type chloroplasts with lower and narrower grana thylakoid stacks as well as rather large osmiophilic plastoglobuli is quite different compared to shade-type chloroplasts which contain much higher and broader grana stacks and only few small plastoglobuli (Fig. 2).

The Chl *a*/*b* ratio of sun and HL leaves of various trees and crop plants is found in the range of 2.9–3.8 in comparison to values of 2.3–2.8 for shade and LL leaves. The higher carotenoid content of sun leaves and HL-leaves on a

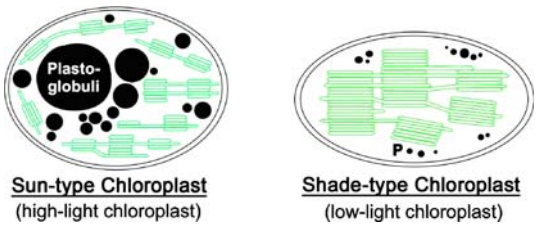

Fig. 2 Differences in ultrastructure, plastoglobuli content, thylakoid frequency, width and stacking degree of thylakoids in sun-type chloroplasts and shade-type chloroplasts of sun and shade leaves of trees, and of leaves of herbaceous plants grown at high-light or low-light conditions. P = Plastoglobuli. (Based on Lichtenthaler 1981; Lichtenthaler et al. 1981a, 1982b, 1982c; Meier and Lichtenthaler 1981)

Chl basis is documented by significantly lower values for the weight ratio of chlorophyll/carotenoids $(a + b)/(x + c)$ (range: 3.6–4.6), as compared to shade and LL-leaves with significantly higher values (range: 4.9–7). Examples of the different quantitative pigment composition are shown for sun and shade leaves of beech and for HL and LL leaves of radish (Table 1). The photosynthetic pigments, Chls *a* and *b* as well as total carotenoids $x + c$, were determined spectrophotometrically (Lichtenthaler 1987) and the individual carotenoids via HPLC (Schindler and Lichtenthaler 1996).

Sun and HL-leaves possess on a total carotenoid basis a higher percentage of β-carotene (36% in both cases), xanthophyll cycle carotenoids (violaxanthin + zeaxanthin + antheraxanthin) (17% and 19 %, respectively), and a lower percentage of lutein and neoxanthin (39% and 37%) compared to shade leaves and LL-leaves with only 13% and 11% of the xanthophyll cycle carotenoids V + A + Z (Table 1). The higher level of β-carotene in sun and HL leaves is also seen in lower values for the ratio *a*/*c* (Chl *a* to β-carotene), and the ratio xanthophylls/β-carotene (*x*/*c*) of 1.8 in both cases as compared to shade and LL leaves with values of 2.6 and 2.8 (Table 1). The lower proportion of lutein and neoxanthin in sun and HL leaves is caused by the fact that these two xanthophylls are bound to the light-harvesting Chl *a*/*b* proteins LHCPs (Lichtenthaler et al 1982a) that are less frequent in chloroplasts of sun leaves and HL-leaves (Lichtenthaler et al. 1982b). In contrast, β-carotene (and with it some α-carotene) is bound to the reaction center pigment proteins of both photosystems, PSI and PSII, which are more frequent on a Chl basis in sun-type chloroplasts. Thus, the percentage of β-carotene among total carotenoids is higher in sun and HL-leaves (31–36%) as compared to shade and LL-leaves (only 26–28%). The level of Z among the three xanthophyll cycle carotenoids V + A + Z in the sun and LL-leaves is relatively low (as shown in Table 1) because the leaf extracts were made in the morning. However, at mid-day on sunny days (beech leaves) and several hours after the start of illumination (radish seedlings) 75–85% of V has, indeed, been de-epoxidated to Z.

Table 1 Differences in the relative levels of chlorophylls and carotenoids and in pigment ratios (weight ratios) between sun and shade leaves of beech (*Fagus sylvatica* L.) in mid-July, and in cotyledons of high-light (HL) and low-light (LL) 8-day-old radish seedlings (*Raphanus sativus* L.)

	Beech		Radish	
	Sun	Shade	HL	LL
Pigment levels				
Chlorophyll a+b	532	409	345	296
Carotenoids x+c	121	65	70	54
β-Carotene (c)	43	18	25	15
Xanthophylls (x)	77	47	45	39
Pigment ratios				
Chl *a*/*b*	**3.3**	2.7	**3.8**	2.8
Chl a/β-carotene, a/c	**9.5**	16.6	**10.9**	14.5
x/c	**1.8**	2.6	**1.8**	2.8
$(a + b)/(x + c)$	**4.4**	6.3	**4.9**	5.5
% Composition of carotenoids				
β-Carotene	**36**	28	**36**	26
Lutein	**39**	49	**37**	54
Neoxanthin	**7**	10	**8**	11
V + A + Z	**17**	13	**19**	11
Zeaxanthin (Z)	4	0	6	0
Antheraxanthin (A)	2	1	1	1
Violaxanthin (V)	11	12	12	10

The pigment levels of fully developed leaves are given in mg m^{-2} leaf area. For a better comparison the pigment ratios and percentage carotenoid composition of sun and HL leaves are presented in bold print. Mean values of 12 determinations from two beech trees of three different years, and six determinations from two repetitions (radish). Standard deviation <7% (pigment levels) and <4% (pigment ratios). The differences are highly significant $p < 0.01$. V + A + Z is the sum of the xanthophyll cycle carotenoids (zeaxanthin + antheraxanthin + violaxanthin), and (a + b)/(x + c) is the weight ratio chlorophylls to carotenoids

A similar de-epoxidation of V to Z at high light conditions at mid-day and considerably higher levels of the xanthophyll cycle carotenoids V + A + Z in sun leaves have also been observed by different authors for various other plants (Demmig-Adams and Adams 1992, 1996; Lichtenthaler and Schindler 1992; Schindler and Lichtenthaler 1996; Thayer and Björkman 1990). Comparing sun and shade tolerant species the percentage of the xanthophyll cycle carotenoids present in the oxidized state as violaxanthin at solar noon was 96–100% for shade grown plants, but only 4–5% for sun grown plants (Thayer and Björkman 1990). In cork oak the pool of V + A + Z is astonishingly high during leaf unfolding, is higher in sun leaves than shade leaves, and increases in sun leaves during

the summer and in response to excess light (Garcia-Plazaola et al. 1997). The high-light induced Z accumulation is in general positively involved in the non-photochemical quenching of chlorophyll *a* fluorescence, heat emission, and protection of the photosynthetic apparatus against photoinhibition by preventing a buildup of excessive excitation energy at the photosynthetic reaction centers (Adams and Demmig-Adams 1994; Brugnoli et al. 1998; Thiele et al. 1996; Young 1991).

De novo biosynthesis and accumulation of β-carotene and zeaxanthin at sudden high irradiance stress

The photosynthetic apparatus of younger leaves can respond to high-light conditions and an exposure to excess light by rapid adaptation responses including formation of new pigments. *Aurea tobacco*: An example of this is shown for an 'aurea' mutant of tobacco (Su/su), which is poor in Chl b and has a much lower total Chl content than regular green tobacco plants. This plant was exposed to high irradiance for 5 h. The accumulation of Z at high irradiance conditions proceeded in two steps: (1) there was a fast de-epoxidation of violaxanthin to zeaxanthin (90% transformation of V into Z), which was completed after 15–20 min, followed by (2) a slow but continuous increase of the Z pool through *de novo* biosynthesis, which doubled the Z and A content and the total V + A + Z pool within 5 h (Table 2). The level of β-carotene increased as well but only 1.33-fold, the total chlorophyll content changed little (increase of 7%), whereas the levels of lutein and neoxanthin were not affected. The level of the doubled V + A + Z pool was also maintained in the subsequent dark phase, but Z and A were epoxidated to V, 60% after 1h and 92% after 16 h continuous darkness. The changes in pigment levels induced by 5 h HL also changed the pigment weight ratios in the typical way known for a HL-adaptation response of the photosynthetic apparatus. The ratio Chl *a*/*b*, relatively high in this tobacco mutant, which is poor in Chl b, increased from 7.0 to 7.7 under this condition, whereas the ratio of chlorophylls to carotenoids $(a + b)/(x + c)$ and Chl *a* to β-carotene (*a*/*c*) decreased. Due to the doubling of the Z levels the ratio of xanthophylls to β-carotene *x*/*c* increased from 2.6 to 3.0 (Table 2). All these changes were typical high irradiance adaptation responses.

Radish seedlings

Also changes in pigment levels of green, well-developed cotyledons took place during exposure of low-light seedlings to a 120 min HL stress. A 2-fold increase occurred in the β-carotene level, and a 1.4-fold increase of the x cycle carotenoids as a result of *de novo* biosynthesis of zeaxanthin (Table 3). The increase in total Chl $a + b$ amounted to only 8% and that of total carotenoids to 23%. These pigment changes caused considerable changes in the pigment ratios, which were strong indicators for a rapid HL-adaptation response of the chloroplasts from LL- to HL-chloroplasts, also known as sun-type chloroplasts (Fig. 2). The ratio Chl *a*/*b* increased from 2.7 to 3.1, whereas all other pigment ratios decreased (Table 3), with the largest decrease of the ratio *a*/*c* from 22.3 to 12.5. After this 120 min HL-exposure the pigment ratios were not yet identical to that of the HL radish plants (see Table 1, radish HL), although the HL-adaptation had progressed rather far.

The two examples given indicate that, at sudden HL stress, the photosynthetic apparatus responds not only with a quick de-epoxidation of V to Z, but also with a fast *de novo* biosynthesis and accumulation of carotenoids, such as Z and β-carotene. The large increase in the Z pool by *de novo* biosynthesis is understood because Z has a particular function in enabling the process of non-photochemical quenching of the absorbed excess light energy. The increase in β-carotene, which is associated with the two photosynthetic reaction centers (Lichtenthaler et al. 1982a, b), is a clear sign that under HL-conditions more photosynthetic reaction centers are formed. These results indicate that the photosynthetic apparatus is fairly reactive to excess light and channels new photosynthates via the chloroplastidic DOXP/MEP pathway (Lichtenthaler 1999;

Table 2 Increase in pigment levels (chlorophylls, carotenoids) and changes in pigment weight ratios of the low-chlorophyll tobacco 'aurea' mutant Su/su grown at medium irradiance (400 μmol m^{-2} s^{-1}) at a 5-h exposure to sudden high irradiance (HL) stress (2,200 μmol m^{-2} s^{-1}) as compared to the control

	Control	+5 h HL	Increase
Pigment levels			
Chlorophyll $a + b$	108.7	116.7	1.07 x
Carotenoids $x + c$	27.9	41.2	1.48 x
β-Carotene (*c*)	7.8	10.4	1.33 x
Xanthophylls (*x*)	20.1	30.8	1.53 x
V + A + Z	**9.7**	**19.4**	**2.00 x**
Pigment ratios			
Chl *a*/*b*	7.1	7.7	1.08 x
Chl *a*/β-carotene, *a*/*c*	12.3	9.9	0.80 x
x/*c*	2.6	3.0	1.15 x
Chls/Cars, $(a + b)/(x + c)$	3.9	2.8	0.72 x

Mean of three determinations, standard deviation <6% (pigments) and <4% (pigment ratios). The doubling of xanthophyll cycle carotenoids is given in bold print. The pigment levels are given in mg m^{-2} leaf area. V + A + Z is the sum of xanthophyll cycle carotenoids (zeaxanthin + antheraxanthin + violaxanthin), and $(a + b)/(x + c)$ is the weight ratio chlorophylls to carotenoids. (Based on Schindler et al. 1994)

Table 3 Successive changes in pigment levels (chlorophylls, carotenoids) and pigment weight ratios in the cotyledons of low-light (LL) 8-day-old radish plants (*Raphanus sativus* L.) grown at medium irradiance (170 µmol m^{-2} s^{-1}) at an exposure to high irradiance (HL) stress (2,200 µmol m^{-2} s^{-1})

	LL-Control	+HL		
		30min	60min	120min
Pigment levels				
Chlorophyll *a* + *b*	**275**	276	285	**297**
Carotenoids x+c	**53**	54	60	**65**
β-Carotene (c)	**9**	12	15	**18**
Xanthophylls (x)	**44**	42	45	**47**
V + A + Z	**16**	17	18	**22**
Zeaxanthin (Z)	**0**	9	13	**18**
Antheraxanthin (A)	**1**	3	2	**2**
Violaxanthin (V)	**15**	5	3	**2**
Pigment ratios				
Chl *a*/*b*	**2.7**	2.8	2.9	**3.1**
Chl *a*/*β*-carotene, *a*/*c*	**22.3**	17.0	13.3	**12.5**
x/c	**4.9**	3.5	3.0	**2.7**
(*a* + *b*)/(*x* + *c*)	**5.2**	5.1	4.9	**4.6**

Within 120 min of HL-exposure the pigment ratios changed from those of LL towards those of HL plants (see Table 1 for HL leaf pigment ratios). The pigment levels are given in mg m^{-2} leaf area. Mean of four determinations, standard deviation <6% (Pigments) and <4% (pigment ratios). V + A + Z is the sum of xanthophyll cycle carotenoids (zeaxanthin + antheraxanthin + violaxanthin), and (*a* + *b*)/(*x* + *c*) is the weight ratio chlorophylls to carotenoids. For a better comparison of the changes the values of the LL-control and those after 120 min HL-exposure are shown in bold print

Lichtenthaler et al. 1997a; see also Fig. 3) of isoprenoid formation into the biosynthesis of additional carotenoids, such as Z and *β*-carotene.

Accumulation of plastoquinone-9 and α-tocopherol at high irradiance

In contrast to carotenoids, chlorophylls, phylloquinone K1, and α-tocoquinone, whose level per leaf area remain practically constant after leaf unfolding, the level of plastoquinone-9 and α-tocopherol steadily increases, preferentially under high irradiance photosynthetic conditions, throughout the vegetation period shown for beech (Lichtenthaler 1971a). Thus, in sun leaves of beech, the level of total plastoquinone-9 is 5.2 times higher at the end of August compared to May, and the level of α-tocopherol increased 3-fold (Table 4A). By contrast, the level of chlorophylls and carotenoids and that of the other leaf prenylquinones (plastidic phylloquinone K1 and α-tocoquinone as well as cytosolic ubiquinone) does not change during the same time period (Table 4A). However, in shade leaves of beech which receive much less light for

Table 4 Changes in the levels (mg m^{-2} leaf area) of plastoquinone-9 and α-tocopherol (A) in sun leaves of beech (*Fagus sylvatica* L.) during the vegetation period (May–August) and (B) in a young light-green leaf (30 days after leaf unfolding) and a three-year-old dark-green leaf of fig tree (*Ficus elastica* Roxb.) as compared to other prenylquinones and pigments. The large increase in plastoquinone-9 and α-tocopherol is contrasted in bold print. (Based on Lichtenthaler 1971a,1969a–d; Lichtenthaler and Weinert 1970)

(A) Beech sun leaf	May	End of August	Increase May/August
Plastoquinone-9	**17**	**88**	**5.2 x**
α -Tocopherol	**14**	**42**	**3.0 x**
Phylloquinone K1	1.2	1.3	–
α-Tocoquinone	2.3	1.8	–
Ubiquinone-9/10	1.7	1.3	–
Carotenoids	35	36	–
(B) Fig tree	Young leaf	Old leaf	Increase young/old
Plastoquinone-9	**11**	**198**	**18 x**
α -Tocopherol	**10**	**200**	**20 x**
Carotenoids	30	70	2.3 x
Chlorophylls	155	350	2.3 x

photosynthesis, the increase during the same time period of May through August is much lower and amounts only to 1.3-fold for plastoquinone-9 and 1.9-fold for α-tocopherol. This accumulation of excess amounts of both prenyllipids also occurs in sun leaves of many other trees and in herbaceous plants, as it becomes obvious when comparing their levels in older and younger leaf tissues (Lichtenthaler 1969c). Leaves of fig tree (*Ficus elastica* Roxb.) are a particular example: in several year old, dark-green leaves the level of plastoquinone-9 is 18-times and that of α-tocopherol is 20-times higher compared to the thin, young, light-green leaf of about 20–30 days after leaf unfolding (Table 4B). On the other hand the level of carotenoids and chlorophylls increased only 2.3 times (Lichtenthaler 1969b; Lichtenthaler and Weinert 1970). In fact, in dark-green sun leaves of beech (at the beginning of September), the concentration of plastoquinone-9 and α-tocopherol considerably exceeds that of Chl *b* (1969c), and in several year old dark-green fig leaves their molar levels were more than two-fold higher than that of Chl *b* (Lichtenthaler and Weinert 1970). These excess amounts of both prenyllipids are deposited in the osmiophilic plastoglobuli of the plastid stroma, whose size increases with increasing leaf age (reviewed in Lichtenthaler 1968). In older fig leaves, especially large plastoglobuli are observed in the chloroplast stroma (Lichtenthaler and Weinert 1970) that were originally described as "magnoglobuli" (Falk 1960). A similar, age-dependent accumulation of plastoquinone-9 and α–tocopherol was observed in the green assimilation parenchyma of the stem tissue of the cactus, *Cereus*

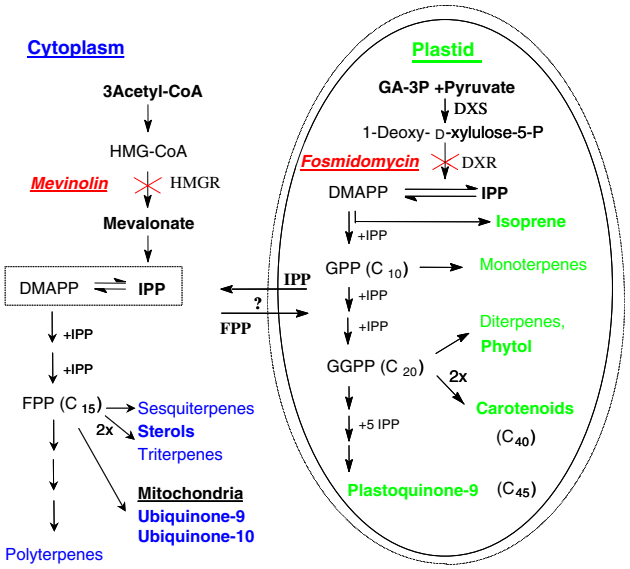

Fig. 3 Compartmentation of the isoprenoid biosynthesis in the plant cell: (1) the plastidic DOXP/MEP pathway (also termed MEP pathway) for the biosynthesis of the active C_5 unit (IPP) for chlorophylls (phytyl side-chain), carotenoids and prenylquinones (isoprenoid side-chains) and (2) the cytosolic acetate/mevalonate pathway of IPP biosynthesis for the formation of sterols and the prenyl side-chain of the mitochondrial ubiquinones (Q-9, Q-10). The specific inhibition of the acetate/mevalonate pathway by *mevinolin* and other statins (target: HMG-CoA reductase) and of the DOXP/MEP pathway by f*osmidomycin* (target: DOXP-reductoisomerase, DXR) is indicated. FPP—farnesyl diphosphate; GPP—geranyl diphosphate; GGPP—geranylgeranyl diphosphate; HMGR—hydroxymethylglutaryl-CoA reductase. (Based on Lichtenthaler et al. 1997a, b; Lichtenthaler 1999, 2000; Zeidler et al. 1998)

peruvianus (L.) Mill., where the 3-year-old dark green stem tissue had 4-fold higher level of plastoquinone-9 and a 2.5-fold increase of α–tocopherol compared to young tissue (Lichtenthaler 1969d). This accumulation was again correlated with the appearance of numerous osmiophilic plastoglobuli (size 0.1–1.5 μm). Plastoquinone-9 accumulated in the plastoglobuli with increasing age is in the reduced hydroquinone form (up to 70–90% plastoquinol-9, PQ-$9H_2$), and becomes partly oxidized during maceration and extraction of leaves with organic solvents. Plastoglobuli are osmiophilic which means they efficiently reduce osmium tetroxide (applied as leaf structure fixans in electron microscopy) to osmium that is deposited in the plastoglobuli. Their strong osmiophilic character is a consequence of the high reducing power of plastoquinol-9 and the antioxidant α-tocopherol, the two major constituents of plastoglobuli lipids.

Both prenyl compounds, plastoquinone-9 and α–tocopherol, can be regarded as photosynthetic products in a similar way as sugar phosphates, starch or triacylglerol lipids formed under photosynthetic conditions. The biosynthesis of their isoprenoid side-chains (the C_{45} nona-prenyl chain of plastoquinone-9 and the C_{20} phytyl chain of α-tocopherol) consumes considerable amounts of ATP and NADPH, since the biosynthesis of one active C_5 isoprenoid unit (IPP) from glyceraldehyde-3-phosphate and pyruvate in the plastidic DOXP/MEP pathway (Lichtenthaler 1999; Lichtenthaler et al. 1997a), shown in Fig. 3, requires 3 ATP and 3 NADPH (Lichtenthaler 2000). Thus, under high irradiance conditions, where the PPFD exceeds the light requirements necessary for the saturation of photosynthetic CO_2 fixation, the biosynthesis of both prenyllipids consumes ATP and NADPH and essentially contributes as one of several mechanisms to the protection of the photosynthetic pigment apparatus from photoinhibition and photooxidation. Analogous to Andy Benson, who, in his numerous photosynthetic $^{14}CO_2$ labeling studies of sugar phosphates and various lipid compounds, frequently published his results as "Path of carbon in photosynthesis," one could call this high-light induced accumulation of the two prenyllipids "Path of carbon into plastoquinone-9 and α-tocopherol." Both prenyllipids can also be considered secondary plant products because their accumulation exceeds by far the level of these prenyllipids necessary for the physiological function in photochemically active thylakoids.

Biosynthesis and emission of volatile isoprene and methylbutenol at high irradiance (Path of photosynthetic carbon into hydrocarbons)

Isoprene (C_5H_8, 2-methyl-1,3-butadiene), a volatile hemiterpene, is emitted by many green plants, including mosses, ferns, gymnosperms and angiosperms (Kesselmeier and Staudt 1999; Sharkey and Yeh 2001). Its emission from plants amounts to hundreds of millions of metric tons to the global atmosphere, the estimations range from 180 to 450 × 10^{12} g carbon per year worldwide (e.g., Rasmussen and Khalil 1998; Sharkey 1996). More organic carbon is lost from plants as isoprene than any other volatile plant molecule (Lerdau et al. 1997). The light and temperature-dependent isoprene emission by leaves preferentially occurs at high rates at temperatures above 28°C and at high irradiance, such as full sun light, when the photosynthesis process with the photosynthetic light reactions and associated electron transport reactions is fully light saturated. Isoprene emission can easily be measured in plant leaves by applying two simple methods, a photometric UV-cuvette test using leaf pieces and through GC-MS (Zeidler and Lichtenthaler 1998). The rapid appearance of ^{13}C-labeling in isoprene from photosynthetically fixed $^{13}CO_2$ (Delwiche and Sharkey 1993; Schnitzler et al. 2004) indicates that isoprene biosynthesis must be closely connected to intermediates of the Calvin–Benson cycle. In fact, the biosynthesis of the volatile C_5 hydrocarbon isoprene proceeds via the plastidic DOXP/

MEP pathway for isoprenoid biosynthesis from GAP and pyruvate (Figs. 3, 4). This has been shown by the specific incorporation of deuterium-labeled 1-deoxy-D-xylulose (^{2}H-DOX) in the form of its xyluloside into isoprene, as verified by gas chromatography combined with mass spectrometry (GC–MS) and via high resolution NMR spectroscopy (Zeidler et al. 1997; Schwender et al. 1997). DOX is rapidly phosphorylated by a cytosolic enzyme into DOXP, the intermediate precursor of the plastidic isoprenoid pathway (Hemmerlin et al. 2006), which can be transported to chloroplasts by the recently discovered plastidic transporter for pentose phosphates, xylulose 5-phosphate translocator (Flügge and Gao 2005). Biosynthesis and emission of isoprene from illuminated plant leaves is efficiently blocked by the herbicide fosmidomycin that inhibits the second enzyme DXR of the plastidic DOXP/MEP pathway of IPP and isoprenoid formation as shown in Fig. 3 (Zeidler et al. 1998). Isoprene is set free from DMAPP in a single enzymatic step via the plastidic isoprene synthase (Silver and Fall 1995), which exists in a thylakoid-bound form (Wildermuth and Fall 1996) and stromal isoforms in the chloroplast (Wildermuth and Fall 1998). The enzyme isoprene synthase is related to monoterpene synthases found in other plants (Sharkey et al 2005). Its K_m is 10–100-fold higher for its dimethylallylic diphosphate substrate DMAPP than related monoterpene synthases for geranyl diphosphate (Wolfertz et al. 2004).

The regulation of the light and temperature dependent isoprene emission apparently proceeds via the relative activity of the DOXP/MEP pathway and possibly via the concentration of DMAPP (Rosenstiel et al. 2002; Wolfertz et al. 2004). Moreover, in grey poplar leaves isoprene synthase and the second enzyme of the DOXP/MEP pathway, 1-deoxyxylulose 5-phosphate reductoisomerase DXR, show distinct seasonal patterns peaking in summer (Mayrhofer et al. 2005), thus suggesting that the metabolic carbon flux through the MEP pathway and isoprene emission are closely intercoordinated. Studies on the natural ^{13}C-carbon isotope composition of isoprene in several plants confirmed that isoprene is synthesized *de novo* from the currently formed primary photosynthates, yet a low percentage of carbon came from another carbon source (Affek and Yakir 2003), possibly from cytosolic pyruvate imported into the chloroplast to be joined with GAP to form DOXP, the first intermediate in the DOXP/MEP pathway (cf. Fig. 4). When in these studies photosynthetic carbon fixation was inhibited by CO_2-free air, the contribution of this alternative carbon source increased. Also, other leaf-internal carbon pools, e.g., starch or xylem-fed labeled glucose, can be used as alternative carbon sources for isoprene emission, especially when, after abscisic acid application, the stomata close and CO_2 for photosynthetic carbon fixation is missing (Schnitzler et al. 2004).

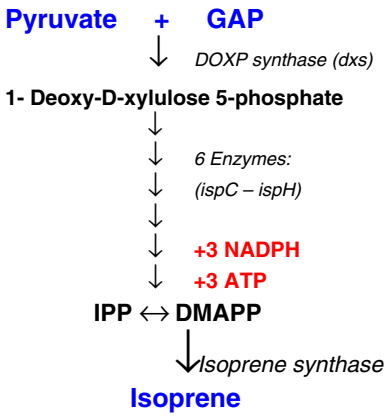

Fig. 4 Biosynthesis of the volatile hemiterpene isoprene in chloroplasts via the DOXP/MEP pathway from pyruvate and glyceraldehyde-3-phosphate (GAP) with indication of the cofactor requirements and the seven enzymes (genes) involved in the biosynthesis of the active isoprenoid C_5 unit IPP. Isoprene is set free from the IPP-isomer dimethylallyl diphosphate (DMAPP) by the plastidic enzyme isoprene synthase

Methylbutenol

In western North America the needles of several pines (*Pinus ponderosa, P. contorta, P. sabiniana*) do not emit isoprene itself but a partially oxidized form, the hemiterpene 2-methylen-3-buten-2-ol (MBO), in a light and temperature-dependent manner (Harley et al. 1998; Schade et al. 2000). Like isoprene, MBO can have a significant impact on the oxidative capacity of the atmosphere through the consumption of hydroxyl radicals. The biosynthesis of the C_5 structure of the volatile MBO proceeds also via the plastidic MEP pathway as has been determined by a high-rate incorporation of deuterium-labeled deoxy-D-xylulose (^{2}H-DOX) into MBO as proven by mass spectrometry (Zeidler and Lichtenthaler 2001). Like isoprene, MBO is formed from DMAPP in one step by the enzyme MBO synthase as shown in Fig. 5. Isoprene synthase and MBO synthase use the same substrate DMAPP, but the chemical mechanism for cleavage of the C_5 carbon structure from the diphosphate of DMAPP is different, thus yielding different end products. MBO emission and photosynthetic rates increased with light intensity and neither process showed light saturation even at PPFD of 2000 μmol m^{-2} s^{-1} (Gray et al. 2002). Although water stress (closure of stomata) reduced the photosynthetic rates, it had no effect on MBO emission. Under water stress the biosynthesis of MBO is apparently supported by the breakdown of starch or by other endogenous carbon sources.

The rather large amounts of isoprene and MBO, emitted at higher irradiances and elevated summer temperatures by herbaceous plants, as well as broadleaf trees and pine forests, derive from spontaneous *de novo* biosynthesis

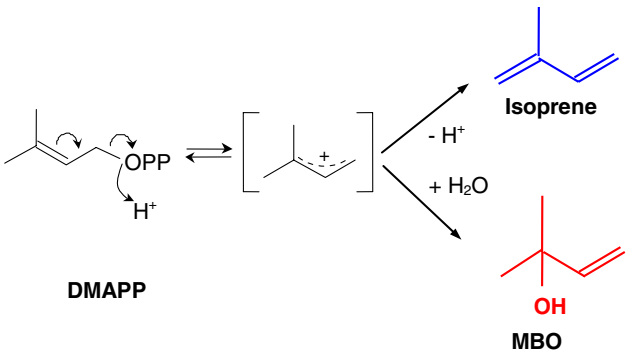

Fig. 5 Scheme of the formation of isoprene and methylbutenol (MBO) from dimethylallyl diphosphate (DMAPP) in leaves and ponderosa pine needles through the activity of isoprene synthase and MBO synthase

under photosynthetic conditions starting from GAP, an intermediate of the Calvin–Benson cycle, and pyruvate (Fig. 3). Pyruvate can be formed in the chloroplast, at least in some plants such as spinach, directly from photosynthetically fixed carbon (Schulze-Siebert et al. 1984; Schulze-Siebert and Schulz 1987) via 3-phosphoglyceric acid, the first product of the Calvin–Benson cycle, but can be imported in intact plants from the cytosol as well. However, pyruvate is also formed as a byproduct of the ribulosebisphosphate carboxylase/oxygenase activity (Andrews and Kane 1991). The possibility that at higher temperatures and a certain shortage of CO_2, i.e., the best conditions for isoprene emission, the ribulosebisphosphate carboxylase/oxygenase may give a higher yield of pyruvate is feasible, but has yet to be investigated.

The biosynthesis and emission of both volatile plant hemiterpenes depend on the chloroplastidic production of DMAPP. In fact, plant species with the highest potential for isoprene and MBO production also exhibit an elevated light-dependent production of DMAPP (Rosenstiel et al. 2002). The physiological meaning of the emission of isoprene and MBO is not yet clear. Isoprene provides the leaves with a certain thermotolerance against heat damage (Sharkey and Singsaas 1995). In addition, by functioning as a potential scavenger of radicals both volatile hemiterpenes can protect thylakoid lipids and other chloroplast constituents from ozone and other reactive oxygen species (e.g,. Affek and Yakir 2002; Loreto and Velikova 2001; Loreto et al. 2001), thereby preventing photooxidation of the photosynthetic apparatus at high irradiance conditions. One also has to consider that the enhanced *de novo* biosynthesis of isoprene and MBO requires a continuous supply of ATP and NADPH being formed in the photosynthetic light reactions (cf. Fig. 4), a process keeping the two photosynthetic photosystems 'busy' and intact by avoiding overreduction and photooxidative damage at excess light conditions. Thus, isoprene and methylbutenol emissions may be a 'safety valve', similar to the process of photorespiration, to protect the photosynthetic pigment apparatus with its photosystems and light-harvesting pigment-proteins against photooxidation. The only disadvantage is that this isoprene emission is a waste of the previously photosynthetically fixed reduced carbon. Although the ATP and NADPH consumption through isoprene and MBO biosynthesis should not be overestimated, it contributes, besides other mechanisms, to the stability of the photosynthetic pigment apparatus under high irradiance conditions. Recently Rosenstiel et al. (2004) proposed a new hypothesis of why plants emit isoprene: the isoprene synthase converting DMAPP to isoprene and pyrophosphate would prevent DMAPP to rise to such high levels that would unnecessarily sequester phosphate. In any case, the available data show that under high incident photon fluxes and elevated temperatures rather high amounts of photosynthetically fixed carbon are channeled into the two hemiterpenes isoprene and MBO.

Path of photosynthetic carbon into cytosolic sterols

Plants and algae with two isoprenoid pathways

In plants the C_5 carbon building blocks of all terpenoids, the two isomers IPP and DMAPP, are derived from two independent cellular biosynthetic pathways that are localized in two different compartments, the chloroplast and the cytosol, as shown in Fig. 3 (Lichtenthaler et al. 1997b; Lichtenthaler 1999). The plant sterols as regular constituents of the cytosolic biomembranes are usually synthesized via the cytosolic acetate/mevalonate pathway of IPP and plant isoprenoid biosynthesis. The biosynthesis of sterols via that pathway can be efficiently blocked by mevinolin, cerivastatin and other statins that have been shown to be efficient and specific inhibitors of the cytosolic HMG-CoA-reductase of plants, the key enzyme of the MVA pathway (Bach and Lichtenthaler 1983a, b; Schindler et al. 1985). Plastidic isopentenyl diphosphate (IPP) biosynthesis can, however, contribute to the biosynthesis of the cytosolic sterol biosynthesis by providing isoprenoid C_5 units that are exported to the cytosol. Under photosynthetic light conditions, direct carbon flow from CO_2 to IPP takes place via the photosynthetically formed substrates glyceraldehyde-3-phosphate (GAP) and pyruvate. The IPP product is subsequently transported as a C_5 isoprenoid unit from chloroplasts and used to form cytosolic sterols. Thus, in the red alga *Cyanidium* a large part of the deuterium-label of [1-^{2}H] DOX, a precursor of plastidic IPP, was not only incorporated into phytol, but also into the cytosolic sterols

(Schwender et al. 1997). This has been further documented by labeling experiments using ^{14}C-DOX as a precursor of the plastidic DOXP/MEP pathway and tritium labeled mevalonolactone (^{3}H-MVL), a substrate readily used by the cytosolic acetate/MVA pathway of isoprenoid biosynthesis (Schwender et al. 2001). As expected ^{14}C-DOX was incorporated at high rates into plastidic phytol (side-chain of chlorophylls) and ^{3}H-MVL was incorporated into the cytosolic sterols of two algae and a higher plant (*Lemna gibba*), which possess both cellular pathways for isoprenoid biosynthesis (Table 5). To our surprise the label of ^{14}C-DOX showed up in the cytosolic sterols (to a high extent in the two algae and to a lower extent in *Lemna*) indicating that isoprenoid C_5 units must have been exported from the chloroplast to the cytosol, and there they must have been used for the biosynthesis of FPP and the C_{30} isoprenoid sterols. In the alga *Mesostigma viride*, ^{14}C-DOX was incorporated into sterols even at a higher rate than ^{3}H-MVL, the proper substrate of the cytosolic MVA isoprenoid pathway. By contrast, the label of ^{3}H-MVL showed up in all three photosynthetic organisms tested but only to a very low extent in the plastidic isoprenoid phytol (Table 5). The incorporation of ^{14}C-DOX into phytol of *Klebsormidium, Mesostigma* and *Lemna* was 95, 107 and 23 times higher, respectively, than the incorporation of ^{3}H-MVL. These data indicate the presence of cross-talk between the two isoprenoid pathways of the plant cell. They also show that under physiological conditions the transport of isoprenoid compounds proceeds almost exclusively into one direction from chloroplasts to the cytosol, but not from the cytosol to the chloroplasts or, if so, only at extremely low rates. That the rate of ^{14}C-DOX incorporation into sterols was not as high in the higher plant *Lemna gibba* as in the two algae (Table 5) is paralleled by the fact that less ^{3}H-MVL was also incorporated into sterols, thus indicating that the rate of sterol formation in *Lemna* at the time of investigation was much lower than in the two algae. Yet, also in this case, ^{14}C-DOX was used at 60% the incorporation rate of the cytosolic MVA pathway precursor. These observations provide clear evidence of cross-talk exists between the two isoprenoid pathways of the plant cell. Further, a major part of the isoprenoid C_5 units for cytosolic sterol biosynthesis comes from the chloroplast, indicating that there apparently occurs carbon flow from CO_2 via the Calvin–Benson cycle, GAP, and pyruvate into plastidic IPP and finally into cytosolic sterols under photosynthetic conditions. A recently described plastidic transporter for pentose phosphates, the xylulose 5-phosphate translocator, can transport DOXP but not IPP (Flügge and Gao 2005). However, Bick and Lange (2003) demonstrated in isolated spinach chloroplast and envelope membrane vesicles the transport of IPP and geranyl diphosphate (GPP) in a plastid-to-cytosol direction by a plastidial proton symport system. Lower rates were observed with DMAPP, farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP). These data suggest that plastid envelope membranes possess a unidirectional proton symport system for the export of specific isoprenoid intermediates involved in the metabolic cross-talk between plastidial and cytosolic isoprenoid biosynthesis.

Algae with only one IPP pathway

Very special examples for the unidirectional flow of isoprenoid C_5 units from chloroplasts to the cytosol are given by Chlorophyta, green algae, such as *Chlorella*, *Chlamydomonas* and *Scenedesmus*, where all sterols are synthesized via the plastidic DOXP/MEP pathway (Schwender et al. 1996). These algae lost their cytosolic acetate/MVA pathway during evolution (Schwender et al. 2001) and are fully dependent on the plastidic isoprenoid pathway. For this reason, such green unicellular algae are best suited in the search for transporters for the export of isoprenoid C_5 compounds from chloroplasts to the cytosol. This flow of ^{14}C radioactivity from photosynthetically fixed $^{14}CO_2$ into cytosolic sterols in unicellular green alga had been seen in 1963, though not published.[2] The inverse situation and the only exception of all photosynthetic organisms, where both cytosolic sterols and also photosynthetic isoprenoids (carotenoids, phytol, side-chain of prenylquinones) are synthesized via the cytosolic MVA pathway, is *Euglena* (Disch et al. 1998). In this organism the DOXP/MEP pathway of isoprenoid biosynthesis was apparently lost during evolution in the course of a secondary endosymbiontic event (Lichtenthaler 1999). Therefore, *Euglena* is best qualified for studies on import mechanisms of isoprenoid C_5 or even C_{15} compounds from the cytosol into the chloroplast.

[2] As post-doc in Berkeley at beginning of December 1963, I actually detected the rapid flow of 14C-label from photosynthetically fixed $^{14}CO_2$, not only into the plastidic isoprenoids β-carotene, plastoquinone-9, phylloquinone K1 and chlorophylls, but also into cytosolic sterols in *Chlorella* after an exposure time of 2 min. In this experiment *Chlorella* suspensions were fed $^{14}CO_2$ in the original 'Lollipop' vessel set up by Andy Benson in Melvin Calvin's photosynthesis laboratory in Berkeley in the early 1950s for studies of the path of carbon in photosynthesis. Due to my return to Germany at the end of December 1963, this work could not be continued. However, feeding 13C-labeled glucose to illuminated *Chlorella* suspensions in my Karlruhe laboratory 42 years later led to the detection of the chloroplastidic DOXP/MEP pathway for isoprenoid biosynthesis and its responsibility for the biosynthesis of the cytosolic sterols in unicellular green algae (Schwender et al. 1996). (H. K. Lichtenthaler, March 2007).

Table 5 Labeling of the plastidic isoprenoid phytol (side-chain of chlorophylls) and the cytosolic sterols from precursors of the plastidic DOXP/MEP pathway and the cytosolic mevalonate pathway in two algae and a higher plant (*Lemna gibba*) under photosynthetic conditions

Organism/Isoprenoid	Applied precursor		Ratio
	^{14}C-DOX	^{3}H-MVL	^{14}C/^{3}H
Klebsormidium flaccidum			
Phytol	**257.7**	2.7	95.4
Sterols	114.2	**174.9**	0.7
Mesostigma viride			
Phytol	**1600.0**	14.9	107.4
Sterols	618.9	**535.7**	1.2
Lemna gibba			
Phytol	**359.6**	15.4	23.4
Sterols	28.3	**46.5**	0.6

Applied were ^{14}C-labeled deoxyxylulose (^{14}C-DOX) and tritium-labeled mevalonolactone (^{3}H-MVL). The radioactivity of phytol and sterols is given in decays per per minute. The labeling of the cytosolic sterols from ^{14}C-DOX indicates export of isoprenoid units from the chloroplast to the cytosol. (Based on Schwender et al. 2001)

Cross-talk between both cellular isoprenoid pathways

That cross-talk exists in the plant cell between the plastidic and cytosolic isopentenyl diphosphate (IPP) providing pathways, as described above and shown in Table 5, has been confirmed by various other observations and authors. When applying specific inhibitors of both isoprenoid pathways, mevinolin for the MVA pathway, and fosmidomycin for the DOXP/MEP pathway, Hemmerlin et al. (2003) used for the first time the term, 'cross-talk', for this cooperation between both isoprenoid pathways: 1-deoxy-D-xylulose (DOX), the dephosphorylated first precursor of the plastidial DOXP/MEP pathway, complemented growth inhibition by mevinolin in the low mM concentration range, whereas growth inhibition by fosmidomycin of TBY-2 cells could only partially be overcome by MVA. Another investigation using labeled precursors of the non-mevalonate DOXP/MEP pathway and the cytosolic MVA pathways showed that, in the case of volatile terpenoids being formed in the chlorophyll-free epidermis of snapdragon petals, the DOXP/MEP pathway provides not only the IPP precursors for the plastidic monoterpene synthesis, but also for cytosolic sesquiterpene biosynthesis (Dudareva et al. 2005). Also, in this research, the transport of IPP or C_5 isoprenoid compounds occurred unidirectionally from the plastids to the cytosol. A certain cross-talk, i.e., cooperation between both cellular isoprenoid pathways, had been previously described in an earlier review (Lichtenthaler 1999). Thus, when studying the ^{13}C-labeling of the diterpene ginkgolide from ^{13}C-glucose, three isoprenoid units were found to be labeled via the MVA pathway and the fourth isoprenoid unit via the DOXP/MEP pathway (Schwarz 1994). In addition, in the liverwort *Heterocyphus planus*, the first three isoprene units of phytol and other diterpenes showed some label applied from ^{13}C-MVA, whereas the fourth unit was not labeled (Nabeta et al. 1995, 1997). Both observations pointed to the transfer of cytosolic farnesyl diphosphate (FPP) or a C_{15} isoprenoid compound into the plastid compartment, where it was condensed with a DOXP/MEP pathway derived IPP to the C_{20} isoprenoid phytol. Also, an observation in chamomile indicated cross-talk between both isoprenoid pathways: in labeling studies of sesquiterpenes the first two C_5-units were derived from ^{13}C-glucose via the DOXP/MEP pathway, and the third C_5-unit was labeled by either the plastidic or the MVA pathway (Adam et al. 1998). In *Arabidopsis* seedlings gibberelins are predominantly synthesized through the DOXP/MEP pathway, whereas the MVA pathway plays a major role in the biosynthesis of campesterol (Kasahara et al. 2002). However, consistent with some cross-talk between the two isoprenoid pathways, phenotypic defects caused by the block of the MVA and MEP pathways were partially rescued by exogenous application of the MEP and MVA precursors, respectively. A further observation showed that mevalonic acid partially restores chloroplast and etioplast development in *Arabidopsis* lacking the non-mevalonate pathway (Nagata et al. 2002). A certain type of cross-talk was also found in cell cultures of *Catharanthus roseus*, where sitosterol was predominantly labeled via the cytosolic MVA pathway, while the plastidic phytol was labeled to a higher extent (ca. 60%) via the DOXP/MEP pathway but also via the MVA pathway (ca. 40%) (Schuhr et al. 2003). However, one has to consider in this respect that photoheterotrophic cell cultures usually exhibit rather limited photosynthetic performance and are not really representative for the metabolite flow in fully green intact plants. In carrot leaves and carrot roots monoterpenes are synthesized exclusively via the DOXP/MEP pathway, whereas sesquiterpenes are generated by the classical MVA pathway as well as the DOXP/MEP pathway (Hampel et al. 2005).

Although cross-talk between both cellular isoprenoid pathways and compartments is principally possible and evident from the research reviewed above, the question arises if a block of one isoprenoid pathway can be compensated for by the C_5 metabolites of the second isoprenoid pathway in the other compartment. In the early work on mevinolin effects on plants by Bach and Lichtenthaler (1982, 1983a, b), the cytosolic MVA biosynthesis was blocked by the inhibitor mevinolin, whereby the cellular sterol biosynthesis was inhibited and the plants finally died. However, the accumulation of chlorophylls (phytol), carotenoids, and plastoquinone-9 in chloroplasts in these

plants was not affected. This oberservation demonstrated that plastids could not export adequate amounts of isoprene C_5-units or higher prenyl homologues to support sufficient cytosolic sterol biosynthesis required for growth. Moreover, in an *Arabidopsis* mutant, where a nuclear gene (*CLA1*) apparently encoding for the first enzyme DOXP synthase of the plastidic DOXP/MEP pathway, is missing (Mandel et al. 1996), the development of chloroplasts and the growth of plants is blocked. This observation shows that the cytosolic isoprenoid pathway cannot sufficiently provide the missing amounts of C_5 isoprenoid precursors to guarantee a normal chlorophyll (phytyl side-chain) and carotenoid formation required for chloroplast development. From all these data it appears that a cross-talk between both isoprenoid pathways is possible in principle and can experimentally be demonstrated using specific inhibitors and substrates of both pathways. Yet one has to consider that, in such experiments, the concentration of the applied precursor compounds is usually much higher than the endogenous cellular pool sizes of undisturbed cells. This then can indicate an export or import of isoprenoid C_5 units from or to plastids that may not proceed under normal physiological conditions.

In summary, a full or adequate compensation of the missing activity of one isoprenoid pathway by the second isoprenoid pathway of the other cell compartment apparently does not occur under physiological standard conditions of growth. Thus, what remains from presently available data is a unidirectional relatively high flow of plastidic C_5 isoprenoid units into cytosolic sterol biosynthesis under photosynthetic conditions (Table 5, and Schwender et al. 2001). Further, in non-green snapdragon flowers (Dudareva et al. 2005) and in carrots (Hampel et al. 2005), there is flow into cytosolic sesquiterpene presumably mediated by the plastidial unidirectional proton symport system (Bick and Lange 2003).

Concluding remarks

Plants possess their system for the biosynthesis of IPP and isoprenoid formation in chloroplasts, the DOXP/MEP pathway, that operates independently of the cytosolic acetate/MVA pathway. This DOXP/MEP pathway is required to supply the IPP C_5 units needed for the synthesis of functional compounds in the photosynthetic apparatus, such as carotenoids, prenyl side-chains of chlorophylls, plastoquinone-9, α-tocopherol and phylloquinone K1. This plastidic isoprenoid pathway is also involved in chloroplast adaptation response to high or low light conditions by providing the isoprenoid C_5 building blocks to form sun- and shade-type chloroplasts that are characterized by particular chlorophyll *a*/*b* ratios and carotenoid and prenylquinone composition. At high photon flux densities the DOXP/MEP pathway also participates in the relatively rapid *de novo* biosynthesis of high additional amounts of the xanthophyll cycle carotenoids (zeaxanthin, antheraxanthin) that protect the photosynthetic apparatus from photoinhibition as well as photooxidation. In sun-exposed leaves the DOXP/MEP pathway supplies the active C_5 IPP units for the continuous accumulation of plastoquinone-9 and α-tocopherol in the osmiophilic plastoglobuli. At high irradiances the chloroplast DOXP/MEP pathway efficiently operates and uses photosynthetically formed ATP, NADPH, and newly fixed carbon to provide a major part of the C_5 IPP units necessary for the biosynthesis of the cytosolic sterols. In addition, starting with intermediates of the Calvin–Benson cycle, the chloroplastid isoprenoid pathway, serves in the biosynthesis and emission or accumulation of volatile isoprenoids, such as isoprene, methylbutenol, monoterpenes, diterpenes (see Fig. 3) and particular sesquiterpenes all of which can be regarded as both direct primary photosynthetic products and secondary plant products or natural products. The data reviewed above indicate that, depending on the light and temperature conditions, enormous amounts of freshly fixed photosynthetic carbon flow into various volatile and non-volatile isoprenoid compounds. Thus, the chloroplast isoprenoid biosynthesis via the IPP forming pathway appears to be a 'metabolic valve' for regulating photosynthetic carbon flow as well as a fine tuning for chloroplast and cell metabolism. This chloroplast isoprenoid pathway consumes large amounts of photosynthetically formed ATP and NADPH, and may also serve as a 'safety valve' in order to avoid overreduction and photoinhibition of the photosynthetic apparatus. Plants can emit also other, non-isoprenoid, volatile organic compounds.[3]

Epilogue

Andy Benson performed truly pioneering work over several decades on a wide range of scientific topics, including elucidating the path of carbon in photosynthesis leading to various organic compounds (e.g., sugar phosphates, plant glycerolipids, galactolipids, sulfolipids), the isolation and function of the chloroplast envelope, and,

[3] In addition to isoprenoid compounds, plants emit substantial amounts of phytogenic volatile organic compounds (PVOCS) comprising alkanes, alkenes, alcohols, aldehydes, ethers, esters and carboxylic acids (e.g., Penuelas and Llusia 2004). Plants not only metabolize methanol as shown in a paper co-authored by Andy Benson (Gout et al. 2000), but they also emit substantial amounts of methanol (Nonomura and Benson 1992a, b) via stomates (Nemecek-Marshall et al. 1995) during the early stages of leaf expansion due to pectin demethylation (see review by Fall and Benson, 1996).

finally, on metabolism and emission of methanol from plants. His achievements were brilliant. Andy, I extend my warmest wishes on the occasion of your 90th birthday. I am proud to have you as a colleague and a friend. May you celebrate many more happy and healthy birthdays.

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