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Athanasakoglou, Anastasia; Kampranis, Sotirios C.

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Research review paper

Diatom isoprenoids: Advances and biotechnological potential

Anastasia Athanasakoglou¹, Sotirios C. Kampranis*

Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark



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ABSTRACT

Diatoms are among the most productive and ecologically important groups of microalgae in contemporary oceans. Due to their distinctive metabolic and physiological features, they offer exciting opportunities for a broad range of commercial and industrial applications. One such feature is their ability to synthesize a wide diversity of isoprenoid compounds. However, limited understanding of how these molecules are synthesized have until recently hindered their exploitation. Following comprehensive genomic and transcriptomic analysis of various diatom species, the biosynthetic mechanisms and regulation of the different branches of the pathway are now beginning to be elucidated. In this review, we provide a summary of the recent advances in understanding diatom isoprenoid synthesis and discuss the exploitation potential of diatoms as chassis for high-value isoprenoid synthesis.

1. The unusual physiology of diatoms provides molecules with high biotechnological potential

Diatoms (Superphylum: Heterokonta/Stramenopiles, class: Bacillariophyceae) (Cavalier-Smith, 2018) are unicellular photosynthetic eukaryotes that include approximately 100,000 species distributed worldwide, in almost all aquatic habitats (Armburst, 2009; Malviya et al., 2016). With 40% estimated contribution to the marine primary production, they are one of the most significant groups of phytoplankton in contemporary oceans (Falkowski, 2002; Field et al., 1998; Nelson et al., 1995). Their ecological success has prompted research on their evolution and metabolism and unveiled a unique combination of features that are particularly useful for a range of potential biotechnological applications (Allen et al., 2011; Bozarth et al., 2009; Damsté et al., 2004; Kooistra et al., 2007; Obata et al., 2013).

Many of the unique features of diatoms are the result of their distinctive evolution. Unlike cyanobacteria, green algae, and red algae, diatoms arose from the heterokont lineage that evolved through secondary endosymbiosis, when a heterotrophic eukaryote engulfed a red/green algal related cell. (Falkowski et al., 2004; Yoon et al., 2004). This incorporation has shaped a multi-sourced genetic background that provided them with a complex and flexible metabolism, and a broad diversity of metabolites. Among them, there are different isoprenoids (Stonik and Stonik, 2015). This group of ubiquitous secondary metabolites comprises a family of biotechnologically relevant molecules with well-known applications in pharmaceutical, cosmetics, food and

fuel industries. Well-known examples are the anti-cancer drug taxol and the widely-used antimalarial agent artemisinin (George et al., 2015; Tippmann et al., 2013). Due to the broad interest in these molecules, remarkable effort has been put into engineering heterologous isoprenoid production, with many isoprenoids currently being produced in microbial hosts (Ajikumar et al., 2010; Ignea et al., 2018; Meadows et al., 2016; Ro et al., 2006; Vickers et al., 2017; Zhou et al., 2015). Novel isoprenoid structures are continuously characterized and alternative microbial platforms for sustainable synthesis of biotechnologically relevant molecules are always on the spotlight (Lauersen, 2018; Vavitsas et al., 2018).

Diatoms are a good source of isoprenoids and in addition to well-known, conserved structures, species-specific molecules have also been reported (Table 1). Marine isoprenoids in general are often characterized by distinct properties compared to their counterparts originating from terrestrial organisms (Stonik and Stonik, 2015). With increasing information from genomic and transcriptomic data being integrated in recent years (Keeling et al., 2014), there has been considerable progress in the elucidation of different branches of diatom isoprenoid biosynthesis.

In this review, we summarize the advances on the elucidation of isoprenoid pathways in diatoms and present metabolic engineering efforts to improve the biosynthetic yields of isoprenoids of interest in diatom platforms. By providing this comprehensive overview, we aim to highlight the potential for bioproduction of diatom-specific molecules in heterologous hosts, such as yeast, bacteria, or other algae, and

* Corresponding author.

E-mail address: soka@plen.ku.dk (S.C. Kampranis).¹ Present address: Swiss Federal Institute of Aquatic Science and Technology, Eawag, 8600 Dübendorf, Switzerland.

Table 1
Main isoprenoids identified in diatoms and their applications/properties.

	Isoprenoid	Applications/Properties	Reference
Carotenoids	Fucoxanthin	Anti-oxidant, anti-obesity, anti-inflammatory	Maeda (2015), Sachindra et al. (2007), Tan and Hou (2014), Zhang et al. (2015)
	β -carotene	Anti-oxidant	Russell and Paiva (1999)
	Diatoxanthin, diadinoxanthin	Health promoting agents	Sathasivam and Ki (2018)
Sterols	Phytosterols, ergosterol	Various health promoting effects	Kim and Ta (2011), Li et al. (2015), Moreau et al. (2002)
HBIs	C25 isomers	Sea-ice proxies, cytostatic effects, anti-HIV	Belt and Müller (2013), Collins et al. (2013)
	C30 isomers	Biofuels	Niehaus et al. (2011)
Other	Domoic acid	Neurotoxin	Brunson et al. (2018)

discuss the possibility for the development of diatoms into dedicated platforms for the industrial-level production of isoprenoids.

2. Diatoms synthesize diverse isoprenoids

Isoprenoid structures reported from diatoms are characterized by a structural complexity that has not been observed in algal or terrestrial organisms and a number of interesting isoprenoids, either conserved or species-specific, have been isolated. Considering the enormous diversity that characterizes diatoms, the evidence for significant horizontal gene transfer between diatoms and bacteria (Amin et al., 2012), and that the majority of diatom species has not been chemically characterized, it is probable that additional interesting isoprenoid structures will be revealed by ongoing or future studies.

The components of diatoms' photosynthetic machinery, including carotenoids, have attracted considerable interest as they facilitate high photosynthetic efficiency, photoprotection and photoacclimation. Their main light harvesting pigment, fucoxanthin, is an oxygenated xanthophyll derived from β -carotene. The latter, together with other xanthophylls, like diadinoxanthin and diatoxanthin, participates in non-photochemical quenching that facilitates the dissipation of excess light energy as heat (Kuczynska et al., 2015). Fucoxanthin has a distinctive structure consisting of an unusual allenic bond, 9 conjugated double bonds, a 5,6-monoepoxide and additional oxygenic functional groups. Each of these features is believed to contribute to the broad range of biological properties of the molecule (Zhang et al., 2015), including its anti-oxidant (Sachindra et al., 2007), anti-obesity (Maeda, 2015) and anti-inflammatory (Kim et al., 2010; Tan and Hou, 2014) effects. The deacetylated derivative of fucoxanthin, fucoxanthinol, has shown anti-neoplastic activity and good potential for use in both prevention and treatment of different types of cancer (Martin, 2015). The low toxicity levels of these molecules (Kadekar et al., 2008) make them safe pharmaceutical ingredients, facilitating applications in human health. Two other diatom specific xanthophylls, diatoxanthin and diadinoxanthin (Lohr and Wilhelm, 1999), also contain allenic bonds and acetyl- and epoxy- groups in their structures, features that may convey potential bioactivities (Sathasivam and Ki, 2018).

Sterols are molecules that are found in all eukaryotic organisms, as membrane structural components (Dufourc, 2008). Brassicasterol, the main sterol of *Phaeodactylum tricornutum*, was recently shown to be part of the lipid monolayer on the surface of lipid droplets formed under nitrogen stress (Lupette et al., 2019). Apart from free sterols, many microalgal species, including different diatom species, contain conjugated forms of these molecules. *Thalassiosira pseudonana* was found to contain most of its sterols in esterified form, while more than 50% of the sterols in *Chaetoceros calcitrans* are glycoconjugated (steryl glucosides and acylated steryl glucosides). *Haslea ostrearia* also contains 23,23-dimethylcholesterol-5-en-3 β -ol in its glycoconjugated form (Véron et al., 1998). Although the biological roles of such molecules are not fully understood, they most probably participate in cellular stress response mechanisms by regulating the action of hormonal and environmental signals (Stonik and Stonik, 2018). Steryl glycosides also play a role in biosynthesis of polysaccharides in plants (Grille et al., 2010; Peng et al., 2002), while sterol sulfates in *Skeletonema marinoi*

regulate cell death (Gallo et al., 2017).

A distinctive characteristic that highlights the diversity within diatoms, is the heterogeneity of their sterol content. A study on 106 diatom species, representing all major groups, revealed that they contain a variety of C27-C31 sterols, none of which is unique or representative of species or order (Rampen et al., 2010). Even though all structures reported have been identified in other algal classes, the fact that diatoms can produce ergosterol (typically found in fungi) as an intermediate in the sterol biosynthetic pathway of *P. tricornutum* (Fabris et al., 2014), cholesterol (typically found in animals) as the major sterol of *Cylindrotheca fusiformis* and *Nitzschia closterium*, and different other phytosterols, depending on the species, highlights the complexity of the evolution of their biosynthesis. In addition, diatoms also produce steroidal ketones, however their biological functions are currently unknown (Rampen et al., 2010).

Highly Branched Isoprenoids (HBIs) is a unique group of isoprenoids, reported in specific diatom genera, namely *Haslea*, *Rhizosolenia*, *Pleurosigma*, *Navicula* and *Berkeleya*. The most common structures have 25 or 30 carbon atoms and 1–6 double bonds (Belt et al., 2000). However, several monocyclic compounds, in addition to epoxides, alcohols, thiolanes and thiophenes, have also been reported (Belt et al., 2000, 2006; Massé et al., 2004a, 2004b). It has been proposed that these molecules serve as membrane constituents and synthetic HBIs were shown to form stable vesicles in a pH-dependent manner. Branched phosphates show lower water permeability comparing to non-branched analogues and structural parameters such as the position of double bonds can further affect robustness of the vesicles (Gotoh et al., 2006). Even though their exact biological roles remain elusive, their applications are well established. They are extensively used as sea-ice proxies in paleoenvironmental studies. Sea-ice is a major catalyst on oceanic and atmospheric processes and, as a consequence, on global climate. Using climate models based on HBI content, it is possible to reconstruct changes in sea-ice coverage, interpret past climate conditions and predict future climate states (Belt and Müller, 2013; Collins et al., 2013). In addition, due to their long, branched structure, they have been considered as good candidates for biofuel production through the hydrocracking process, similar to botryococenes, branched isoprenoids produced by the green algae *Botryococcus braunii* (Hillen et al., 1982; Niehaus et al., 2011). Production of other isoprenoids, like farnesene, in heterologous systems, such as yeast, are already being exploited as a renewable form of fuel with production titers reaching 130 g/L (Meadows et al., 2016). Because HBIs are more saturated, hence more energy dense, but still derived from the same precursor (farnesyl diphosphate; FPP), they could offer a promising alternative to farnesene in this production system. Specific C25 HBIs have shown *in vitro* cytostatic effects against human lung cancer cell lines, activity that depends on the degree of unsaturation, with the most unsaturated being the most potent (Rowland et al., 2001b).

Finally, domoic acid, a molecule synthesized from the isoprenoid precursor geranyl diphosphate, is a mammalian neurotoxin produced in algal blooms of *Pseudo-nitzschia* diatoms. Domoic acid can have severe health effects both on aquatic life and human health. The recent elucidation of its biosynthesis aims to provide better monitoring and risk

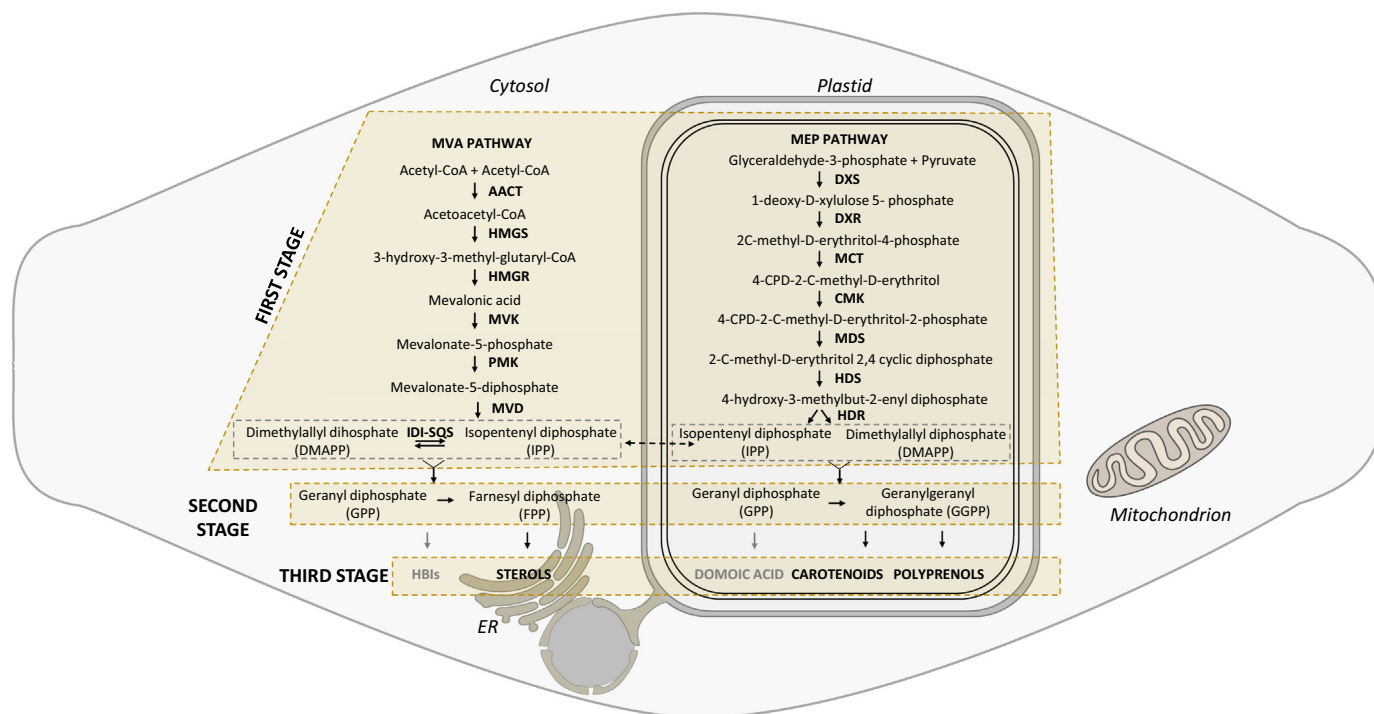


Fig. 1. The three stages of isoprenoid biosynthesis in diatoms. AACT; Acetyl-CoA c-acetyltransferase, HMGS; hydroxy-methylglutaryl-CoA synthase, HMGR; hydroxyl-methylglutaryl-CoA reductase, MVK; mevalonate kinase, PMK; phospho-mevalonate kinase, MVD; mevalonate diphosphate decarboxylase, DXS; 1-deoxy-D-xylulose 5-phosphate synthase, DXR; 1-deoxy-D-xylulose 5-phosphate reductoisomerase, MCT; 2-C-methyl-D-erythritol-4-phosphate-cytidyltransferase, CMK; 4-diphosphocytidyl-2c-methyl-d-erythritol kinase, MDS; 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase, HDS; (E)-4-hydroxy-3-methylbut-2-enyl diphosphate synthase, HDR; 4-hydroxy-3-methylbut-2-en-1-yl diphosphate reductase, IDI-SQS; isopentenyl diphosphate isomerase fused to squalene synthase, GPPS; geranyl diphosphate synthase, FPPS; farnesyl diphosphate synthase, GGPPS; geranylgeranyl diphosphate synthase.

assessment of harmful diatom blooms by establishing the environmental conditions that induce its synthesis and excretion (Brunson et al., 2018).

3. Isoprenoid biosynthesis in diatoms through a metabolic engineering perspective

Isoprenoid biosynthesis is a well-studied biochemical pathway in all domains of life. In general, it can be divided into three stages (Fig. 1). The early stage, during which the universal C5 building blocks, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), are synthesized via the mevalonate (MVA) and the methylerythritol phosphate (MEP) pathways. The central stage, which involves the linear condensation of the isoprene units for the formation of prenyl diphosphates of different lengths. And the late stage, in which the prenyl diphosphates are allocated to different branches of the pathway, where they are further modified to give rise to final products (carotenoids, sterols, etc.) (Vranová et al., 2012). In the following section, we will focus on the differences observed in diatoms in these three stages, comparing to other organisms. In each stage, we will outline both tested and potential metabolic engineering efforts towards increased bioproduction of specific isoprenoid compounds in different expression systems. To enable unambiguous identification of the enzyme used in each example discussed below, we are providing NCBI accession numbers in parenthesis next to the protein name.

3.1. Precursor supply via the MVA and MEP pathways

In contrast to other algal classes, which possess the MEP pathway but may have lost the MVA route for IPP and DMAPP synthesis (Lohr et al., 2012), diatoms seem to have retained both. This first became evident during isotope tracer experiments (Cvejić and Rohmer, 2000; Massé et al., 2004a, 2004b) and was later confirmed by transcriptomic

sequencing of several species (Athanasakoglou et al., 2019; Di Dato et al., 2015; Fabris et al., 2014). The two pathways generate precursors that are allocated to different isoprenoid end-products (Cvejić and Rohmer, 2000; Massé et al., 2004a, 2004b; Zhang et al., 2009). In other studied organisms (bacteria, fungi, mammals), the consensus is that sterols are synthesized in the cytosol via MVA generated precursors, while the MEP pathway is responsible for the synthesis of carotenoids, phytol and other plastidic molecules. This pattern is followed by the diatom species *P. tricornutum* and *Nitzschia ovalis* that synthesize their main sterols -epibrassicasterol and cholestadienol, respectively- via the MVA pathway using acetate as carbon source, as demonstrated by experiments with labelled acetate under mixotrophic conditions. The same species produce phytol using CO₂ that is converted to IPP/DMAPP via the MEP route (Cvejić and Rohmer, 2000). The centric diatom *Rhizosolenia setigera* also uses the cytosolic pathway to synthesize sterols and HBIs. On the contrary, in the pennate diatom *H. ostrearia*, HBIs and β-sitosterol incorporate precursors from the MEP pathway (Massé et al., 2004a, 2004b). In *T. pseudonana*, there is complementarity between the two pathways in the generation of precursors for sterol synthesis, under fast growing, nitrogen-replete culture conditions (Zhang et al., 2009). Taken together, these data indicate that precursor allocation in diatoms might be species specific and/or that external conditions might affect the regulation of the two pathways. Recently, our group proposed that possible crosstalk between the MVA and MEP pathways and transport of intermediate metabolites between plastids and cytosol is likely involved in the synthesis of sterols in *H. ostrearia* (Athanasakoglou et al., 2019). Evidence for a similar crosstalk has already accumulated in higher plants (Bick and Lange, 2003; Hemmerlin et al., 2003a; Laule et al., 2003; Skorupinska-Tudek et al., 2008); and recently, three previously unrelated enzymes were found to participate in the regulation of the isoprenoid pathway. An isopentenyl phosphate kinase (IPK) and two nudix hydrolases regulate the levels of IP and IPP by catalyzing active phosphorylation/

dephosphorylation reactions. Perturbation of the IPP:DMAPP ratio affects the yields of both MVA and MEP derived isoprenoids, suggesting an involvement in the crosstalk mechanism between the two pathways (Henry et al., 2015, 2018). IPK and nudix hydrolase homologues seem to be expressed in different diatom species and, thus, a similar regulatory process may contribute to the transport of precursors between subcellular compartments in diatoms and requires further investigation. (Athanasakoglou et al., 2019).

A distinctive feature of most diatoms is that they possess an isopentenyl diphosphate isomerase (IDI) that is fused to a squalene synthase, the enzyme that catalyzes the first committed step towards sterol biosynthesis (Davies et al., 2015). This fusion has been characterized in the species *P. tricornutum* (reconstructed from XP_002180941.1 and XP_002180940.1), *R. setigera* (MF351614) and *H. ostrearia* (AYV97147.1) (Athanasakoglou et al., 2019; Fabris et al., 2014; Ferriols et al., 2017). In the latter species, the enzyme seems to be localized at the endoplasmic reticulum (ER) membrane, where an FPP synthase (AYV97141.1) is also present. This co-localization supports previous speculations on the formation of a metabolic complex that facilitates the efficient channeling of precursors to sterol biosynthesis (Athanasakoglou et al., 2019; Davies et al., 2015).

Enzymes involved in the MVA and MEP pathways are among the first targets of any metabolic engineering effort, as the supply of precursors is a key point of consideration. Extensive research and diverse experimental evidence on plant, bacterial and cyanobacterial isoprenoid biosynthesis has shown that a main bottleneck of the MEP pathway is the step catalyzed by 1-deoxy-D-xylulose 5-phosphate synthase (DXS) (Cordoba et al., 2009; Han et al., 2013; Kudoh et al., 2014). Other enzymes, like 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MDS), 4-hydroxy-3-methylbut-2-en-1-yl diphosphate reductase (HDR) and IDI have also been reported to have a rate-limiting role in the pathway, but this role varies among different organisms and conditions (Botella-Pavía et al., 2004; Carretero-Paulet et al., 2006; Cordoba et al., 2009; Mahmoud and Croteau, 2001; Veau et al., 2000; Walter et al., 2000). Consistently, DXS appears to also play a crucial role in diatoms' MEP pathway. In light-stress experiments in *P. tricornutum*, dark-adapted cultures transferred to light showed a constant increase of DXS mRNA levels. This induction was proposed to increase flux of precursors and boost synthesis of carotenoids, which are molecules with photo-protective roles. Introduction of *PtDXS* (XP_002176386.1) in *E. coli* resulted in a 1.5-fold increase in β -carotene formation, an intermediate in the carotenoid pathway. When the same gene was overexpressed in *P. tricornutum*, fucoxanthin synthesis increased 2.4 times comparing to wild type strains, while other intermediates of the pathway, namely diadinoxanthin and β -carotene, were also accumulated at high levels (Eilers et al., 2016a). These experiments further confirmed the rate-limiting role of DXS in diatoms and initiated efforts on the metabolic engineering of *P. tricornutum* for isoprenoid biosynthesis.

The enzyme responsible for the reductive dehydroxylation of (E)-4-hydroxy-3-methylbut-2-enyl diphosphate (HMBPP) to IPP and DMAPP is HDR. As it was previously stated, diatoms possess a cytosolic IDI, the enzyme that isomerizes IPP to DMAPP, in a fusion with a squalene synthase (IDI-SQS). While some species have an additional IDI that is probably targeted to the plastids, in other species a second IDI is absent (Ferriols et al., 2017). If these observations are true, HDR must play a central role in isoprenoid synthesis in the plastids, regulating the ratio of IPP and DMAPP for downstream reactions. The ratio commonly reported for bacteria is 4:1–6:1 (Gräwert et al., 2011; Rohdich et al., 2002; Xiao et al., 2008). The plant pathogenic bacterium *Burkholderia glumae* that lacks an IDI produces a 2:1 ratio of IPP and DMAPP (Kwon et al., 2013). A recent study on *Ginkgo biloba* HDR, which produces an exceptional ratio of 16:1, showed that a single catalytic residue (F212) at the active site of the enzyme is critical for the product formation. This residue mediates the proton transfer to the allyl anion intermediate before the product release (Shin et al., 2017). Diatoms seem to have the

F212 conserved, however further and comparative studies on HDR enzymes from different species are essential to specify the exact IPP:DMAPP ratio produced. If this is not sufficient to support the plastidic isoprenoid synthesis, then those species that do not encode an independent IDI must have evolved specific mechanisms for the isomerization of IPP to DMAPP in plastids, such as the dual targeting of IDI-SQS or alternative splicing of the IDI-SQS transcript. Achieving an optimal IPP:DMAPP ratio is important for metabolic engineering of cells for the production of specific isoprenoids, because depending on the target compound a different ratio is required. For example, sesquiterpenoids or sterols require a 2:1 ratio, while diterpenoids or carotenoids a 3:1.

Studies on the MVA pathway in diatoms, from metabolic engineering perspective, are less. Hydroxy methyl glutaryl reductase (HMGR), the enzyme that catalyzes the conversion of HMG-CoA to mevalonic acid, has been shown to be the most critical component of this pathway in several other organisms. During the heterologous expression of plant triterpenoid biosynthesis genes in *P. tricornutum*, HMGR and IDI-SQS were among the genes that were found constantly upregulated (D'Adamo et al., 2018). This makes them prime candidates for overexpression in future pathway optimization efforts.

3.2. Synthesis of prenyl diphosphates in different subcellular compartments

After their synthesis, IPP and DMAPP are condensed by prenyltransferases to form longer chain diphosphate molecules. Based on biochemical characterization, localization predictions and phylogenetic analysis, our group has shed light on the core steps of isoprenoid biosynthesis, which in *H. ostrearia* are mediated by a set of at least 4 prenyltransferases (Athanasakoglou et al., 2019). A farnesyl diphosphate synthase (*HoPTS1*; AYV97141.1) produces the FPP that is used in sterol synthesis. A homologous enzyme (AKH49589.1) was previously characterized in *R. setigera* and was found to additionally supply the precursors of HBIs (Ferriols et al., 2015). Two GGPP synthases, one possibly cytosolic (*HoPTS3*; AYV97143.1) and one chloroplastic (*HoPTS5*; AYV97145.1), likely participate in cytosolic isoprenoid-related reactions and carotenoid synthesis, respectively. Finally, a polyprenyl synthase (*HoPTS2*; AYV97142.1), likely localized in the chloroplast, contributes to the synthesis of polyprenyl diphosphates, such as those that give rise to the side chain of plastoquinone. This core set of prenyltransferases is conserved in almost all sequenced diatoms and phylogenetic analysis revealed that these enzymes have diverse evolutionary origin, reflecting the multiple endosymbiotic events that gave rise to diatoms (Athanasakoglou et al., 2019). A GPP synthase has not been identified in *H. ostrearia* but a GPP synthase-like genes have been identified in *Pseudo-nitzschia* species and are likely involved in the synthesis of domoic acid (Di Dato et al., 2015).

A better understanding of these central steps is essential for a better design of metabolic engineering strategies to produce isoprenoids in diatoms. As prenyl diphosphates are synthesized in a linear manner and each serves as a precursor for more than one branches of the pathway, metabolic engineering interventions cannot be limited only to increasing precursor synthesis by overexpression of the corresponding prenyltransferase, but they must also channel the metabolic fluxes towards the desired branch of the pathway. Diatoms have evolved ways of achieving this channeling in accordance to their metabolic needs and offer interesting examples for the engineering process. In light acclimation experiments in *P. tricornutum*, GGPPS (XP_002178555) and phytoene synthase (XP_002178776.1), the enzymes catalyzing the first steps towards carotenoid biosynthesis, were found to be upregulated for 12–48 h after light exposure, with similar expression ratio values (Nymark et al., 2009). This coordinated upregulation enables channeling of substrates towards photo-protective carotenoid synthesis. Metabolic channeling is also mediated by the formation of enzymatic fusions or complexes, which provide more efficient catalysis by ensuring that substrates do not diffuse in the cell. The presence of fusion

enzymes in diatoms is relatively frequent. A triosephosphate-isomerase/glyceraldehyde-3-phosphate dehydrogenase, a UDP-glucose-pyrophosphorylase/phosphoglucomutase and a glucose-6-phosphate-dehydrogenase/6-phosphogluconate-dehydrogenase in carbohydrate metabolism have been predicted to exist as fusions in the genome of *P. tricornutum* (Fabris, 2014; Kroth et al., 2008). However, in all of the aforementioned enzymatic combinations, the enzymes catalyze consecutive reactions. Of particular interest is the fusion between IDI and SQS, two enzymes that catalyze non-consecutive reactions. The putative complex formation between IDI-SQS with FPPS is an effective way to channel precursors towards sterols. At the same time, the same enzyme has regulatory role in HBI profile in *R. setigera* (Athanasakoglou et al., 2019; Davies et al., 2015; Ferriols et al., 2017). A final consideration for the redirection of the fluxes into the desired pathway branch is the knockdown of genes that compete for the same precursors in other branches. Such efforts have not yet been applied to the isoprenoid pathway of diatoms but have been proven efficient in increasing the pool of specific prenyl diphosphate substrates in the green algae *Chlamydomonas reinhardtii*. In their effort to engineer *C. reinhardtii* for (*E*)- α -bisabolene production, a sesquiterpene derived from FPP, Wichmann and co-workers created SQS knockdown strains. These strains showed improved bisabolene production due to decreased flux of FPP towards sterols (Wichmann et al., 2018). This work could serve as an example in future diatom metabolic engineering projects.

3.3. Carotenoid biosynthesis proceeds via two xanthophyll cycles

The main photosynthetic pigment in diatoms is fucoxanthin. The route to its biosynthesis has been partially established in *P. tricornutum* and the enzymes involved in the initial steps have been identified and characterized by heterologous expression in *E. coli* (Fig. 2) (Dambek et al., 2012). The pathway starts with the 'head-to-head' condensation of two geranylgeranyl diphosphate (GGPP) molecules by a phytoene synthase (PSY; XP_002178776.1). A PSY enzyme from *H. ostrearia* (AYV97146.1) has been characterized using the *Saccharomyces cerevisiae* expression system (Athanasakoglou et al., 2019). The produced phytoene is then converted to lycopene through a series of desaturation reactions and intermediates of ζ -carotene and prolycopene. The cyclization of both ends of lycopene to β -ionone rings results in β -carotene formation. The remaining steps of the pathway have yet to be elucidated but the intermediate substrates towards fucoxanthin have been identified with substrate enrichment methods (Dambek et al., 2012).

Based on these studies, both β -ionone rings of β -carotene are hydroxylated at positions 3 and 3', leading to the synthesis of zeaxanthin. However, in the diatom genomes investigated so far (*P. tricornutum*, *T. pseudonana*), the genes responsible for these hydroxylations (β -carotene hydroxylases) are missing or appear partial. It is, thus, hypothesized that this step in diatoms is catalyzed by promiscuous enzymes involved in other pathways, such as cytochrome P450s (CYPs) (Coesel et al., 2008). Given the recent discovery of an alternative form of the squalene epoxidase in *P. tricornutum*, which belongs to the fatty acid hydroxylase superfamily (Pollier et al., 2018), a similar scenario may be relevant for the β -carotene hydroxylase in diatoms. After zeaxanthin formation, a xanthophyll cycle that is regulated by light conditions initiates. Under low light or in the dark, zeaxanthin is successively epoxidated at positions 5,6 and 5',6' to yield antheraxanthin and eventually violaxanthin. This reaction is catalyzed by zeaxanthin epoxidases ZEP2 (XP_002176935) and ZEP3 (XP_002178367). Upon strong light exposure, violaxanthin de-epoxidase (VDE; XP_002178643) catalyzes the conversion of violaxanthin to zeaxanthin (Eilers et al., 2016b). In downstream reactions of carotenoid biosynthesis one of the epoxy rings of violaxanthin opens and rearranges to allenic double bonds to form neoxanthin. This is a branching point of the pathway. Neoxanthin serves as substrate to both fucoxanthin and the second major, light dependent xanthophyll cycle, that of diadinoxanthin-diatoxanthin. High light conditions promote de-epoxidation of diadinoxanthin to diatoxanthin to achieve dissipation of excess light energy. This step is mediated by violaxanthin de-epoxidase (VDE; XP_002178643) also reported as diadinoxanthin de-epoxidase (DDE) (Lavaud et al., 2012). Due to their important roles in diatoms' xanthophyll cycles, zeaxanthin epoxidases (ZEP) and violaxanthin de-epoxidases (VDE) have been investigated in many studies (Coesel et al., 2008; Depauw et al., 2012). However, the thorough understanding of the cycles and the exact enzymatic mechanisms are yet unknown.

The important properties of fucoxanthin and its precursors have already prompted metabolic engineering efforts towards optimization of their production yields in *P. tricornutum*. The first target has been PSY, the gatekeeper enzyme that controls metabolic fluxes towards carotenoid synthesis in plants (Fray et al., 1995; Rodríguez-Villalón et al., 2009), bacteria (Iwata-Reuyl et al., 2003), cyanobacteria (Schäfer et al., 2006) and fungi (Wöstemeyer et al., 2005). Similar to DXS, this enzyme is under light-dependent transcriptional regulation. DXS and PSY co-upregulation under light stress facilitates the synthesis of photoprotective carotenoids, as DXS controls the flux towards the MEP

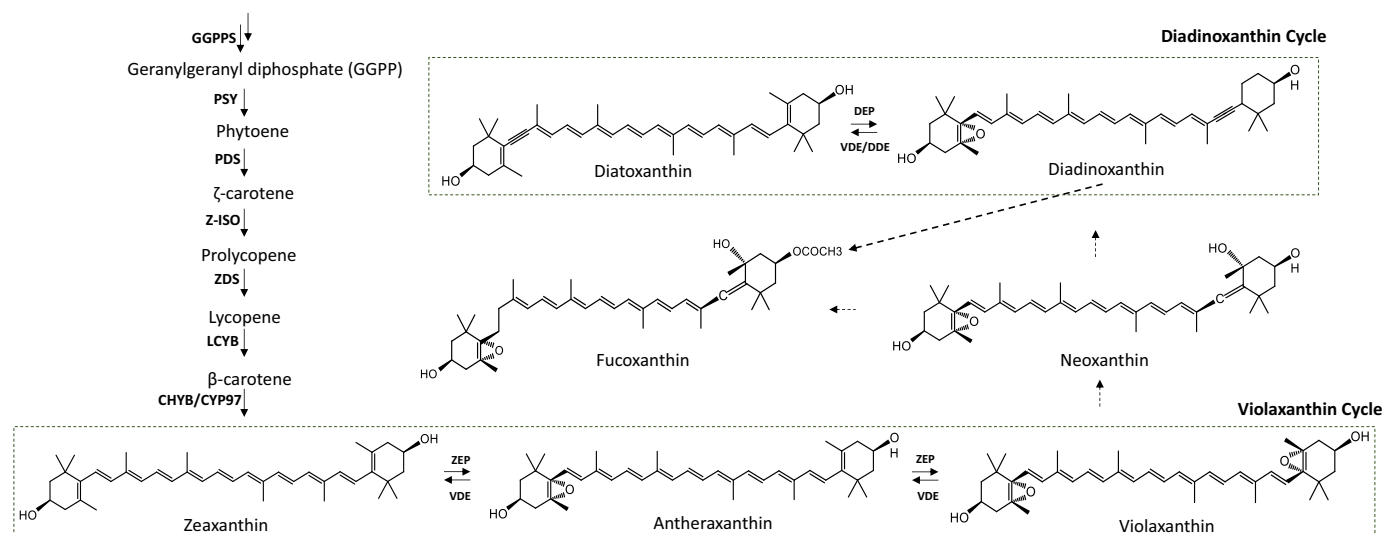


Fig. 2. Carotenoid biosynthesis in diatoms. Adapted from (Dambek et al., 2012). GGPPS; geranylgeranyl diphosphate synthase, PSY; phytoene synthase, PDS; phytoene desaturase, Z-ISO; ζ -carotene isomerase, ZDS; ζ -carotene desaturase, LCYB; lycopene beta cyclase, CHYB/CYP97; beta carotene hydroxylase/cytochrome P450, ZEP; zeaxanthin epoxidase, VDE; violaxanthin de-epoxidase, DDE; diadinoxanthin de-epoxidase, DEP; diatoxanthin epoxidase.

Table 2
Metabolic engineering approaches on diatom carotenoid genes in homologous and heterologous expression systems.

Overexpressed diatom gene (accession number)	Product	Fold-increase (Compared to wild-type)	Product titer	Expression System	Reference
<i>dxs</i> (XM_002176350.1)	β -carotene	1.5	0.6 mg/g dw	<i>E. coli</i>	Eilers et al. (2016a)
	Fucoxanthin	2.4	24.2 mg/g dw	<i>P. tricornutum</i>	
	Diadinoxanthin	4.3	3.05 mg/g dw	<i>P. tricornutum</i>	
	β -carotene	3.3	1.0 mg/g dw	<i>P. tricornutum</i>	
<i>psy</i> (XM_002178740.1)	Phytoene	1.6	0.9 mg/g dw	<i>P. tricornutum</i>	Eilers et al. (2016a)
	Fucoxanthin	1.8	18.4 mg/g dw	<i>P. tricornutum</i>	
	Diadinoxanthin	1.4	1.3 mg/g dw	<i>P. tricornutum</i>	
	β -carotene	2	0.6 mg/g dw	<i>P. tricornutum</i>	Kadono et al. (2015)
	Fucoxanthin	1.45	50 μ g/10 ⁸ cells	<i>P. tricornutum</i>	

pathway and PSY channels the precursors to the carotenoid pathway. In two separate studies, when a copy of *psy* was introduced into the *P. tricornutum* genome, the *psy* transcript levels increased by 6.6-fold, and up to 1.8-fold improvement in fucoxanthin production and 1.6-fold increase in phytoene accumulation were observed (Eilers et al., 2016a; Kadono et al., 2015) (Table 2). The lack of linear correlation between transcript level and product formation suggested that flux through *psy* may be limited by other regulatory mechanisms (Eilers et al., 2016a). Even though these studies showed promising results, combined metabolic engineering interventions, such as overexpression of two or more genes or overexpression in combination with competitive pathway knockdowns, are still to be implemented.

3.4. A chimeric pathway provides a wide diversity of sterols

There is huge structural diversity of sterols in diatoms. Sterol biosynthesis typically uses precursors derived from the MVA pathway and starts from the condensation of two FPP molecules and formation of squalene. A squalene synthase (SQS) catalyzes the condensation of two FPP molecules and formation of squalene. A squalene epoxidase (SQE) catalyzes the oxidation of squalene, using molecular oxygen and FAD as cofactor. This oxidation step leads to the synthesis of 2,3-epoxysqualene, which is then cyclized by an oxidosqualene cyclase type enzyme (OSC). In plants, this enzyme is cycloartenol synthase (CAS), which provides cycloartenol, the precursor of the various phytosterols. In fungi and animals, the OSC enzyme is lanosterol synthase (LAS), and the produced lanosterol is converted to ergosterol and cholesterol, respectively (Benveniste, 2004).

In diatoms, both phytosterols and animal/fungal molecules are present (Rampen et al., 2010). Depending on the species, either cycloartenol or lanosterol have been observed, leading to the assumption that there is species differentiation both on the composition and on the enzymatic steps that lead to the synthesis of the different sterols. Detailed information in terms of gene identification and characterization has only been reported for *P. tricornutum* (Fig. 3). Using *in silico* reconstruction of the MVA pathway and the subsequent steps towards sterol biosynthesis, Fabris and co-workers showed that the diatom synthesizes its main sterols, brassicasterol (24-methylcholest-5,22-dien-3 β -ol) and campesterol (24-methylcholest-5-en-3 β -ol), through cycloartenol, with ergosterol as intermediate. This observation led to the assumption that *P. tricornutum* employs a chimeric organization for sterol biosynthesis, combining both plant-like and fungal-like features (Fabris et al., 2014). A similar organization has been proposed for sterol synthesis in *Chlamydomonas reinhardtii* (Brumfield et al., 2017). Like other marine organisms, diatoms lack a conserved SQE and possess an extended form of OSC. Even though the gene that encodes for a typical SQE is missing in diatoms, the product of squalene oxidation, 2,3-epoxysqualene, is present (D'Adamo et al., 2018; Fabris et al., 2014). This has prompted the testing of alternative enzymes that could catalyze this reaction (Fabris et al., 2014) and led to the recent identification of an alternative SQE (AltSQE; AYI99264.1) in the genome of *P. tricornutum* that was characterized in complementation assays, using an SQE-deficient yeast. The AltSQE is widespread not only among other diatom species but also within members of other eukaryotic lineages,

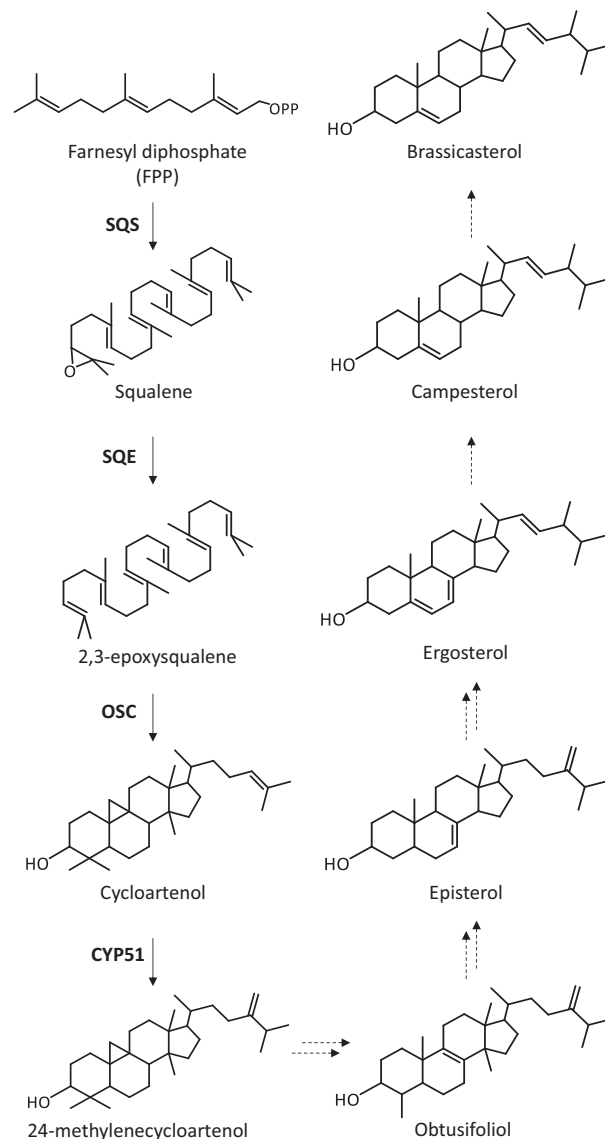


Fig. 3. Sterol biosynthesis in the diatom *P. tricornutum*. Adapted from (Fabris et al., 2014). SQS; squalene synthase, SQE; squalene epoxidase, OSC; oxidosqualene cyclase, CYP51; cytochrome P450.

giving variability in a previously considered well-conserved pathway (Pollier et al., 2018). In the steps that follow the oxidation of squalene, a sterol methyltransferase methylates cycloartenol at C-24, producing 24-methylene-cycloartenol, which is converted to obtusifoliol through three consecutive reactions. Subsequently, a CYP catalyzes the removal of the methyl group of obtusifoliol and generates a double bond between C-14 and C-15, yielding (4 α)-methyl-(5 α)-ergosta-8,14,24(28)-

trien-3 β -ol. Several downstream steps are required for ergosterol synthesis, which is finally converted to campesterol and brassicasterol, the two main phytosterols of *P. tricornutum*. For all these steps, candidate genes were identified based on homology (Fabris et al., 2014). However, the complete elucidation of the pathway, in terms of enzymatic characterization, is still missing and investigations on other diatom species are also lacking.

Recent metabolic engineering efforts in *P. tricornutum* aimed at the production of the plant triterpenoid betulin and gave useful insight into the regulation of diatoms' sterol pathway (D'Adamo et al., 2018). The betulin biosynthetic pathway uses the same precursor (2,3 oxidosqualene) used for the synthesis of sterols. Expression of the betulin biosynthetic genes drew precursors from sterol synthesis and, in order to compensate this perturbation, diatoms induced the expression of key MVA pathway genes, including HMGR, IDI-SQS and hydroxy-methylglutaryl-CoA synthase (HMGS). Surprisingly, the expression levels of CAS did not change significantly, even though other enzymes that catalyze downstream reactions showed upregulation. Thus, CAS is another enzyme that can be targeted for overexpression in future metabolic engineering efforts.

3.5. Synthesis of species-specific isoprenoids: highly branched isoprenoids and domoic acid

HBIs are molecules of high importance in paleoenvironmental studies and they show great potential for different biotechnological applications. However, although a number of studies has suggested possible mechanisms by which these molecules may be synthesized, the genetic and biochemical basis of their biosynthesis remains elusive. Early work on the pathway by Masse and coworkers (Massé et al., 2004a, 2004b), used isotopic precursor labelling experiments and inhibitors of the MVA and MEP pathway to identify which isoprenoid route provides the precursors for HBI biosynthesis. The results of this analysis were different in the two diatom species investigated. A strain of *R. setigera* that produces both C25 and C30 HBIs, incorporated precursors generated by the MVA pathway. In contrast, in *H. ostrearia*, C25 HBIs were synthesized via MEP derived precursors. Initial hypothesis proposed that the condensation of two FPP (C15) molecules in a head-to-middle (1'-6') orientation gives rise to C30 HBIs, while a similar condensation that involves one FPP (C15) and one GPP (C10) molecule likely results in C25 isomers (Fig. 4a) (Ferriols et al., 2015). As *H. ostrearia* was found to also produce functionalized HBIs, like epoxides and alcohols (Belt et al., 2006), other isoprenoid precursors were also suggested as candidate substrates (for example C10 linalool or linaloyl diphosphate or C15 farnesol). Alternatively, the functional groups on these HBIs could be added at later steps in the pathway by different enzymes. Similar 'head-to-middle' condensation reactions have been reported in plants by prenyltransferases using DMAPP as substrate (Demissie et al., 2013; Hemmerlin et al., 2003b; Rivera et al., 2001). In 2015, an FPP synthase (AKH49589.1) from *R. setigera* was isolated and characterized and its in vivo inhibition resulted in reduced HBI synthesis. These results indicate that at least one of the precursors of HBIs in *R. setigera* is FPP (Ferriols et al., 2015). The same group, a few years later, investigated the involvement of the IDI-SQS (MF351614) enzyme in HBI biosynthesis. Although, the fusion does not seem to be directly involved in the pathway, the authors concluded that it probably has a regulatory role on the HBI profile synthesis. The identification of independently transcribed IDI and SQS in C30 HBI strains of *R. setigera* and IDI-SQS fusion in C25 HBI producing strains of the same species and of *H. ostrearia* supports a role for this fusion in the regulation of the GPP pool, which is required for C25, but not for C30, HBIs (Ferriols et al., 2017).

The recent elucidation of the biosynthetic pathway of the neurotoxin domoic acid revealed yet another interesting feature of diatoms. The domoic acid biosynthetic genes are clustered together in the genome of *Pseudo-nitzschia multiseriata*. This organization is typical in

prokaryotic genomes but not common in eukaryotes. A total of 3 genes in this cluster catalyze the synthesis of domoic acid. A terpene cyclase (DabA; AYD91073.1) catalyzes the N-prenylation of L-glutamic acid with GPP, a reaction that results in N-geranyl-L-glutamic acid (L-NGG) formation. The reaction likely takes place in the chloroplast as dabA has a chloroplastic target peptide. Three consecutive oxidation reactions at the 7'-methyl of L-NGG by DabD (AYD91074.1) enzyme produce the intermediate 7'-carboxy-LNGG, which is then cyclized by DabC (AYD91075.1) to isodomoic acid A. A final isomerization reaction converts the latter to domoic acid, with the isomerase being currently unknown (Brunson et al., 2018).

From an evolutionary perspective, the synthesis of both HBIs and domoic acid are distinctive cases, as they are only produced by specific species. The fact that domoic acid production is encoded by a gene cluster raises speculations that the corresponding genes have been acquired from prokaryotes through horizontal gene transfer. Investigations on the physiological roles of these molecules and on the evolutionary advantage that led to the acquisition of their biosynthetic pathways can shed more light into their origin.

4. Engineering diatoms as platforms for isoprenoid biosynthesis

Isoprenoids have numerous industrial applications and high economic value. For example, the global market of carotenoids alone reached \$1.23 billion in 2015, and is expected to increase to \$1.81 billion by 2022 (BCC-Research The Global Market Outlook (2016-2022), 2017). Extraction of isoprenoids from many natural sources is not sustainable, while their chemical synthesis is not economically viable. In addition, there is increasing consumer awareness for green chemicals. Biotechnologically exploitable natural producers have in some cases successfully been used. For example, the astaxanthin-producing microalgal species *Haematococcus pluvialis* produces particularly high amounts of this xanthophyll and is already commercially exploited (Olaizola, 2000). Moreover, various traustochytrids have also shown potential for squalene or carotenoid production (Xie et al., 2017). However, not all compounds of interest are available from natural producers at levels that are industrially relevant. Thus, the exploitation of microbially derived isoprenoids and the engineering of microbial platforms for improved yields has been pursued. *S. cerevisiae* and *E. coli* are the best platforms currently available for this purpose. However, it is not always possible (due to structural complexity) or economically feasible to produce certain isoprenoids in these microbial hosts. There is, therefore, a continuous interest to develop alternative platforms to overcome such limitations. Of particular interest are photosynthetic microorganisms that can utilize sunlight and CO₂ for growth, without the need for expensive media or complex cultivation systems (Davies et al., 2015; Lauersen, 2018; O'Neill and Kelly, 2017; Vavitsas et al., 2018). The recent reconstruction of a plant isoprenoid pathway in *P. tricornutum* (D'Adamo et al., 2018) revealed the potential of diatoms as an alternative chassis for isoprenoid synthesis.

There are several features of diatoms that make them an attractive platform for the light-driven synthesis of interesting isoprenoids (Falcatore and Bowler, 2002). Being rich sources of isoprenoids themselves, diatoms already dedicate increased carbon resources into the synthesis of these molecules. Diatoms grow well both on photobioreactors (Wang and Seibert, 2017) and in outdoor open systems (Lebeau and Robert, 2003) and their growth rates are not affected by high density (diatom blooms) (Hildebrand et al., 2012). With respect to heterologous gene expression, diatoms offer a significant advantage. Their flexible metabolism, as shaped by their complex evolutionary history, combines features from multiple lineages. Thus, it has been possible to successfully express in diatoms genes from diverse kingdoms, including plants (D'Adamo et al., 2018; Zulu et al., 2017), animals (Hempel et al., 2011b; Hempel and Maier, 2012), fungi (Zulu et al., 2017) or bacteria (Hempel et al., 2011a).

The complex evolutionary history of diatoms has also created a

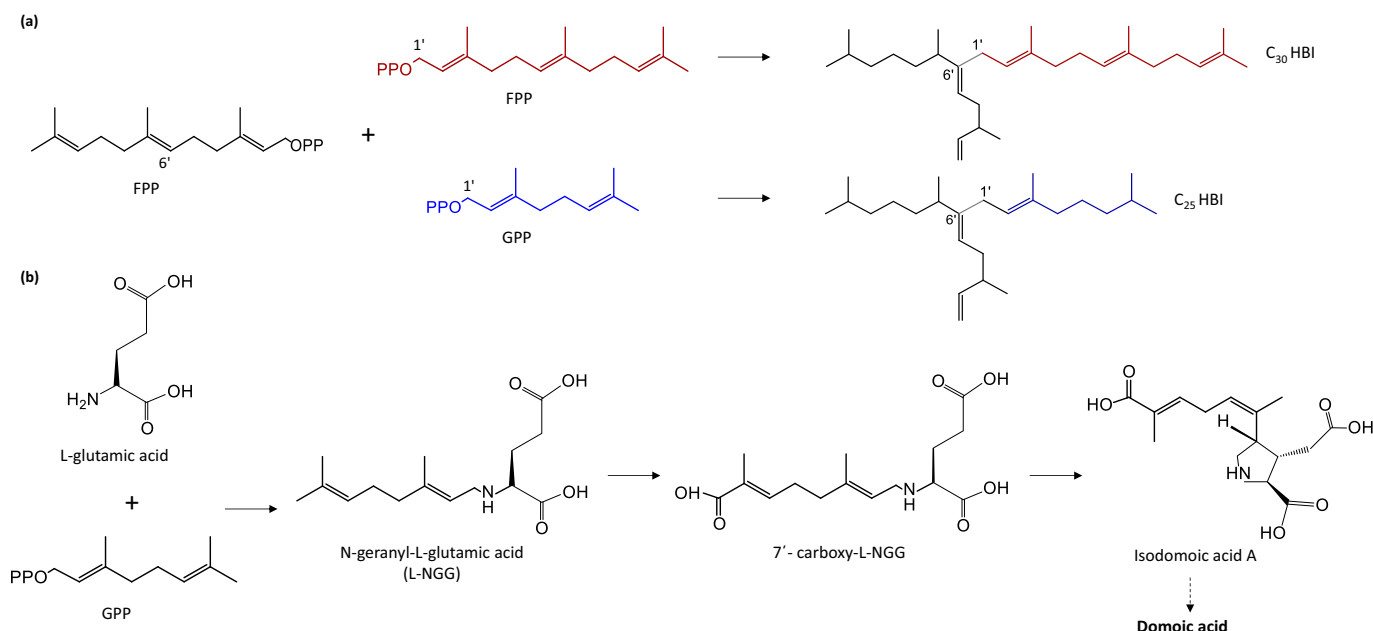


Fig. 4. (a) Hypothetical scheme for highly branched isoprenoid biosynthesis. Adapted from (Ferriols et al., 2015). (b) Domoic acid biosynthesis. Adapted from (Brunson et al., 2018). Colors are used to indicate the different backbones; farnesyl diphosphate (FPP) in red, geranyl diphosphate (GPP) in blue. HBI; highly branched isoprenoid. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

unique subcellular organization. Their plastids are surrounded by four membranes, with the outer being continuous with the ER membrane. Between the second and third membrane of diatoms' plastids, there is an additional subcellular compartment. This is the periplastidial compartment (PPC) (Bolte et al., 2009; Flori et al., 2016; Moog et al., 2011) that could be used to target specific pathways and isolate them from competing reactions or separate toxic intermediates. Several studies in other expression systems have achieved significant improvements in the production of specific isoprenoids by targeting their synthesis in organelles (Avalos et al., 2013; Hammer and Avalos, 2017; Huttanus and Feng, 2017; Lv et al., 2016; Malhotra et al., 2016). The close proximity of diatoms' PPC to the ER and chloroplast, in combination with possible transportation of pathway intermediates between these compartments, may offer a considerable advantage for engineering compartmentalized isoprenoid synthesis. Another possibility of exploitation of compartmentalization in diatoms lies in their ability to form lipid droplets under nutrient limitation stress, as it has been demonstrated for different species (Maeda et al., 2017). Although the large majority of molecules accumulating in these cytoplasmic compartments are lipids, a more detailed recent study on their architecture revealed possible accumulation of the carotenoid pathway precursor β -carotene (Lupette et al., 2019). Even though the proteomics analysis performed in this study did not indicate targeting of carotenoid pathway enzymes in the lipid droplets, accumulation of this important intermediate offers advantage in future metabolic engineering projects for carotenoid bioproduction in *P. tricornutum*. By targeting downstream enzymes of the pathway in lipid droplets, different products of interest could be synthesized without perturbation of the chloroplastic pool of β -carotene that is essential for vital functions related to photosynthesis and photoprotection. This is a well-described strategy, naturally followed by the green algal species *Haematococcus pluvialis* that converts β -carotene into astaxanthin in cytoplasmic lipid droplets (Grünewald et al., 2001).

One key aspect in metabolic engineering projects is the connection of the pathway of interest with the central metabolism and the understanding of regulatory mechanisms affecting gene expression and precursor supply. Interesting observations have been reported for the sterol biosynthetic pathway and its connection to lipid synthesis. In an effort to identify drugs that trigger fatty acids and triacylglycerol

accumulation in *P. tricornutum*, Conte and co-workers found a number of molecules that target MVA and sterol pathway enzymes among the compounds identified (Conte et al., 2018). Since the triacylglycerol and sterol pathway use the same precursor, acetyl-CoA, any effort towards upregulation of one of the two pathways should take into account the interconnection between the two. Sterol regulation mechanisms have been investigated in detail in mammals, fungi and plants and critical regulatory elements have been characterized. Similar efforts in microalgae would be essential for the design of rational strategies for improved sterol yields (Jaramillo-Madrid et al., 2019).

The efficacy of genetic manipulation of model diatom species is another factor that facilitates their development into platforms for isoprenoid bioproduction. A first essential step for successful heterologous pathway expression is the efficient introduction of recombinant genes. In diatoms, various transformation methods have been developed and applied, including particle bombardment (Zaslavskaja et al., 2000), electroporation (Zhang and Hu, 2014), and bacterial conjugation, which allows integration of large DNA fragments (Karas et al., 2015). One limitation of these methods is that DNA integration occurs in a random manner. This could lead to transgenic lines with major differences in the expression levels of introduced genes and may create undesirable off-target effects (Huang and Daboussi, 2017; Lauersen, 2018). These problems have recently been tackled with the development of plasmid-based expression tools (Karas et al., 2015; Slattery et al., 2018). Another requirement for metabolic engineering is the ability to generate gene knock-outs. This is essential in order to downregulate competitive pathways or host factors that have a negative regulatory effect on heterologous pathways and/or to re-direct fluxes towards desirable branches. In diatoms genome editing tools are continuously advancing (Kroth et al., 2018). The first efforts were based on the use of meganucleases (Daboussi et al., 2014) and transcription activator-like effector nucleases (TALENs) (Weyman et al., 2015) with specific DNA binding activities. A CRISPR/Cas9 system has also been adapted in various model species for less laborious generation of stable targeted gene mutations (Hopes et al., 2016; Nymark et al., 2016). As assembly of complex pathways requires multiple genetic modifications at once, a strategy that simultaneously introduces multiple ribonucleoprotein complexes has been developed to enable fast strain

manipulation (Serif et al., 2018).

With these advances offering improved results in transgene expression and genome editing, there is a continuous need for endogenous regulatory elements to manipulate foreign gene expression. The most widely used promoter in *P. tricornutum* is the light-regulated promoter of the fucoxanthin chlorophyll *a/c*-binding protein gene (FCPA/LHCF1) (Zaslavskaja et al., 2000). The nitrate reductase (NR) gene promoter (Niu et al., 2012; Poulsen and Kröger, 2005) has been applied for inducible transgenic manipulation upon transfer from an ammonium- to a nitrate-containing medium. Other commonly used elements include the constitutive histone 4 (H4) (De Riso et al., 2009) and elongation factor 2 (EF2) promoters (Seo et al., 2015) and iron-responsive promoters (Yoshinaga et al., 2014). Two novel *P. tricornutum* promoters that are either constitutively active in the presence or absence of nitrate or upregulated under N starvation have also been characterized (Adler-Agnon (Shemesh et al., 2017).

The effect of environmental stimulants that can direct fluxes into specific pathways or branches are also an approach to consider. Application of mechanical stimuli to *Chaetoceros decipiens* cultures was shown to result in upregulation of isoprenoid biosynthesis (Amato et al., 2017). Temperature and salinity affect HBI biosynthesis in *H. ostrearia* and *R. setigera*, altering the distribution of specific isomers (Rowland et al., 2001a, 2001b). Furthermore, carotenoid biosynthesis is induced under light stress conditions in many diatoms studied (Dambek et al., 2012).

A main limitation regarding the use of algal cells, including diatoms, as platforms for light-driven isoprenoid production lies on economic barriers created by the still low production yields (D'Adamo et al., 2018), unexplored downstream purification and processing methods to obtain final products, and infrastructure operating costs (Lauersen, 2018). Yet, the fact that it is possible to exploit almost all the biomass produced during diatom cultivation, either by using it as animal/aquaculture feed or by converting it to bio-crude oil through thermochemical procedures (Hildebrand et al., 2012; Zulu et al., 2018) can be exploited to tackle economic barriers. Additional features to consider in this direction is the increased lipid content of many diatoms as well as their ability to mitigate CO₂ and pollutants. This opens up the possibility of using them for other processes, in parallel with value-added compound bioproduction. The design of simultaneous isoprenoid synthesis with biofuel production, power generation or wastewater remediation, will improve the cost/profit balance and increase the appeal of diatoms on future biotechnological projects (Wang and Seibert, 2017).

5. Conclusions and future perspectives

Diatoms use isoprenoid pathways with distinctive organization to synthesize unique molecules with important applications. Recent advances on the elucidation of biosynthetic pathways of isoprenoids together with the development of advanced techniques for genetic manipulation have brought diatoms' isoprenoids into the spotlight of biotechnological research. There is now potential on the future exploitation of diatom species as chassis for value-added compound synthesis and metabolic engineering for the heterologous production of diatom-specific compounds.

Developing diatoms as a next-generation photosynthetic platform for isoprenoid bioproduction, is still at an early stage. Even though our knowledge on how isoprenoids are synthesized in this microalgal group has significantly advanced in the past decade, there are branches of the pathway that remain unstudied. The elucidation of those can further improve our understanding of the whole pathway and determine species-specific differentiations. Beyond that, what seems to be essential for future metabolic engineering efforts is the thorough understanding of the regulatory mechanisms that govern isoprenoid biosynthesis in diatoms. Accumulated evidence on high degree of genomic and metabolic diversity within different species indicates multiple possibilities

for manipulation of enzymes and pathways to improve resource fluxes towards desirable routes. This directs to comparative studies that will identify conditions and guide interventions for optimized gene expression and specific product synthesis. These studies will not only allow the efficient engineering of an alternative chassis for isoprenoid production, but can add upon existing knowledge on the peculiar, yet exciting evolution of diatoms.

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