EVOLUTION OF CAROTENOID AND ISOPRENOID BIOSYNTHESIS IN PHOTOSYNTHETIC AND NON-PHOTOSYNTHETIC ORGANISMS

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Abstract

Sterols and carotenoids are typical representatives of the group of isoprenoid lipids in plants. All isoprenoids are synthesized by condensation of the two active C_5-units: dimethylallyl diphosphate, DMAPP, and isopentenyl diphosphate, IPP. Like animals, higher plants form their sterols via the classical cytosolic acetate/mevalonate (MVA) pathway of IPP biosynthesis. Plants as photosynthetic organisms, however possess a second, non-mevalonate pathway for IPP biosynthesis, the DOXP/MEP pathway. The latter operates in the chloroplasts and is responsible for the formation of carotenoids and all other plastidic isoprenoid lipids (phytol, prenylquinones). Although there exists some cooperation between both IPP producing pathways, one can never fully compensate for the other. Thus, in *higher plants* sterols are primarily made via the MVA pathway and carotenoids via the DOXP pathway. This also applies to several algae groups, such as *red algae* and *Heterokontophyta*.

In the large and diverging group of 'Green Algae' the situation is more complex. The more advanced evolutionary groups (*Charales, Zygnematales)* possess, like higher plants, both IPP forming pathways and represent an evolutionary link to these. In contrast, the proper *Chlorophyta*, often single cell organisms (*Chlorella, Scenedesmus, Trebouxia*), represent a separate phylum and synthesize sterols and carotenoids via the DOXP pathway whereas the MVA pathway is lost. The common ancestor of both groups, *Mesostigma viride*, again exhibits both IPP pathways. In the photosynthetic *Euglenophyta* the situation is inverse, both the sterols and the carotenoids are formed exclusively via the MVA pathway, the DOXP pathway is lost during the secondary endosymbiosis. Also *Fungi* synthesize sterols and carotenoids via the MVA pathway. Animals possess only the MVA pathway for sterol biosynthesis. In contrast, the malaria parasite *Plasmodium* and other *Apicomplexa* have lost the MVA pathway and synthesize their isoprenoids only via the DOXP pathway of their plastid-type apicoplast. In evolutionary terms the DOXP/MEP pathway shows up first in photosynthetic and heterotrophic *bacteria*, whereas *Archaea* possess the MVA pathway. The early anoxigenic *photosynthetic bacteria* (one photosynthetic light reaction) and the later *Cyanobacteria* (two light reactions and oxigenic photosynthesis) that form a link to the endosymbiontic chloroplasts contain the DOXP/MEP pathway. The latter is also present in many heterotrophic *pathogenic bacteria*. Some bacteria possess, in addition to the DOXP/MEP pathway, some genes of the MVA pathway that they obtained apparently by lateral gene transfer. A few others have evidently lost the DOXP/MEP pathway and acquired the MVA pathway. Some members of the *Streptomycetes,* in turn, have both IPP producing routes, one for 'housekeeping' (DOXP/MEP pathway) and the other (MVA pathway) for synthesis of secondary isoprenoid products.

When viewing the evolutionary trends it is clear that 1) the two pathways of IPP biosynthesis evolved independently, 2) lateral gene transfer has occurred especially on the bacteria level, 3) primary endosymbiosis has taken place and secondary endosymbiosis partially with differing results, and 4) a loss of the genes of the DOXP pathway took place in some organisms and in others a loss of the genes for the MVA pathway. On the basis of the available evidence an evolutionary view of IPP formation is presented.

1. Introduction

Plants, animals and microorganisms contain various primary and partially also secondary isoprenoid compounds that are made of the C_5-units of 'active isoprene', known today as isopentenyl diphosphate (IPP). The 'biogenetic isoprene rule' was first detected in 1885 by Wallach [65] and the head-to-tail addition of the 'active C_5 units' was pointed out by Ruzicka [52, 53]. In the early 1950s acetate [42] and acetyl-CoA [44] were detected as precursors, as well as mevalonic acid (MVA) as an intermediate [69] and finally isopentenyl-diphosphate (IPP) as the active cellular biosynthetic C_5-unit [14]. For more than three decades it had been believed that all isoprenoids of living cells were made via this acetate/MVA pathway. Despite the fact that more and more inconsistencies in the labeling of plastidic isoprenoids (carotenoids, phytol, plastoquinone-9) showed

up, as reviewed by Lichtenthaler [35, 40], the acetate/MVA pathway was regarded as the only biosynthetic pathway for IPP biosynthesis in living organisms.

In the early 1990s when labeling with ^{13}C-glucose and applying high resolution NMR spectroscopy a second, biochemically fully independent, non-mevalonate IPP-biosynthesis pathway, known today as DOXP/MEP pathway, was detected: first in bacteria [51], then in all photosynthetic oxygen evolving organisms, such as green algae [38, 56, 57] and higher plants [6, 39, 40, 76]. This demonstrated that green plants (with the exception of some green algae) possess two IPP synthesizing pathways, the acetate/MVA pathway in the cytosol (e.g. for sterols) and the DOXP/MEP pathway in the plastids (e.g. for carotenoids and phytol). This was confirmed by various other authors as reviewed by Lichtenthaler [36] and Rohmer [50].

Heterotrophic organisms such as *Archaea*, fungi and animals do not possess the DOXP/MEP pathway of IPP biosynthesis, they synthesize their IPP and isoprenoids via the acetate/MVA pathway. The larger part of bacteria contains the DOXP/MEP pathway, whereas a few others possess the MVA pathway and a few *Streptomycetes* contain even both pathways as reviewed in [10]. Higher plants with their chloroplasts that are derived from cyanobacteria-like endosymbionts possess the DOXP/MEP pathway for carotenoid biosynthesis and the MVA pathway for sterol biosynthesis. This applies for several algae groups as well [36, 17]. However, the photosynthesising organism *Euglena* does not possess the DOXP/MEP pathway, whereas in *Chlorophyta* the cytosolic MVA pathway is missing [58, 60]. Moreover, the malaria parasite *Plasmodium*, as a heterotrophic organism, unexpectedly exhibits the DOXP/MEP pathway [29]. So what are the principles for the distribution of both IPP producing pathways in living organisms, where did the DOXP/MEP and the MVA pathway originate from? This topic is shortly reviewed here.

2. The MVA and the DOXP/MEP pathways and their inhibition

The present knowledge of the biochemical enzymatic steps of the two independent IPP producing pathways is shown in Fig. 1. The classical acetate/MVA starts from 3 acetyl-CoA, requires 6 enzymes, 2 NADPH and 3 ATP to finally yield isopentenyl-diphosphate. All enzymes have been cloned from plants. The regulatory step is the HMG-CoA reductase (enzyme 3) that can specifically be blocked by the statin mevinolin as first shown for plants in [8, 9], whereby the active part of the inhibitor mevinolin is a structural analogue of the endogenous substrate intermediate as shown in Fig. 2. The final product of this pathway IPP is transferred by IPP isomerase to its isomer dimethylallyl diphosphate (DMAPP). The latter is the starter molecule for terpenoid biosynthesis to which one or several IPP molecules are added in a head-to-tail addition response depending on the final terpenoid product.

In contrast, the non-mevalonate, plastidic DOXP/MEP pathway of IPP biosynthesis starts from pyruvate and glycerinaldehyde-3-phosphate, comprises 7 enzymes, requires 3 ATP equivalents (ATP or CTP), 3 NADPH and yields in the last enzymatic step, catalysed by HMBPP reductase (Lyt B), both substrates, IPP and its isomer DMAPP (usually in a ratio of 5:1 or 3:1), as indicated in Fig. 1. The first enzyme is the DOXP synthase yielding DOXP that is reduced by DOXP reductoisomerase to MEP [59]. An essential regulatory step of the DOXP/MEP pathway is this DOXP reductoisomerase that can efficiently be blocked by fosmidomycin and its derivative FR-900098 as is independently shown for plants [59, 71] and for bacteria [31]. Fosmidomycin is a structural analogue to 2-C-methylerythrose 4-phosphate, the intermediate in the enzymic reaction of DXR, as shown in Fig. 2. The seven enzymes involved in the DOXP/MEP pathway have been isolated and their genes (*dxs, dxr, ygbP, ychB, ygbB, gcpE* and *lytB*) have been cloned in plants and bacteria. This has been summarized for the first 5 enzymes in Table 2 (see below). Enzyme 3 (*ygbP*) catalyses an activation of MEP by CTP to form CDP-methyl-D-erythritol [27, 48]. The function of HMBPP synthase (*gcpE*) in the DOXP/MEP pathway was demonstrated by several authors [2, 13, 61, 47]. Evidence for the 7th enzyme HMBPP reductase (*lytB*) came from several groups [1, 3, 25, 49].

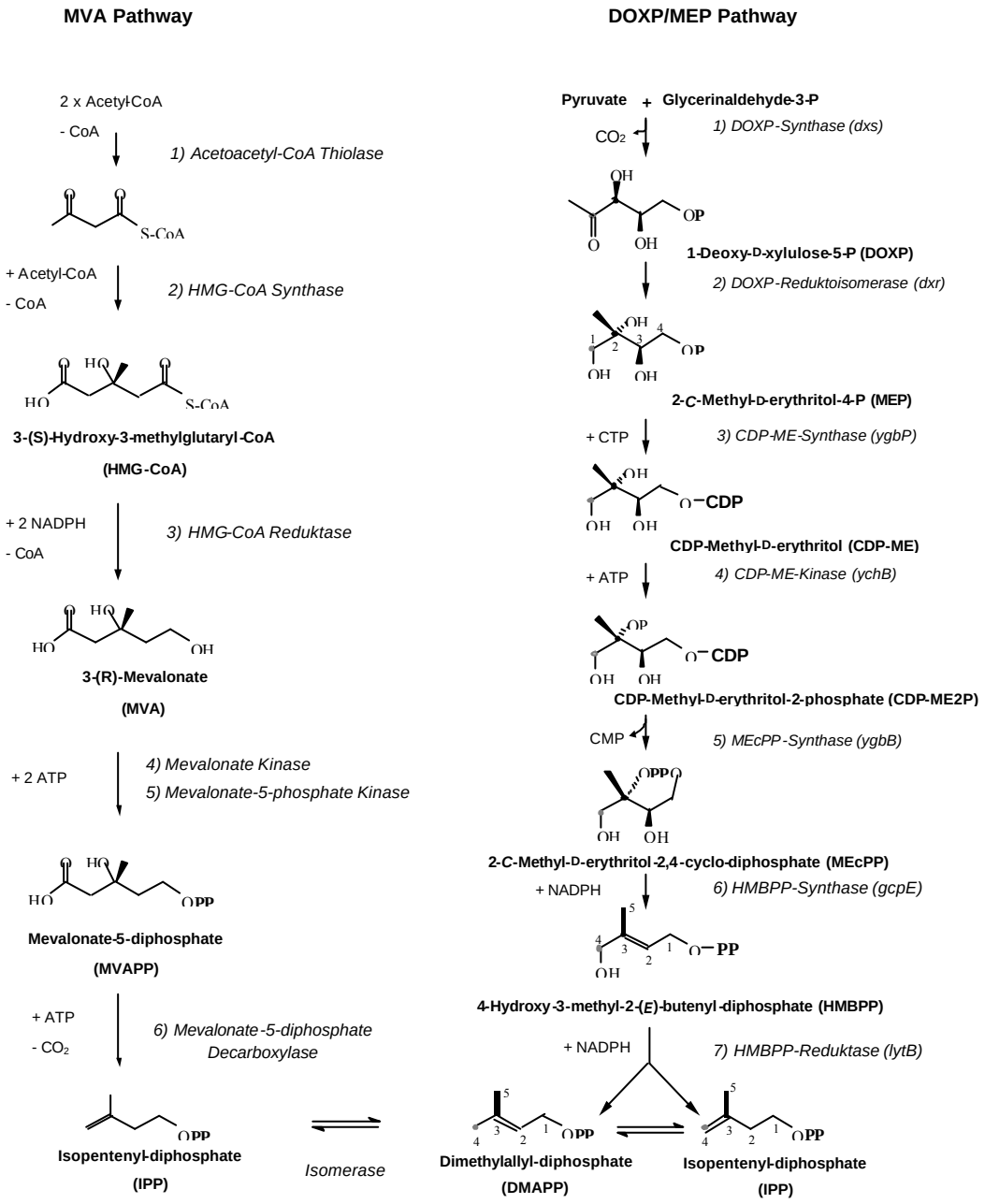

Figure 1. Scheme of the two pathways for isopentenyl diphosphate (IPP) and isoprenoid biosynthesis: the acetate/mevalonate pathway (MVA) and the 1-deoxy-D-xylulose-5-phosphate/methyl-D-erythrithol (DOXP/MEP pathway). The enzymes of both pathways are numbered. In the plastidic DOXP/MEP pathway the genes of the corresponding enzymes are indicated in parenthesis.

3. Cross-talks between the plastidic DOXP/MEP and the cytosolic MVA pathway of IPP biosynthesis in plants

In the cells of higher plants and several algae groups the two IPP producing biochemical pathways operate in parallel. The MVA pathway in the cytoplasm is responsible for the biosynthesis of sterols, sesquiterpenes, polyterpenes and can efficiently be blocked by mevinolin, whereas the accumulation of plastidic isoprenoids is not affected [9]. The DOXP/MEP pathway of IPP formation operates in the chloroplast and the other plastid forms and provides the C_5-units for the biosynthesis of carotenoids, phytol (side-chain of chlorophylls), the nona-prenyl side-chain of plastoquinone-9, as well as for isoprene emission [70] and other plastidic isoprenoids as indicated in Fig. 3. This DOXP/MEP pathway and consequently the biosynthesis of carotenoids, phytol etc., can efficiently be blocked by the herbicide fosmidomycin [71] whereas the biosynthesis of the cytosolic sterols is not affected.

In view of the two cellular IPP producing pathways, the question arises whether IPP or any other isoprenoid chain produced by the IPP pathway of one cellular compartment can be used by the isoprenoid biosynthesis machinery of the other compartment. Can the two IPP pathways complement each other if necessary? In other words, is there a cross-talk between the IPP pathways in the cytosol and that in the plastids? These questions can be answered by studying the incorporation of intermediates of each IPP pathway, such as labeled MVA and labeled DOXP or its non-phosphorylated form DOX, into typical plastidic isoprenoids, such as ß-carotene or phytol (chlorophyll). The results of such a study for two algae and a higher plant, all of which possess both IPP forming pathways, is shown in Table 1.

TABLE 1. Contribution of the DOXP/MEP pathway and the MVA pathway to isoprenoid biosynthesis in the two green algae *Klebsormidium* (‚Charophyceae') and *Mesostigma* (‚Prasinophyceae') and in the higher plant *Lemna gibba* L. The incorporation of precursors of the DOXP/MEP pathway [2-^{14}C]-1deoxy-D-xylulose (^{14}C-DOX) and the MVA pathway [5-^{3}H]-mevalonolactone (^{3}H -MVL) into the plastidic phytol and the cytosolic sterols was studied [60].

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** (SAG 50-1)*			
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

As expected the applied ^{14}C-DOX is incorporated into the phytol chain of chlorophyll in the two 'green alga' *Klebsormidium* and *Mesostigma* to a very high extent, whereas the incorporation of the ^{3}H-mevalonolactone (^{3}H-MVL) into the phytol fraction occurred only in trace amounts (Table 1). In fact, the incorporation rate of ^{14}C-DOX into phytol was 95 and 107 times higher in both algae than that of the ^{3}H-MVL label. In the higher plant *Lemna* the preferred incorporation of ^{14}C-DOX into phytol was, however, only 23.4 times higher than that of ^{3}H-MVL. In contrast to phytol, the labeling of the cytosolic sterols by ^{3}H-MVL proceeded at higher rates as expected. However, the sterol fraction was also labeled from ^{14}C-DOX to a relatively high degree in the two green algae (Table 1), whereas the sterols of the higher plant showed only some labeling by ^{14}C-DOX. The results clearly demonstrate that in the two green algae the plastidic pathway DOXP/MEP contributes considerably to the biosynthesis of sterols, however only to some extent in the higher plant *Lemna*. In contrast, the MVA -pathway only contributes little to the biosynthesis of the plastidic isoprenoid phytol.

Also several early observations indicate at least some exchange or cooperation between both isoprenoid pathways. One example is the very low labeling rate of plastidic isoprenoids from applied ^{14}C-MVA, whereas sterols are labeled at high rates. This had already been detected in 1958 by Goodwin [23] and was noticed by a number of other authors, see review [40]. Moreover, in the ^{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 C_5-unit in a different way [55] which is now known as labeling pattern of the DOXP/MEP pathway [39, 40]. In the liverwort *Heteroscyphus* the first three isoprene units of phytol were shown to be labeled from ^{13}C-MVA whereas the fourth C_5-unit was not labeled [45]. Both observations point to the import of a cytosolic farnesyl diphosphate (FPP) or a non-phosphorylated isoprenoid C_{15}-unit into the plastid to which a fourth plastidic IPP (derived from the DOXP/MEP pathway) was added. In our labeling studies of phytol and carotenoids from ^{13}C-glucose in algae and higher plants, performed in the light under photosynthesis conditions, we did not detect such an import of FPP into the plastid. However, one has to consider that a low labeling of a plastidic isoprenoid via the cytosolic pathway of clearly less than 10 % would not have been seen in the NMR spectra.

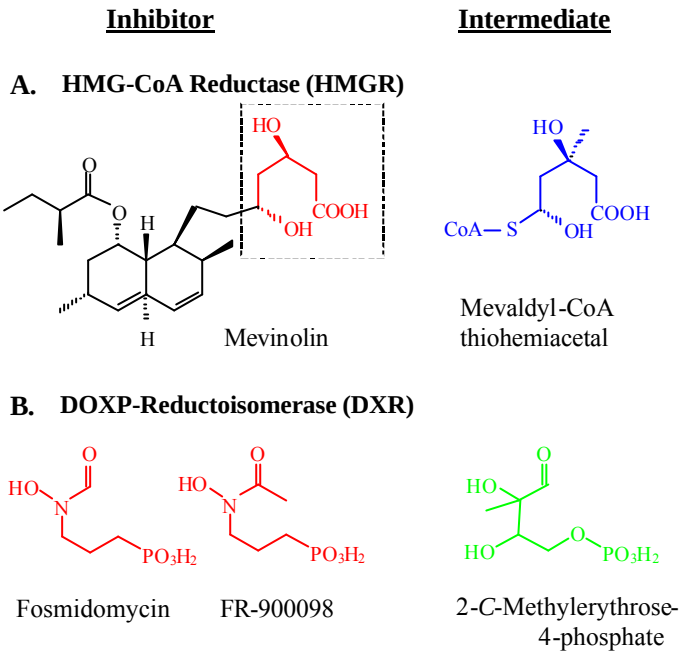

Figure 2. Inhibitors of the acetate/MVA pathway (Mevinolin) and of the DOXP/MEP pathway (fosmidomycin and its methyl derivative FR-900098) for biosynthesis of isopentenyl diphosphate (IPP). The structural analogy of the active inhibitor (site) with the natural enzyme intermediates is indicated.

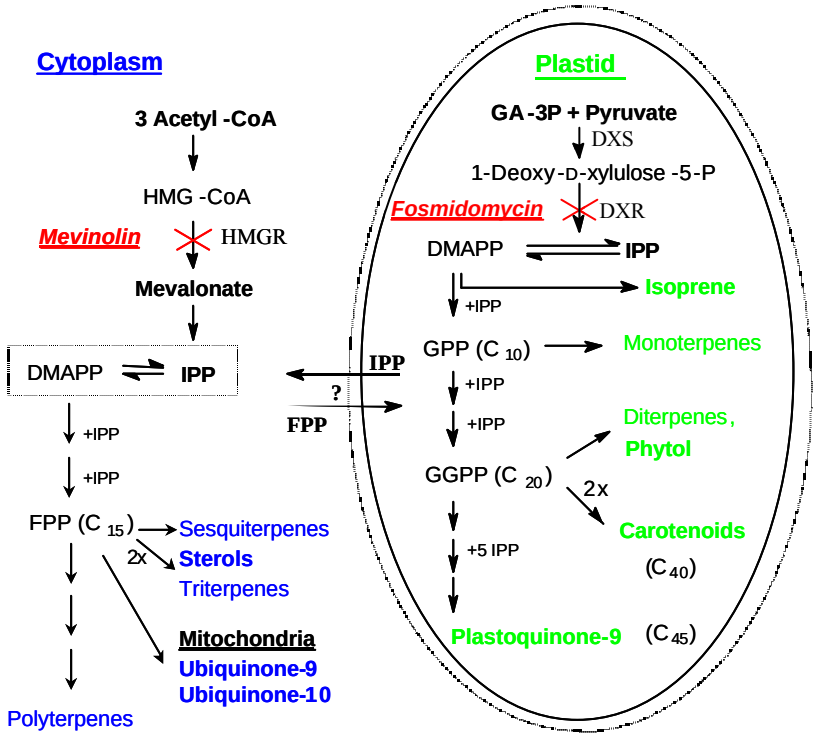

Figure 3. Compartmentation of IPP and isoprenoid biosynthesis in higher plants between cytosol (MVA pathway) and the plastid (DOXP/MEP pathway). The specific block of the enzyme HMG-CoA reductase (HMGR) by the antibiotic mevinolin (a statin) and of the DOXP reductoisomerase (DXR) in the plastid by fosmidomycin is indicated (based on [36]). IPP = isopentenyl diphosphate, DMAPP = dimethylallyl diphosphate, GPP = geranyl diphosphate, FPP = farnesyl diphosphate, GGPP = geranylgeraniol diphosphate.

Another possibility to detect cross-talk is to compare the biosynthesis of selected plastidic and cytosolic isoprenoids when one of the two IPP pathways is blocked by inhibitors, such as shown in Fig. 2. This has been done with cell cultures of green tobacco cells where the uptake of inhibitors and substrates works better than in intact whole plants. Also, this investigation demonstrated that the sterols, normally labeled via the MVA

pathway, can be formed via the DOXP/MEP pathway when the cytosolic MVA pathway is blocked [26]. During an inhibition of the DOXP/MEP route of tobacco TBY-2 cells by fosmidomycin an incorporation of [2-^{13}C]-MVA into plastoquinone-9 was observed.

A similar attempt using fosmidomycin and a mevinolin-type statin as an inhibitor of the MVA pathway, was made in *Arabidopsis* seedlings [34]. Inhibition of the cytosolic MVA pathway caused only a transient reduction of sterol levels indicating that the plastidic DOXP/MEP pathway might partially compensate for the lack of cytosolic IPP needed for the biosynthesis of sterols.

In any case, the currently available data of labeling and inhibitory studies of several laboratories demonstrate that there exists a substantial cross-talk between both cellular IPP biosynthesis pathways. Especially the plastidic DOXP/MEP pathway delivers isoprenoid building units for the biosynthesis of sterols in the cytosol. It may contribute to the formation of other cytosolic isoprenoids, such as polyterpenes, as well; however, this has yet to be investigated. The inverse cooperation, the import of isoprenoid building blocks into the chloroplast, apparently proceeds to a much lower degree than the export. The whole exchange apparently depends on the physiological state and the developmental stage of the plant cells. At high photosynthetic rates it sounds reasonable that the chloroplast exports isoprenoid building blocks for the cytosolic isoprenoid formation. Only in the case of non-green plant tissues the partial import of cytosolic isoprenoid chains into the plastid might play a certain role.

Despite all cross-talk indications it appears to be clear that in intact plants neither the cytosolic MVA nor the plastidic DOXP/MEP pathway of IPP formation can fully compensate the other isoprenoid route when one of the two is partially blocked or operates at a low rate. What isoprenoid compounds can be imported from the cytosol or exported from the plastid is not known at all. The question if they are prenyl diphosphates, such as IPP, GPP or FPP, or rather non-phosphorylated isoprenoid chains, which would be more likely, cannot be answered. Transporters for isoprenoid intermediates or prenyl diphosphates in the plastid envelope have not yet been detected, but this is certainly a promising research topic.

4. Distribution of the DOXP/MEP and MVA pathways in photosynthetic algae and bacteria

The compartmentalization of the isoprenoid biosynthesis with the cytosolic MVA pathway and the plastidic DOXP/MEP pathway of IPP formation (Fig. 3) exists in all higher plants tested so far [35, 36, 40]. This is further emphasized by the presence of all the genes required for the performance of the DOXP/MEP pathway in higher plants as indicated for the first 5 enzymes [37] in Table 2. The enzymes of the MVA pathway are present as well [7]. The genes of the DOXP/MEP pathway are bound to the nucleus, yet the proteins operate in the plastids. In contrast to the bacterial DOXP/MEP enzymes, the plant enzymes possess an additional transit peptide sequence that directs them to their proper organelle, the plastid.

4.1. *Photosynthetic algae*

In the various photosynthetic algae groups possessing a differential photosynthetic pigment apparatus with either chlorophyll a and b (usually addressed as 'green algae' but representing a polyphyletic group), chlorophyll a and phycobilisomes (red algae, rhodophytes) or with chlorophyll a and c (heterokontophytes), we found the same dichotomy of the cellular isoprenoid biosynthesis as in higher plants: the MVA pathway for biosynthesis of the cytosolic sterols and the DOXP/MEP pathway for the biosynthesis of carotenoids, phytol and isoprene [17, 36, 39, 58, 70]. This also applies to the marine diatoms *Nitschia* and *Phaeodactylum* belonging to the heterokontophytes [15]. One of two exceptions of this rule was found in the case of the chlorophytes *Chlorella*, *Scenedesmus* and *Chlamydomonas* [56, 57], where not only the plastidic isoprenoids are made via the DOXP/MEP pathway but also the cytosolic sterols. A detailed further investigation of this unexpected phenomenon resulted in the detection that the commonly termed polyphyletic group 'green algae' consists of the *Streptophyta* (including higher plants) with both IPP pathways, and the proper *Chlorophyta* that have lost their MVA pathway for IPP and isoprenoid biosynthesis [60]. This is indicated in Fig. 4. Chlorophyta have also lost certain cytosolic enzymes of their sugar metabolism [54]. Moreover, chlorophyta also form a separate group from the streptophyta based on differences in the 18S-rRNA composition and ultra-structural characteristics of their cells and flagellae [21]. Further evidence for the absence of the MVA pathway is the fact that statins (mevinolin, cerivastatin) do not inhibit the growth of chlorophyta and also that genes of the MVA pathway could not be detected [60]. In addition, in the pigment-free chlorophyte *Prototheca wickerhamii* ergosterol is synthesized via the DOXP/MEP pathway [72]. The prasinophyte *Mesostigma viride* that is a common precursor of Chlorophyta and Streptophyta contains both the MVA and the DOXP/MEP pathway for IPP biosynthesis (Fig. 4) indicating that chlorophytes and streptophytes, including higher plants, are derived from the same

evolutionary ancestor. Another exception from the existence of two IPP routes in the cell are the Euglenophytes. In the flagellate *Euglena gracilis* sterols and the plastidic isprenoids (carotenoids, phytol) are formed via the classical acetate/MVA pathway [17]. *Euglena* originated from a colorless flagellate that incorporated a green alga (possibly a chlorophyte) in a secondary endosymbiosis, during which the plastidic DOXP/MEP pathway of the endosymbiont was lost (see Fig. 5). A secondary endosymbiosis may not necessarily lead to a loss of the IPP producing pathway of the endosymbiont as is seen in the example of the heterokontophytes in which both IPP producing pathways were maintained (see Fig. 5).

TABLE 2. Distribution of the genes for the first five enzymes of the DOXP/ MEP pathway in plants, protozoa and eubacteria (based on [37, 41]).

Organism	dxs	dxr	ygbP	ychB	ygbB
Plants					
Arabidopsis thaliana	*Q38854*	*AJ242588*	*AF230737*	*AAC32234*	*AAF07360*
Capsicum annuum	*O78327*				
Chlamydomonas reinhardtii	*O81954*				
Mentha piperita	*O64904*	*AF116825*		*AF179283*	
Oryza sativa	*O22567*				
Protozoan parasite					
Plasmodium falciparum	*O96694*	*O96693*	+	+	*AAC71873*
Toxoplasma grandii	+	+	+	+	+
Eubacteria					
Photosynthetic Bacteria:					
Synechocystis sp.	*P73067*	*Q55663*	*P74323*	*P72663*	*P73426*
Synechoccocus leopoliensis	*Y18874*	*AJ250721*			
Chlorobium tepidum	+	+	+	+	
Rhodobacter capsulatus	*P26242*		*Q08113*		*Q08113*
Pathogenic Bacteria:					
Escherichia coli	*P77488*	*P45568*	Q46893	P24209	P36663
Haemophilus influenzae	*P45205*	*P44055*	*O05029*	*P45271*	*P44815*
Helicobacter pylori	*Q9ZM94*	*AAD05777*	*AAD5981*		*O25664*
Chlamydia pneumoniae	*Q9Z6J9*	*Q9Z8J8*	*AAD18718*	*AE001363*	+
Mycobacterium tuberculosis	*O07184*	*Q10798*	*P96864*	+	*P96863*
Vibrio cholerae	+	+	+	+	+

Recently research has concentrated on the Chlorarachniophytes (*Bigelowiella natans*), green amoeboflagellate algae that acquired their plastid by a secondary endosymbiosis of a green alga [5]. Like the plastids of heterokonts, haptophytes, apicomplexa and cryptomonads, their plastids are surrounded by four envelope membranes, whereas the plastids of euglenids and dinoflagellates are surrounded by only three envelope membranes [5]. In contrast to heterokonts, the photosynthetic apparatus of *Bigelowiella* contains chlorophyll a and b as do euglenids, green algae and higher plants, and must be derived from a 'green alga'. It is of great interest to know if *Bigelowiella* contains both IPP producing pathways, the DOXP/MEP and MVA pathway, or if it has lost one of them during or after the secondary endosymbiosis of a green alga.

A very special taxonomic group are the *Apicomplexa* comprising the sporozoa parasites *Plasmodium falciparum* and *Toxoplasma gondii*. These contain a special cell organelle, the apicoplast, a non-green plastid with a genome similar to that of chloroplasts of green algae. The apicomplexa arose from a secondary endosymbiosis (the apicoplast envelope consists of 4 membranes) by either the incorporation of a green alga [22] or a red alga [4, 68] (see also Fig. 5) whereby the plastid obtained an envelope of four membranes. Whether Apicomplexa, Heterocontophyta, Haptophyta, Cryptophyta and Dinoflagellates have a common ancestor [4, 68] is still a matter of debate. In any case, *Plasmodium* possesses the DOXP/MEP pathway of IPP and isoprenoid biosynthesis [29], and its development can be blocked by fosmidomycin whereas the MVA pathway is missing. This also applies to *Toxoplasma gondii* as has been recently established [19]. Cyanobacteria possess the DOXP/MEP pathway of IPP formation as has been shown for *Synechocystis* [17, 40, 46]. This is in agreement with the endosymbiosis theory of chloroplasts according to which cyanobacteria-like photosynthetic organisms were taken up by a flagellate in a primary endosymbiosis as is shown in Fig. 5.

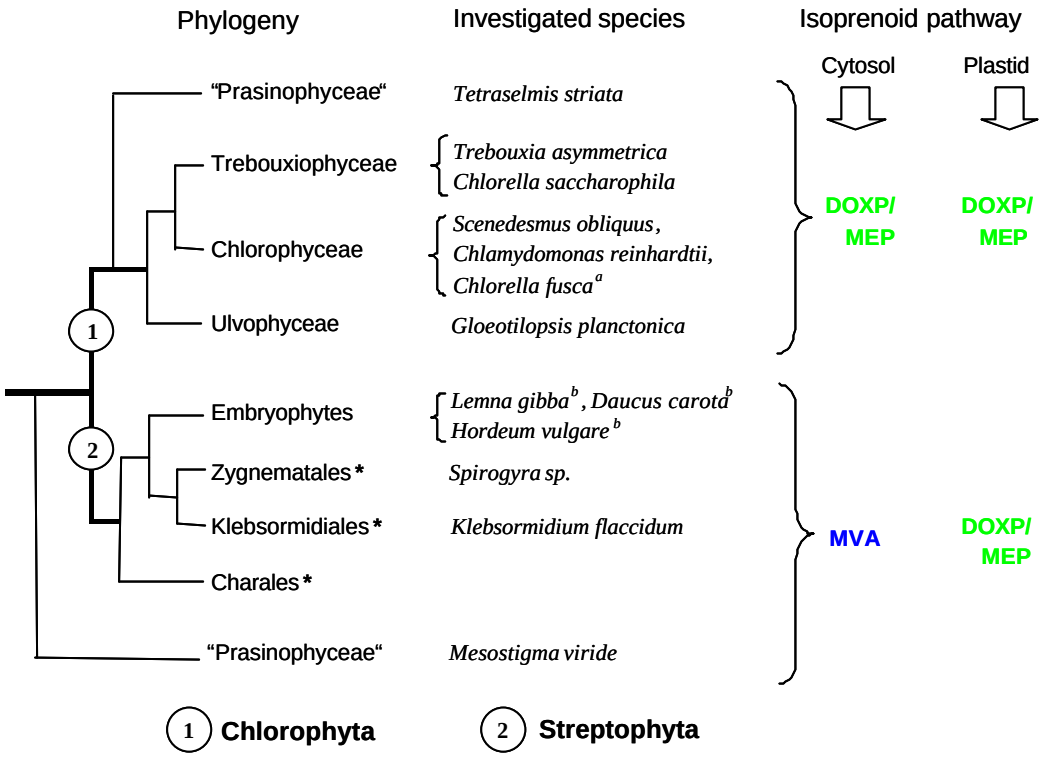

Figure 4. Distribution of the DOXP/MEP pathway and the MVA pathway of IPP biosynthesis in Chlorophyta (1) and Streptophyta, including higher plants (2). The distribution correlates with the current phylogeny of green algae and higher plants [21] based on 18S-rRNA sequences, ultrastructural characteristics of flagellate cells and of cell mitosis. The chlorophyta have lost the MVA pathway, whereas the common ancestor *Mesostigma viride* has both IPP pathways. * These clades of 'green algae' are summed up by most of the recent authors as 'Charophyceae', but are not monophyletic. [a,b] IPP and isoprenoid biosynthesis of these organisms has been studied before [17, 40].

4.2. *Photosynthetic bacteria*

In contrast to plastids, mitochondria as symbiontic bacterial organisms have lost their capacity for their own isoprenoid biosynthesis [43] and are dependent on the cytosolic MVA pathway for the synthesis of the isoprenoid chains of ubiquinones -9 and -10 [18]. The photosynthetic *Cyanobacteria* possess two photosynthetic light reactions (photosystems 1 and 2), split water and evolve oxygen as do the different algae groups and higher plants. They perform an oxigenic photosynthesis. A more primitive photosynthesis with only one light reaction is found on one hand in the green sulfur photosynthetic bacteria, such as *Chlorobium tepidum* (Chlorobacteriaceae) (see Table 2), and on the other hand in the purple bacteria such as *Rhodobacter*. Both photosynthetic phototroph bacteria have bacteriochlorophylls, perform an anoxigenic photosynthesis (no oxygen evolution) and possess the DOXP/MEP pathway for the biosynthesis of their carotenoids and isoprenoid chains (ubiquinones, menaquinones, phytol) as is indicated in Table 2, has been demonstrated for *Rhodobacter capsulatus* [24], and is also outlined in [10]. The MVA pathway does not occur in these photosynthetic phototroph bacteria. Thus, from all photosynthetic organisms the DOXP/MEP pathway of IPP biosynthesis shows up first in the prokaryotic photosynthetic bacteria with only one light reaction. Since the cyanobacteria represent photosynthetic organisms, with a higher complexity of their photosynthetic apparatus, their two photosystems apparently derive from Chlorobacteriaceae (photosystem 1) and purple bacteria (photosystem 2), in evolutionary terms they must have emerged later than the photosynthetic bacteria with only one light reaction and anoxigenic photosynthesis. Thus, the origin of the DOXP/MEP pathway in chloroplasts of algae and higher plants goes back to the early photosynthetic bacteria as is summarized in Fig. 5.

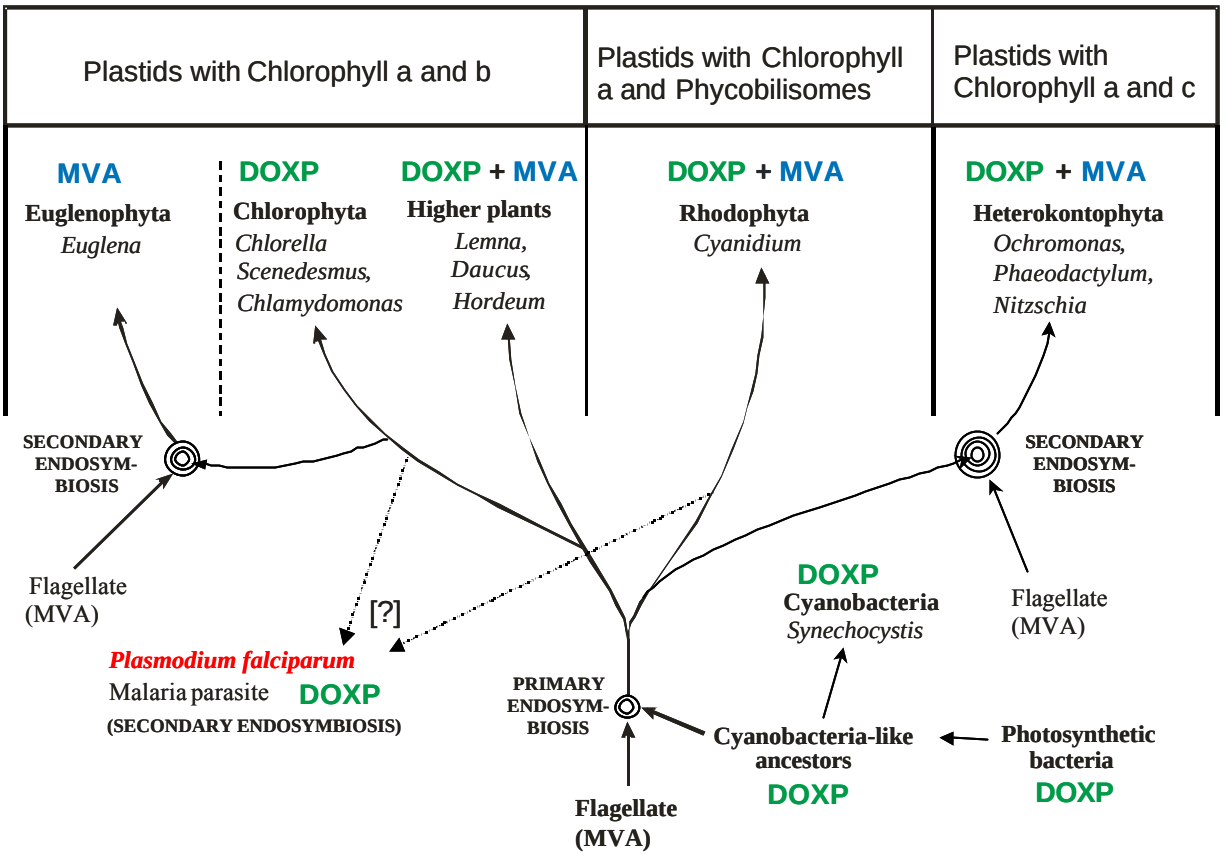

Figure 5. Putative evolution of photosynthetic bacteria, algae and higher plants with particular emphasis on the occurrence of the DOXP and/or the MVA pathway for IPP-biosynthesis. During evolution the MVA pathway for IPP-biosynthesis was lost in Chlorophyta, whereas the DOXP pathway was lost in Euglenophyta. The scheme lists also the malaria parasite (*Plasmodium falciparum*) that contains the DOXP pathway in a plastid-like apicoplast, and originated by incorporation of either a red or green alga in a secondary endosymbiosis process. The scheme is based on [36].

5. Distribution of the DOXP/MEP pathways in Bacteria.

According to our present knowledge the DOXP/MEP and MVA pathways of IPP formation is a very early biosynthesis pathway that originated during the evolution of bacteria. From there the DOXP/MEP pathway was transferred via photosynthetic cyanobacteria-like ancestors of cyanobacteria by primary and later also secondary endosymbiosis to algae and higher plants. It is of interest in this respect that the sequences of the plant genes of the DOXP/MEP pathways differ to a higher degree from that of the cyanobacteria than from other bacteria [32] indicating that the presently existing cyanobacteria have gone through a long evolutionary development.

Today most of the members of the bacteria, such as E. coli, use exclusively the DOXP/MEP pathway of IPP formation. The photosynthetic cyanobacteria belong to this group, as well as green sulphur bacteria (*Chlorobium*), the purple bacteria within the proteobacteria (e.g. *Rhodobacter*), and many non-photosynthetic bacteria, such as members of the Aquificales, Thermotogales, Chlamydiae, Bacteroides and Gram-positive ones with either low or high G+C as reviewed in [10]. An exception from this general rule is a minority of bacteria that 1) either lost the DOXP/MEP pathway and replaced it by the MVA pathway or 2) acquired, in addition to the DOXP/MEP route, the full or part of the genes of the MVA pathway. All this apparently occurred by lateral gene transfer, a process that happened rather frequently during the evolution of the large diverging phylum of bacteria. Thus *Borrelia burgdorferi*, *Myxococcus fulvus* [10], *Paracoccus zeaxanthinifaciens* [20], some Gram-positive bacteria with low G+C content [67], including *Lactobacillus plantarum* and the green phototroph non-sulfur bacterium *Chloroflexus aurantiacus* [10], belong to the first group and only possess the MVA pathway. The second group is represented by members of the Gram-positive bacteria with a high G+C content, i.e. several *Streptomyces* species possessing both the MVA and the DOXP/MEP route. In *Streptomyces aeriouvifer* primary isoprenoids, such as the electron carrier menaquinone, are formed during the exponential growth phase via the DOXP/MEP pathway, whereas in the stationary phase the antibiotic naphpertin is synthetized via the acetate/MVA pathway [62]. The accumulation of naphpertin could be blocked by the mevinolin-like statin pravastatin without affecting the growth of the bacterium. A similar partition of isoprenoid biosynthesis in primary isoprenoids (DOXP/MEP pathway) and secondary isoprenoids via the MVA route has also been

detected in the actinomycete *Actinoplanes* [63]. Bacteria evidently were the play ground of the evolution in those cases where a full or partial loss of gene sequences and the acquirement of others by lateral gene transfer has occurred. Thus, the DOXP/MEP route is present and some genes of the MVA route and vice versa. This has been reviewed in detail in [10]. The DOXP/MEP pathway of isoprenoid biosynthesis and its genes are also found in many pathogenic bacteria, e.g. those causing lung disease, tuberculosis, leprosy, cholera, and ulcer, i.e. *Chlamydia pneumonia*, *Mycobacterium tuberculosis*, *M. leprae*, *Vibrio cholerae* and *Helicobacter pylori*, respectively, as is shown in Table 2 and [41].

Within the *Bacteria* the phylogenesis of the different groups is presently unresolved. Thus, except for Aquifacales and Thermotogales, that had branched off relatively early from the common unknown ancestors, it is not known which group is more advanced or more primitive than others. The photosynthetic bacteria with only one photosystem (one light reaction) and the mandatory anaerobics must have developed earlier than the cyanobacteria with two photosystems that evolve oxygen. However this is not reflected in the tree of bacteria usually shown [e.g. 12]. One can assume that during the evolution various photosynthetic bacteria have lost their pigment apparatus and with it their competence for photosynthetic quantum conversion. Therefore, the question arises which of the present heterotrophic bacteria are derived from former photoautotrophic bacteria. Are pathogenic bacteria, such a set of former phototropic bacteria that survived the loss of the photosynthetic (photo-autotrophic) competence by turning into pathogens that live and multiply in a host organism? Or did they originate from other earlier heterotrophic bacteria? Such questions are summarized in Fig. 6 and must be a matter of further research. Also, the possible relationships between photosynthetic bacteria and early non-pathogenic bacteria have to be investigated.

6. Isoprenoid biosynthesis in Archaea and Eucarya

Heterotrophic eukaryotes, such as animals and fungi [16, 66], only use the MVA pathway of IPP and isoprenoid biosynthesis. The chloroplast containing photo-autotrophic plants and most algae groups possess in their cytosol the MVA pathway being used for biosynthesis of sterols, sesquiterpenes [see reviews 35, 36] and the side-chain of the mitochondrial ubiquinones [18], whereas the plastids contain the DOXP/MEP pathway of IPP formation. Thus, all *Eucarya* possess the MVA pathway, except for the proper chlorophyta within the 'green algae' that have lost this pathway [60].

The prokaryotic *Archaea* (formerly archaebacteria) form an independent evolutionary group that developed in their own way, independently of *Bacteria* and *Eucarya*. Like non-photosynthetic eukaryotes, archaea possess only the MVA pathway to synthesize their isoprenoids [11]. All archaea have the genes for HMG-CoA synthase, HMG-CoA reductase and the mevalonate kinase, whereas any further genes for the formation of IPP are missing [11, 32, 64]. Evidently, the genes of the enzymes for the transformation of MVA phosphate to IPP show no similarities to those of other organisms with the MVA pathway. Also, the usual IPP isomerase was not found in the genome of Archaea, but instead a 'class 2' IPP isomerase resembling that found in the bacterium *Streptomyces* [30]. Genes of the DOXP/MEP pathway were not found in Archaea, except for the sequence of a CDP-ME synthase in *Pyrococcus honkoshii* which seems to have been acquired by lateral gene transfer, possibly from a bacterium [10]. On the basis of various other biochemical and genetic informations the Archaea are somewhat closer to the Eucarya than the Bacteria [11, 33]. A putative scheme of a phylogenetic tree of the evolution of Archaea, Bacteria and Eucarya, originally based on the analysis of rRNA, is shown in a modified form in Fig. 7 and summarizes the presently known distribution of the DOXP/MEP and the MVA pathways of IPP formation.

Acknowledgements: I wish to thank Martin Knapp and Dr. Christian Müller for assistance during the preparation of the manuscript.

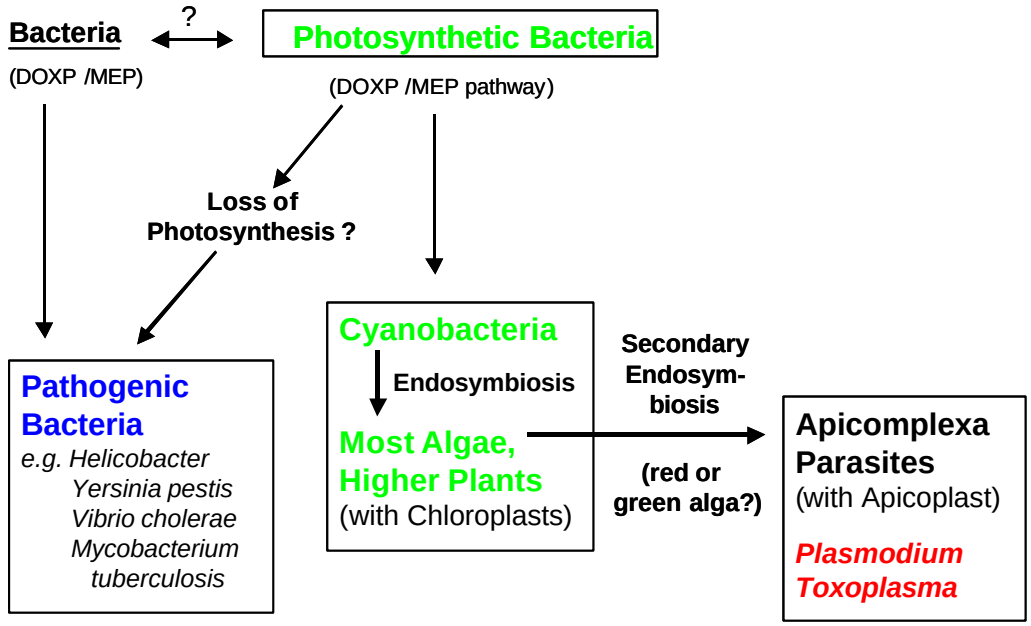

Figure 6. Putative evolution and transfer of the DOXP / MEP pathway of IPP biosynthesis from early heterotrophic or photosynthetic bacteria to pathogenic bacteria, cyanobacteria and by endosymbiosis to algae, higher plants and the parasitic apicomplexa.

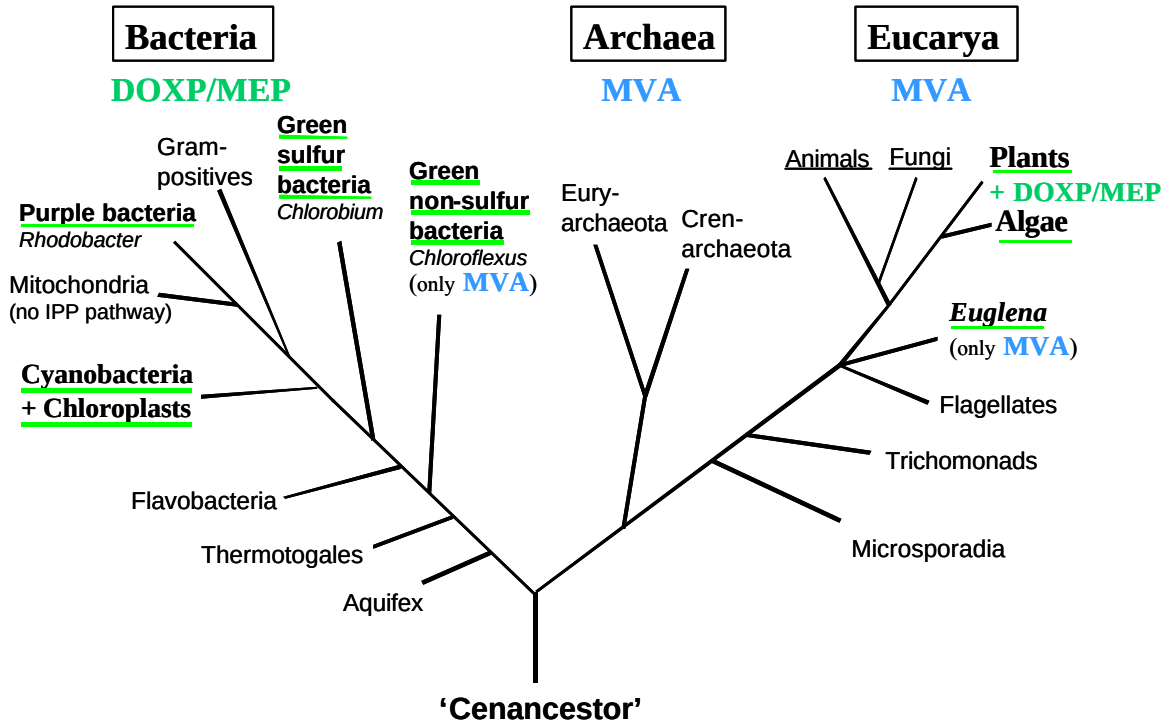

Figure 7. Simplified phylogenetic tree of the domains *Bacteria* , *Archaea* and *Eucarya* with indication of the presently known predominant distribution of the **DOXP/MEP** and the **MVA** pathways of IPP and isoprenoid biosynthesis. There are a few exceptions from this scheme, e.g. Chlorophyta (only DOXP/MEP pathway) and Apicomplexa (only DOXP/MEP pathway), whereas some Bacteria can have also the MVA pathway. The photosynthetic Bacteria and Eucarya are underlined by a green bar. (Branch lengths have no particular meaning in this tree).

7. References

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