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Review

Isoprenoid biosynthesis in eukaryotic phototrophs: A spotlight on algae

Martin Lohr[a,*], Jörg Schwender[b], Jürgen E.W. Polle[c,*]

[a] Institut für Allgemeine Botanik, Johannes Gutenberg-Universität, 55099 Mainz, Germany
[b] Biology Department, Brookhaven National Laboratory, Upton, NY 11973, USA
[c] Department of Biology, Brooklyn College of CUNY, 2900 Bedford Ave, Brooklyn, NY 11210, USA

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ABSTRACT

Isoprenoids are one of the largest groups of natural compounds and have a variety of important functions in the primary metabolism of land plants and algae. In recent years, our understanding of the numerous facets of isoprenoid metabolism in land plants has been rapidly increasing, while knowledge on the metabolic network of isoprenoids in algae still lags behind. Here, current views on the biochemistry and genetics of the core isoprenoid metabolism in land plants and in the major algal phyla are compared and some of the most pressing open questions are highlighted. Based on the different evolutionary histories of the various groups of eukaryotic phototrophs, we discuss the distribution and regulation of the mevalonate (MVA) and the methylerythritol phosphate (MEP) pathways in land plants and algae and the potential consequences of the loss of the MVA pathway in groups such as the green algae. For the prenyltransferases, serving as gatekeepers to the various branches of terpenoid biosynthesis in land plants and algae, we explore the minimal inventory necessary for the formation of primary isoprenoids and present a preliminary analysis of their occurrence and phylogeny in algae with primary and secondary plastids. The review concludes with some perspectives on genetic engineering of the isoprenoid metabolism in algae.

Contents

1. Introduction to isoprenoid metabolism in plants and algae

Isoprenoids – also known as terpenes or terpenoids – are essential components of all living organisms. Consequently, the ability to synthesize isopentenyl diphosphate (IPP), the basic 5-carbon structure necessary for isoprenoid formation, is ubiquitous

* Corresponding author.
E-mail addresses: lohr@uni-mainz.de (M. Lohr), jpolle@brooklyn.cuny.edu (J.E.W. Polle).

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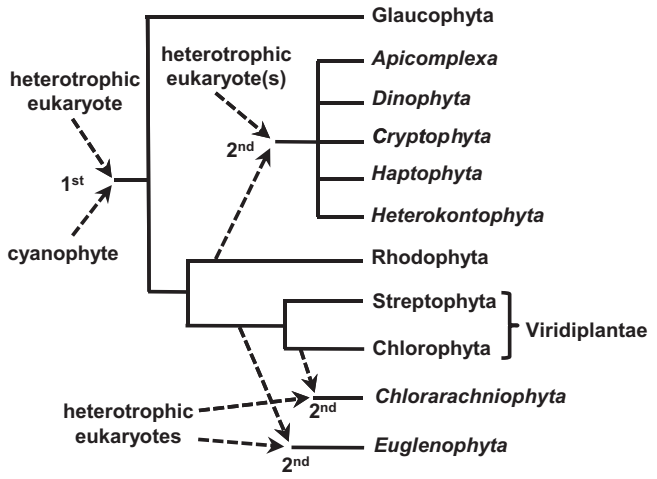

Fig. 1. Phylogenetic relations of the major algal groups that are discussed in the review. Dashed arrows indicate primary (1st) or secondary (2nd) endosymbiotic events. Names of algal groups that evolved from secondary endosymbiotic events are italicized. The non-photosynthetic Apicomplexa are included because they contain reduced plastids (apicoplasts). See text for further details.

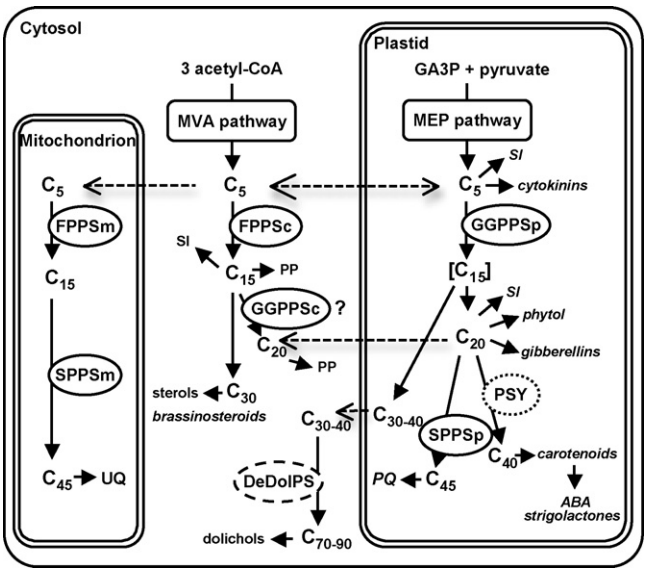

Fig. 2. Compartmentation of isoprenoid biosynthesis in Streptophyta (charophyte algae and embrophytes) based on experimental data and *in silico* analyses as discussed in the text. Dashed arrows indicate putative transport of isoprenoid precursors across organellar membranes. Names of isoprenoid products specific to eukaryotic phototrophs are italicized. Abbreviations of enzymes are: DeDolPS dehydrodolichyl diphosphate synthase; FPPSc cytosolic farnesyl diphosphate synthase; FPPSm mitochondrial farnesyl diphosphate synthase; GGPPSc cytosolic geranylgeranyl diphosphate synthase; GGPPSp plastidic geranylgeranyl diphosphate synthase; PPPS polyprenyl diphosphate synthase; PSY phytoene synthase; SPPSm mitochondrial solanesyl diphosphate synthase; SPPSp plastidic solanesyl diphosphate synthase. Solid circles indicate *trans*-prenyltransferases, dashed circle indicates *cis*-prenyltransferase, dotted circle indicates other enzyme. Other Abbreviations: ABA abscisic acid; GA3P glyceraldehyde-3-phosphate; PP protein prenylation; PQ plastoquinone; SI secondary isoprenoids; UQ ubiquinone.

in archaebacteria, eubacteria and eukaryotes [1–3]. Key events in the evolution of eukaryotic phototrophs were the repeated fusions of whole cells of unrelated organisms in a process termed endosymbiosis. In this way, the mitochondria present in almost all extant eukaryotes are derived from ancient proteobacteria [4], whereas the plastids are derived from an ancient cyanobacterial endosymbiont [5,6]. Given the fundamental roles of isoprenoids in the core metabolism of life on earth, it is safe to assume that each of the (endo-)symbiotic partners had its own enzymatic machinery for isoprenoid formation. During the genomic and metabolic integration of host and endosymbiont, their cellular machineries were streamlined by loss of redundant pathways and the underlying genetic information [6–8]. Thus, animal cells contain only one set of genes for IPP formation. In the cells of land plants, however, the legacies of both the eukaryotic host and the cyanobacterial endosymbiont are still traceable, because the plastids fully retained their capacity for isoprenoid formation [9,10] (Section 2.1). Conversely, green algae demonstrate that the original contribution of the eukaroytic host cell to isoprenoid metabolism can be minimized and that the enzymatic capacity of the "prokaryotic" plastid is sufficient to meet the isoprenoid demands for all cellular compartments [3,9,10] (Section 2.3). Algal groups that acquired their plastids by secondary endosymbiosis between a heterotrophic eukaryote as host and a phototrophic eukaryote as endosymbiont, likely have an even more complex evolutionary history of isoprenoid metabolism (Section 2.5).

1.1. Algal evolution and the core of isoprenoid metabolism

Untangling the phylogenomics of isoprenoid biosynthesis in eukaryotic algae requires basic knowledge of their evolutionary histories. Land plants and green algae, collectively termed Viridiplantae (Latin for "green plants"), acquired their plastids by the same endosymbiotic event. Two other extant algal phyla, the red algae (Rhodophyta) and the Glaucophyta, likely also emerged from this so called primary endosymbiosis starring a heterotrophic eukaryote as host and a phototrophic cyanobacterium as endosymbiont (Fig. 1) [11–13]. The Viridiplantae are comprised of two major evolutionary lineages that split early: the Chlorophyta (green algae sensu stricto, including Chlorophyceae, Ulvophyceae, Trebouxiophyceae and most taxa of the polyphyletic Prasinophyceae) and the Streptophyta (charophyte algae and embryophytes) [14–17].

Algae with primary plastids were preyed upon by other heterotrophic eukaryotes and in some cases became eukaryotic endosymbionts that ultimately were reduced to their plastids. Several cases of such secondary endosymbioses are documented: green algae served as endosymbionts at least two times, giving rise to the Euglenophyta (e.g. *Euglena gracilis*) and the Chlorarachniophyta (e.g. *Bigelowiella natans*) [18,19]. Algal groups such as the haptopflagellates (Haptophyta), the dinoflagellates (Dinophyta) and the heterokont algae (Heterokontophyta or Stramenopiles), that include the diatoms (Bacillariophyceae) and brown algae (Phaeophyceae), acquired their plastids probably by secondary endosymbiosis involving a red algal organism. There is an ongoing debate on how many times such a secondary endosymbiosis between a heterotrophic host and a red alga took place [5,6,19–21].

The major primary isoprenoids with functions probably conserved in all eukaryotes are: (i) sterols, controlling fluidity of the ER and plasma membranes by ordering the lipid phase and participating in membrane-related processes such as endocytosis (reviewed in [22]); (ii) the C_{15} and C_{20}-polyprenyls used for protein prenylation, thereby recruiting many cytosolic proteins to membranes [23,24]; (iii) medium-chain polyprenyls (9–11 C_5-units) that are part of ubiquinones, essential components of the mitochondrial electron transport chain [25]; and (iv) the long-chain polyprenyl dolichol as sugar-carrier-lipid used in protein glycosylation in the eukaryotic ER [26] (Fig. 2).

The primary metabolites of eukaryotic phototrophs include additional isoprenoids such as (i) phytol as a part of chlorophylls, tocopherols and phylloquinones [27], (ii) the various carotenoids, that are essential as functional and structural components of

the photosynthetic apparatus [28]; and (iii) the medium-chain polyprenyl that is part of the photosynthetic electron carrier plastoquinone. Finally, isoprenoids serve as precursors or components of various phytohormones, namely cytokinins, gibberellins, abscisic acid, strigolactones and brassinosteroids [29–32].

As depicted in Fig. 2, the isoprenoids that are generally conserved in eukaryotes are synthesized in the cytosol or mitochondria, whereas most of the plant-specific primary isoprenoids (italicized names in Fig. 2) are made in the plastid. Exceptions are the brassinosteroids, that derive from the cytosolic sterols, and the dolichols which in plants appear to be generated by a concerted effort of plastid and cytosol ([33]; see also Section 2.1). The involvement of the plastid in the supply of the geranylgeranyl diphosphate used in the prenylation of cytosolic proteins emerges as another intriguing exception ([34]; see also Section 3.1).

Apart from phytohormones, these primary isoprenoids are common to all plants and algae and define the core of the isoprenoid metabolism in plants. However, plants have not stopped here. Most plant isoprenoids are secondary metabolites with functions in defense against herbivores and pathogens or the attraction of pollinators or seed dispersers [35,36]. These metabolites have a high chemical diversity, and many of these compounds are limited to certain plant genera or families. Some of these secondary plant terpenoids are promising candidates for pharmaceutical or nutraceutical applications. Prominent examples with pharmaceutical use are the anti-malarial artemisinin from *Artemisia annua* and paclitaxel (Taxol®) from *Taxus brevifolia* [37] used in cancer chemotherapy. Among the high-value isoprenoids from algae are the secondary carotenoids, which accumulate in various chlorophytes under adverse environmental conditions and are not involved in photosynthesis. Examples are β-carotene in *Dunaliella salina* or astaxanthin in *Haematococcus pluvialis* [38]. The biotechnological production of secondary isoprenoids of commercial interest is still in development, and algae may be established as useful platforms for large-scale production of isoprenoids.

2. Pathways and regulation of isopentenyl diphosphate biosynthesis

2.1. The baseline: MVA and MEP pathways and their regulation in streptophytes

The bioynthesis of isoprenoids can be divided into three major stages: (i) the formation of active isoprene (IPP or DMAPP, the central building blocks of all isoprenoids); (ii) the linear condensation of isoprene units to polyprenyl diphosphates of varying chain lengths; and (iii) further modification of the polyprenyl diphosphates that are metabolized to a plethora of isoprenoid end products with various functions. Two pathways are known for the biosynthesis of IPP and DMAPP, the mevalonate (MVA) pathway and the methylerythritol phosphate (MEP) pathway. Depending on the organism, either one of the two pathways is present exclusively or both pathways may act in different compartments within the same cell.

The MVA pathway was discovered in the 1950s and long regarded as the only route for the synthesis of isoprenoids [39–41]. Six different enzymes participate in the formation of IPP (Fig. 3) [41,42]. A seventh enzyme, the isopentenyl diphosphate isomerase (IDI), is required to generate DMAPP from IPP. Although the pathway begins with condensation of three molecules of acetyl-CoA, the committed step towards IPP is the phosphorylation of mevalonate by mevalonate kinase (MVK).

The MVA pathway operates in many photosynthetic organisms such as the higher plants [9,43] (Fig. 2), providing the precursors for cytosolic sterols and brassinosteroids as well as for polyprenyls used for protein prenylation and mitochondrial ubiquinone [43–46]. Most of the enzymes involved in the MVA pathway are soluble proteins, with the exception of 3-hydroxy-3-methylglutaryl CoA reductase (HMGR), that is anchored in the membranes of the endoplasmic reticulum [42,47,48]. The general consensus that the MVA pathway operates in the cytosol has been challenged by recent studies showing a peroxisomal location of AACT and IDI in plant cells and the presence of peroxisomal targeting motifs in the other enzymes of the MVA pathway [49]. For mammals, there is evidence that the peroxisomes may contain a full set of enzymes of the MVA pathway including IDI ([50,51] and references therein).

The MEP pathway has only recently been fully elucidated [9,52,53]. In contrast to the MVA pathway, the last enzyme in the MEP pathway (Fig. 3), the 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (HDR), produces both IPP and DMAPP as final products [54]. Although both isomers are made, so far no eukaryotic phototroph is known that lacks IDI, indicating an essential role in providing a balanced supply of IPP and DMAPP [55]. For example, in streptophytes such as *A. thaliana*, IDI is located in mitochondria, cytosol, and the chloroplasts [56]. The MEP pathway is localized in the plastids [10,57] and produces the precursors for plastidic isoprenoids such as carotenoids, phytol, phytohormones, and polyprenyls for plastoquinone [43,45,58,59].

The eight enzymes of the MEP pathway [9,53,60] are nucleus-encoded and post-translationally imported into the plastids as indicated by the presence of typical N-terminal transit peptides in all enzymes [61,62]. Biochemical [45,58,63] and genomic data [64] have revealed the presence of the MEP pathway in many organisms containing plastids, regardless of the presence of a functional process of oxygenic photosynthesis. This would be expected, because cyanobacteria use the MEP pathway for the biosynthesis of isoprenoids, indicating that the cyanobacterial ancestor of all plastids introduced the MEP pathway to the plant cell [65].

As the MVA and the MEP pathways operate simultaneously in streptophyte cells (Fig. 2) [9,66–69], questions arose about the regulation of metabolic fluxes among different cellular compartments and the contribution of one or both pathways to the synthesis of various isoprenoid compounds. In general, each pathway contributes precursors preferentially to the major isoprenoids synthesized in the compartment in which the pathway is located. However, isotope labeling [58,63,70] and inhibitor studies [71,72] suggest that the exchange of precursors between cytosol and chloroplasts is possible in both directions. In some cases, the precursor exchange between compartments is significant as demonstrated by the biosynthesis of dolichol, which is a 'mosaic' isoprenoid containing C_5-units from both pathways [33].

In general, export of precursor prenyl diphosphates from the chloroplast into the cytosol appears to be favored over import, as it was discovered that in higher plants import from the cytosol is not sufficient to counterbalance genetic disruption of the MEP pathway [73,74]. Metabolite exchange studies using isolated chloroplasts from three different plant species indicated export rates of prenyl diphosphates in the following order: IPP and geranyl diphosphate at the highest rates, farnesyl diphosphate and DMAPP at low rates, and geranylgeranyl diphosphate was not transported [75]. However, no specific transporters for isoprenoid molecules have yet been identified in plastid envelope membranes despite intensive past [76] and recent [75,77,78] studies on transport as well as proteomic approaches [57,79–81]. Consequently, the arrows in Fig. 2 are not linked to any specific transporters, but rather signify the existence of metabolite trafficking.

Besides isoprenoid trafficking, knowledge about the regulation of metabolic fluxes through the MVA and MEP pathways is also

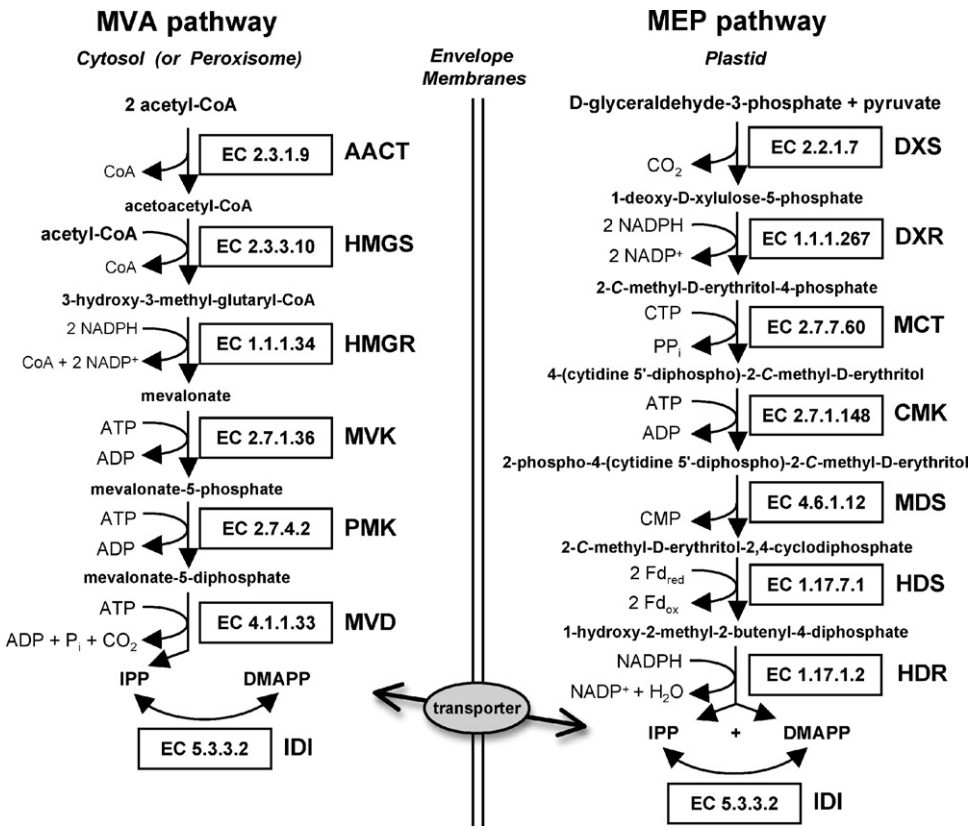

Fig. 3. Biosynthesis of isopentenyl diphosphate (IPP) in Streptophyta with the MVA pathway operating in the cytosol and the MEP pathway operating in the plastid (see text for further explanations). Abbreviations of enzymes are according to Refs. [201,202]: AACT acetoacetyl-CoA thiolase; CMK 4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol kinase; DXR 1-deoxy-D-xylulose 5-phosphate reductoisomerase; DXS 1-deoxy-D-xylulose 5-phosphate synthase; HDR 4-hydroxy-3-methylbut-2-en-1-yl diphosphate reductase; HDS 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase; HMGS 3-hydroxy-3-methylglutaryl-CoA synthase; HMGR 3-hydroxy-3-methylglutaryl-CoA reductase; IDI isopentenyl diphosphate:dimethylallyl diphosphate isomerase; MCT 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase; MDS 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase; MVD mevalonate-5-diphosphate decarboxylase; MVK mevalonate kinase; PMK 5-phosphomevalonate kinase.

limited. As signaling between cellular compartments is essential to coordinate metabolic flux [82], cross-talk between the MVA and MEP pathways for fine tuning of the IPP/DMAPP supply is likely to occur. This idea is supported by observations that in higher plants, depending on the biotic or abiotic stress factor, either the MVA or the MEP pathway contributes to the biosynthesis of specific isoprenoids [33,70,83]. These results also indicated that both pathways may be independently regulated. Moreover, both pathways are probably coupled to the redox state of the cell due to the fact that: (i) the biosynthesis of IPP/DMAPP requires NADPH; and that (ii) the 4-hydroxy-3-methylbut-2-enyl diphosphate synthase (HDS) in the MEP pathway draws electrons directly from ferredoxin [84] (Fig. 3). However, more research is needed to elucidate the coordination between the MVA and the MEP pathways.

3-Hydroxy-3-methylglutaryl-CoA reductase (HMGR) has been identified as the major rate controlling step in the MVA pathway of higher plants [83,85]. The picture is more complex for the MEP pathway, with the major rate-limiting enzymes believed to be 1-deoxy-D-xylulose 5-phosphate synthase (DXS) [85,86], 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) [73], and HDR [73,87]. These enzymes are often encoded by multi-gene families, with one gene product responsible for housekeeping and other copies providing the products for the synthesis of secondary isoprenoids, as shown for DXS [86,88–90], DXR [91], and HDR [92,93]. Further, a concerted transcriptional regulation of genes coding for MEP pathway enzymes and genes coding for enzymes of downstream reactions, such as in the carotenoid biosynthetic pathway, was observed [85,88,94,95]. Notably, the isoprenoid metabolism appears to be cross-regulated also with the level of other metabolites such as sugars [83].

In summary, good progress has been made concerning regulation of the MVA and the MEP pathways in higher plants, but some important questions remain, as e.g.:

(1) What is the nature of the transporter(s) for prenyl diphosphates?
(2) How is the metabolic flux of precursor molecules to carotenoids versus phytol for chlorophyll formation regulated?
(3) How does coupling of isoprenoid biosynthesis to carotenoid storage in chromoplasts impact metabolic flux through the pathways?
(4) To what extend is chromatin remodelling involved in the control of isoprenoid biosynthesis [96,97]?

2.2. MVA and MEP pathways in algae—variations of a theme

In phototrophs with primary plastids, the MVA pathway should have been contributed by the eukaryotic host cell during the primary endosymbiotic event. In this scenario (Fig. 2), which is visible in the streptophytes, the MVA pathway functions in the cytosol at the same time as the MEP pathway is active within the plastids [9,66–69]. In contrast, for algal groups originating from secondary endosymbiosis, the origin of the MVA pathway may not be so clear, as it could have been provided by the secondary eukaryotic host or have possible contributions from the primary host cell.

Table 1
Distribution of the MVA and the MEP pathways in examples of different oxygenic phototrophic species.

Phylogeny, organism	Pathway		Evidence
	MVA	MEP	
Cyanobacteria	–	+	Various genomic
Synechocystis sp. PCC 6714	–	+	Biochemical[g]
Primary endosymbiosis			
Glaucophyta – Glaucocystophytes			
Cyanophora paradoxa	+	+	Cloning[c], EST[a]
Rhodophyta – Red Algae			
Cyanidioschyzon merolae	–	+	Genomic[b]
Galdieria sulphuraria	+	+	Cloning[c], Biochemical[e]
Cyanidium caldarium	+	+	Biochemical[g]
Viridiplantae – Green Lineage			
Chlorophyta – Green Algae			
Chlorophyceae			
Chlamydomonas reinhardtii	–	+	Genomic[n],
Scenedesmus obliquus	–	+	Biochemical[d,e,g], EST[a]
Trebouxiophyceae			
Chlorella fusca	–	+	Biochemical[g]
Coccomyxa sp. *C-169*	–	+	Genomic[n]
Prasinophyceae			
Ostreococcus lucimarinus	–	+	Genomic[n]
Tetraselmis striata	–	+	Biochemical[i]
Streptophyta – Streptophytes			
Mesostigma viride	+	+	Biochemical[i], Genomic[c]
Embryophytes – Land plants	+	+	Biochemical[f], various genomic
Secondary endosymbiosis			
Euglenophyta			
Euglena gracilis	+	+	Biochemical[g,j], EST[a,k]
Chlorarachniophyta			
Bigelowiella natans	+	+	EST[a]
Heterokontophyta – Stramenopiles			
Chrysophyceae			
Ochromonas danica	+	+	Biochemical[g]
Bacillariophyceae – Diatoms			
Phaeodactylum tricornutum	+	+	Biochemical[h], Genomic[n]
Nitzschia ovalis	+	+	Biochemical[h]
Pelagophyceae			
Aureococcus anophagefferens	+	+	Genomic[n]
Haptophyta – Haptoflagellates			
Isochrysis galbana	?	+	EST[a]
Emiliania huxleyi	+	+	Genomic[n]
Cryptophyta – Cryptomonads			
Guillardia theta	?	+	EST[k]
Alveolata – Alveolates			
Dinophyta – Dinoflagellates	?	+	Various EST[k,o]
Apicomplexa (Non-photosynthetic, apicoplast)			
Plasmodium falciparum	–	+	Biochemical[l], Genomic[m]

This table is not comprehensive in that for chlorophytes only exemplary species were listed. '+' indicates proof of existence of pathway. '–' indicates absence of the pathway. '?' indicates unknown if present.

[a] Protist database, Canada (http://amoebidia.bcm.umontreal.ca/pepdb/searches/welcome.php).
[b] Genome Project (http://merolae.biol.s.u-tokyo.ac.jp/).
[c] Ref. [98].
[d] Ref. [59].
[e] Ref. [58].
[f] Ref. [44].
[g] Ref. [45].
[h] Ref. [123].
[i] Ref. [63].
[j] Ref. [120].
[k] Ref. [64].
[l] Ref. [206].
[m] PlasmoDB (http://PlasmoDB.org).
[n] US DOE Joint Genome Institute.
[o] Refs. [125–128].

A summary of the currently known distribution of the MVA and the MEP pathways in oxygenic phototrophs is presented in Table 1. The evolutionary aspects of isoprenoid biosynthesis have still not been fully elucidated for all algal groups, but further genomic sequencing of species is expected to provide more genomic evidence for distribution of the MVA and MEP pathways. In brief, it appears that the MVA pathway was lost several times in various (sub)groups of algae, whereas it can be hypothesized that the MEP pathway once acquired was essential for further survival of the organism. A more detailed account of the current state of research on algae is presented below.

2.3. Green algae (Chlorophyta): loss of the MVA pathway

There is ample evidence that in green algae the MVA pathway has been abandoned and that the MEP pathway supplies the build-

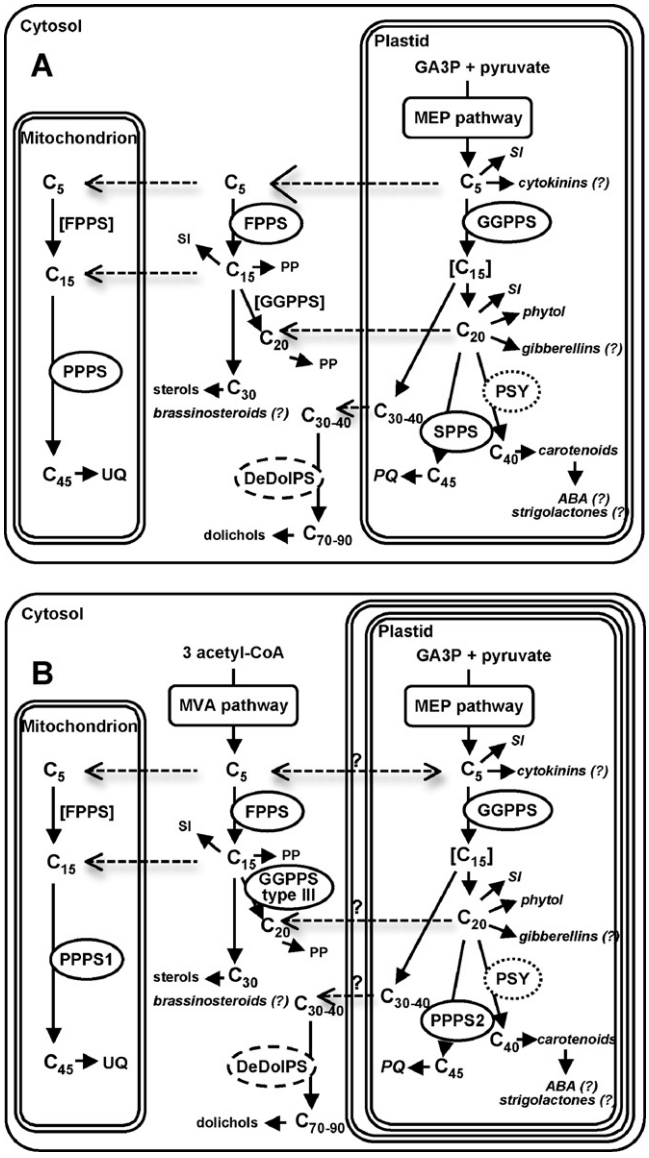

Fig. 4. Hypothetical compartmentation of isoprenoid biosynthesis in eukaryotic algae based on biochemical data and *in silico* analyses of genome sequences as discussed in the text. (A) Only the MEP pathway operates in the plastids of green algae and the red alga *Cyanidioschyzon merolae* with the consequence that the chloroplast provides the precursor molecules for all isoprenoids. (B) In heterokont algae (e.g. diatoms) derived from a secondary endosymbiosis, the MVA pathway operates in the cytosol and the MEP pathway functions in the plastids. Dashed arrows indicate putative transport of isoprenoid precursors across organellar membranes. See text for further explanations. For abbreviations, see legend of Fig. 2. Solid circles indicate *trans*-prenyltransferases, dashed circles indicates *cis*-prenyltransferase, dotted circles indicated other enzyme, brackets indicate putatively missing enzymes.

ing blocks of all cellular isoprenoids (Fig. 4A). The first biochemical evidence came through labeling studies [45,59,63], which were later corroborated by genomic evidence [98,99]. The confirmation of the sole presence of the MEP pathway in the chlorophytes means that plastids must export large amounts of isoprenoid precursor molecules for the formation of sterols in the cytosol/endoplasmic reticulum and of prenylquinones in the mitochondria (Fig. 4A). Consequently, it appears reasonable to assume that green algae had to increase the capacity of IPP/DMAPP export, possibly by new or more efficient transporters. A specific IPP/phosphate antiporter similar to the well-known triose phosphate/phosphate antiporter [100] may exist in the chloroplast envelope membrane, balancing export of an IPP molecule with import of two phosphates to maintain phosphate homeostasis. Such a transporter may be specific for green algae and may have developed very early after the split of chlorophytes and streptophytes, allowing the host cell to tap into the plastidic IPP pool as a pre-requisite to the loss of its MVA pathway.

Moreover, chlorophytes may have evolved novel regulatory mechanisms that ensure a balanced flux through the different downstream pathways such as carotenoids and sterols. This appears particularly important for green algae that accumulate large amounts of secondary carotenoids in cytosolic oil bodies. There is only limited information regarding the export of the necessary precursor molecules from the plastid. In *H. pluvialis*, ß-carotene is exported from chloroplasts into cytosolic oil bodies [101]. Another unicellular green alga, *Botryococcus braunii*, excretes botryococcene (a triterpene made from two molecules of farnesyl diphosphate) to the extracellular matrix [102,103], but no specific transport mechanisms have yet been reported.

All enzymes of the MEP pathway appear to be encoded by single-copy genes in the Chlorophyceae *Chlamydomonas reinhardtii* and *Volvox carteri*, as well as in the Prasinophyceae *Ostreococcus tauri*, and *Ostreococcus lucimarinus* [64]. Preliminary investigations of other green algal genomes indicated single-copy genes also for the Trebouxiophyceae *Chlorella variabilis* NC64A and *Coccomyxa* sp. C-169 (Polle and Lohr, unpublished). The MEP pathway in green algae has probably been best investigated in *C. reinhardtii* [61,95,104], indicating transcriptional regulation at the level of the enzymes DXS, DXR, HDR and IDI. In the unicellular alga *Dunaliella salina*, HDR is transcriptionally upregulated under various stress conditions [87]. In conclusion, the rate-limiting steps of the MEP pathway can be considered identical for streptophytes and green algae, although the MEP pathway is the only IPP biosynthetic pathway in green algae.

Unlike the other chlorophytes investigated so far, the carotenogenic alga *Haematococcus pluvialis* has a cytosolic and a plastidic IDI [105]. It appears that the massive de-novo production of IPP/DMAPP under stress-induced carotenogenic conditions in *H. pluvialis* requires specific up-regulation of the second gene copy for production of secondary carotenoids [105]. One may speculate that, as with higher plants, some green algae regulate the flux of secondary isoprenoids by differential expression of members of small gene families of enzymes for specific steps in the MEP pathway. Similar to *H. pluvialis* [106–108], many other green algae are able to accumulate secondary carotenoids in cytosolic lipid bodies [109], but these algae have not yet been investigated at the genomic level.

In contrast to *H. pluvialis*, *D. salina* accumulates secondary carotenoids in lipid globules in the chloroplast [110,111]. How the extensive biosynthesis of isoprenoid molecules is coupled to metabolite storage either in plastids of higher plants [94] and algae such as *D. salina* [108] or in cytosolic oil bodies such as in *H. pluvialis* [107] is still unknown. Currently, it is hypothesized that the regulation of such over-accumulation of secondary carotenoids in green algae occurs through photosynthetic redox control [112]. It is further proposed that concerted regulation exists between lipid biosynthesis and secondary carotenoid biosynthesis [113,114], but the details of the regulatory mechanisms are unknown.

2.4. Glaucophyta and red algae (Rhodophyta): the MVA pathway—keep it or drop it?

For the glaucophyte *Cyanophora paradoxa*, four of the six MVA genes have been cloned from cDNA, and the genome of the rhodophyte *Galdieria sulphuraria* contains homologs of all MVA genes [98]. In addition, there is biochemical evidence for the operation of both the MVA and the MEP pathways in *G. sulphuraria* [58]. Therefore, the scheme in Fig. 2 is also representative of the metabolic condition in glaucophytes and some red algae. However,

the genome of *Cyanidioschyzon merolae*, a close relative of *G. sulphuraria*, contains only a HMGS gene, while genes encoding enzymes for all subsequent reactions in the MVA pathway are absent. The predicted HMGS protein has an N-terminal extension that may target it to the mitochondria [115], indicating that the putative HMGS is involved in other metabolic processes than isoprenoid biosynthesis. Therefore, *C. merolae* is an example of an independent loss of the MVA pathway in rhodophytes [98]. The pathways shown in Fig. 4A would be assumed to be representative of this alga.

The absence of the MVA pathway in *C. merolae* may offer the opportunity to search by comparative genomic analyses of *C. merolae* and *G. sulphuraria* for genes encoding putative transporters involved in the export of isoprenoid precursors from the plastid to the cytosol. However, such a strategy is hampered by the yet incomplete coverage of the *G. sulphuraria* genome and by a large expansion of the gene families encoding putative carbohydrate and ion transporters in *G. sulphuraria* [116]. Additional information may be gained by biochemical analyses of the sterol and carotenoid content in the two rhodophytes, which could unravel differences that are related to their different modes of supplying isoprene building blocks.

In brief, currently little is known about isoprenoid biosynthesis in Glaucophyta and Rhodophyta. As more red algal genomes are being sequenced (*Porphyra purpurea* and *P. umbilicalis* at the US DOE Joint Genome Institute and *Chondrus crispus* at the Station Biologique Roscoff), we will learn more about the frequency of MVA loss among rhodophytes. As for now, the following questions arise:

(1) What is the distribution of the MVA and the MEP pathways in the Glaucophyta?
(2) Is the presence of the MVA pathway in Rhodophyta the rule or the exception?
(3) Does *C. merolae* use the same transport mechanism/transporters as the chlorophytes or has it evolved a different mechanism for the export of isoprenoid precursors from the plastid to the cytosol?

2.5. The MVA pathway in algae with secondary plastids—a genetic patchwork?

The primary endosymbiosis has been estimated to have occurred more than 1.5 billion years ago, whereas the secondary endosymbiotic events are assumed to have occurred around 1.3 billion years ago [117]. The multiple endosymbiotic events in the evolution of algae with secondary plastids resulted in the repeated merging of pathways as well as in retargeting of enzymes [6,8]. Consequently, a mosaic of genes from different source organisms may emerge for the enzymes involved in isoprenoid biosynthesis.

Specifically for the MVA pathway, the evolutionary history of the contributing enzymes may be more complex, as probably both the secondary host cell and the eukaryotic endosymbiont used the MVA pathway for the biosynthesis of cytosolic isoprenoids. Extant algae with a secondary plastid still have a cytosolic MVA pathway (Table 1 and Fig. 4B), which may have been inherited completely from the secondary host cell. Alternatively, the pathway may represent a mosaic of genes from both the primary and the secondary eukaryotic host. Such a scenario is indicated by recent phylogenetic analyses of the MVA pathway in eukaryotes [41], but needs to be subtantiated by broader sampling of algal taxa.

For many algae, the evolution of the MVA pathway may turn out to be even more complex. A replacement of the secondary plastid with a tertiary plastid by uptake of a secondary edosymbiosis-derived alga has been documented for several dinoflagellate species [6]. In these algae, three different eukaryotic host cells may have contributed genes to the extant nuclear genome. Moreover, algae with secondary plastids of red algal origin (such as diatoms, haptoflagellates or cryptophytes) may have harbored a green algal endosymbiont during their early evolution, that later was replaced by the red algal endosymbiont [64,118,119]. In such a serial endosymbiosis, again three different eukaryotic host cells may have contributed to the cellular gene pool. It is also important to keep in mind, that differences exist between the eukaryotic and the archaeal MVA pathways [41,42]. As only relatively few genomes of algae have been sequenced, it remains to be seen if there exist any enzymes in the diverse groups of algae that are of archaeal origin, possibly contributed by horizontal gene transfer.

Labeling experiments with *Euglena gracilis* [120] and EST data [64] provided evidence that both the MVA and the MEP pathways exist in Euglenophytes. As expected, the sterols were derived from the MVA pathway [45]. Plastidic carotenoids incorporated isoprene units derived from both pathways [120,121]. In contrast to what might be expected for plastid-localized phytol, the precursors for phytol biosynthesis were supplied solely by the MVA pathway [45,120], strongly suggesting an import of large amounts of precursor molecules from the cytosol to the plastids. However, the exact nature of the precursor molecules (C_5, C_{10}, C_{15}, C_{20}) and their proposed transporters are currently unkown.

EST data for the chlorarachniophyte *Bigelowiella natans* (Protist EST Program, Canada) indicate that the MVA pathway may be present (Table 1). Currently, the genome of *Bigelowiella natans* CCMP2755 is in the pipeline at the U.S. DOE Joint Genome Institute (http://www.jgi.doe.gov/genome-projects/). Therefore, novel information regarding evolutionary aspects of isoprenoid biosynthesis in these algae can be expected to be revealed soon.

For the phylum Heterokontophyta, all algae investigated so far contain both the MVA and the MEP pathways (Table 1), indicating that the situation shown in Fig. 4B applies. However, the significance of the two additional plastid membranes in heterokonts for a putative exchange of isoprenoid precursors between plastid and cytosol is not known. As expected, in the chrysophyte *Ochromonas danica* plastidic carotenoids and phytol are derived from the MEP pathway [45]. The MEP pathway also seems to be the main source for carotenoids [122] and phytol [123,124] in diatoms. However, species-specific differences in the contribution of both pathways to the formation of certain secondary isoprenoids have been reported: In the centric diatom *Rhizosolenia setigera*, the highly branched isoprenoids (HBI) are synthesized by the MVA pathway, whereas in the pennate *Haslea ostrearia*, HBI are produced principally by the MEP pathway [124].

Data on the biosynthesis of isoprenoids by either of the two pathways are sparse for the other major algal groups. The genomic analysis of the haptophyte *Emiliania huxleyi* (http://www.jgi.doe.gov/genome-projects/) indicates that both the MVA and the MEP pathways are operative (Table 1; [64]). For the Cryptophyta, the genome of *Guillardia theta* strain CCMP2712 is being sequenced at JGI (http://www.jgi.doe.gov/genome-projects/), and three genes encoding putative enzymes of the MEP pathway have been identified from EST sequences of this alga [64].

There exist no data on the presence of the MVA and MEP pathways in photosynthetic dinophytes with secondary plastids. However, EST data from the non-photosynthetic dinoflagellates *Crypthecodinium cohnii* [125], *Oxyrrhis marina* [126] and *Perkinsus marinus* [127,128] suggest that they still harbor relict plastids with a functional MEP pathway. Intriguingly, the malaria parasite *Plasmodium falciparum* (Apicomplexa) that is related to dinoflagellates and contains a relict secondary plastid termed apicoplast, uses the apicoplast-based MEP pathway as the only source of IPP [129–131]. It is not known, whether a complete loss of the MVA pathway in *Plasmodium* was only possible owing to its parasitic lifestyle or can also occur in photoautotrophic algae with secondary plastids.

In summary, it appears that the MEP pathway is essential for all plastid-bearing organisms, regardless whether the plastid

has lost its photosynthetic function and the ability to synthesize carotenoids. Moreover, all phototrophic algae with secondary plastids, that have been investigated to date, maintain the MVA pathway. Many basic questions remain regarding isoprenoid biosynthesis in oxygenic phototrophs originating from secondary endosymbiotic events, e.g.:

(1) What is the evolutionary history of the genes of the MVA pathway in the different algal groups originating from secondary endosymbiosis?
(2) How many times, if at all, has the MVA pathway been lost in phototrophic algae with secondary plastids, and what are the prerequisites for the loss?
(3) How do the additional plastid membranes affect a putative exchange of isoprenoid precursors between plastid and cytosol?
(4) Is the contribution of two different eukaryotic hosts to the genome of algae with secondary plastids also apparent in the phylogeny of the enzymes involved in sterol biosynthesis [132]?

3. Formation of linear polyprenyls by prenyltransferases: a key step in precursor allocation

3.1. Defining a core set of prenyltransferases in plants and algae

Prenyltransferases (also termed isoprenyl diphosphate synthases) generate linear isoprenoids with different chain lengths that ultimately determine the allocation of IPP/DMAPP to different isoprenoid end products. Prenyltransferases catalyze the sequential 1′-4 condensation of IPP molecules with DMAPP or another allylic prenyl diphosphate as acceptor [133,134]. Two classes of unrelated chain-elongating enzymes are known: *trans*- or (*E*)-prenyltransferases generating molecules that contain only *trans*-/(*E*)-double bonds, and *cis*–or (*Z*)-prenyltransferases forming a *cis*-/(*Z*)-double bond in each IPP unit that is added [135–137].

Only a limited number of *cis*-prenyltransferase family enzymes have been studied in detail [137,138]. The genome of *A. thaliana* encodes ten putative long-chain *cis*-prenyltransferases [139]. Two gene products were functionally characterized that catalyze the formation of dehydrodolichyl diphosphates with 15–18 isoprene units [139–141]. These dehydrodolichyl diphosphate synthases (DeDolPS) can be assumed as essential to all plants and algae, as they supply the immediate precursor of dolichol. To the best of our knowledge, the occurrence of *cis*-prenyltransferases in algae has not been investigated so far.

The *trans*-prenyltransferases are an extended protein family [135] with several members involved in the formation of essential polyprenyls in the core metabolism of isoprenoids in plants and algae (Figs. 2 and 4A and B). Farnesyl diphosphate synthase (FPPS) generates the C_{15}-isoprenoid farnesyl diphosphate necessary for sterol biosynthesis and protein prenylation. In addition, farnesyl diphosphate serves as the primer for the biosynthesis of dolichols and the isoprenoid moiety for polyprenyl quinones. The latter reaction is catalyzed by solanesyl diphosphate synthase (SPPS), yielding the C_{45}-moiety of ubiquinones and plastoquinones.

Geranylgeranyl diphosphate synthase (GGPPS) catalyzes the formation of the C_{20}-molecule geranylgeranyl diphosphate that is also used for protein prenylation and is the common precursor of phytol and of carotenoids in the plastids. Plants contain type II-GGPPS that generates geranylgeranyl diphosphate from IPP and DMAPP, whereas the type III-GGPPS from animals and fungi can only use the farnesyl diphospate supplied by FPPS as the acceptor for IPP [135]. The nuclear genome of *A. thaliana* contains 12 paralogous genes encoding putative GGPPS enzymes [62]. Two of the potential GGPPS isozymes were localized in the chloroplast, two others in the endoplasmic reticulum, and one protein was imported to the mitochondria [142]. A functional proof of GGPPS activity, however, has been gained only for plastidic isoenzymes [143–150] and potentially for one mitochondrial isoform [151]. Intriguingly, a recent report shows that in the tobacco BY-2 cell line the geranylgeranyl moiety for prenylation of cytosolic proteins is provided by the MEP pathway in the non-photosynthetic plastids [34]. If this observation can be extended to photosynthetic cells of tobacco and other plant/algal species, the cytosolic GGPPS (GGPPSc in Fig. 2) may be dispensable in photosynthetic eukaryotes.

Geranyl diphosphate synthase (GPPS) is mainly implicated in the biosynthesis of secondary monoterpenes [35,152] and therefore would not meet our definition of an essential polyprenyltransferase. However, downregulation of expression of (homodimeric) GPPS in tomato and in *A. thaliana* resulted in dwarfed plants with a decreased content of gibberellins, indicating an involvement of the enzyme in gibberellin biosynthesis [153] and arguing for an essential role of GPPS at least in higher plants. Intriguingly, the enzyme from *A. thaliana* originially described as a GPPS [152] catalyzes the *in vitro* formation of multiple *trans*-polyprenyls ranging from C_{25} to C_{45}, prompting its reassignment as a polyprenyl diphosphate synthase (PPPS [154]). Moreover, the genomes of *A. thaliana* and many other seed plants also encode a protein closely related to the small subunit (SSU) of the heterodimeric GPPS, that in spearmint (*Mentha piperita*) supplies geranyldiphosphate in the production of secondary isoprenoids [155]. The large subunit (LSU) of the heterodimeric GPPS has high sequence similarity to GGPPS. In an *in vitro* assay using IPP and DMAPP as substrates, the GPPS-LSU from hop (*Humulus lupulus*) generated geranylgeranyl diphosphate as the major product, whereas LSU-SSU heterodimers preferentially synthesized geranyl diphosphate [155]. Similar results were obtained with the corresponding proteins from snapdragon (*Antirrhinum majus*) *in vitro* [156] and *in vivo* [157]. These observations suggest that the major *in situ*-function of the homomeric GPPS [152,154] is the formation of long-chain polyprenyls such as solanesyl diphosphate used in the biosynthesis of plastoquinone. The GPPS-SSU mediates the formation of geranyl diphosphate for monoterpenes by altering the product specificity of GGPPS. Consequently, homomeric GPPS/PPPS would join our list of essential/ubiquitous polyprenyl transferases.

Multiple candidate genes for the different polyprenyltransferases have been identified in several algal genomes [64,99,158]. Still, the occurrence and distribution of these enzymes across the major algal groups has not been studied in detail. One reason may be the sequence similarity of the different family members that complicates the *in silico* prediction of function and consequently the correct annotation of newly discovered sequences. However, the pattern of isoprenoids common to all plants and algae allows speculating on the minimal inventory of polyprenyltransferases that may be necessary to support the core metabolism of isoprenoids (Figs. 2 and 4A and B). The minimum set of cytosolic transferases would include FPPS, GGPPS with the reservations noted above, and potentially also DeDolPS. That of plastids would comprise another GGPPS and SPPS (homomeric “GPPS” with PPPS-activity), and that of mitochondria additional FPPS and SPPS enzymes, comprising a total of one *cis*- and six *trans*-prenyltransferases. The validity of this prediction is corroborated by the presence of multiple copies of FPPS [159,160] or SPPS [161–163] in land plants, that localize to different compartments. It should be kept in mind, however, that these predictions are based on the simplistic assumptions, that: (i) no significant exchange of polyprenyls occurs between cytosol, plastids and mitochondria; (ii) none of the polyprenyltransferases is targeted to multiple compartments; and (iii) each polyprenyltransferase uses a single substrate as the initial acceptor for IPP and catalyzes the formation of a single product. As exceptions to each of

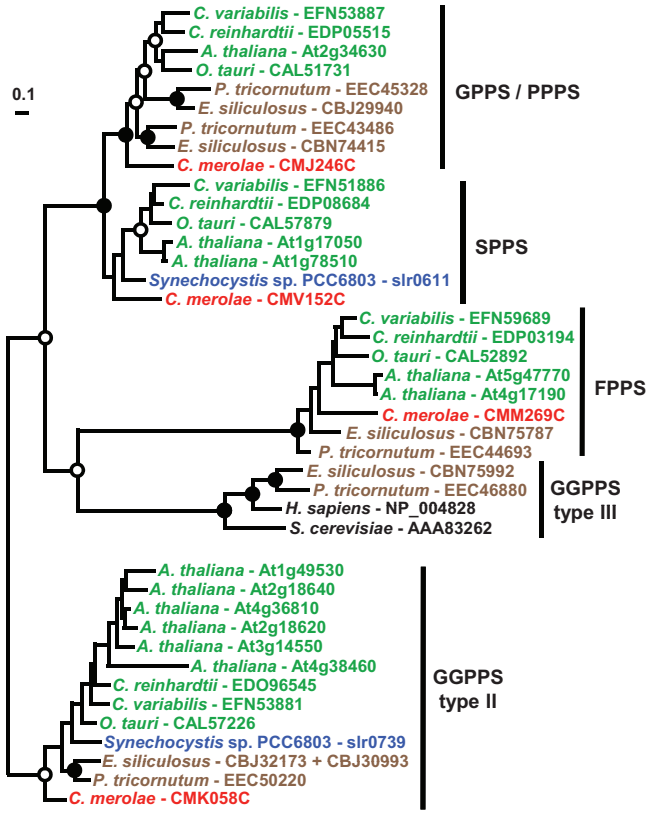

Fig. 5. Phylogenetic tree of the *trans*-prenyltansferases from selected oxygenic phototrophs. Maximum likelihood (RAxML) tree constructed from a protein alignment including 321 amino acid positions. GenBank accessions of sequences are provided behind species names. Black dots indicate ≥90% levels and white dots indicate 70–90% levels of bootstrap support by analyses with RaxML (100 replicates) [203] and PHYML (100 replicates) [204], and of Bayesian posterior probabilities using MrBayes [205], respectively. GPPS geranyl diphosphate synthase; for other abbreviations, see legend of Fig. 2 and text. In the on-line version of this figure, sequences from Cyanobacteria are in blue, from Viridiplantae in green, from Rhodophyta in red, from Heterokontophyta in brown, and from other organisms in black.

the three assumptions have been repeatedly documented, a complement of less than six *trans*-prenyltransferases may be sufficient for the biosynthesis of all essential isoprenoids.

As a first attempt to evaluate our predictions, we searched the publicly available genome data of the rhodophyte *C. merolae*, the green algae *Ostreococcus tauri*, *C. reinhardtii* and *Chlorella variabilis*, and the heterokont algae *Phaeodactylum tricornutum* and *Ectocarpus siliculosus* for *trans*-prenyltransferase candidates. We analysed the phylogenetic relations of these proteins to prenyltransferases from *A. thaliana* and selected other organisms. All algae that were examined contain unique candidates for type-II GGPPS and for FPPS (Fig. 5). The supply of both plastid and cytosol with geranylgeranyl diphosphate and of both cytosol and mitochondria with farnesyl diphosphate may be achieved either by dual targeting of GGPPS and FPPS or by transport of their polyprenyl products across the envelope membranes of plastids and mitochondria, respectively.

Green and red algae additionally possess orthologs of SPPS and of the homodimeric "GPPS" from *A. thaliana* that has PPPS-activity [154]. These two prenyltransferases may supply solanesyl diphosphate for ubiquinone and plastoquinone (Fig. 4A), respectively. The heterokont algae appear to completely lack orthologs of SPPS. Instead, they contain two paralogs with close affiliation to the GPPS/PPPS of Viridiplantae. Again, one of the two paralogs may supply solanesyl diphosphate for plastoquinone biosynthesis in plastids, while the other may sustain the biosynthesis of ubiquinone in mitochondria. Notably, the heterokont algae contain a second GGPPS gene encoding a fungal/animal type-III GGPPS. Our preliminary phylogenetic analyses in Fig. 5 suggest that this gene was inherited from the secondary eukaryotic host cell and that the enzyme may still be localized in the cytosol, possibly supplying geranylgeranyl diphosphate for protein prenylation (Fig. 4B).

To lend further support to the phylogenetic analyses and predicted function of the various prenyltransferases in algae, we are currenly searching for prenyltransferase genes in additional algal genomes and for the presence of potential organellar targeting sequences in the various prenyltransferases (Lohr, unpublished). The general characteristics of the N-terminal targeting sequences for the import of proteins into plastids are conserved among the Viridiplantae, red algae and glaucophytes [164]. However, the targeting peptides that govern the transport of proteins into primary plastids have no conserved primary sequence and share some features with mitochondrial targeting sequences [165], that may even result in dual targeting of proteins [166]. Therefore, it is not possible to reliably assign a protein as plastidic or mitochondrial based solely on *in silico* analyses. In algae with secondary plastids such as the diatoms, the transport of proteins across the four plastid membranes is facilitated by a bipartite targeting sequence that is comprised of an N-terminal endoplasmic reticulum-type signal peptide followed by a transit peptide typical of proteins imported into primary plastids [167]. This difference allows the unambiguous assignment of most nucleus-encoded organellar proteins to either mitochondria or plastids, making sequence-based predictions of the subcellular location of proteins in these algae a particularly powerful tool (see e.g. [168]).

3.2. Regulation of precursor allocation by prenyltransferases: some examples

Prenyltransferases occupy a central position in the biosynthetic network of isoprenoid biosynthesis. They can be regarded as shunting switches that control the partitioning of precursors from the cellular isoprene pools to various branches of isoprenoid metabolism. Thus, a better understanding of prenyltransferase regulation in algae will aid in engineering algal isoprenoid metabolism for the production of high-value isoprenoids.

The distribution of the various prenyltransferases between cytosol, plastids and mitochondria establishes a regulatory framework, as discussed in the previous section. Within a cellular compartment, prenyltransferase activities can be modulated in various ways. One possibility is by transcriptional regulation, and a concerted expression of prenyltransferases and enzymes catalyzing downstream reactions is often observed. Examples for this type of regulation are the tightly linked expression of GGPPS and phytoene synthase (PSY, Figs. 2 and 4) in the biosynthesis of carotenoids in *C. reinhardtii* [95] or the concerted expression of GPPS-SSUs with monoterpene synthases for the production of secondary monoterpenes in *Humulus lupulus* [155]. A related mechanism is the duplication of prenyltransferase genes and their tandem arrangement together with genes for downstream enzymes. In the nuclear genome of *A. thaliana*, several putative GGPPS genes are arranged in a cluster and contiguous with terpene synthase genes [169], indicating a repeated tandem duplication of the GGPPS and terpene synthase genes.

Another very effective way to channel precursors into a specific pathway is by formation of complexes of sequential metabolic enzymes [170,171]. The formation of multienzyme complexes has been proposed for several steps in the biosynthesis of carotenoids [172], and there is experimental evidence for a metabolite channeling between GGPPS and PSY from plants [173], which may ensure the preferential allocation of precursors towards carotenogenesis. The binding of GPPS-SSU proteins to GGPPS that results in the preferential formation of geranyl diphosphate instead of

geranylgeranyl diphosphate [155,157] can be viewed as a related mechanism of (re)directing precursors into specific pathways. It remains to be investigated to what extent the various regulatory mechanisms observed in land plants contribute to the coordinated allocation of isoprenoid precursors by polyprenyltransferases in algae.

4. Perspectives

4.1. Basic research

As indicated above, many open questions remain regarding basic research on algal isoprenoids. The fundamental question of the distribution and evolution of the isoprenoid biosynthetic pathways in the different lineages of oxygenic phototrophs is expected to be resolved at a much more detailed level in the near future due to the rate at which genomic and transcriptomic data are currently being published. Several algal genomes are at various stages of sequencing and others have ongoing EST projects [174]. Such genome analyses are expected to help (re)define the position of some early-diverging algal groups in the green lineage, as for example the detection of the MVA pathway in the green alga *Mesostigma viride* supported its phylogenetic affiliation to early diverging streptophytes [98].

Other important issues that need to be addressed in the different algal groups are the mode and the magnitude of transport of precursor isoprenoid molecules across organellar membranes and the regulation and metabolic integration of isoprenoid biosynthesis. Although the genomic studies provide a baseline for gene discovery, further targeted systems biology approaches are necessary to discover common and/or group specific transport as well as regulatory mechanisms governing not only primary, but specifically secondary isoprenoid metabolism.

4.2. Engineering of isoprenoid metabolism in algae

As many isoprenoids have applications in animal feed, human nutrition, cosmetics, and biofuels, current research on algal isoprenoid metabolism is driven by applied research that is aimed at increasing productivities as well as alterations in profiles obtained by algae. With the recent strong interest in use of microalgae for biofuel production, genetic engineering of lipid metabolism in microalgae appears to be one of the major thrust areas of applied microalgal research [175–178]. In principle, algae have great potential to serve as cell factories for the production of high-volume IPP/DMAPP based biofuels molecules as well as for high-value specialty compounds such as ketocarotenoids. However, most unicellular green algae such as *D. salina* [110] or *H. pluvialis* [107] over-accumulate these secondary carotenoids only when the cells are exposed to abiotic stress, resulting in the concomitant accumulation of storage lipids and a strong reduction or even cessation of growth. Some retardation of growth appears unavoidable, because the accumulation of storage lipids requires a massive input of energy and carbon but at the same time supplies the lipophilic compartment that is necessary for carotenoid accumulation. This "conflict of interests" applies to the accumulation of other high-value metabolites as well. Therefore, obtaining sufficiently high levels of special isoprenoid molecules in conjunction with growth reductions that are still tolerable will be a major challenge regarding metabolic engineering of algae. Manipulation of metabolic flux by increasing flux, shifting flux, or introduction of new functionalities will require a thorough understanding of the basic isoprenoid metabolism of the chosen species. Strategies will have to take into account that metabolic and regulatory networks differ between land plants, green algae, red algae, and the various groups of algae originating from secondary endosymbiosis.

Examples for recent approaches on general metabolic engineering in microalgae include attempts to redirect metabolic flux from carbohydrates to lipids by eliminating starch biosynthesis [179,180]. These approaches were only met with partial success, as the level of lipids in starchless mutants did not increase during the logarithmic growth phase. Only when cells entered the stationary phase of growth or were exposed to abiotic stress in the form of nutrient deprivation, more lipids were made than in the wild type cells. These results demonstrated that a prediction of redirection of metabolic flux is not straightforward and that reduction of carbon flux into carbohydrates may not necessarily have an impact on secondary isoprenoid metabolism.

The choice of suitable algal strains for metabolic engineering is another factor that may have significant impact on the results [181], but even more important is an understanding of the complexity of regulation of isoprenoid biosynthesis at the molecular level. This became apparent in many studies on manipulation of carotenoid content/composition in higher plants [73,182–184]. In principle, it might be expected that metabolic engineering in algae should be easier to accomplish than in higher plants, because algae lack the necessity for organ and plant development regulated by plant hormones. Nevertheless, metabolic engineering in algae may not be as straightforward as anticipated. Recent attempts to down-regulate phytoene synthase by antisense RNA [185] or the heterologous expression of a ketolase gene from *H. pluvialis* in *C. reinhardtii* [186] resulted in much smaller changes of the carotenoid profile than expected. Moreover, some of the the isoprenoid hormones known from higher plants may also have regulatory functions in algae [187], which has been a neglected area of research. Therefore, metabolic engineering of isoprenoid metabolism in algae may hold further surprises.

Overall, a variety of key strategies for pathway engineering within the isoprenoid metabolism in algae could be considered, which may require combinatorial approaches targeting the interface between primary and secondary isoprenoid metabolism. One strategy for engineering is the re-direction and enhancing of metabolic flux into an endogenous downstream product. This approach will draw more molecules into the pathway of choice, but may result in depletion of precursor molecule pools leading to negative side effects [182]. A successful example for this strategy is the heterologous expression of a phytoene synthase gene from *D. salina* in *C. reinhardtii*, resulting in an increased carotenoid content [188].

Another possibility is the downregulation of particular enzymes leading to the accumulation of a precursor molecule in an existing pathway. This was demonstated in the green alga *D. salina*, where phytoene accumulated instead of ß-carotene when inhibitors of the phytoene desaturase were administered [189,190]. However, down-regulation of phytoene desaturase in *C. reinhardtii* by antisense RNA did not dramatically change the carotenoid profile, although the PDS expression level was reduced to less than 10% [185].

A third approach to increase the content of specific isoprenoid molecules would be an increased supply of IPP/DMAPP by up-regulation of the MVA and/or the MEP pathways. In addition, an enhanced expression of prenyltransferases could result in higher levels of isoprenoids in specific downstream pathways. These strategies have been successfully applied in land plants by targeting the MVA pathway (HMGR [191]), the MEP pathway (DXS [192]; DXR [191]) and the prenyl transferases [193].

In addition to manipulating endogenous enzymes, new pathways may be introduced to harness algae as cell factories for production of designer isoprenoids. This was demonstrated for higher plants by introduction of ketocarotenoid production [194,195]. For cyanobacteria, the successful, introduction of new pathways was demonstrated by the production of isoprene [196]

and β-caryophyllene [197] in *Synechocystis* sp. PCC 6803. Overall, genetic engineering with cyanobacteria, specifically *Synechocystis*, may serve as a model for eukaryotic oxygenic phototrophs.

Recently, a novel engineering approach has been taken, which advocates the creation of intracellular metabolic sinks in plants [198]. For algae, even bypassing of intracellular sinks by milking [199] or by excretion [200] has been proposed. Enhancing the intracellular metabolic sink capacity may become feasible for microalgae, because recently the green algal homolog of the *Or* gene, which in cauliflower is involved in the differentiation of proplastids or other non-colored plastids into carotenoid-rich chromoplasts [198], was cloned from *C. reinhardtii* [95]. Similar to the conversion of chloroplasts into chromoplasts occurring in streptophytes, the green alga *D. salina* can over-accumulate ß-carotene in plastidic carotene globules to up to 10% of the cellular dry weight [111]. If it should turn out that an algal homolog of the *Or* gene is involved in the accumulation of secondary isoprenoids in *D. salina*, it may also be possible to increase the carotenoid content in other algae by (over-)expression of the *Or* gene.

In conclusion, the age of metabolic engineering of algae has just begun. With the gaps in our knowledge on isoprenoid biosynthesis in the various algal groups narrowing and more genomic data becoming available, it should be possible to use algae as cell factories for the production of any isoprenoid molecule desired. For this to become reality, however, it will be necessary to go beyond the one-gene-at-a-time approaches and pursue a more global strategy of metabolic engineering.

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