Annu. Rev. Plant Physiol. Plant Mol. Biol. 1999. 50:47–65

THE 1-DEOXY-D-XYLULOSE-5-PHOSPHATE PATHWAY OF ISOPRENOID BIOSYNTHESIS IN PLANTS

Hartmut K. Lichtenthaler
Botanisches Institut (Plant Physiology and Biochemistry), University of Karlsruhe, D-76128 Karlsruhe, Germany;
e-mail: Hartmut.Lichtenthaler@bio-geo.uni-karlsruhe.de

KEY WORDS: carotenoid biosynthesis, chloroplast metabolism, isopentenyl diphosphate, isoprene formation, non-mevalonate IPP formation

ABSTRACT

In plants the biosynthesis of prenyllipids and isoprenoids proceeds via two independent pathways: (*a*) the cytosolic classical acetate/mevalonate pathway for the biosynthesis of sterols, sesquiterpenes, triterpenoids; and (*b*) the alternative, non-mevalonate 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway for the biosynthesis of plastidic isoprenoids, such as carotenoids, phytol (a side-chain of chlorophylls), plastoquinone-9, isoprene, mono-, and diterpenes. Both pathways form the active C_5-unit isopentenyl diphosphate (IPP) as the precursor from which all other isoprenoids are formed via head-to-tail addition. This review summarizes current knowledge of the novel 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway for isopentenyl diphosphate biosynthesis, apparently located in plastids. The DOXP pathway of IPP formation starts from D-glyceraldehyde-3-phosphate (GA-3-P) and pyruvate, with DOXP-synthase as the starting enzyme. This pathway provides new insight into the regulation of chloroplast metabolism.

CONTENTS

1040-2519/99/0601-0047$08.00

INTRODUCTION

The biosynthesis of plant isoprenoids, carotenoids, phytol, sterols, plastoquinone-9 as well as monoterpenes, sesquiterpenes, diterpenes, or polyterpenes seemed to have been well understood since the late 1950s (36). Labeling experiments with ^{14}C-labeled substrates indicated that the photosynthetic plants and algae form their isoprenic C_5-unit (IPP) and all isoprenoids—as in animal systems and fungi—via the acetate/mevalonate (MVA) pathway (23–25, 34, 35, 70), although some observations were not in agreement with the MVA pathway. For example, photosynthetically fixed $^{14}CO_2$ was rapidly incorporated into the plastidic isoprenoids (carotenoids, phytol, plastoquinone-9), whereas ^{14}C-labeled acetate and MVA were readily incorporated into the cytosolic sterols, but only at low rates into the plastidic isoprenoids (8, 9, 21, 22, 27, 34, 37). Moreover, mevinolin, a highly specific inhibitor of the HMG-CoA reductase, efficiently inhibited the cytosolic sterol and ubiquinone accumulation, but did not affect the accumulation of phytol, carotenoids, and plastoquinone-9 in plastids (4–6, 13, 58). In addition, isolated plastids could not make IPP from MVA (44). The discovery that the isoprenoid hopanoids (sterol surrogates) of certain eubacteria are formed via a non-MVA pathway (53, 55) was the starting point in 1993 for the author's group to re-investigate the biosynthesis of plastidic isoprenoids, in cooperation with Michael Rohmer (Strasbourg), and Frieder W. Lichtenthaler (Darmstadt). Applying ^{13}C- and ^{2}H-labeling techniques, NMR spectroscopy, and GC-MS analyses, it was shown that green algae (chlorophyta), higher plants, and other algal groups synthesize their plastidic isoprenoids including isoprene via the novel 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway (3, 38, 39, 41, 42, 63–68, 75). This pathway is also involved in the biosynthesis of various other terpenoids (16, 17, 30, 46).

^{13}C-LABELING OF PLASTIDIC ISOPRENOIDS FROM [1-^{13}C]GLUCOSE

The ^{13}C-labeling of ß-carotene, lutein, phytol, and the nona-prenyl chain of plastoquinone-9 in green algae and higher plants, grown photoheterotrophically on [1-^{13}C]glucose, provided a ^{13}C-labeling pattern (Figure 1) that was not in agreement with the formation of the IPP precursor unit from acetate and MVA (39–42, 63–68). The IPP C_5-units of these plastidic isoprenoids did not exhibit the expected ^{13}C-enrichment in the three C-atoms C-2, C-4, and C-5, but rather showed labeling in the two C-atoms C-1 and C-5 (Figure 1). This finding clearly indicated the existence of a completely different IPP biosynthesis pathway in green algae and higher plants for the biosynthesis of plastidic isoprenoids. Examination of the ^{13}C-labeling pattern of cytosolic sterols from [1-^{13}C]glucose revealed that green algae (*Scenedesmus, Chlorella, Chlamydomonas*) exhibited the same non-MVA labeling pattern for sterols as for plastidic isoprenoids

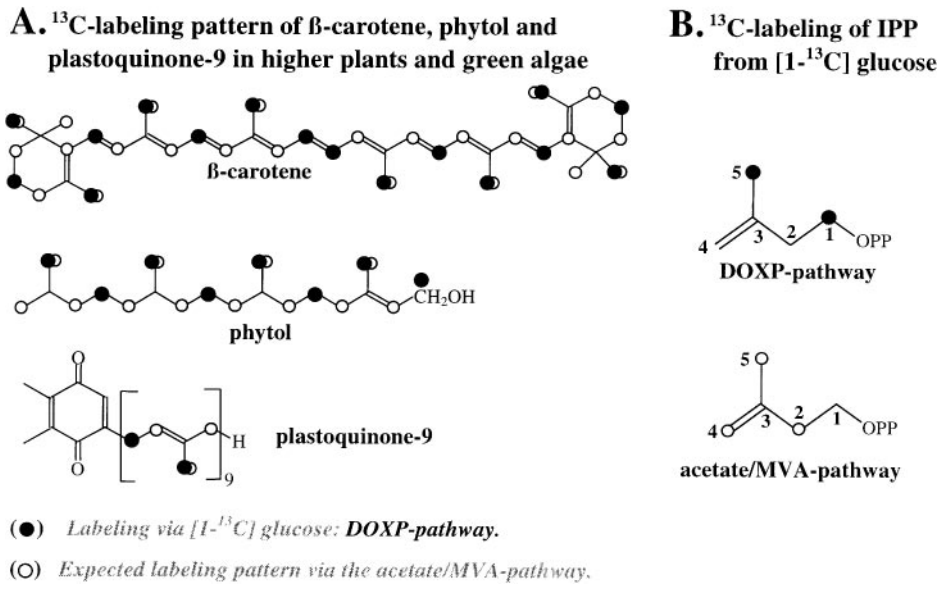


Figure 1 Labeling patterns in (*A*) plastidic isoprenoids of higher plants and various algae when supplied with [1-^{13}C]glucose. (*B*) Two labeling patterns in isopentenyl diphosphate (IPP) resulting from the DOXP pathway (*upper*) and the MVA-pathway (*lower*). The labeling of the plastidic isoprenoids and IPP proceeded only via the DOXP pathway; the expected labeling via the MVA pathway could not be detected. *Black circles*: Labeling of C-atoms from [1-^{13}C]glucose via the DOXP pathway; *white circles*: expected labeling of C-atoms via the acetate/MVA pathway of IPP formation.

(12, 65, 67), whereas in higher plants (barley, carrot, duckweed) sitosterol was labeled via the classical acetate/MVA pathway (38, 41). Thus, unicellular green algae only have the DOXP pathway for IPP formation, whereas higher plants possess two different IPP biosynthesis pathways (36, 38).

THE DOXP PATHWAY OF IPP BIOSYNTHESIS

The 1-Deoxy-D-Xylulose-5-Phosphate Synthase, First Enzyme of the DOXP Pathway

The starting substrates of the DOXP pathway are glyceraldehyde-3-phosphate (GA-3-P) and pyruvate. In a thiamin-dependent transketolase-type reaction, a C_2-unit derived from pyruvate (hydroxyethyl-thiamine) is transferred to GA-3-P, whereby DOXP is formed (Figure 2). This step is catalyzed by the enzyme DOXP-synthase, or DXS. For photosynthetic organisms this enzymic step was first verified in green algae by extensive ^{13}C-NMR studies using various glucoses, ^{13}C-labeled at different C-atoms, and uniformly labeled [U-$^{13}C_6$]glucose (63–68). It was subsequently also demonstrated in higher plants applying labeling from [1-^{13}C]glucose (39, 41). The labeling pattern of the C_5-units of IPP (Figure 1) is identical to that found in eubacteria (53–55). Further evidence for this initial step was the efficient incorporation of 1-deoxy-D-xylulose (DOX) into plastidic isoprenoids (3, 68, 75) (see below). As final proof for

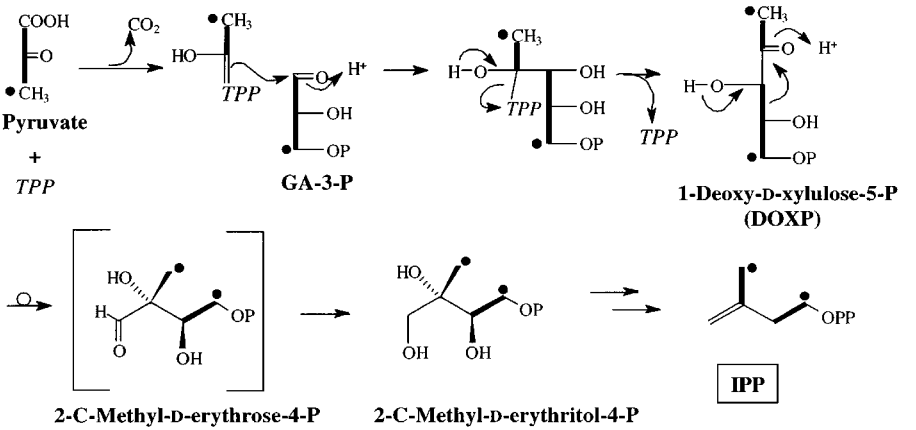


Figure 2 Steps and possible intermediates in the thiamin (TPP)-dependent biosynthesis of isopentenyl diphosphate (IPP) from pyruvate and GA-3-P. The label arising from [1-^{13}C]glucose in the final product IPP is marked by *black circles*. The DOXP pathway requires an intramolecular rearrangement of the carbon atoms in the step following 1-deoxy-D-xylulose-5-phosphate. 2-C-methyl-D-erythrose-4-P and 2-C-methyl-D-erythritol-4-P are possible intermediates. The further enzymatic steps and intermediates are not yet known.

DOXP-synthase as the starting step, it was demonstrated that a plant DOXP-synthase of *Mentha* (33) and a bacterial DOXP-synthase of *Escherichia coli* (43, 69), both overexpressed in *E. coli*, form DOXP from GA-3-P and pyruvate.

C-Skeleton Rearrangement

In further steps that are not yet fully clarified DOXP is transformed into IPP, possibly via 2-C-methyl-D-erythrose-4-phosphate and 2-C-methyl-D-erythritol-4-phosphate (Figure 2). These steps from DOXP to IPP require several reductases, dehydratases, and a kinase, and as co-factors possibly 3 NADPH and one ATP. This transformation of DOXP to IPP is based on an intramolecular C-skeleton rearrangement, whereby the C_2-unit of DOXP, originating from pyruvate, is inserted between the C-atoms C-1 and C-2 of GA-3-P (Figure 2). The incorporation of the complete C_2- and C_3-units from glucose into IPP was shown by the $^{13}C/^{13}C$ coupling constants of the NMR spectra seen after growing *Scenedesmus* on uniformly labeled [U-^{13}C]glucose (63, 67). Whether IPP is formed as the first isoprenoid C_5-unit or its isomer DMAPP in the DOXP pathway is unresolved.

1-Deoxy-D-Xylulose as Precursor Substrate

Evidence for DOXP as the first intermediate in the alternative IPP biosynthesis pathway came from the specific incorporation of deuterium (d)-labeled [1-$^2H_1$]deoxy-D-xylulose (d-DOX) and its methyl-glycoside (methyl-d-DOX) into the plastidic isoprenoid phytol in green algae (*Scenedesmus, Chlamydomonas*), a red alga (*Cyanidium*), and a higher plant (*Lemna*) (68), as well as into isoprene (*Populus, Chelidonium, Salix*) (68, 75, 76) as analyzed by NMR and/or GC-MS spectra. ^{13}C-MVA, when applied at a high concentration to a leaf, can be incorporated into isoprene and phytol, albeit to a lower extent (68). Plants and most algae apparently have the capacity to readily hydrolyze the applied xyluloside methyl-d-DOX to the free pentulose d-DOX, and to phosphorylate it to DOXP as the endogenous intermediate that is incorporated into the final isoprenoid. The transfer of methyl-d-DOX and d-DOX via IPP into isoprene and phytol (68) is additional evidence for the C-skeleton rearrangement in the DOXP pathway occurring in one of the steps after the formation of DOXP. The specific incorporation of a ^{13}C-labeled DOX into ß-carotene of *Catharanthus* (3), and of double ^{13}C-labeled DOX into ubiquinone of *E. coli* (52) provides additional corroboration.

2-C-Methyl-D-Erythritol-4-Phosphate as a Possible Intermediate

The further enzymic steps in the biosynthesis of IPP from DOXP have not yet been established in plants. One highly probable candidate is 2-C-methyl-D-erythritol-4-phosphate, which, after further reduction, dehydration and

phosphorylation steps should yield IPP or DMAPP (Figure 2). When applied to plants or green algae, however, deuterium-labeled methyl-erythritol (14) was not incorporated into isoprenoids or isoprene (76), possibly due to the lack of a kinase that could convert 2-C-methyl-D-erythritol to its phosphate being the putative endogenous intermediate. In bacteria, by contrast, this deuterium-labeled methyl-erythritol is incorporated into the prenyl side-chain of menaquinone and ubiquinone at a low rate (15), and DOXP is transformed in *E. coli* to 2-C-methyl-D-erythritol-4-phosphate in the presence of NADPH by a reducto-isomerase (32). In *Corynebacterium*, methyl-D-erythritol-2,4-cyclodiphosphate is accumulated and marked from ^{13}C-glucose according to the DOXP pathway (14). In *Liriodendron*, a ^{13}C-labeled 1-DOX was converted into 2-C-methyl-D-erythritol (56): A 2-C-methyl-erythronolactone has been detected in higher plants (18, 31, 60). DOXP could possibly yield this lactone after oxidation and benzilic acid rearrangement. Although the intermediates following 2-C-methyl-D-erythritol-4-phosphate in the DOXP pathway of IPP-formation have not yet been identified, their structure can be presumed to be two reduction and dehydration steps, with one phosphorylation step being involved in these final steps of IPP formation.

COMPARTMENTATION OF IPP AND ISOPRENOID BIOSYNTHESIS IN HIGHER PLANTS

In their IPP and isoprenoid biosynthesis, there is a dichotomy in higher plants, one related to the plastid and the other to the cytosol (36, 41). The acetate/MVA pathway, producing IPP for sterol biosynthesis (11, 38, 41), proceeds in the cytosol and can be inhibited by mevinolin (4, 5, 13, 58). Sesquiterpenes are formed in the cytosol (2, 7), as well as polyterpenes by a consecutive chain elongation (Figure 3) (19). Given the existence of the DOXP pathway of IPP formation, polyterpene biosynthesis requires investigation to determine if it is solely based on the MVA pathway or is partly dependent on the DOXP pathway.

LOCALIZATION OF THE DOXP PATHWAY IN PLASTIDS

The plastid, in turn, is the site of the DOXP pathway of IPP formation (see below). This IPP biosynthesis starts from GA-3-P, an intermediate in the photosynthetic carbon reduction cycle, and pyruvate, which can be formed within the plastid from 3-phosphoglyceric acid. The DOXP pathway delivers isoprene (68, 75, 76), carotenoids (3, 38, 39, 41), phytol, and the nona-prenyl chain of plastoquinone-9 (3, 11, 38, 39, 41) as well as mono- (2, 17) and diterpenoids (7, 16, 30, 62), as indicated in Figure 3. This IPP and isoprenoid pathway can

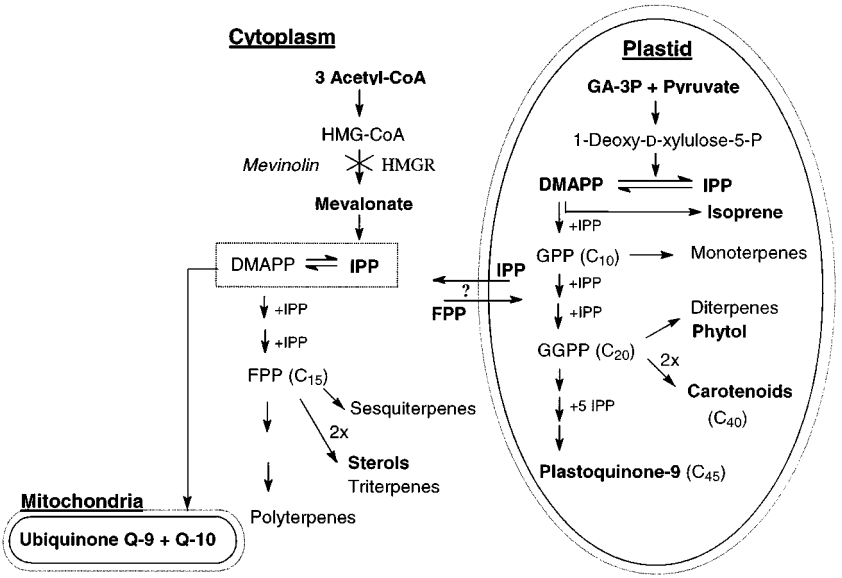


Figure 3 Suggested compartmentation of IPP and isoprenoid biosynthesis in higher plants between cytosol (acetate/MVA pathway) and plastids (DOXP pathway). The specific inhibition of the cytosolic HMG-CoA reductase (HMGR) by the antibiotic mevinolin (4–6, 13) is indicated. The nona- and deca-prenyl chain formation of ubiquinones Q-9 and Q-10 apparently proceeds in mitochondria from cytosolic IPP. Abbreviations used: DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosophate; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate.

easily be labeled from photosynthetically fixed $^{14}CO_2$, as Goodwin et al observed some 40 years ago (8, 21, 22).

At least the final steps of the biosynthesis of the plastidic isoprenoids proceed in chloroplasts (35). Supporting evidence for the plastid localization of the DOXP pathway is the observation that the light-dependent emission of isoprene is formed from DMAPP (74) within the chloroplast (68, 75, 76). Furthermore, the biosynthesis of thiamine and pyridoxal occurs in chloroplasts (28, 29). The DOXP pathway is also present in the cyanobacterium *Synechocystis* for biosynthesis of phytol and ß-carotene (Table 1) (12, 38, 49). If cyanobacteria are progenitors of chloroplasts, they could have conserved their originally bacterial DOXP pathway of IPP biosynthesis during co-evolution with the eukaryotic host cells. Moreover, the fact that the genes for DOXP synthase in *Arabidopsis* and *Chlamydomonas* possess a plastid transit peptide sequence (40, 47) is strong evidence for the localization of the DOXP pathway in plastids.

Table 1 Formation of isoprenoids in plants and photosynthetic organisms via the acetate/mevalonate (MVA) or the new 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway of IPP formation. The data were obtained by determining the ^{13}C-labeling pattern of the isoprenoids from ^{13}C-glucoses or from deuterium- or ^{13}C-labeled 1-deoxy-D-xylulose via ^{13}C-NMR or mass spectrometry. PQ-9 = plastoquinone-9.

Organism	Isoprenoid	IPP pathway	References
Cyanobacteria			
Synechocystis PCC 6714	Phytol, ß-carotene	DOXP	12, 38
Green algae			
Scenedesmus obliquus	Phytol, ß-carotene, lutein,	DOXP	42
	Plastoquinone-9, Chondrilla-	DOXP	63, 65
	sterol, ergost-7-enol,	DOXP	66, 67
	Ubiquinone-10	DOXP	12
Chlorella fusca	Phytol, ß-carotene	DOXP	38
	Chondrillasterol	DOXP	12
Chlamydomonas	Phytol, ß-carotene	DOXP	38, 39
reinhardtii	Chondrillasterol	DOXP	12
Red algae			
Cyanidium caldarium	Phytol	DOXP	68
	Ergosterol	MVA	12
Heterokontophyta			
Ochromonas danica	Phytol	DOXP	68
	Ergosterol	MVA	12
Euglenophyta			
Euglena gracilis	Phytol	MVA	68
	Ergosterol	MVA	12
Liverworts			
Riccocarpus natans	Ricciocarpin A (sesquiterpene)	MVA	72
	Phytol	DOXP	1
Conocephalum conicum	Bornylacetate (monoterpene)	DOXP	72
	Phytol	DOXP	1
	Cubebanol (sesquiterpene)	MVA	1
Higher plants			
Carotenoids, phytol, isoprene, sterols			
Lemna gibba	Phytol, ß-carotene, PQ-9,	DOXP	38–41
	Sitosterol, stigmasterol,	MVA	38–41
	campesterol	MVA	38–41
Daucus carota	Phytol,	DOXP	38–41
	Sitosterol, stigmasterol,	MVA	38–41
	campesterol	MVA	38–41
Hordeum vulgare	Phytol	DOXP	38–41
	Sitosterol	MVA	38–41

(*Continued*)

Table 1 (*Continued*)

Organism	Isoprenoid	IPP pathway	References
Populus nigra	Isoprene (hemiterpene)	DOXP	75, 76
Chelidonium maius	Isoprene	DOXP	75, 76
Salix viminalis	Isoprene	DOXP	75, 76
Catharanthus roseus	Phytol, carotene	DOXP	3
	Sitosterol	MVA	3
Lycopersicon esculentum	Lycopene	DOXP	64
Nicotiana tabacum	Plastoquinone-9	DOXP	11
	Sitosterol, stigmasterol	MVA	11
	Ubiquinone-10	MVA	11
Mono-, sesqui- or diterpenoids			
Ginkgo biloba	Ginkgolide A (diterpene)	DOXP	62
Taxus chinensis	Taxol (diterpene)	DOXP	16
Marrubium vulgare	Marrubiin (diterpene)	DOXP	30
Mentha x piperita	Menthone (monoterpene)	DOXP	17
Mentha pulegium	Pulegone (monoterpene)	DOXP	17
Pelargonium graveolens	Geraniol (monoterpene)	DOXP	17
Thymus vulgaris	Thymol (monoterpene)	DOXP	17
Matricaria recutita	Sesquiterpenes	DOXP[a]	2
Hordeum vulgare	Sesquiterpenoid derivative	DOXP	46
Salvia officinalis	Kauren (diterpene)	DOXP	*b*
Eucalyptus globulus, *Clematis vitisalba*	Volatile mono-, sesqui- and diterpenes	DOXP	*b*

[a]Primarily DOXP pathway, third C_5-unit also via MVA pathway.
[b]J Piel & W Boland, personal communication.

COOPERATION BETWEEN THE TWO IPP PATHWAYS OF HIGHER PLANTS

Whether the two cellular IPP pools cooperate and exchange IPP or other prenyl diphosphates, such as GPP, FPP or GGPP, is unresolved at present (Figure 3). Several observations suggest at least some exchange. One example is the low labeling rates of plastidic isoprenoids from applied ^{14}C-MVA. In ^{13}C-labeling of the diterpene ginkgolide from ^{13}C-glucose, three isoprene units were found to be labeled via the MVA pathway, and the fourth isoprene unit via the DOXP pathway (62). In the liverwort *Heteroscyphus*, the first three isoprenic units of phytol showed some label from applied ^{13}C-MVA, whereas the fourth unit was not labeled (50, 51). Both observations point to the transfer of a cytosolic FPP into the plastid where FPP was condensed with a DOXP-derived IPP. In our ^{13}C-labeling studies of phytol and carotenoids from ^{13}C-glucoses, we detected no such import of FPP into the plastid.

Some export of IPP or GPP from plastids into the cytosol may occur, yet such a transfer cannot proceed to a large extent, as deduced from inhibitor studies with mevinolin. When cytosolic MVA and sterol biosynthesis were blocked by the inhibitor mevinolin (4–6, 58), transfer of IPP or higher prenyl homologues from the chloroplast was insufficient for cytosolic sterol biosynthesis although labeling experiments with ^{13}C-MVA and deuterium-labeled DOXP in algae demonstrated some export of IPP or other prenyl diphosphates from the plastids (68). Also, recent studies in chamomile indicated in sesquiterpenes the first two C_5-units were derived from ^{13}C-glucose via the DOXP pathway, and the third C_5-unit was labeled by either the DOXP or the MVA pathway (1). Future research must define at what physiological conditions and developmental stages the plastidic DOXP-dependent biosynthesis of IPP, isoprene, monoterpene, diterpene (phytol), and tetraterpenes (carotenoids) is fully autonomous or partially dependent on the cytosolic IPP pathway, and vice versa.

BIOSYNTHESIS OF THE PRENYL SIDE-CHAINS OF UBIQUINONES

Mitochondria, which contain ubiquinones with prenyl side-chains (34, 58, 60), apparently do not possess their own IPP biosynthesis pathway. Their prenyl chain biosynthesis is dependent on cytosolic IPP formation (11) (see below). Plant mitochondria contain ubiquinone-9 (Q-9) and ubiquinone-10 (Q-10) (57, 59). The final steps of ubiquinone biosynthesis, the prenylation of the benzoquinone nucleus, apparently proceed in the mitochondria. The accumulation of sterols and ubiquinones was strongly mevinolin inhibited (4, 5, 13), which suggests that formation of the prenyl side-chains of ubiquinones is dependent on cytosolic IPP biosynthesis. Moreover, labeled MVA-5-P was not incorporated by mitochondria isolated from higher plants, whereas IPP was (45). In higher plants the mitochondrial ubiquinone biosynthesis is dependent on the cytosolic IPP formation. It has recently been shown in non-green tobacco cell cultures that sterols and the prenyl side-chain of Q-10 came from the same IPP pool synthesized via MVA (11) (Figure 3).

In green algae, however, not only the plastidic isoprenoids are formed via the DOXP pathway, but so too are the cytosolic sterols (63, 65–68). With ^{13}C-labeled glucose it was demonstrated that the deca-prenyl chain of ubiquinone Q-10 in *Scenedesmus* is also synthesized via the DOXP pathway (12).

DISTRIBUTION OF THE DOXP-PATHWAY IN ALGAE

The DOXP pathway for IPP biosynthesis is widely distributed in photosynthetic organisms, such as algae and higher plants, and is required for the synthesis

of plastidic isoprenoids (Table 1). This pathway also occurs in cyanobacteria (12), in several green algae (36, 63, 65, 66), the red alga *Cyanidium* (12, 68), and in the chrysophyte *Ochromonas* (12, 68). In *Cyanidium* and *Ochromonas*, the cytoplasmic sterols are formed via the classical MVA pathway as in higher plants (12, 49, 68). In contrast, the unicellular green algae tested synthesize not only their plastidic isoprenoids, but also their sterols via the DOXP pathway (38, 42, 63, 65, 66).

In *Euglena*, the situation is inverse; both the plastidic phytol and the cytoplasmic ergosterol are ^{13}C-labeled from glucose via the MVA pathway (12, 49, 68). When [2-^{13}C]MVA is supplied to *Euglena*, a large amount of the label shows up in ergosterol, and to a lesser degree in plastidic phytol (49, 68). These recent results confirm the very early labeling studies of *Euglena* ß-carotene via the MVA pathway (71). *Euglena* may have lost the DOXP pathway during the genetic rearrangement after the second endosymbiotic event (Figure 4). In contrast, in *Ochromonas*, which is believed to represent a secondary endosymbiotic event (73), the plastidic DOXP pathway was conserved. Green algae, in turn, seem to have lost their cytosolic MVA pathway of IPP formation. This suggests that during the evolution of various extant algal groups different strategies of genetic and metabolic organization took place.

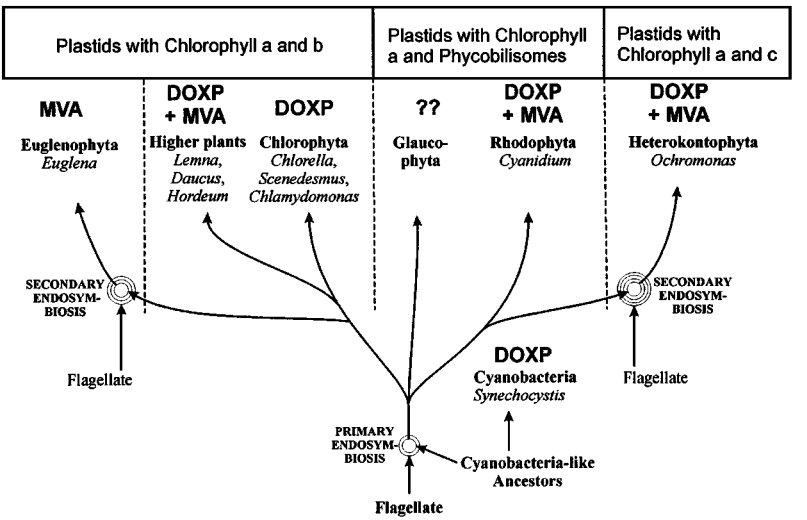


Figure 4 Putative evolution of some photosynthetic algae and higher plants with indication of the presence of one or both types of isopentenyl diphosphate (IPP) biosynthesis: MVA and/or DOXP pathway. Primary and secondary endosymbiotic events (73) leading to chloroplasts with an envelope consisting of 2, 3, or 4 biomembranes are indicated.

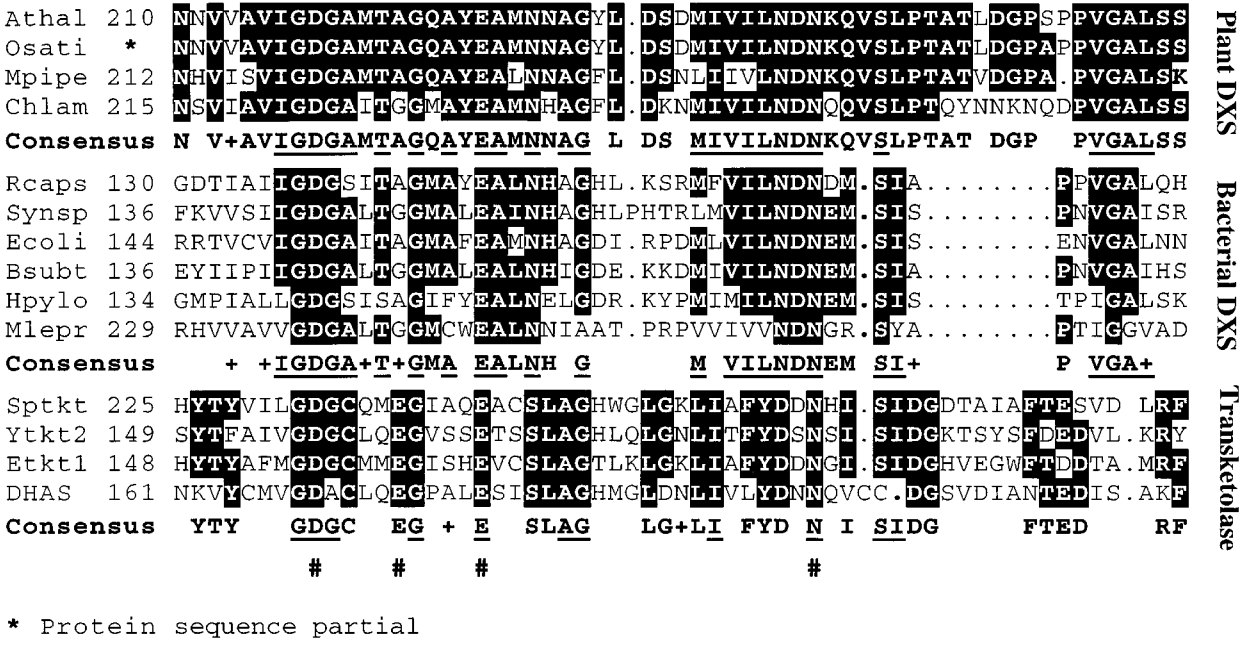
Low labeling of phytol with [2-^{13}C]MVA was observed in *Cyanidium* and *Ochromonas* (49, 68), but not in the green alga *Scenedesmus*. When applying intermediates of the DOXP pathways, such as [1-^{2}H]DOX to *Cyanidium* and *Ochromonas*, the deuterium label showed up not only in phytol, but also in ergosterol (49, 68), indicating that in both algae some exchange may exist between the two IPP pools of different biosynthetic origin. Incorporation of minor label of methyl[1-$^{2}H_1$]DOX into phytol and ergosterol of *Euglena* (49, 68) is thought to be caused by a breakdown of d-DOX.

THE DOXP PATHWAY AND BIOSYNTHESIS OF TERPENOIDS

The new DOXP pathway for IPP biosynthesis has now been established unequivocally in eubacteria (53–55), cyanobacteria (12, 49), various algal groups (42, 63, 65, 66), and higher plants (3, 16, 17, 30, 41, 68). In higher plants, it is further responsible for the formation of the volatile hemiterpene isoprene (68, 75, 76), diterpenes, such as ginkgolides (62), taxol (16), marrubiin (30), the monoterpenes menthone (17), and borneol (72); for the secondary carotenoid lycopene in tomato fruits (36, 64); for sesquiterpenoid biosynthesis in chamomile (2) and in mycorrhizal barley roots (46) and the volatile mono-, sesqui- and diterpenoids of several flowers (Table 1). Although the DOXP pathway for IPP and terpenoid biosynthesis is widely distributed in higher plants (Table 1), it has yet to be determined whether the basic carbon skeleton of the numerous other plant terpenoids is derived from the MVA or plastidic DOXP pathway, or by a cooperation of both.

GENES OF 1-DEOXY-D-XYLULOSE-5-PHOSPHATE SYNTHASE (DXS)

1-Deoxy-d-xylulose-5-phosphate synthase (dxs) is the first enzyme of the DOXP pathway to be characterized at the enzymatic and molecular level. DXS of *E. coli* is a transketolase-like enzyme with a molecular weight of about 65 KDa (43, 69). It is one of a distinct family of DXS-like protein sequences that have been found in several bacteria and plants (See Figure 5). The DXS are highly conserved and share sequences of a special class of transketolases (Figure 5). The sequence motif (between "VILNDN" and "VGAL") allows the bacterial and plant DXS sequences to be distinguished from each other. One of these DXS-like genes is *CLA1* (47), a single copy gene that is positively regulated by light. The protein sequence includes a predicted chloroplast transit peptide. Thus, *CLA1* is likely a plastidic enzyme with a key function in pigment biosynthesis (47). A



* Protein sequence partial

Figure 5 Alignments of transketolase-type DXS sequences of plants and bacteria (or DXS homologous open reading frames) and of transketolases in the range of the thiamine building site. The putative thiamine binding-site includes **GDG**$X_{7\text{-}8}$**E**$X_{3\text{-}4}$**A**$X_{11\text{-}13}$**N** (26). # denotes residues involved in TPP-binding in yeast transketolase (48); *dots* denote the absence of amino acid residues; the *boldface* and *shaded* characters denote amino acid residues that are highly conserved. Conserved residues are indicated in each group with the symbol +. If a residue of the consensus occurs in more than one group, it is underlined. Athal, *Arabidopsis thaliana CLA1* (accession number U27099) (33); Osati, *Oryza sativa* (af024512); Mpipe, *Mentha* x *piperita* (af019383) (33); Chlamy, *Chlamydomonas reinhardtii* (aj007559); Rcaps, *Rhodobacter capsulatus* (z11165); Synsp, *Synechocystis sp*. (d90903); Ecoli, *Escherichia coli* (u82664); Bsubt, *Bacillus subtilis* (d84432); Hpylo, *Helicobacter pylori* (ae000552); Mlepr, *Mycobacterium leprae* (u15181); Sptkt, *Spinacia oleracea* chloroplast transketolase (L76554); Ytkt2, Yeast transketolase 2 (p33315); Etkt1, *E. coli* transketolase 1 (p27302); DHAS, *Hansenula polymorpha* formaldehyde transketolase (p06834).

highly similar gene was recently cloned from *Mentha piperita* and the expressed protein was shown to be active in DOXP synthesis (33). The *Mentha* gene sequence predicts 68% identical amino acid residues with those of *CLA1*.

In our laboratory a cDNA clone was isolated from *Chlamydomonas*, which is highly similar to *CLA1* (85% homologous amino acid residues) and with a predicted plastid transit peptide. We also detected a second DXS-homologous sequence in *Chlamydomonas*. Thus, *Chlamydomonas* appears to have two different DXS genes, possibly for cytosolic and plastidic IPP formation. Whether the plant DXS has a regulatory role in IPP and isoprenoid biosynthesis is not yet clear. One could expect several isoforms of DXS, assuming that biosynthesis of photosynthetic isoprenoids, of essential oils in oil glands, and of terpenoid phytoalexins are dependent on particular DXS activities.

BRANCH POINTS WITH OTHER CHLOROPLAST BIOSYNTHETIC PATHWAYS

The early observations that ^{14}C-labeled CO_2, GA-3-P, and pyruvate are better precursors of plastidic isoprenoids than ^{14}C-acetate or ^{14}C-MVA (8, 9, 21, 22, 27) are now being clarified with the operation of the DOXP pathway of IPP formation, in which case GA-3-P and pyruvate are direct substrates of the DOXP synthase. $^{14}CO_2$ is rapidly transferred into 3-phosphoglyceric acid (3-PGA) and GA-3-P via photosynthetic CO_2 assimilation (Figure 6). The quick formation of IPP and DMAPP from CO_2 via GA-3-P and pyruvate also explains the rapid isoprene emission under heat stress conditions (74) and the fast labeling and emission of isoprene from photosynthetically fixed $^{13}CO_2$ (10). Exogenously applied ^{14}C-acetate is quickly incorporated into fatty acids via the plant's plastidic de novo fatty acid synthetase (e.g. 20), but not into carotenoids and other plastid isoprenoids, since acetate is not a substrate of the DOXP pathway. DOXP, in turn, is an intermediate not only in the plastidic IPP biosynthesis, but also in the synthesis of thiamine and pyridoxal (28, 29). Pyruvate, in turn, is an essential branch point of the plastid metabolism; it serves as substrate of the DOXP pathway, of acetyl-CoA formation, and de novo fatty acid biosynthesis, and is also required for the biosynthesis of valin, leucin, and isoleucin (61) (Figure 6). Whether pyruvate is made in plastids from 3-PGA or arises as a byproduct of the ribulosebisphosphate carboxylase activity (2a) or may be delivered, in part, from the cytosol, has yet to be determined. Moreover, phosphoenol pyruvate (PEP) is a substrate of the shikimic acid pathway that, in plants, also occurs in plastids. Thus, there are many branch points in the use and metabolite flow of the primary photosynthetic products 3-PGA and GA-3-P to the various end products that require a fine regulation of chloroplast metabolism.

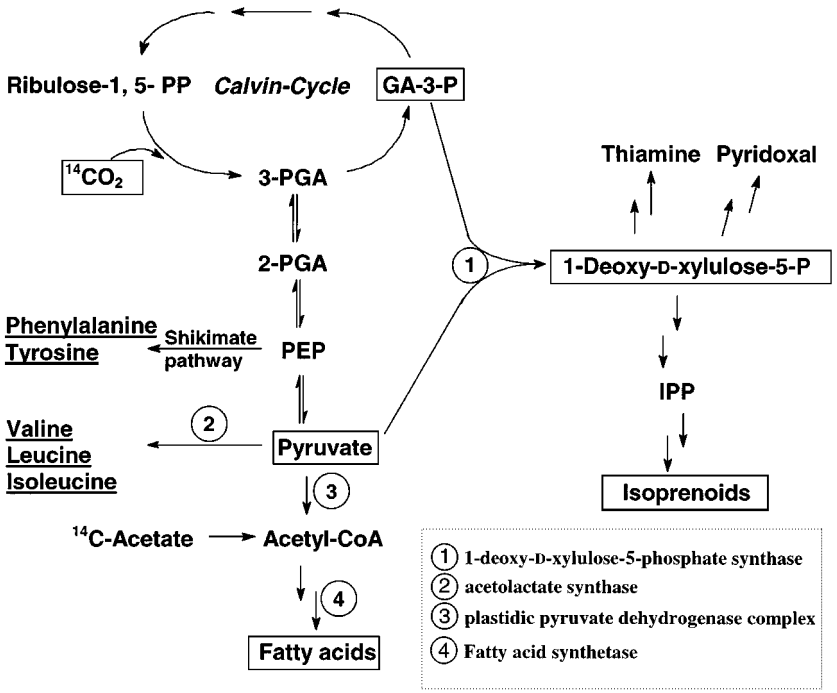


Figure 6 Metabolic pathways and branch points in plastids. The flow of metabolites from the photosynthetic reductive pentosephosphate cycle (Calvin cycle) into different end products, such as IPP, plastidic isoprenoids, isoprene, fatty acids, amino acids as well as thiamine and pyridoxal, is indicated. The central role of GA-3-P and pyruvate in the formation of 1-deoxy-D-xylulose-5-phosphate, IPP, and plastidic isoprenoids is emphasized.

CONCLUSION AND OUTLOOK

The incorporation studies over the past four years demonstrated that the DOXP pathway of IPP and isoprenoid biosynthesis is widely distributed in photosynthetic organisms. Future research should be directed to (*a*) elucidating the individual enzymatic steps between DOXP and IPP, (*b*) characterizing the corresponding genes and enzymes, and (*c*) evaluating the regulation of the DOXP pathway with respect to other metabolic pathways in chloroplasts. Finally, the possibility must also be examined of a partial cooperation of the two IPP yielding cellular pathways, the MVA and DOXP routes, in the biosynthesis of plant terpenoids. Enzymes of the DOXP pathway represent targets for new inhibitors. We may therefore anticipate the development of novel herbicides against plants and algae as well as antibacterial substances against pathogenic

bacteria possessing the DOXP pathway. In fact, fosmidomycin has now been described as the first herbicide blocking the DOXP pathway (77).

ACKNOWLEDGMENTS

A major part of the work described here was supported by a grant from the German Research Council, DFG Bonn, which is gratefully acknowledged. Part of the work was performed in cooperation with Frieder W. Lichtenthaler, Darmstadt/Germany, and Michel Rohmer, Strasbourg/France. I thank my PhD students Jörg Schwender, Johannes Zeidler, and Christian Müller for assistance; Antonella Barelli-Kummer for typing the manuscript; and Gabrielle Johnson for language assistance.

Literature Cited

1. Adam KP, Thiel R, Zapp J, Becker H. 1998. Involvement of the mevalonic acid pathway and the glyceraldehyde-pyruvate pathway in terpenoid biosynthesis of the liverworts *Riccio carpus natans* and *Conocephalum conicum*. *Arch. Biochem. Biophys.* 354:181–87
2. Adam KP, Zapp J. 1998. Biosynthesis of the isoprene units of chamomile sesquiterpenes. *Phytochemistry* 48:653–59

2a. Andrews TJ, Kane HJ. 1991. Pyruvate is a by-product of catalysis by ribulosebisphoshate carboxylase/oxygenase. *J. Biol. Chem.* 266:9447–52

3. Arigoni D, Sagner S, Latzel C, Eisenreich W, Bacher A, Zenk MH. 1997. Terpenoid biosynthesis from 1-deoxy-D-xylulose in higher plants by intramolecular skeletal rearrangement. *Proc. Natl. Acad. Sci. USA* 94:10600–5
4. Bach TJ, Lichtenthaler HK. 1982. Mevinolin, a highly specific inhibitor of microsomal 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase of radish plants. *Z. Naturforsch. Teil C* 37:46–50
5. Bach TJ, Lichtenthaler HK. 1982. Inhibition of mevalonate biosynthesis and plant growth by the fungal metabolite mevinolin. See Ref. 74b, pp. 515–21
6. Bach TJ, Lichtenthaler HK. 1983. Inhibition by mevinolin of plant growth, sterol formation and pigment accumulation. *Physiol. Plant.* 59:50–60
7. Bohlmann J, Meyer-Gauen G, Croteau R. 1998. Plant terpenoid synthases: molecular biology and phytogenetic analysis. *Proc. Natl. Acad. Sci. USA* 95:4126–33
8. Braithwaite GD, Goodwin TW. 1960. Studies on carotenogenesis. 27. Incorporation of [2-^{14}C]acetate, DL-[2-^{14}C] mevalonate and $^{14}CO_2$ into carrot-root preparations. *Biochem. J.* 76:194–97
9. Braithwaite GD, Goodwin TW. 1960. Studies on carotenogenesis. 25. The incorporation of [1-^{14}C]acetate, [2-^{14}C]acetate and $^{14}CO_2$ into lycopene by tomato slices. *Biochem. J.* 76:1–5
10. Delwiche CF, Sharkey TD. 1993. Rapid appearance of ^{13}C in biogenic isoprene when $^{13}CO_2$ is fed to intact leaves. *Plant Cell Environ.* 16:587–91
11. Disch A, Hemmerlin A, Bach TJ, Rohmer M. 1998. Mevalonate-derived isopentenyl diphoshate is the biosynthetic precursor of ubiquinone prenyl side-chain in tobacco BY-2 cells. *Biochem. J.* 331:615–21
12. Disch A, Schwender J, Müller C, Lichtenthaler HK, Rohmer M. 1998. Mevalonate versus glyceraldehyde phosphate pathway for isoprenoid biosynthesis in unicellular algae and the cyanobacterium *Synechocystis*. *Biochem. J.* 333:381–88
13. Döll M, Schindler S, Lichtenthaler HK, Bach TJ. 1984. Differential inhibition by mevinolin of prenyllipid accumulation in cell suspension cultures of *Silybum marianum* L. See Ref. 68a, pp. 277–80
14. Duvold T, Bravo JM, Pale-Grosdemange C, Rohmer M. 1997. Biosynthesis of 2-C-methyl-D-erythritol, a putative C_5 intermediate in the mevalonate-independent

pathway for isoprenoid biosynthesis. *Tetrahedron Lett.* 38:4769–72
15. Duvold T, Cali P, Bravo JM, Rohmer M. 1997. Incorporation of 2-C-methyl-D-erythritol, a putative isoprenoid precursor in the mevalonate-independent pathway, into ubiquinone and menaquinone of *Escherichia coli*. *Tetrahedron Lett.* 38:6181–84
16. Eisenreich W, Menhard B, Hylands PJ, Zenk MH, Bacher A. 1996. Studies on the biosynthesis of taxol: The taxane carbon skeleton is not of mevalonoid origin. *Proc. Natl. Acad. Sci. USA* 93:6431–36
17. Eisenreich W, Sagner S, Zenk MH, Bacher A. 1997. Monoterpenoid essential oils are not of mevalonate origin. *Tetrahedron Lett.* 38:3889–92
18. Ford CW. 1981. A new lactone from water-stressed chickpea. *Phytochemistry* 20:2019–20
19. Gershenzon J, Croteau RB. 1993. Terpenoid biosynthesis: the basic pathway and formation of monoterpenes, sesquiterpenes and diterpenes. See Ref. 48a, pp. 339–88
20. Golz A, Focke M, Lichtenthaler HK. 1994. Inhibitors of *de novo* fatty acid biosynthesis in higher plants. *J. Plant Physiol.* 143:426–33
21. Goodwin TW. 1958. Incorporation of $^{14}CO_2$, [2-^{14}C]acetate and [2-^{14}C]mevalonic acid into ß-carotene in etiolated maize seedlings. *Biochem. J.* 68:26–27
22. Goodwin TW. 1958. Studies in carotenogenesis 25. The incorporation of $^{14}CO_2$, [2-^{14}C]acetate and [2-^{14}C]mevalonic acid into ß-carotene by illuminated etiolated maize seedlings. *Biochem. J.* 70:612–17
23. Goodwin TW. 1965. Regulation of terpenoid biosynthesis in higher plants. In *Biosynthetic Pathways in Higher Plants*, ed. JB Pridham, T Swain, pp. 57–71. London: Academic
24. Goodwin TW. 1977. The prenyllipids of the membranes of higher plants. In *Lipids and Lipid Polymers in Higher Plants*, ed. M Tevini, HK Lichtenthaler, pp. 29–47. Berlin: Springer-Verlag
25. Goodwin TW. 1981. Biosynthesis of plant sterols and other triterpenoids. See Ref. 70, pp. 444–80
26. Hawkins CF, Borges A, Perham RN. 1989. A common structural motif in thiamin pyrophosphate-binding enzymes. *FEBS Lett.* 255:77–82
27. Heintze A, Görlach J, Leuschner C, Hoppe P, Hagelstein P, et al. 1990. Plastidic isoprenoid synthesis during chloroplast development. Change from metabolic autonomy to division-of-labor stage. *Plant Physiol.* 93:1121–22
28. Julliard JH. 1992. Biosynthesis of the pyridoxal ring (vitamin B6) in higher plant chloroplasts and its relationship with the biosynthesis of the thiazol ring (vitamin B1). *C.R. Acad. Sci. Ser.* 314: 285–90
29. Julliard JH, Douce R. 1991. Biosynthesis of the thiazole moiety of thiamin (vitamin B1) in higher plant chloroplasts. *Proc. Natl. Acad. Sci. USA* 88:2041–45
30. Knöss W, Reuter B, Zapp J. 1997. Biosynthesis of the labdane diterpene marrubiin in *Marubium vulgare* via a non-mevalonate pathway. *Biochem. J.* 326: 449–54
31. Kringstad R, Singsaas AO, Rusten G, Baekkemoen G, Paulsen BS, Nordal A. 1980. 2-C-methylaldotetronic acid present in plants. *Phytochemistry* 19:543–45
32. Kuzuyama T, Takahashi S, Watanabe H, Seto H. 1998. Direct formation of 2-methyl-D-erythritol 4-phosphate from 1-deoxy-D-xylulose 5-phosphate by 1-deoxy-D-xylulose 5-phosphate reductoisomerase, a new enzyme in the non-mevalonate pathway to isopentenyl diphosphate. *Tetrahedron Lett.* 39:4509–12
33. Lange B, Wildung M, McCaskill R, Croteau R. 1998. A family of transketolases that directs isoprenoid biosynthesis via a mevalonate-independent pathway. *Proc. Natl. Acad. Sci. USA* 95:2100–4
34. Lichtenthaler HK. 1987. Functional organization of carotenoids and prenylquinones in the photosynthetic membrane. In *The Metabolism, Structure and Function of Plant Lipids*, ed. P Stumpf, JB Mudd, WD Nes, pp. 63–73. New York: Plenum
35. Lichtenthaler HK. 1993. The plant prenyllipids including carotenoids, chlorophylls and prenylquinones. See Ref. 48a, pp. 427–70
36. Lichtenthaler HK. 1998. The plant's 1-deoxy-D-xylulose-5-phospate pathway for biosynthesis of isoprenoids. *Fett/Lipid.* 100:128–38
37. Lichtenthaler HK, Bach TJ, Wellburn AR. 1982. Cytoplasmic and plastidic isoprenoid compounds of oat seedlings and their distinct labeling from ^{14}C-mevalonate. See Ref. 74b, pp. 489–500
38. Lichtenthaler HK, Rohmer M, Schwender J. 1997. Two independent biochemical pathways for isopentenyl diphosphate and isoprenoid biosynthesis in higher plants. *Physiol. Plant.* 101:643–52
39. Lichtenthaler HK, Rohmer M, Schwender

J, Disch A, Seemann M. 1997. A novel mevalonate-independent pathway for the biosynthesis of carotenoids, phytol and prenyl chain of plastoquinone-9 in green algae and higher plants. See Ref. 74a, pp. 177–79
40. Lichtenthaler HK, Schwender J. 1998. The 1-deoxy-D-xylulose-5-phosphate pathway for biosynthesis of carotenoids and plastidic isoprenoids. See Ref. 56a, pp. 419–24
41. Lichtenthaler HK, Schwender J, Disch A, Rohmer M. 1997. Biosynthesis of isoprenoids in higher plant chloroplasts proceeds via a mevalonate independent pathway. *FEBS Lett.* 400:271–74
42. Lichtenthaler HK, Schwender J, Seemann M, Rohmer M. 1995. Carotenoid biosynthesis in green algae proceeds via a novel biosynthetic pathway. See Ref. 47a, pp. 115–18
43. Lois LM, Campos N, Putra SR, Danielsen K, Rohmer M, Boronat A. 1998. Cloning and characterization of a gene from *Escherichia coli* encoding a transketolase-like enzyme that catalyzes the synthesis of D-1-deoxyxylulose 5-phosphate, a common precursor for isoprenoid, thiamin, and pyridoxol biosynthesis. *Proc. Natl. Acad. Sci. USA* 95:2105–10
44. Lütke-Brinkhaus F, Kleinig H. 1987. Formation of isopentenyl diphosphate via mevalonate does not occur within etioplasts or etiochloroplasts of mustard (*Sinapis alba* L.) seedlings. *Planta* 171:401–11
45. Lütke-Brinkhaus F, Liedvogel B, Kleinig H. 1984. On the biosynthesis of ubiquinones in plant mitochondria. *Eur. J. Biochem.* 141:537–41
46. Maier W, Schneider B, Strack D. 1998. Biosynthesis of sesquiterpenoid cyclohexane derivatives in mycorrhizal barley roots proceeds via the glyceraldehyde-3-phosphate/pyruvate pathway. *Tetrahedron Lett.* 39:521–24
47. Mandel MA, Feldmann KA, Herrera-Estrella L, Rocha-Sosa M, Leon P. 1996. *CLA1*, a novel gene required for chloroplast development, is highly conserved in evolution. *Plant J.* 9:649–58
47a. Mathis P, ed. 1995. *Photosynthesis: From Light to Biosphere*. Amsterdam: Kluwer
48. Meshalkina L, Nilsson U, Wikner C, Kostikowa T, Schneider G. 1997. Examination of thiamin diphosphate binding site in yeast transketolase by site-directed mutagenesis. *Eur. J. Biochem.* 244:646–52
48a. Moore TS, ed. 1993. *Lipid Metabolism in Plants*. Boca Raton, FL: CRC
49. Müller C, Schwender J, Disch A, Rohmer M, Lichtenthaler FW, Lichtenthaler HK. 1998. Occurrence of the 1-deoxy-D-xylulose-5-phosphate pathway of isopentenyl diphosphate biosynthesis in different algae groups. See Ref. 56a, pp. 425–28
50. Nabeta K, Ishikawa T, Okuyama H. 1995. Sesqui- and diterpene biosynthesis from ^{13}C labeled acetate and mevalonate in cultured cells of *Heteroscyphus planus*. *J. Chem. Soc. Perkin. Trans.* 1:3111–15
51. Nabeta K, Kawae T, Saitoh T, Kikuchi T. 1997. Synthesis of chlorophyll α and ß-carotene from ^{2}H and ^{13}C-labeled mevalonates and ^{13}C-labeled glycin in cultured cells of liverworts *Heteroscyphus planus* and *Lophocolea heterophylla*. *J. Chem. Soc. Perkin Trans.* 1:261–67
52. Putra SR, Lois LM, Campos N, Boronat A, Rohmer M. 1998. Incorporation of [2,3-$^{13}C_2$]- and [2,4-$^{13}C_2$]-D-deoxyxylulose into ubiquinone of *Escherichia coli* via the mevalonate-independent pathway for isoprenoid biosynthesis. *Tetrahedron Lett.* 39:23–26
53. Rohmer M, Knani M, Simonin P, Sutter B, Sahm H. 1993. Isoprenoid biosynthesis in bacteria: a novel pathway for early steps leading to isopentenyl diphosphate. *Biochem. J.* 295:517–24
54. Rohmer M, Seemann M, Horbach S, Bringer-Meyer S, Sahm H. 1996. Glyceraldehyde 3-phosphate and pyruvate as precursors of isoprenic units in an alternative non-mevalonate pathway for terpenoid biosynthesis. *J. Am. Chem. Soc.* 118:2564–66
55. Rohmer M, Sutter B, Sahm H. 1989. Bacterial sterol surrogates. Biosynthesis of the side chain of bacteriohopanetetrol and of a carbocyclic pseudopentose from ^{13}C-labeled glucose in *Zymomonas mobilis*. *J. Chem. Soc. Chem. Commun.*, pp. 1471–72
56. Sagner S, Eisenreich W, Fellermeier M, Latzel C, Bacher A, Zenk MH. 1998. Biosynthesis of 2-C-methyl-D-erythritol in plants by rearrangement of the terpenoid precursor 1-deoxy-D-xylulose 5-phosphate. *Tetrahedron Lett.* 39:2091–94
56a. Sanchez J, Cerda-Olmedo E, Martinez-Force E, eds. 1998. *Advances in Plant Lipid Research*. Univ. Sevilla: Secretariado Publ.
57. Schindler S. 1984. Verbreitung und Konzentration von Ubichinon-Homologen in Pflanzen. *Karlsr. Contrib. Plant Physiol.* 12:1–240
58. Schindler S, Bach TJ, Lichtenthaler HK.

1985. Differential inhibition by mevinolin of prenyllipid accumulation in radish seedlings. *Z. Naturforsch. Teil C* 40:208–14

59. Schindler S, Lichtenthaler HK. 1984. Comparison of the ubiquinone homologue pattern in plant mitochondria and their possible prokaryotic ancestors. See Ref. 68a, pp. 273–76
60. Schramm RW, Tomaszewska B, Petersson G. 1979. Sugar-related hydroxy acids from *Phaseolus* and *Trifolium* species. *Phytochemistry* 18:1393–94
61. Schulze-Siebert D, Heinecke D, Scharf H, Schultz G. 1984. Pyruvate derived amino acids in spinach chloroplasts. *Plant Physiol.* 76:465–71
62. Schwarz MK. 1994. *Terpenbiosynthese in Ginkgo biloba*. PhD thesis. Eidgenoss. Tech. Hochsch., Zürich
63. Schwender J. 1995. Untersuchungen zur Biosynthese der Isoprenoide bei der Grünalge *Scenedesmus obliquus* mittels ^{13}C-Isotopenmarkierung. *Karlsr. Contrib. Plant Physiol.* 31:1–85
64. Schwender J, Lichtenthaler HK. 1998. Biosynthesis of lycopene in tomato fruits proceeds via the non-mevalonate isoprenoid pathway. See Ref. 56a, pp. 429–32
65. Schwender J, Lichtenthaler HK, Disch A, Rohmer M. 1997. Biosynthesis of sterols in green algae (*Scenedesmus, Chlorella*) according to a novel, mevalonate-independent pathway. See Ref. 74a, pp. 180–82
66. Schwender J, Lichtenthaler HK, Seemann M, Rohmer M. 1995. Biosynthesis of isoprenoid chains of chlorophylls and plastoquinone in *Scenedesmus* by a novel pathway. See Ref. 47a, pp. 1001–4
67. Schwender J, Seemann M, Lichtenthaler HK, Rohmer M. 1996. Biosynthesis of isoprenoids (carotenoids, sterols, prenyl side-chains of chlorophyll and plastoquinone) via a novel pyruvate/glyceraldehyde-3-phosphate non-mevalonate pathway in the green alga *Scenedesmus*. *Biochem. J.* 316:73–80
68. Schwender J, Zeidler J, Gröner R, Müller C, Lichtenthaler HK, et al. 1997. Incorporation of 1-deoxy-D-xylulose into isoprene and phytol by higher plants and algae. *FEBS Lett.* 414:129–34

68a. Siegenthaler PA, Eichenberger W, eds. 1984. *Structure, Function and Metabolism of Plant Lipids*. Amsterdam: Elsevier
69. Sprenger GA, Schörken U, Wiegert T, Grolle S, de Graaf AA, et al. 1997. Identification of a thiamin-dependent synthase in *Escherichia coli* required for the formation of 1-deoxy-D-xylulose-5-phosphate precursor to isoprenoids, thiamin, and pyridoxol. *Proc. Natl. Acad. Sci. USA* 94:12857–62
70. Spurgeon SL, Porter JW. 1981. Introduction. In *Biosynthesis of Isoprenoid Compounds*, ed. JW Porter, SL Spurgeon, 1:1–46. New York: Wiley
71. Steele JW, Gurin S. 1960. Biosynthesis of ß-carotene in *Euglena gracilis*. *J. Biol. Chem.* 235:2778–85
72. Thiel R, Adam KP, Zapp J, Becker H. 1997. Isopentenyl diphosphate biosynthesis in liverworts. *Pharm. Pharmacol. Lett.* 7:103–5
73. Van den Hoek C, Mann DG, Jahns HM. 1995. *Algae, An Introduction to Phycology*. Cambridge: Cambridge Univ. Press
74. Wildermuth MC, Fall R. 1996. Light-dependent isoprene emission. *Plant Physiol.* 112:171–82

74a. Williams JP, Khan MU, Lem NW, eds. 1997. *Physiology, Biochemistry and Molecular Biology of Plant Lipids*. Dordrecht: Kluwer

74b. Wintermans JFGM, Kuiper PJC, eds. 1982. *Biochemistry and Metabolism of Plant Lipids*. Amsterdam: Elsevier

75. Zeidler JG, Lichtenthaler HK, May HU, Lichtenthaler FW. 1997. Is isoprene emitted by plants synthesized via the novel isopentenylpyrophosphate pathway? *Z. Naturforsch. Teil C* 52:15–23
76. Zeidler JG, May HU, Lichtenthaler FW, Lichtenthaler HK. 1998. Isoprene emitted by plants is formed via the 1-deoxy-D-xylulose phosphate pathway of isopentenyl diphosphate biosynthesis. See Ref. 56a, pp. 446–49
77. Zeidler JG, Schwender J, Müller C, Wiesner J, Lichtenthaler HK, et al. 1998. Inhibition of the non-mevalonate 1-deoxy-D-xylulose-5-phosphate pathway of plant isoprenoid biosynthesis by fosmidomycin. *Z. Naturforsch.* 53C:980–86