



## **Ameliorating microalgal OMEGA production using omics platforms**

Downloaded from: <https://research.chalmers.se>, 2025-07-02 14:06 UTC


Citation for the original published paper (version of record):

Mariam, I., Bettiga, M., Rova, U. et al (2024). Ameliorating microalgal OMEGA production using omics platforms. Trends in Plant Science, 29(7): 799-813.  
<http://dx.doi.org/10.1016/j.tplants.2024.01.002>

N.B. When citing this work, cite the original published paper.

## Review

## Ameliorating microalgal OMEGA production using omics platforms

Iqra Mariam,<sup>1</sup> Maurizio Bettiga,<sup>2,3</sup> Ulrika Rova,<sup>1</sup> Paul Christakopoulos,<sup>1</sup> Leonidas Matsakas,<sup>1</sup> and Alok Patel <sup>1,\*</sup>

Over the past decade, the focus on omega ( $\omega$ )-3 fatty acids from microalgae has intensified due to their diverse health benefits. Bioprocess optimization has notably increased  $\omega$ -3 fatty acid yields, yet understanding of the genetic architecture and metabolic pathways of high-yielding strains remains limited. Leveraging genomics, transcriptomics, proteomics, and metabolomics tools can provide vital system-level insights into native  $\omega$ -3 fatty acid-producing microalgae, further boosting production. In this review, we explore ‘omics’ studies uncovering alternative pathways for  $\omega$ -3 fatty acid synthesis and genome-wide regulation in response to cultivation parameters. We also emphasize potential targets to fine-tune in order to enhance yield. Despite progress, an integrated omics platform is essential to overcome current bottlenecks in optimizing the process for  $\omega$ -3 fatty acid production from microalgae, advancing this crucial field.

Carbon skeleton with myriad benefits:  $\omega$ -3 fatty acids

The carboxylic acids of hydrocarbon chains with the occurrence of more than one double bond are referred to as polyunsaturated fatty acids (hereafter referred to as ‘PUFAs’). These PUFAs can be further classified into two groups: PUFAs with the first double bond at the third carbon from the methyl end ( $\omega$  carbon) are  $\omega$ -3 fatty acids, whereas fatty acids with a double bond at the sixth position are  $\omega$ -6 fatty acids [1]. Linoleic acid (LA; C18:2) and alpha-linolenic acid (ALA; C18:3) are **essential fatty acids** (see Glossary) ( $\omega$ -6 and  $\omega$ -3, respectively) and are recommended in dietary supplements. As suggested by the National Institutes of Health (NIH) Office of Dietary Supplements, an adult human should intake 1.1–1.6 g of ALA daily<sup>1</sup>. By contrast, adult humans are recommended 3–6 g per day of LA, which is desaturated to  $\gamma$ -linolenic acid by  $\Delta$ -6 desaturase and further converted to another  $\omega$ -6 fatty acid, arachidonic acid (ARA; C20:4) [2]. ARA can be further metabolized by three distinctive sets of enzymes [cyclooxygenases (COXs), lipoxygenases (LOXs), and cytochrome P450 (CYP)] to generate prostaglandins, leukotrienes, hydroxyeicosatetraenoic acids (HETs), among others, which act as therapeutics in cancers, cardiovascular and inflammatory diseases [3]. ALA, eicosapentaenoic acid (EPA; C20:5), and docosahexaenoic acid (DHA; C22:6) are the major  $\omega$ -3 fatty acids. In humans, the rate of conversion of ALA to EPA or DHA is limited (<8% to EPA and <4% to DHA) [4,5]. Thus, along with ALA, EPA and DHA are also recommended as part of a healthy diet, with a daily intake of 200–500 mg of EPA and DHA combined<sup>ii</sup>.

ALA is involved in ameliorating obesity, diabetes, cardiovascular diseases, and cancers [6]. Additionally,  $\omega$ -3 fatty acids are structural constituents of the cell membrane, regulating fluidity and permeability [7,8]. DHA is a major structural component of neurons and outer segments of retina and, therefore, is essential for normal neurotransmission and visual function [9]. Similarly, high-dose administration of EPA was found to improve cognitive symptoms in patients with attention deficit hyperactivity disorder (ADHD) [10]. Deficiencies in EPA or DHA result in altered flexibility of

## Highlights

Long-chain polyunsaturated fatty acids comprising omega ( $\omega$ )-3 and  $\omega$ -6 are essential fatty acids with myriad health benefits.

Marine microalgae, such as *Phaeodactylum*, *Nannochloropsis*, and *Cryptocodinium*, have emerged as a potential source of  $\omega$ -3 fatty acids, which comprise 50–70% of their total fatty acid content.

Although there is a plethora of studies on bioprocess optimization, the system-level information of these microalgae remains restricted to model microalgae.

Omics studies have moonlighted the bioprocess optimization by identifying the presence of CAZymes, xylose isomerase, and other pathways for utilization of unconventional carbon sources, such as glycerol.

Similarly, transcriptomics and metabolomics studies have aided understanding of the effect of various abiotic factors, enabling the unraveling of several molecular mechanisms for  $\omega$ -3 fatty acid production.

<sup>1</sup>Biochemical Process Engineering, Division of Chemical Engineering, Department of Civil, Environmental, and Natural Resources Engineering, Luleå University of Technology, SE-971 87 Luleå, Sweden

<sup>2</sup>Department of Life Sciences – LIFE, Division of Industrial Biotechnology, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden

<sup>3</sup>Innovation Unit, Italbiotec Srl Società Benefit, Milan, Italy

\*Correspondence: alok.kumar.patel@tu.se (A. Patel).

the cell membrane, which affects the function of membrane proteins, such as voltage-gated ion channels and enzymes, subsequently attenuating neurotransmission [11]. During the recent coronavirus 2019 (COVID-19) pandemic, one of the clinical features of the disease in patients was the ‘**cytokine storm**’ [12]. EPA and DHA were found to be effective in modulating the concentrations of interleukin (IL)-6, IL-1, or tumor necrosis factor (TNF) $\alpha$ , key cytokines provoking cytokine storms. Both these  $\omega$ -3 fatty acids are converted by LOX and COX enzyme systems to generate anti-inflammatory metabolites, which further bind to their receptors and elicit anti-inflammatory changes in cells [13].

With the increasing awareness and demand for,  $\omega$ -3 fatty acids, the market value of these nutraceuticals reached US\$ 4.1 billion in 2019 and is expected to grow at a compound annual growth rate (CAGR) of 13%, reaching US\$8.5 billion by 2025<sup>iii</sup>. Plant oils, such as flaxseed, soybean, and canola oils are rich in ALA [14], whereas the major sources of EPA and DHA are fish and other seafood [15]. However, to meet the increasing demand for  $\omega$ -3 fatty acids and maintain aquatic ecosystems, microbial platforms have been repeatedly evaluated for production of PUFAs. Studies suggest that fish enriched in PUFAs obtain them through feeding on microalgae and other protists [16]. Thus, over the past few years, several reviews highlighting the microbial potential of synthesizing  $\omega$ -3 have been published (summarized in Table 1).

The major  $\omega$ -3 fatty acid-producing microalgae among the Chromista are marine diatoms, dinoflagellates, and Eustigmatophyceae. For example, DHA and EPA account for 0.7–1% and 13.0–34.1%, respectively of the total fatty acid (TFA) content of *Phaeodactylum tricornutum*; by contrast, *Cryptothecodinium cohnii* contains only DHA (up to 60% of TFAs) [17], whereas *Nannochloropsis* species have only EPA (14–30% of TFAs) [18]. *Emiliania huxleyi* and *Isochrysis galbana* are members of the Coccolithophyceae and are DHA-producing strains, accounting for 9.2% and 13.1% of TFAs, respectively [19,20].

Among Chlorophyta, which are more closely related to land plants, the most promising strains reported for EPA production are *Dunaliella* (21.4% of TFAs), *Chlamydomonas* (19.2%), *Haematococcus* (5.8 mg g<sup>-1</sup>), and *Botryococcus* (6.2 mg g<sup>-1</sup>) [20–23]. By contrast, ALA is commonly found in most microalgal species, including *Chlorella* and *Scenedesmus* [24–26]. In thraustochytrids, such as *Aurantiochytrium* and *Schizochytrium*, which are often misidentified as microalgae, DHA comprises ~50% of their total lipid [27,28]. Modulating cultivation parameters, such as CO<sub>2</sub>, organic carbon supplementation and nutrient deprivation, enhances the  $\omega$ -3 fatty acid content; however, hurdles remain on the path to attaining  $\omega$ -3 fatty acid yields at a commercial level. Using multi-omics to gain insights into the microalgal system could overcome these hurdles by providing a holistic understanding of biosynthetic routes, crosstalk among various metabolic pathways, and the relevant regulatory nodes. These regulatory targets could then be modified to further enhance the biosynthesis of these nutraceuticals.

### $\omega$ -Fatty acid biosynthesis: the elongation and desaturation cycle

Acetyl CoA carboxylase (ACCase) and type-II fatty acid synthase (FAS) are conserved enzymes responsible for *de novo* fatty acid synthesis in plastids [29]. The acetyl-CoA pool in plastids is carboxylated by ACCase to generate malonyl CoA, which is then loaded to the acyl carrier protein (ACP) via enzyme malonyl acetyl transferase (MAT) to form malonyl-ACP, which serves as a substrate for FAS (Figure 1, Key figure). Type-II FAS is an iterative multi-enzyme protein comprising ketoacyl-ACP synthase (KAS), ketoacyl-ACP reductase (KAR), hydroxyacyl-ACP dehydratase (HAD), and enoyl-ACP reductase (EAR) [30]. Either palmitic acid (C16:0) or stearic acid (C18:0) is formed as the end product of iterative cycles by FAS. Stearic acid can be converted to oleic

### Glossary

**Biofuel:** fuel obtained from biomass derived from either plants or agricultural waste, such as molasses and bagasse.

**Calvin-Benson-Bassham (CBB)**

**cycle:** involves carbon fixation by RuBisCO to generate glyceraldehyde-3-phosphate, which then enters into gluconeogenesis to generate glucose.

**cDNA libraries:** collections of cloned cDNA from a sample generated using mRNA as template.

**Cytokine storm:** physiological response in humans during which the immune system releases an excessive number of small proteins, called cytokines, which are involved in cell signaling.

**De-epoxidation:** reverse of the epoxidation reaction (conversion of carbon-carbon double bonds to epoxide, an ether molecule with a three-membered ring).

**Desaturation:** removal of hydrogen atoms from two adjacent carbon atoms in a fatty acid to generate a double bond.

**Digalactosyldiacylglycerol (DGDG):** glycerolipid with acyl chains bonded to glycerol at the first and second carbons along with a polar carbohydrate head comprising two galactose units linked via a (1→6) bond.

**Essential fatty acids:** fatty acids that are not synthesized by the human body and are recommended to be included in a healthy diet.

**Genomics:** study of the total or part of the genomic sequence of an organism.

**Metabolic modeling:** mathematical model that comprises all the reactions, genes, proteins, and metabolites of an organism; metabolic models can be simulated to predict desired fluxes in a pathway.

**Metabolomics:** the study of metabolites (small molecules, such as organic acids, cofactors, lipids, and sugar) and their interaction within a cell or an environment.

**Phenomics:** the systematic study of traits that comprise a phenotype.

**Phylogenetically:** evolutionary relatedness of organisms, as determined by phylogenomics, the study of evolutionary relatedness among a group of organisms determined using traits, morphology, or DNA and protein sequences.

**Proteomics:** the study of the interactions, function, composition, and structures of proteins in an organism.

acid (C18:1) by stearyl-acyl carrier protein (ACP)  $\Delta 9$ -desaturase or acyl-CoA  $\Delta 9$ -desaturase, which is transferred from chloroplasts to the endoplasmic reticulum (ER) to undergo **desaturation** and elongation [31]. Initial desaturation in ER occurs via  $\Delta 12$ -desaturase for the formation of LA, the first  $\omega$ -6 fatty acid, which may be further desaturated by  $\Delta 15$ -desaturase, generating ALA ( $\omega$ -3). These fatty acids are dehydrogenated by  $\Delta 6$ -desaturase and diverted into  $\omega$ -6 and  $\omega$ -3 pathways to undergo elongation and desaturation to form either ARA or EPA [32]. In the pennate diatom *P. tricornutum*, intermediates from both  $\omega$ -pathways contribute to the formation of EPA [33]. However, in certain EPA-accumulating strains, such as *Nannochloropsis* spp., *Monodus subterraneus*, and *Porphyridium cruentum*, the  $\omega$ -6 pathway dominates and, thus,  $\Delta 17$ -desaturase mediates conversion of ARA to EPA [34–36]. EPA is further converted to docosapentaenoic acid (DPA; C22:5) and DHA via  $\Delta 5$  elongase and  $\Delta 4$  desaturase enzymes [37].

In contrast to microalgae, bacteria and thraustochytrids utilize an additional oxygen-independent fatty acid synthase [i.e., polyketide synthase (PKS)] for production of PUFA [38]. The anaerobic PKS comprises multiple catalytic domains, such as ketoacyl synthase, ketoacyl reductase,

**RNAi:** a biological process in which RNA molecules are involved in sequence-specific suppression of gene expression by double-stranded RNA, through translational or transcriptional repression.

**Triacylglycerol (TAG):** neutral lipid with fatty acids attached to all three carbons of the glycerol molecule.

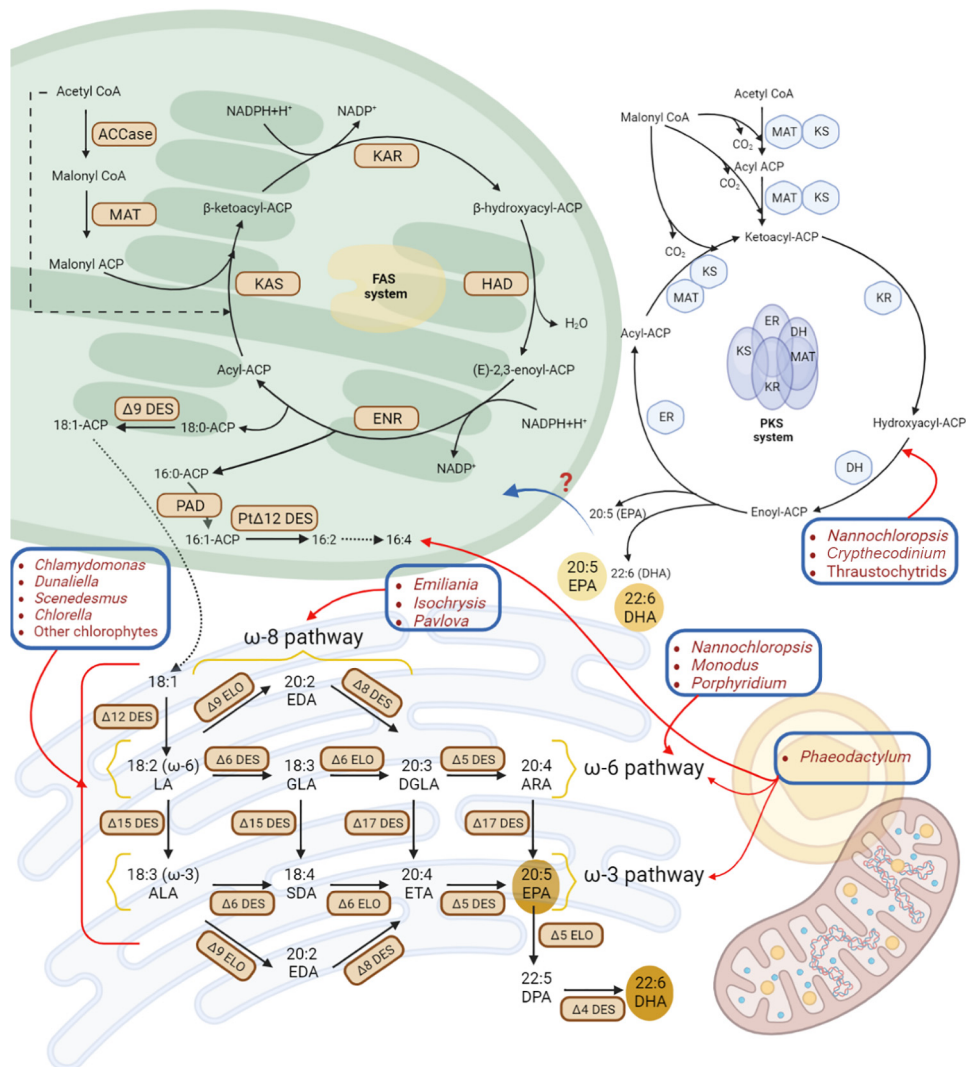
**Transcriptomics:** the study of the abundance of mRNA expressed within an organism (i.e., its transcriptome).

Table 1. Highlights from published reviews discussing relevance of microalgal-derived  $\omega$ -3 fatty acids

Title	Journal	Year	Topics covered	Omics	Host	Refs
Biotechnological production of omega-3 fatty acids: current status and future perspectives	Frontiers in Microbiology	2023	Relevance, potential source, overview of lipid synthesis pathway, and genetic tools	No	Plants, yeast, bacteria, microalgae	[116]
New perspectives of omega-3 fatty acids from diatoms	Systems Microbiology and Biomanufacturing	2023	Current state of production, sources, and market trends	No	Diatoms	[117]
Recent advances in enhancing the production of long chain omega-3 fatty acids in microalgae	Critical Reviews in Food Science and Nutrition	2023	Bioprocess optimization and genetic engineering	No	Microalgae	[118]
Benefits of supplementation with microbial omega-3 fatty acids on human health and the current market scenario for fish-free omega-3 fatty acid	Trends in Food Science & Technology	2023	Relevance to human health, bioprocess optimization, and genetic engineering	No	Microalgae	[119]
Could microalgae be a strategic choice for responding to the demand for omega-3 fatty acids? A European perspective	Trends in Food Science & Technology	2022	Bioprocess optimization and genetic engineering	No	Microalgae	[18]
Emerging prospects of microbial production of omega fatty acids: recent updates	Bioresource Technology	2022	Bioprocess optimization and genetic engineering	No	All microbes	[120]
Microalgae as sources of omega-3 polyunsaturated fatty acids: biotechnological aspects	Algal Research	2021	Bioprocess optimization, downstream processing, and genetic engineering	No	Microalgae	[121]
Cellular engineering strategies toward sustainable omega-3 long chain polyunsaturated fatty acids production: state of the art and perspectives	Biotechnology Advances	2020	Genetic engineering	No	Fungi and microalgae	[1]
Engineering microbes to produce polyunsaturated fatty acids	Trends in Biotechnology	2019	Relevance, potential source, overview of lipid synthesis pathway, and genetic engineering	No	All microbes	[122]
Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production	Microbial Cell Factories	2012	Health benefits, potential source, extraction, and metabolic engineering	No	Microalgae	[123]

## Key figure

Schematic of the routes of omega ( $\omega$ )-fatty acid biosynthesis in microalgae



Trends in Plant Science

**Figure 1.** Conventional C16:0 and 18:1 fatty acids are synthesized in the chloroplast and then exported to the endoplasmic reticulum (ER) for elongation and desaturation. Elongation/desaturation varies significantly among different microalgae and occur via the  $\omega$ -3,  $\omega$ -6, or  $\omega$ -8 pathways. An additional polyketide synthase (PKS) system is present in some microalgae, which produces eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) through iterative cycles of elongation and desaturation in the cytoplasm. Red lines indicate the presence of different pathways in the specified microalgae, blue boxes indicate specific pathways in microalgal species, dashed arrows indicate multi-step reactions, whereas (?) indicates unknown routes for import of EPA/DHA into ER. Abbreviations: ACCase, acetyl CoA carboxylase; ACP, acyl carrier protein; ALA,  $\alpha$ -linolenic acid; ARA, arachidonic acid; DES, desaturase; DGLA, dihomogamma-linolenic acid; DPA, docosapentaenoic acid; EDA, eicosadienoic acid; ELO, elongase; ENR/ER, enoyl reductase; ETA, eicosatetraenoic acid; FAS, fatty acid synthase; GLA, gamma-linolenic acid; HAD/DH, hydroxyacyl dehydratase; KAR/KR, ketoacyl reductase; KAS/KS, ketoacyl synthase; LA, linoleic acid; MAT, malonyl/acetyl transferase; PAD, palmitoyl ACP- $\Delta 9$  desaturase; Pt $\Delta 12$  DES, plastidial  $\Delta 12$  desaturase; SDA, stearidonic acid. Figure created with BioRender (biorender.com).



dehydratase, and enoyl reductase (Figure 1). Although PKS is similar to FAS, it lacks one or more catalytic subunits, leading to the formation of a longer acyl chain [39]. This pathway for  $\omega$ -3 fatty acid utilizes less reducing power and energy as the intermediates are simultaneously desaturated and elongated, generating PUFAs efficiently [40].

### Genomics: identification of alternative routes for $\omega$ -fatty acid synthesis

Along with accumulation of EPA and DHA, the marine haptophyte alga *E. huxleyi* also synthesizes octadecapentaenoic acid (OPA; C18:5) and stearidonic acid (SDA; C18:4), which are not generally reported for related microalgal strains. This suggests that C-18 fatty acids are first elongated and then desaturated at  $\Delta$ 8, followed by introduction of a double bond at  $\Delta$ 5 positions [41].

**Genomics** analysis and functional characterization identified five putative genes in *E. huxleyi* encoding elongases and desaturases responsible for DHA synthesis [42]. Thus, in an alternative pathway, ALA is converted to C20:3 by the catalytic activity of  $\Delta$ 9-elongase, followed by two subsequent desaturation events by  $\Delta$ 8 and  $\Delta$ 5 desaturases to form EPA (Figure 1). Final chain elongation and desaturation by  $\Delta$ 5 elongase and  $\Delta$ 4 desaturase, respectively, leads to the formation of DHA [43]. The C18  $\Delta$ 9-elongase has also been identified in the DHA-accumulating microalgae *I. galbana* and *Pavlova salina* using **cDNA libraries** [44,45]. Additionally, both microalgae have a functional  $\Delta$ 6-desaturase, with accumulation of intermediate fatty acids from both  $\Delta$ 9-elongase and  $\Delta$ 6-desaturase suggesting that EPA synthesis occurs via both conventional and alternative routes. *P. tricornutum* has a unique plastidial  $\Delta$ 12 desaturase that contributes to formation of the unique hexadecatrienoic acid (16:3  $\Delta$ 6,9,12) in chloroplasts, which is further extended to C16:4 by  $\omega$ -3 fatty acid desaturase (FAD) [46].

Although PKS for PUFA production is functionally characterized in bacteria and thraustochytrids, functional annotation of the marine heterokont *Nannochloropsis oceanica* CCMP1779 genome identified a homolog of PKS [46], which was **phylogenetically** similar to fungal PKS [47]. Furthermore, Balzano *et al.* identified 22 putative genes encoding PKS across *Nannochloropsis* sp., which can be classified into four clades on the basis of the sequence alignment of the KAS-PKS domain [48]. Clade-I PKS comprises iterative multidomain enzymes, which might be involved in PUFA production, whereas clade-II enzymes have only acyl transferase and KAS domains and are involved in formation of long-chain hydroxylated fatty acids [48]. The nonphotosynthetic microalga *C. cohnii* has both aerobic desaturation pathways and genes encoding PKS clusters [17]. Interestingly,  $\Delta$ 12 and  $\Delta$ 15 desaturases are absent in *C. cohnii* and studies with inhibitors targeting  $\Delta$ 5 and  $\Delta$ 6 desaturases did not find any alteration of the fatty acid profile. This evidence suggests that *C. cohnii* uses PKS for production of DHA, although further research is required to support this hypothesis [49]. Apart from identifying alternative routes, omics studies have also identified various transcription factors regulating PUFA biosynthetic genes (Box 1).

### 'Omics' technologies and $\omega$ -fatty acid synthesis

Using omics tools has revolutionized various research fields, such as identifying cancer markers, novel therapeutic targets in antimicrobial resistance, or generation of stress-tolerant plants [50–52]. Microalgal omics has mainly focused on **biofuel** production and is used for understanding the effect of climate change, such as ocean acidification, on microalgal physiology [53]. However, integrated omics studies focused on  $\omega$ -3 fatty production remain in the development stage. Table 2 details omics studies identifying key genes responsible for  $\omega$ -3 fatty acid production in microalgae.

### Photosynthesis-mediated remodeling of $\omega$ -fatty acids

A major proportion of  $\omega$ -fatty acids is allocated to galactolipids [monogalactosyldiacylglycerol (MGDG) and **digalactosyldiacylglycerol (DGDG)**] in the thylakoid membrane. These lipids

### Box 1. Transcriptional regulation of $\omega$ -fatty acid synthesis

Transcription factors (TFs) regulate gene expression by binding to *cis* elements through their DNA-binding domains and either activating or repressing transcription. Rapidly advancing omics technologies have identified several TFs that regulate metabolic genes in microalgae [108]. Various TF families, such as bZIP and MYB, have been identified to be involved in modulating lipid pathways. For example, in *Chlamydomonas reinhardtii*, the bZIP2 expression profile was positively correlated with diacylglycerol acyltransferase during nitrogen deprivation, suggesting its role in TAG accumulation [109]. bZIP77 was also found to regulate blue light-mediated TAG accumulation in *Nannochloropsis oceanica* [110]. Another family, MYB, was found to regulate transport of fatty acids from chloroplast to ER in *C. reinhardtii* during nitrogen deprivation by targeting fatty acid exporters, acyl-ACP thioesterase, and long-chain acyl-CoA synthetase [111]. Hu *et al.* identified 30 TFs involved in lipid pathways in *N. oceanica* IMET1 through gene mining and coexpression analysis [112]. Ajjawi *et al.* generated RNAi-mediated knockdown of the ZnCys TF, and found upregulation of several elongases and desaturases in mutant *Nannochloropsis gaditana*. This suggests either a direct transcriptional control or indirect regulation of conventional elongase-desaturase pathways by ZnCys [113]. MYB modulates the transcription of fatty acid desaturase (FAD8 and FAD3) and its overexpression resulted in a lower PUFA content and reduced membrane fluidity in *Arabidopsis thaliana* [114]. Transcriptomic studies revealed homologs of MYB (i.e., MYB106 and MYB94) in *Phaeodactylum tricornutum*, which were found to be downregulated during the EPA accumulation stage [115]. However, our understanding of the TF-mediated regulation of elongases/desaturases and the PKS pathway for  $\omega$ -fatty acid production remains in its infancy and requires further experimental investigation.

serve as a matrix for the photosynthetic complexes [i.e., photosystem II (PSII) and I (PSI)] and ATP synthase embedded in the thylakoid membrane. Environmental cues such as temperature and light, significantly alter the lipid composition of the thylakoid membrane, thereby affecting oxygenic photosynthesis [54]. Low light intensities, such as  $90 \mu\text{mol m}^{-2} \text{s}^{-1}$  increased the EPA content in *M. subterraneus* [55]. In *P. tricornutum*, high light intensities ( $750 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) were reported to decrease the PUFA content, whereas low irradiance increased it, resulting in EPA comprising 5.7 % of the TFA. A decreased EPA content as well as decreased total PUFA content upon high light irradiance can be attributed to downregulation of  $\Delta 6$  desaturase, the key enzyme in  $\omega$ -fatty acid biosynthesis [56]. By contrast, proteomic insights into *P. tricornutum* exposed to dark stress identified upregulation of  $\Delta 9$  desaturase and EAR, resulting in a higher overall lipid and EPA content [57].

Higher plants and microalgae prevent photodamage resulting from high irradiance through **de-epoxidation** of violaxanthin in the xanthophyll cycle [58]. MGDG in the thylakoid membrane acts as a solvent for these xanthophyll cycle pigments, regulating the conversion of violaxanthin to zeaxanthin [59]. Apart from varying light intensities,  $\omega$ -fatty acids are significantly altered by light wavelength. For instance, multichromatic white light ( $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was identified to increase the EPA content in *Nannochloropsis* spp, because red light enhanced the absorption ability of PSII, while PSI was improved by blue light. The highest EPA productivity for *N. oceanica* CY2 ( $13.24 \text{ mg l}^{-1}$  per day) was achieved in a photobioreactor equipped with both blue and red light-emitting diodes [60]. **Transcriptomic** studies identified upregulation of  $\Delta 6$  and  $\Delta 9$  desaturases in the presence of blue light ( $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), further supporting the enhanced EPA accumulation in *Nannochloropsis gaditana* [61]. Blue light is sensed by aureochrome proteins, which contain a light-oxygen voltage-sensing domain along with a DNA-binding domain, thereby modulating gene transcription in response to light. Reduced expression of aureochrome genes was associated with lower transcript abundance of blue light-induced  $\Delta 12$ ,  $\Delta 9$ ,  $\Delta 6$  and  $\Delta 5$  desaturases in *N. oceanica* CCMP1779 [62].

### Omics to identify key players of abiotic factor-mediated $\omega$ -fatty acid synthesis

Modulation of various parameters, such as carbon, nitrogen, light, and temperature, has been reported to affect PUFA production in microalgae. Figure 2 highlights all the relevant nodes augmenting  $\omega$ -3 fatty acid production under the influence of various abiotic factors. Photosynthetic microalgae fix atmospheric  $\text{CO}_2$  using the **Calvin–Benson–Bassham (CBB) cycle** to glyceraldehyde-3-

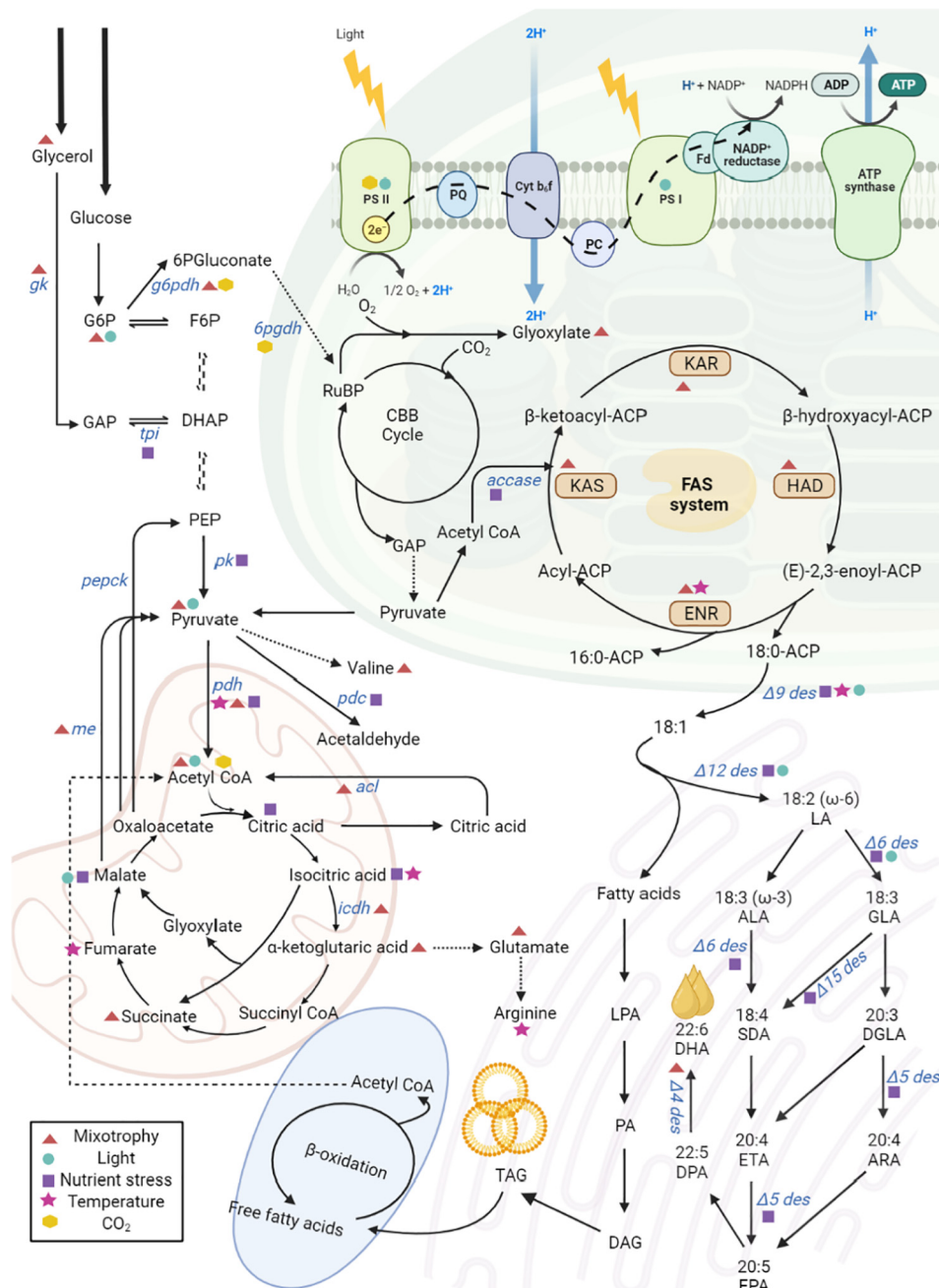
Table 2. Omics studies to identify relevant hubs for  $\omega$ -fatty acid production<sup>a</sup>

Microalga	Culture condition	Omics platform	Identified targets	Refs
<i>Nannochloropsis gaditana</i>	Blue light	Transcriptomics	$\Delta 6$ and $\Delta 12$ desaturase	[61]
		Metabolomics	Malic acid	
<i>Nannochloropsis oceanica</i>	Blue light	Transcriptomics	Aureochrome 3	[62]
<i>Chlamydomonas</i> sp. ICE-L	Cold stress	Transcriptomics	$\Delta 9$ desaturase	[124]
<i>N. oceanica</i> IMET1	Nitrogen starvation	Transcriptomics	Acyl-ACP thioesterase, $\Delta 9$ -FAD, pyruvate decarboxylase, pyruvate kinase	[125]
<i>Nitzschia laevis</i>	Mixotrophy (glucose)	Metabolomics	$\alpha$ -Ketoglutarate, pyruvate, valine	[79]
		Transcriptomics	KAS, HAD	
<i>Scenedesmus</i> sp. NREL 46B-D3	Cold stress	Metabolomics	Fumarate, arginine, ornithine	[69]
<i>Scenedesmus</i> sp. NREL 46B-D3, <i>Lobosphaera bisecta</i>	Cold stress, nitrogen starvation	Transcriptomics	ENR, $\Delta 7$ desaturase, Myb, $\Delta 6$ and $\Delta 5$ desaturase, elongase	[57]
<i>Phaeodactylum tricornutum</i>	Light	Transcriptomics	$\Delta 5$ , $\Delta 6$ , and $\Delta 12$ desaturase	[126]
<i>Neodesmus</i> sp. UTEX 2219-4	Osmotic stress	Transcriptomics	ACCase, $\Delta 15$ desaturase, pyruvate dehydrogenase, triose phosphate isomerase	[127]
<i>P. tricornutum</i>	Dark conditions	Proteomics	Pyruvate dehydrogenase, ENR	[128]
<i>Isochrysis galbana</i>	Nitrogen starvation and acetate supplementation	Proteomics	$\Delta 9$ and $\Delta 12$ desaturase	[129]
<i>N. gaditana</i>	Nitrogen and phosphorus starvation	Proteomics	$\Delta 5$ desaturase	[71]
<i>P. tricornutum</i>	Dark conditions	Proteomics	Enoyl ACP reductase, $\Delta 9$ -desaturase	[57]
<i>Nannochloropsis oculata</i>	Chemical mutagenesis	Proteomics	Fatty acid desaturase type 2, lipid droplet surface protein	[130]
<i>P. tricornutum</i>	Nutrient deprivation	Proteomics	TAG lipase	[75]
			Stearoyl-ACP desaturase, malonyl-CoA:ACP transacylase, 3-oxoacyl-[acyl-carrier-protein] synthase	[74]
			Lipid droplet protein	[131]
<i>Cryptocodinium cohnii</i>	Adaptive laboratory evolution	Proteomics	Medium-chain acyl-(acyl-carrier-protein) hydrolase, fructose-bisphosphate aldolase	[132]
<i>Nitzschia laevis</i>	Silica supplementation	Metabolomics	Citrate, isocitrate, malate	[128]
<i>Nitzschia closterium</i>	Red light and mixotrophy (glucose)	Metabolomics	Glucose-6-phosphate, pyruvate, acetyl-CoA	[129]
<i>I. galbana</i>	Temperature stress	Metabolomics	Oxaloacetic acid, citric acid, $\alpha$ -ketoglutaric acid, succinic acid, fumaric acid, and malic acid	[133]
<i>Nannochloropsis oceanica</i>	Gibberellic acid, salicylic acid, and malic acid	Metabolomics	ATP, NADPH, NADP, and NADH	[134]
<i>C. cohnii</i>	Nitrogen feeding	Metabolomics	D-ribose-5-phosphate	[135]
<i>I. galbana</i>	Temperature and light	Metabolomics	Homarine, dimethylsulfoniopropionate, and glycerol	[133]
<i>C. cohnii</i>	Starch-deficient mutant	Metabolomics	Tagatose	[136]
<i>Nannochloropsis salina</i>	Cold stress	Metabolomics	Isocitrate, glutamate	[137]
<i>Skeletonema marinoi</i>	Temperature stress	<b>Phenomics</b>	Chloroplast fatty acid transporter	[138]

<sup>a</sup>Abbreviations: ACCase, acetyl CoA carboxylase; ACP, acyl carrier protein; ENR, enoyl reductase; FAD,  $\omega$ -3 fatty acid desaturase; HAD, hydroxyacyl-ACP dehydratase; KAS, ketoacyl-ACP synthase.

phosphate, which is further converted to acetyl-CoA and directed toward lipid synthesis [63]. Microalgae can survive at a CO<sub>2</sub> concentration of 0.02% and certain species are able to grow efficiently at high CO<sub>2</sub> concentrations (e.g., 5%) [64]. Therefore, an optimal CO<sub>2</sub> concentration is required for attaining a maximum carbon-to-biomass conversion efficiency. High CO<sub>2</sub> (HC) supplementation





Trends in Plant Science

**Figure 2. Metabolic cues identified for omega (ω)-fatty acid biosynthesis under different cultivation parameters.** Microalgae modulate their physiology in the presence of various abiotic factors. The schematic highlights the relevant genes and the crosstalk among various pathways that modulate ω-fatty acid production under the influence of light, mixotrophy, CO<sub>2</sub> supplementation, nutrient stress, and temperature stress. Abbreviations: 6pgdh, 6-phospho-gluconate dehydrogenase; ACCase, acetyl-CoA carboxylase; acl, ATP-citrate lyase; ACP, acyl carrier protein; ALA, α-linolenic acid; ARA, arachidonic acid; CBB, Calvin–Benson–Bassham; DAG, diacylglycerol; des, desaturases; DGLA, dihomo-γ-linolenic acid; DHAP, dihydroxyacetone phosphate; DPA, docosapentaenoic acid; ENR/ER, enoyl reductase; ETA, eicosatetraenoic acid; F6P,

(Figure legend continued at the bottom of the next page.)

(i.e., 30 000 ppm) was reported to enhance biomass productivity (up to twofold) in *N. gaditana* with an increase in the PUFA content. Analysis of the fatty acid composition identified ALA and LA as the major contributors to the elevated PUFA content in this species [65]. Similarly, in *Pavlova lutheri*, a positive correlation between CO<sub>2</sub> concentration, ALA, and ARA was observed, whereas a negative correlation was found for EPA and DHA [66]. An accumulated pool of acetate in HC as identified by gas chromatography-mass spectrometry (GC-MS) **metabolomics** suggests the flux of carbon toward lipid synthesis in *M. gaditana*. Additionally, increased PSII efficiency was observed in HC, which leads to higher NADPH production required for desaturases involved in PUFA production [65]. Apart from directly influencing the lipid biosynthesis and desaturation pathways, CO<sub>2</sub> supplementation was also found to increase the mRNA expression of glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) in *P. tricornutum* [67]. G6PDH and 6PGDH are NADPH-generating enzymes of the oxidative pentose phosphate pathway in the cytosol, thereby providing reducing equivalents for lipid pathways.

Nutrient starvation has been extensively used in several microalgal strains to enhance **triacylglycerol (TAG)** accumulation [68]. However, in contrast to biofuel, nitrogen starvation was found to decrease  $\omega$ -3 fatty acid content in several native producers. The expression profile of various desaturases, such as  $\Delta$ 5 and  $\Delta$ 6 desaturases, were lower under nitrogen-limited conditions in *Eustigmatos vischeri* JHsu-01, *Chromochloris zofingiensis*, *N. gaditana*, and *Neodesmus* sp. 2219-4, thereby reducing the  $\omega$ -3 fatty acid content [69–73]. Several proteins involved in central energy metabolism and photosynthesis were differentially abundant during nitrogen starvation in the proteomic data sets of *P. tricornutum* [74]. A multi-omics analysis of nitrogen-starved *P. tricornutum* revealed regulation at each level (i.e., transcript, protein, and metabolite). Upregulation of transcripts for  $\Delta$ 6 and  $\Delta$ 9 desaturases coupled with abundant TAG lipase protein and an accumulated pool of betaine lipids and hydroxylated EPA suggest remodeling of membrane lipids to TAG during periods of nitrogen starvation [75].

The unsaturation level of MGDG imparts fluidity to the membrane, which enables the microalgae to thrive in a wide temperature range. **Proteomics** analysis revealed upregulation of  $\Delta$ 9 desaturase at low temperature (i.e., 7°C), in accordance with enhanced EPA production for *Xanthonema hormidioides* [76]. By contrast, PUFAs along with several elongases, pyruvate dehydrogenase, and PKS, were abundant at 28°C (at both the transcript and protein level) in *Auxenochlorella protothecoides* UTEX 2341 [77].

#### Mixotrophic supply of additional carbon skeletons for $\omega$ -fatty acid production

A major bottleneck faced by  $\omega$ -fatty acid production from photosynthetic microalgae is the lower biomass compared with that of heterotrophic hosts, such as *C. cohnii* and *Schizochytrium limacinum*. Supplying additional organic carbon along with photosynthesis can boost biomass production and make the process economically viable. Compared with autotrophy, supplementation with acetic acid was found to enhance EPA productivity in *Navicula saprophila*, *Rhodomonas salina*, and *Nitzschia* sp. The maximum EPA content was observed for *N. saprophila*, which was 41% higher compared with an autotrophic model [78]. In the marine diatom *Nitzschia laevis*, addition of 5 g l<sup>-1</sup> glucose substantially enhanced the EPA yield. Metabolomic profiling in the presence

---

fructose-6-phosphate; g6pdh, FAS, fatty acid synthase; G6P, glucose 6-phosphate; glucose-6-phosphate dehydrogenase; GAP, glyceraldehyde-3-phosphate; gk, glycerol kinase; GLA,  $\gamma$ -linolenic acid; icdh, isocitrate dehydrogenase; KAR/KR, ketoacyl reductase; KAS/KS, ketoacyl synthase; LA, linoleic acid; LPA, lysophosphatidic acid; me, malic enzyme; PA, phosphatidic acid; pdc, pyruvate decarboxylase; PC, plastocyanin; pdh, pyruvate dehydrogenase; PEP, phosphoenol pyruvate; pepck, phosphoenolpyruvate carboxykinase; pk, pyruvate kinase; PS, photosystem; RuBP, Ribulose-1,5-bisphosphate; SDA, stearidonic acid; TAG, triacylglycerol; tpi, triose phosphate isomerase. Figure created with BioRender ([biorender.com](https://www.biorender.com)).

of glucose identified accumulation of pyruvate,  $\alpha$ -ketoglutarate, and valine, which might contribute to the enhanced biomass [79]. Furthermore, transcriptomics revealed significant upregulation of KAS, KAR, HAD, Elongation of Very Long Chain Fatty Acid Proteins (ELOVL), and 3-ketoacyl-CoA synthase (KCS), in the presence of glucose. The latter two enzymes are responsible for extending palmitoyl-CoA and stearoyl-CoA to PUFAs [79].

Although glucose is an ideal carbon source, which can be metabolized by all species, it may elevate the input cost. Therefore, alternative carbon sources are frequently screened for microalgal cultivation. The pennate diatom *P. tricornutum* was reported to utilize variable carbon sources, such as fructose, mannose, lactose, and glycerol. The biomass, lipid, EPA, and pigment content were higher when glycerol and fructose were used as carbon sources [80]. Villanova *et al.* extensively studied the effect of mixotrophy with glycerol on *P. tricornutum* using multi-omics and **metabolic modeling** [81–83]. Glycerol was found to mimic responses similar to nitrogen limitation, thereby increasing TAG accumulation, although both photosynthetic activity and biomass were unaffected. Flux balance analysis predicted enhanced fluxes in the reaction associated with the oxidative pentose phosphate pathway, tricarboxylic acid (TCA) cycle, and glycolysis upon glycerol uptake [82]. Furthermore, accumulation of pyruvate suggests enhanced respiration in the presence of glycerol in *P. tricornutum* [82]. Although no significant difference was observed in the efficiency of PSII (i.e.,  $F_v/F_m$ ) in *P. tricornutum*, in *Chlamydomonas reinhardtii* and *Chromochloris zofingiensis*, photosynthetic pigments, thylakoid membrane proteins, and PSII efficiency were reduced in the presence of organic carbon substrates. Thus, the microalgal cells rely significantly on organic carbon substrates during mixotrophic growth and channel these sugars toward lipid synthesis [82,84,85].

An evolved glucose-tolerant strain of the heterotrophic microalga *C. cohnii* that can utilize  $45 \text{ g l}^{-1}$  glucose was characterized by the abundance of glycerol, glutamic acid, malonic acid, and succinic acid compared with an unevolved strain. These metabolites are reported to maintain the redox balance in cells and prevent substrate inhibition in *C. cohnii* [86]. During the DHA accumulation stage of *C. cohnii* fermentation, transcription of the genes encoding PKS and  $\Delta 9$  and  $\Delta 4$  desaturase was upregulated, as was that for mitochondrial pyruvate dehydrogenase and ATP-citrate lyase, generating an acetyl-CoA pool for lipid synthesis. Additionally, transcription of genes encoding enzymes involved in generating reducing power, such as malic enzyme and isocitrate dehydrogenase, was substantially higher during the lipid synthesis stage [17].

### Challenges in integrated omics ‘iOMICS’

Although the generation of large data sets using omics has advanced over the past few years, utilizing a single platform fails to provide a complete understanding of the biological system [87]. For instance, the abundant lipid precursor acetyl CoA observed in the metabolomics data sets might highlight upregulation of glycolysis, followed by pyruvate dehydrogenase or the fatty acid oxidation pathway. Additionally, there are several other pathways, such as branched-chain amino-acid metabolism, that generate acetyl CoA. Thus, to understand the dynamics of acetyl CoA metabolism, it is relevant to integrate metabolomics data sets with the transcriptome and proteome. Furthermore, these transcripts are induced by several cultivation parameters and are tightly regulated by transcription factors or other epigenetic modifications. Thus, transcriptomics coupled with ChIP-seq or identifying methylation at CpGs using Illumina's MethylationEPIC BeadChip arrays will provide a complete picture [88]. Understanding regulation at the protein level using phosphoproteomics, protein–protein interactions, and allosteric regulation could correlate with the metabolomic data, thus aiding the unveiling of phenotypic outcomes [89]. Integration of these multivariate omics data sets is insightful, yet several challenges, such as missing values and variable distribution in single omics or among different data sets, exist [90]. Furthermore,

heterogeneity in data sets with large differences in measured biomolecules, compared with the number of samples along with complex and noisy biological data, are additional challenges [91]. Several machine learning algorithms have eased the data integration process and been successfully used for various clinical studies [92]. Several user-friendly tools, such as Mapman, MixOmics, and 3Omics, have been developed for understanding interactive multi-omics data sets [93–95]. Additionally, this information can be utilized for generating models that can be trained and modulated to obtain a particular phenotype, such as high  $\omega$ -3 fatty acid yields.

### Boosting $\omega$ -fatty acid synthesis by fine-tuning regulatory nodes

In this review, we highlight how multi-omics platforms have been used to identify the key players involved in  $\omega$ -fatty acid synthesis and have identified new directions to enhance the production of these nutraceuticals. Furthermore, using synthetic biology approaches to fine-tune the production by overexpression of a positive regulator or knockout of a repressor can enhance yields in native  $\omega$ -fatty acid-producing strains [96]. For instance, as discussed previously, transcriptomics identified high correlation among  $\Delta$ 6 desaturase and EPA content in the presence of blue light in *N. gaditana* and *N. oceanica*. Yang *et al.* overexpressed this lipogenic desaturase and reported enhanced EPA content and photosynthetic efficiency in *N. oceanica* [97]. Alternatively, heterologous expression of certain regulatory genes can enrich the PUFA content in the fatty acid profile of high-yielding strains. In *Dunaliella salina*, heterologous expression of  $\Delta$ 6 desaturase from *Thalassiosira pseudonana* increased the EPA yield to 21.3 mg l<sup>-1</sup>, which was 13 times higher compared with wild type [24]. In native  $\omega$ -fatty acid-producing strains, overexpression of the gene identified using omics platform, encoding for  $\Delta$ 5 desaturase, resulted in enhanced EPA yields in *P. tricornutum* and *N. oceanica* [98,99]. Heterologous expression of  $\Delta$ 5 elongase and  $\Delta$ 6 desaturase derived from *Ostreococcus tauri*, resulted in an altered fatty acid profile of *P. tricornutum* and increased the DHA content up to eightfold [100]. Alternatively, overexpression of endogenous DGAT in *P. tricornutum* enhanced the DHA content along with its partitioning in TAG [101].

Apart from the lipid pathways, multi-omics studies highlight crosstalk among other metabolic pathways, which can be engineered to enhance  $\omega$ -fatty acid production. For instance, decarboxylation of oxaloacetate to phosphoenolpyruvate by phosphoenolpyruvate carboxykinase (PEPCK) can be regulated by RNAi-mediated knockdown of *pepck*, which redirects carbon flux toward lipid synthesis in *P. tricornutum* [102]. Malic enzyme (ME) supplies additional NADPH, which is required for lipid synthesis. Thus, overexpression of ME increased lipid yield in *N. salina* and *A. protothecoides* [103,104]. Furthermore, reconstructing lipid pathways in *Brassica napus* (canola) by introducing synthetic expression cassettes comprising *Schizochytrium* sp. ATCC 20888 PKS along with phosphopantetheinyl transferase (PPTase) from *Nostoc* sp. resulted in grains containing 0.7% EPA and 3.7% DHA [105]. Additionally, introduction of metabolic genes, such as those encoding xylose isomerase and  $\beta$ -glucosidases, filled the gap between sustainable production and input cost by enabling the use of cost-effective substrates [84,85].

### Economic viability of microalgal OMEGAs

Large-scale cultivation of microalgae for  $\omega$ -3 fatty acids can provide a sustainable alternative to commercial fish-oil and reduce the aquatic load. However, there are several bottlenecks in the scale-up process, such as high input cost of cultivation, maintaining optimal growth conditions, harvesting, and downstream processing [18]. Furthermore, compared with heterotrophic hosts, such as *C. cohnii* and thraustochytrids, microalgae are slow growing and have a relatively lower  $\omega$ -3 fatty acid content [106]. Exploiting the potential of microalgae to valorize waste effluents into these PUFAs will lower the input cost. Captivating these attributes of microalgae, several companies, such as MiAlgae, are utilizing coproducts from distilleries for sustainable production

of  $\omega$ -3 fatty acids<sup>iv</sup>. Several other commercial suppliers of microalgal-derived  $\omega$ -3 fatty acids include Life's DHA (DSM)<sup>v</sup> and AlgaPrime™ DHA (Corbion)<sup>vi</sup>, which are generally used as dietary supplements and aquafeed. *Nannochloropsis*-derived Almega®PL oil contains 25% EPA and was clinically validated to enhance the  $\omega$ -3 fatty acid index and decrease the cholesterol level in ~120 patients [107].

## Concluding remarks and future perspectives

The ability to modulate their cellular physiology under the influence of various abiotic factors and their ability to grow mixotrophically as well as in dark conditions make microalgae an ideal candidate for commercial  $\omega$ -3 fatty acid production. The complex architecture of the biosynthesis of these value-added fatty acids, with the presence of alternative routes, suggests the need to better understand the crosstalk among the metabolic pathways involved (see [Outstanding questions](#)). Although alternative pathways have been identified through genomics, the preferred route for  $\omega$ -fatty acid production still needs further research. Here, we have highlighted the major regulation at the pyruvate node (Figure 2) under the influence of various factors, suggesting it as the relevant target for synthetic modulation of  $\omega$ -fatty acid production. However, the number of relevant omics studies for native high-yielding strains, such as *Phaeodactylum* and *C. cohnii*, is relatively low. In addition, to address regulation at the post-transcriptional or post-translation level, integration of multiple omics data sets is required to obtain a holistic picture of the microalgal cell. Additionally, establishing a genome-scale metabolic model for  $\omega$ -fatty acid production could suggest routes to maximize production from these microalgae. Thus, omics tools could revolutionize the sustainable production of nutraceutically relevant  $\omega$ -fatty acids using microalgal cell factories.

## Acknowledgments

The authors would like to thank the Swedish Research Council (FORMAS) and Kempestiftelserna, Sweden for supporting this work as part of the projects 'Green and sustainable approach to valorise high saline and oily fish processing effluents for the production of nutraceuticals' (INVENTION; 2020-01028) and 'Boosting the squalene content in thraustochytrids by genetic engineering using CRISPR-Cas9 System to replace the shark-based squalene as an adjuvant for COVID 2019 vaccine' (JCK-2115).

## Declaration of interests

The authors declare no competing interest.

## Resources

<sup>i</sup><https://ods.od.nih.gov/factsheets/Omega3FattyAcids-Consumer/>

<sup>ii</sup>[www.healthline.com/nutrition/how-much-omega-3](http://www.healthline.com/nutrition/how-much-omega-3)

<sup>iii</sup>[www.marketsandmarkets.com/Market-Reports/omega-3-omega-6-227.html](http://www.marketsandmarkets.com/Market-Reports/omega-3-omega-6-227.html)

<sup>iv</sup>[www.mialgae.com](http://www.mialgae.com)

<sup>v</sup>[www.lifesdha.com/en\\_US/home.html](http://www.lifesdha.com/en_US/home.html)

<sup>vi</sup>[www.corbion.com/Products/Algae-ingredients-products/AlgaPrimeDHA](http://www.corbion.com/Products/Algae-ingredients-products/AlgaPrimeDHA)

## References

- Diao, J. *et al.* (2020) Cellular engineering strategies toward sustainable omega-3 long chain polyunsaturated fatty acids production: state of the art and perspectives. *Biotechnol. Adv.* 40, 107497
- National Research Council (US) Subcommittee on the Tenth Edition of the Recommended Dietary Allowances (1989) *Recommended Dietary Allowances: 10th Edition*, National Academies Press (US), Washington (DC) PMID: 25144070
- Wang, B. *et al.* (2021) Metabolism pathways of arachidonic acids: mechanisms and potential therapeutic targets. *Signal Transduct. Target. Ther.* 6, 94
- Brenna, J.T. *et al.* (2009) Alpha-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. *Prostaglandins Leukot. Essent. Fat. Acids* 80, 85–91
- Greupner, T. *et al.* (2018) Effects of a 12-week high- $\alpha$ -linolenic acid intervention on EPA and DHA concentrations in red blood cells and plasma oxylipin pattern in subjects with a low EPA and DHA status. *Food Funct.* 9, 1587–1600
- Yuan, Q. *et al.* (2022) The review of alpha-linolenic acid: sources, metabolism, and pharmacology. *Phytother. Res.* 36, 164–188
- Tallima, H. and El Ridi, R. (2018) Arachidonic acid: physiological roles and potential health benefits - a review. *J. Advert. Res.* 11, 33–41
- Dyall, S.C. *et al.* (2022) Polyunsaturated fatty acids and fatty acid-derived lipid mediators: recent advances in the understanding of their biosynthesis, structures, and functions. *Prog. Lipid Res.* 86, 101165

## Outstanding questions

Understanding why marine microalgae synthesize PUFAs, such as EPA and DHA, while freshwater strains primarily produce ALA, is crucial. Investing the competitive advantages of marine over fresh strains due to their ability to synthesize PUFAs will further advance our understanding of the response of microalgae to various factors.

What is the evolutionary significance of the presence of more than one conventional route for the production of EPA or DHA? This could further highlight the adaptability of microalgae to different environmental conditions or redundancy in metabolic pathways.

Microalgae, such as *Nannochloropsis*, have additional PKS clusters, similar to thraustochytrids. Do they participate in EPA production?

Identifying the transcription factors that regulate enzymes involved in PUFA synthesis, such as elongases, desaturases, and PKS, is fundamental to understanding the genetic control of these pathways.

Elongation of C18:0 to C20:5 or C22:6 occurs in ER, after which these PUFAs are exported back to the chloroplast membrane. How is EPA imported back to the thylakoid membrane? Despite it being an energy-intensive process, investigating the mechanism behind EPA import back to the thylakoid membrane could reveal specific advantages or constraints to microalgae metabolism.

Import of 20:5 might be an ATP-consuming process; why do microalgae use this energy-intensive process rather than extending fatty acids in ER itself? Such information will provide insights into the metabolic compartmentalization of biosynthetic pathway.



9. Bazan, N.G. *et al.* (2011) Docosahexaenoic acid signalolipidomics in nutrition: significance in aging, neuroinflammation, macular degeneration, Alzheimer's, and other neurodegenerative diseases. *Annu. Rev. Nutr.* 31, 321–351
10. Chang, J.P.-C. *et al.* (2019) High-dose eicosapentaenoic acid (EPA) improves attention and vigilance in children and adolescents with attention deficit hyperactivity disorder (ADHD) and low endogenous EPA levels. *Transl. Psychiatry* 9, 303
11. Sinclair, A.J. (2020) Docosahexaenoic acid and the brain - what is its role? *Asia Pac. J. Clin. Nutr.* 28, 675–688
12. Huang, C. *et al.* (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506
13. Szabó, Z. *et al.* (2020) The potential beneficial effect of EPA and DHA supplementation managing cytokine storm in coronavirus disease. *Front. Physiol.* 11, 752
14. Rizzo, G. *et al.* (2023) Promising sources of plant-derived polyunsaturated fatty acids: a narrative review. *Int. J. Environ. Res. Public Health* 20, 1683
15. Gammone, M.A. *et al.* (2018) Omega-3 polyunsaturated fatty acids: benefits and endpoints in sport. *Nutrients* 11, 46
16. Sarker, P.K. *et al.* (2020) Microalgae-blend tilapia feed eliminates fishmeal and fish oil, improves growth, and is cost viable. *Sci. Rep.* 10, 19328
17. Pei, G. *et al.* (2017) De novo transcriptomic and metabolomic analysis of docosahexaenoic acid (DHA)-producing *Cryptocodinium cohnii* during fed-batch fermentation. *Algal Res.* 26, 380–391
18. Magoni, C. *et al.* (2022) Could microalgae be a strategic choice for responding to the demand for omega-3 fatty acids? A European perspective. *Trends Food Sci. Technol.* 121, 142–155
19. Wang, X. *et al.* (2019) Influence of nitrogen limitation on lipid accumulation and EPA and DHA content in four marine microalgae for possible use in aquafeed. *Front. Mar. Sci.* 6, 95
20. Lang, I. *et al.* (2011) Fatty acid profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection. *BMC Plant Biol.* 11, 124
21. Batista, A.P. *et al.* (2013) Comparison of microalgal biomass profiles as novel functional ingredient for food products. *Algal Res.* 2, 164–173
22. Bhosale, R.A. *et al.* (2010) *Dunaliella salina* Teod. as a prominent source of eicosapentaenoic acid. *Int. J. Algae* 12, 185–189
23. Banskota, A.H. *et al.* (2019) Antioxidant properties and lipid composition of selected microalgae. *J. Appl. Phycol.* 31, 309–318
24. Shi, H. *et al.* (2018) Production of eicosapentaenoic acid by application of a delta-6 desaturase with the highest ALA catalytic activity in algae. *Microb. Cell Factories* 17, 7
25. Salama, E.-S. *et al.* (2013) Biomass, lipid content, and fatty acid composition of freshwater *Chlamydomonas mexicana* and *Scenedesmus obliquus* grown under salt stress. *Bioprocess Biosyst. Eng.* 36, 827–833
26. Ördög, V. *et al.* (2013) Lipid productivity and fatty acid composition in *Chlorella* and *Scenedesmus* strains grown in nitrogen-stressed conditions. *J. Appl. Phycol.* 25, 233–243
27. Mariam, I. *et al.* (2021) Delineating metabolomic changes in native isolate *Aurantiochytrium* for production of docosahexaenoic acid in presence of varying carbon substrates. *Algal Res.* 55, 102285
28. Patel, A. *et al.* (2022) A novel bioprocess engineering approach to recycle hydrophilic and hydrophobic waste under high salinity conditions for the production of nutraceutical compounds. *Chem. Eng. J.* 431, 133955
29. De Bhowmick, G. *et al.* (2015) Metabolic pathway engineering towards enhancing microalgal lipid biosynthesis for biofuel application—a review. *Renew. Sust. Energ. Rev.* 50, 1239–1253
30. Shaikh, K.M. *et al.* (2020) Hybrid genome assembly and functional annotation reveals insights on lipid biosynthesis of oleaginous native isolate *Parachlorella kessleri*, a potential industrial strain for production of biofuel precursors. *Algal Res.* 52, 102118
31. Gong, Y. *et al.* (2014) Metabolic engineering of microorganisms to produce omega-3 very long-chain polyunsaturated fatty acids. *Prog. Lipid Res.* 56, 19–35
32. Cohen, Z. *et al.* (1992) Overproduction of gamma-linolenic and eicosapentaenoic acids by algae. *Plant Physiol.* 98, 569–572
33. Domergue, F. *et al.* (2002) Cloning and functional characterization of *Phaeodactylum tricornutum* front-end desaturases involved in eicosapentaenoic acid biosynthesis. *Eur. J. Biochem.* 269, 4105–4113
34. Khozin, I. *et al.* (1997) Elucidation of the biosynthesis of EPA in the microalga *Porphyridium cruentum* (II. Studies with radiolabeled precursors). *Plant Physiol.* 114, 223–230
35. Khozin-Goldberg, I. *et al.* (2002) Biosynthesis of eicosapentaenoic acid (EPA) in the freshwater eustigmatophyte *Monodus subterraneus* (Eustigmatophyceae) 1. *J. Phycol.* 38, 745–756
36. Schneider, J.C. and Roessler, P. (1994) Radiolabeling studies of lipids and fatty acids in *Nannochloropsis* (Eustigmatophyceae), an oleaginous marine alga 1. *J. Phycol.* 30, 594–598
37. Shanab, S.M.M. *et al.* (2018) A review on algae and plants as potential source of arachidonic acid. *J. Advert. Res.* 11, 3–13
38. Metz, J.G. *et al.* (2001) Production of polyunsaturated fatty acids by polyketide synthases in both prokaryotes and eukaryotes. *Science* 293, 290–293
39. Jenke-Kodama, H. *et al.* (2005) Evolutionary implications of bacterial polyketide synthases. *Mol. Biol. Evol.* 22, 2027–2039
40. Sun, X.-M. *et al.* (2019) Enhancement of lipid accumulation in microalgae by metabolic engineering. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1864, 552–566
41. Bell, M.V. and Pond, D. (1996) Lipid composition during growth of motile and coccoid forms of *Emiliania huxleyi*. *Phytochemistry* 41, 465–471
42. Sayanova, O. *et al.* (2011) Identification and functional characterization of genes encoding the omega-3 polyunsaturated fatty acid biosynthetic pathway from the coccolithophore *Emiliania huxleyi*. *Phytochemistry* 72, 594–600
43. Qi, B. *et al.* (2002) Identification of a cDNA encoding a novel C18-Delta(9) polyunsaturated fatty acid-specific elongating activity from the docosahexaenoic acid (DHA)-producing microalga, *Isochrysis galbana*. *FEBS Lett.* 510, 159–165
44. Zhou, X.-R. *et al.* (2007) Isolation and characterization of genes from the marine microalga *Pavlova salina* encoding three front-end desaturases involved in docosahexaenoic acid biosynthesis. *Phytochemistry* 68, 785–796
45. Domergue, F. *et al.* (2003) New insight into *Phaeodactylum tricornutum* fatty acid metabolism. Cloning and functional characterization of plastidial and microsomal delta12-fatty acid desaturases. *Plant Physiol.* 131, 1648–1660
46. Vieler, A. *et al.* (2012) Genome, functional gene annotation, and nuclear transformation of the heterokont oleaginous alga *Nannochloropsis oceanica* CCMP1779. *PLoS Genet.* 8, e1003064
47. Poliner, E. *et al.* (2015) Transcriptional coordination of physiological responses in *Nannochloropsis oceanica* CCMP1779 under light/dark cycles. *Plant J.* 83, 1097–1113
48. Balzano, S. *et al.* (2019) Biosynthesis of long chain alkyl diols and long chain alkenols in *Nannochloropsis* spp. (Eustigmatophyceae). *Plant Cell Physiol.* 60, 1666–1682
49. de Swaaf, M.E. *et al.* (2003) Analysis of docosahexaenoic acid biosynthesis in *Cryptocodinium cohnii* by <sup>13</sup>C labelling and desaturase inhibitor experiments. *J. Biotechnol.* 103, 21–29
50. Xiao, Y. *et al.* (2022) Multi-omics approaches for biomarker discovery in early ovarian cancer diagnosis. *EBioMedicine* 79, 104001
51. Yow, H.-Y. *et al.* (2022) Optimizing antimicrobial therapy by integrating multi-omics with pharmacokinetic/pharmacodynamic models and precision dosing. *Front. Pharmacol.* 13, 915355
52. Zargar, S.M. *et al.* (2021) Physiological and multi-Omics approaches for explaining drought stress tolerance and supporting sustainable production of rice. *Front. Plant Sci.* 12, 803603
53. Lin, W.-R. *et al.* (2019) Challenges and opportunity of recent genome editing and multi-omics in cyanobacteria and microalgae for biorefinery. *Bioresour. Technol.* 291, 121932
54. Hernández, M.L. and Cejudo, F.J. (2021) Chloroplast lipids metabolism and function. A redox perspective. *Front. Plant Sci.* 12, 712022
55. Cohen, Z. (1994) Production potential of eicosapentaenoic acid by *Monodus subterraneus*. *J. Am. Oil Chem. Soc.* 71, 941–945
56. Conceição, D. *et al.* (2020) The effect of light intensity on the production and accumulation of pigments and fatty acids in *Phaeodactylum tricornutum*. *J. Appl. Phycol.* 32, 1017–1025
57. Bai, X. *et al.* (2016) Proteomic analyses bring new insights into the effect of a dark stress on lipid biosynthesis in *Phaeodactylum tricornutum*. *Sci. Rep.* 6, 25494

58. Perin, G. *et al.* (2023) Modulation of xanthophyll cycle impacts biomass productivity in the marine microalga *Nannochloropsis*. *Proc. Natl. Acad. Sci. U. S. A.* 120, e2214119120
59. Goss, R. and Latowski, D. (2020) Lipid dependence of xanthophyll cycling in higher plants and algae. *Front. Plant Sci.* 11, 455
60. Chen, C.-Y. *et al.* (2015) Enhancing the production of eicosapentaenoic acid (EPA) from *Nannochloropsis oceanica* CY2 using innovative photobioreactors with optimal light source arrangements. *Bioresour. Technol.* 191, 407–413
61. Patelou, M. *et al.* (2020) Transcriptomic and metabolomic adaptation of *Nannochloropsis gaditana* grown under different light regimes. *Algal Res.* 45, 101735
62. Poliner, E. *et al.* (2022) Aureochromes maintain polyunsaturated fatty acid content in *Nannochloropsis oceanica*. *Plant Physiol.* 189, 906–921
63. Kareya, M.S. *et al.* (2022) Valorization of carbon dioxide (CO<sub>2</sub>) to enhance production of biomass, biofuels, and biorenewables (B<sup>3</sup>) in *Chlorella saccharophila* UTEX247: a circular bioeconomy perspective. *Biofuels Bioprod. Biorefin.* 16, 682–697
64. Wang, Y. *et al.* (2015) The CO<sub>2</sub> concentrating mechanism and photosynthetic carbon assimilation in limiting CO<sub>2</sub>: how *Chlamydomonas* works against the gradient. *Plant J.* 82, 429–448
65. Kareya, M.S. *et al.* (2020) Photosynthetic carbon partitioning and metabolic regulation in response to very-low and high CO<sub>2</sub> in *Microchloropsis gaditana* NIES 2587. *Front. Plant Sci.* 11, 981
66. Carvalho, A.P. and Maccata, F.X. (2005) Optimization of omega-3 fatty acid production by microalgae: crossover effects of CO<sub>2</sub> and light intensity under batch and continuous cultivation modes. *Mar. Biotechnol.* 7, 381–388
67. Wu, S. *et al.* (2015) Enzyme activity highlights the importance of the oxidative pentose phosphate pathway in lipid accumulation and growth of *Phaeodactylum tricornutum* under CO<sub>2</sub> concentration. *Biotechnol. Biofuels* 8, 78
68. Shaikh, K.M. *et al.* (2019) Molecular profiling of an oleaginous trebouxiophyceal alga *Parachlorella kessleri* subjected to nutrient deprivation for enhanced biofuel production. *Biotechnol. Biofuels* 12, 182
69. Chang, W.C. *et al.* (2016) Comparative transcriptome analysis reveals a potential photosynthate partitioning mechanism between lipid and starch biosynthetic pathways in green microalgae. *Algal Res.* 16, 54–62
70. Janssen, J.H. *et al.* (2020) Time-dependent transcriptome profile of genes involved in triacylglycerol (TAG) and polyunsaturated fatty acid synthesis in *Nannochloropsis gaditana* during nitrogen starvation. *J. Appl. Phycol.* 32, 1153–1164
71. Hulatt, C.J. *et al.* (2020) Proteomic and transcriptomic patterns during lipid remodeling in *Nannochloropsis gaditana*. *Int. J. Mol. Sci.* 21, 6946
72. Wu, T. *et al.* (2021) Characterization of fatty acid desaturases reveals stress-induced synthesis of C18 unsaturated fatty acids enriched in triacylglycerol in the oleaginous alga *Chromochloris zofingiensis*. *Biotechnol. Biofuels* 14, 184
73. Xu, J. *et al.* (2020) Lipid accumulation and eicosapentaenoic acid distribution in response to nitrogen limitation in microalga *Eustigmatos vischeri* JHsu-01 (Eustigmatophyceae). *Algal Res.* 48, 101910
74. Longworth, J. *et al.* (2016) Proteome response of *Phaeodactylum tricornutum*, during lipid accumulation induced by nitrogen depletion. *Algal Res.* 18, 213–224
75. Remmers, I.M. *et al.* (2018) Orchestration of transcriptome, proteome and metabolome in the diatom *Phaeodactylum tricornutum* during nitrogen limitation. *Algal Res.* 35, 33–49
76. Gao, B. *et al.* (2023) The growth, lipid accumulation and adaptation mechanism in response to variation of temperature and nitrogen supply in psychrotrophic filamentous microalga *Xanthonema hormidioides* (Xanthophyceae). *Biotechnol. Biofuels Bioprod.* 16, 12
77. Xing, G. *et al.* (2018) Integrated analyses of transcriptome, proteome and fatty acid profilings of the oleaginous microalga *Auxenochlorella protothecoides* UTEX 2341 reveal differential reprogramming of fatty acid metabolism in response to low and high temperatures. *Algal Res.* 33, 16–27
78. Kitano, M. *et al.* (1997) Changes in eicosapentaenoic acid content of *Navicula saprophila*, *Rhodomonas salina* and *Nitzschia* sp. under mixotrophic conditions. *J. Appl. Phycol.* 9, 559–563
79. Mao, X. *et al.* (2021) Transcriptomics and metabolomics analyses provide novel insights into glucose-induced trophic transition of the marine diatom *Nitzschia laevis*. *Mar. Drugs* 19, 426
80. Cerón García, M.C. *et al.* (2006) Mixotrophic production of marine microalga *Phaeodactylum tricornutum* on various carbon sources. *J. Microbiol. Biotechnol.* 16, 689–694
81. Villanova, V. *et al.* (2021) Boosting biomass quantity and quality by improved mixotrophic culture of the diatom *Phaeodactylum tricornutum*. *Front. Plant Sci.* 12, 642199
82. Villanova, V. *et al.* (2017) Investigating mixotrophic metabolism in the model diatom *Phaeodactylum tricornutum*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 372, 20160404
83. Villanova, V. and Spetea, C. (2021) Mixotrophy in diatoms: molecular mechanism and industrial potential. *Physiol. Plant.* 173, 603–611
84. Vidotti, A.D.S. *et al.* (2020) Analysis of autotrophic, mixotrophic and heterotrophic phenotypes in the microalgae *Chlorella vulgaris* using time-resolved proteomics and transcriptomics approaches. *Algal Res.* 51, 102060
85. Roth, M.S. *et al.* (2019) Regulation of oxygenic photosynthesis during trophic transitions in the green alga *Chromochloris zofingiensis*. *Plant Cell* 31, 579–601
86. Li, X. *et al.* (2017) Metabolomic analysis and lipid accumulation in a glucose tolerant *Cryptocodinium cohnii* strain obtained by adaptive laboratory evolution. *Bioresour. Technol.* 235, 87–95
87. Subramanian, I. *et al.* (2020) Multi-omics data integration, interpretation, and its application. *Bioinform. Biol. Insights* 14, 1177932219899051
88. Graw, S. *et al.* (2021) Multi-omics data integration considerations and study design for biological systems and disease. *Mol. Omics* 17, 170–185
89. Misra, B.B. *et al.* (2018) Integrated Omics: tools, advances, and future approaches. *J. Mol. Endocrinol.* D62, R21–R45
90. Pinu, F.R. *et al.* (2019) Systems biology and multi-omics integration: viewpoints from the metabolomics research community. *Metabolites* 9, 76
91. Kang, M. *et al.* (2022) A roadmap for multi-omics data integration using deep learning. *Brief. Bioinform.* 23, bbab454
92. Flores, J.E. *et al.* (2023) Missing data in multi-omics integration: recent advances through artificial intelligence. *Front. Artif. Intell.* 6, 1098308
93. Thimm, O. *et al.* (2004) MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *Plant J.* 37, 914–939
94. Rohart, F. *et al.* (2017) mixOmics: an R package for 'omics feature selection and multiple data integration. *PLoS Comput. Biol.* 13, e1005752
95. Kuo, T.-C. *et al.* (2013) 3Omics: a web-based systems biology tool for analysis, integration and visualization of human transcriptomic, proteomic and metabolomic data. *BMC Syst. Biol.* 7, 64
96. Muñoz, C.F. *et al.* (2021) Genetic engineering of microalgae for enhanced lipid production. *Biotechnol. Adv.* 52, 107836
97. Yang, F. *et al.* (2019) Harnessing the lipogenic potential of  $\Delta 6$ -desaturase for simultaneous hyperaccumulation of lipids and polyunsaturated fatty acids in *Nannochloropsis oceanica*. *Front. Mar. Sci.* 6, 682
98. Poliner, E. *et al.* (2018) A toolkit for *Nannochloropsis oceanica* CCMP1779 enables gene stacking and genetic engineering of the eicosapentaenoic acid pathway for enhanced long-chain polyunsaturated fatty acid production. *Plant Biotechnol. J.* 16, 298–309
99. Peng, K.-T. *et al.* (2014) Delta 5 fatty acid desaturase upregulates the synthesis of polyunsaturated fatty acids in the marine diatom *Phaeodactylum tricornutum*. *J. Agric. Food Chem.* 62, 8773–8776
100. Hamilton, M.L. *et al.* (2014) Metabolic engineering of *Phaeodactylum tricornutum* for the enhanced accumulation of omega-3 long chain polyunsaturated fatty acids. *Metab. Eng.* 22, 3–9
101. Haslam, R.P. *et al.* (2020) Overexpression of an endogenous type 2 diacylglycerol acyltransferase in the marine diatom *Phaeodactylum tricornutum* enhances lipid production and omega-3 long-chain polyunsaturated fatty acid content. *Biotechnol. Biofuels* 13, 87

102. Yang, J. *et al.* (2016) Knockdown of phosphoenolpyruvate carboxykinase increases carbon flux to lipid synthesis in *Phaeodactylum tricornutum*. *Algal Res.* 15, 50–58
103. Jeon, S. *et al.* (2021) Enhancement of lipid production in *Nannochloropsis salina* by overexpression of endogenous NADP-dependent malic enzyme. *Algal Res.* 54, 102218
104. Yan, J. *et al.* (2019) Engineering a malic enzyme to enhance lipid accumulation in *Chlorella protothecoides* and direct production of biodiesel from the microalgal biomass. *Biomass Bioenergy* 122, 298–304
105. Walsh, T.A. *et al.* (2016) Canola engineered with a microalgal polyketide synthase-like system produces oil enriched in docosahexaenoic acid. *Nat. Biotechnol.* 34, 881–887
106. Barone, G.D. *et al.* (2023) Recent developments in the production and utilization of photosynthetic microorganisms for food applications. *Heliyon* 9, e14708
107. Rao, A. *et al.* (2020) Omega-3 eicosapentaenoic acid (EPA) rich extract from the microalga *Nannochloropsis* decreases cholesterol in healthy individuals: a double-blind, randomized, placebo-controlled, three-month supplementation study. *Nutrients* 12, 1869
108. Bajhaiya, A.K. *et al.* (2017) Transcriptional engineering of microalgae: prospects for high-value chemicals. *Trends Biotechnol.* 35, 95–99
109. Gargouri, M. *et al.* (2015) Identification of regulatory network hubs that control lipid metabolism in *Chlamydomonas reinhardtii*. *J. Exp. Bot.* 66, 4551–4566
110. Zhang, P. *et al.* (2022) Exploring a blue-light-sensing transcription factor to double the peak productivity of oil in *Nannochloropsis oceanica*. *Nat. Commun.* 13, 1664
111. Choi, B.Y. *et al.* (2022) The *Chlamydomonas* transcription factor MYB1 mediates lipid accumulation under nitrogen depletion. *New Phytol.* 235, 595–610
112. Hu, J. *et al.* (2014) Genome-wide identification of transcription factors and transcription-factor binding sites in oleaginous microalgae *Nannochloropsis*. *Sci. Rep.* 4, 5454
113. Ajjawi, I. *et al.* (2017) Lipid production in *Nannochloropsis gaditana* is doubled by decreasing expression of a single transcriptional regulator. *Nat. Biotechnol.* 35, 647–652
114. Raffaele, S. *et al.* (2008) A MYB transcription factor regulates very-long-chain fatty acid biosynthesis for activation of the hypersensitive cell death response in *Arabidopsis*. *Plant Cell* 20, 752–767
115. Rehmanji, M. *et al.* (2022) Elucidation of functional genes associated with long chain-polyunsaturated fatty acids (LC-PUFAs) metabolism in oleaginous diatom *Phaeodactylum tricornutum*. *Hydrobiology* 1, 451–468
116. Qin, J. *et al.* (2023) Biotechnological production of omega-3 fatty acids: current status and future perspectives. *Front. Microbiol.* 14, 1280296
117. Tyagi, R. *et al.* (2023) New perspectives of omega-3 fatty acids from diatoms. *Syst. Microbiol. Biomanuf.*, Published online September 2, 2023. <https://doi.org/10.1007/s43393-023-00202-2>
118. Kumari, A. *et al.* (2023) Recent advances in enhancing the production of long chain omega-3 fatty acids in microalgae. *Crit. Rev. Food Sci. Nutr.* 26, 1–19
119. Karageorgou, D. *et al.* (2023) Benefits of supplementation with microbial omega-3 fatty acids on human health and the current market scenario for fish-free omega-3 fatty acid. *Trends Food Sci. Technol.* 136, 169–180
120. Patel, A.K. *et al.* (2022) Emerging prospects of microbial production of omega fatty acids: Recent updates. *Bioresour. Technol.* 360, 127534
121. Barta, D.G. *et al.* (2021) Microalgae as sources of omega-3 polyunsaturated fatty acids: Biotechnological aspects. *Algal Res.* 58, 102410
122. Ji, X.-J. and Huang, H. (2019) Engineering microbes to produce polyunsaturated fatty acids. *Trends Biotechnol.* 37, 344–346
123. Adarme-Vega, T.C. *et al.* (2012) Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. *Microb. Cell Factories* 11, 96
124. Calhoun, S. *et al.* (2021) A multi-omic characterization of temperature stress in a halotolerant *Scenedesmus* strain for algal biotechnology. *Commun. Biol.* 4, 333
125. Gao, B. *et al.* (2020) Evaluation and transcriptome analysis of the novel oleaginous microalga *Lobosphaera bisecta* (Trebouxiophyceae, Chlorophyta) for arachidonic acid production. *Mar. Drugs* 18, 229
126. Ding, W. *et al.* (2023) Physicochemical and molecular responses of the diatom *Phaeodactylum tricornutum* to illumination transitions. *Biotechnol. Biofuels Bioprod.* 16, 103
127. Kaur, S. *et al.* (2021) Proteomics and expression studies on lipids and fatty acids metabolic genes in *Isochrysis galbana* under the combined influence of nitrogen starvation and sodium acetate supplementation. *Bioresour. Technol. Rep.* 15, 100714
128. Mao, X. *et al.* (2020) High silicate concentration facilitates fucoxanthin and eicosapentaenoic acid (EPA) production under heterotrophic condition in the marine diatom *Nitzschia laevis*. *Algal Res.* 52, 102086
129. Wang, Y. *et al.* (2023) Altering autotrophic carbon metabolism of *Nitzschia closterium* to mixotrophic mode for high-value product improvement. *Bioresour. Technol.* 371, 128596
130. Wan Razali, W.A. *et al.* (2022) Comparative proteomics reveals evidence of enhanced EPA trafficking in a mutant strain of *Nannochloropsis oculata*. *Front. Bioeng. Biotechnol.* 10, 838445
131. Wang, X. *et al.* (2017) A lipid droplet-associated protein involved in lipid droplet biogenesis and triacylglycerol accumulation in the oleaginous microalga *Phaeodactylum tricornutum*. *Algal Res.* 26, 215–224
132. Liu, L. *et al.* (2022) Rewiring the metabolic network to increase docosahexaenoic acid productivity in *Cryptocodinium cohnii* by fermentation supernatant-based adaptive laboratory evolution. *Front. Microbiol.* 13, 824189
133. Aguilera-Sáez, L.M. *et al.* (2019) NMR metabolomics as an effective tool to unravel the effect of light intensity and temperature on the composition of the marine microalgae *Isochrysis galbana*. *J. Agric. Food Chem.* 67, 3879–3889
134. Arumugam, M. *et al.* (2021) Plant growth regulator triggered metabolomic profile leading to increased lipid accumulation in an edible marine microalga. *J. Appl. Phycol.* 33, 1353–1365
135. Liu, L. *et al.* (2018) Nitrogen feeding strategies and metabolomic analysis to alleviate high-nitrogen inhibition on docosahexaenoic acid production in *Cryptocodinium cohnii*. *J. Agric. Food Chem.* 66, 10640–10650
136. Lv, M. *et al.* (2020) Identification and metabolomic analysis of a starch-deficient *Cryptocodinium cohnii* mutant reveals multiple mechanisms relevant to enhanced growth and lipid accumulation. *Algal Res.* 50, 102001
137. Li, J. *et al.* (2014) Choreography of transcriptomes and lipidomes of *Nannochloropsis* reveals the mechanisms of oil synthesis in microalgae. *Plant Cell* 26, 1645–1665
138. Johansson, O.N. *et al.* (2019) Phenomics reveals a novel putative chloroplast fatty acid transporter in the marine diatom *Skeletonema marinoi* involved in temperature acclimation. *Sci. Rep.* 9, 15143