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Fatty Acids from Microalgae: Targeting the Accumulation of Triacylglycerides

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Patricia I. Leonardi

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Abstract

Microalgae were originally considered as sources of long-chain polyunsaturated fatty acids (PUFAs), mainly for aquaculture purposes. However, based on the fact that their fatty acids (FA), stored as triacylglycerides (TAG), can be converted into biodiesel via a transesterification reaction, several microalgal species have emerged over the last decade as promising feedstocks for biofuel production. Elucidation of microalgae FA and TAG metabolic pathways is therefore becoming a cutting-edge field for developing transgenic algal strains with improved lipid accumulation ability. Furthermore, many of the biomolecules produced by microalgae can also be exploited. In this chapter, we describe recent advances in the field of FA and TAG pathways in microalgae, focusing in particular on the enzymes involved in FA and TAG synthesis, their accumulation in lipid droplets, and their degradation. Mention is made of potentially high-value products that can be obtained from microalgae, and possible molecular targets for enhancing FA and TAG production are outlined. A summary is provided of transcriptomics, proteomics, and metabolomics of the above-mentioned pathways in microalgae. Understanding the relation between anabolic and catabolic lipid enzyme pathways will provide new insights into biodiesel production and other valuable biomolecules obtained from microalgae.

Keywords: fatty acids, triacylglycerides, lipid metabolism, microalgae

1. Introduction

Despite the drop in crude oil prices over the last few years, global efforts to develop alternative renewable energy sources continue to be driven by increasing air pollution and growing energy consumption. Extensive research is therefore being conducted in the field of biofuels

[1], which are derived from renewable biological sources. Biodiesel is the main substitute for diesel fuel and can be produced from both edible and non-edible oils. The use of edible oils has generated controversy because of the negative impact on food availability and the environment [2, 3]. As a consequence of these ethical considerations, non-food crops have emerged as a viable alternative for the production of biodiesel [4–6]. However, since non-food crops do not produce sufficient biomatter to feasibly cover the fuel requirements of the world's transport sector, attention is turning to oleaginous microalgae which are able to produce and accumulate large amounts of fatty acids (FA) in the form of triacylglycerides (TAG) that can be converted into biodiesel through a transesterification reaction [2, 3, 7]. Furthermore, some species of oleaginous microalgae can also produce high-value products such as long-chain polyunsaturated fatty acids (docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids), carbohydrates (cellulose, starch), proteins, and other high-value compounds, such as pigments, antioxidants (i.e., β-carotene, astaxanthin), and vitamins, which may have commercial application in various industrial sectors [2, 3, 8, 9]. In addition to their potential as biological factories, the advantage of these photosynthetic microorganisms is that their simple growing requirements (light, CO_2 and nutrients) offer several environmental benefits such as high solar energy conversion efficiency, utilization of saline water, CO_2 sequestration from the air and self-purification if coupled with wastewater treatment [10].

Despite the wide range of metabolites able to be synthesized by microalgae, little is known about the regulation of FA and TAG biosynthetic pathways and their storage and turnover in microalgae. In this chapter, we therefore describe recent advances in these fields and possible high-value co-products that could render the production of biodiesel from microalgae more sustainably. Recent studies on the transcriptomics, proteomics, and metabolomics of the above-mentioned pathways are also outlined. Understanding these metabolic pathways will accelerate the availability of biodiesel and other valuable biomolecules obtained from microalgae.

2. FA and TAG biosynthetic pathways in microalgae

Fatty acids are organic acids containing a carboxylic functional group with an aliphatic chain that can be saturated (SFA), monounsaturated (MUFA), or polyunsaturated (PUFA). The number of carbon atoms can vary, generating short-chain, medium-chain, or long-chain FA.

In plants, the FA biosynthetic pathway occurs in the chloroplasts (**Figure 1**).

As shown in **Figure 1**, the first step in the pathway involves the acetyl-CoA carboxylase (ACCase) which catalyzes the formation of malonyl-CoA from acetyl-CoA and bicarbonate [11]. There is evidence suggesting the presence of genes encoding this enzyme (accA and accD) in *Chlorella pyrenoidosa*. In fact, the transcription of these genes showed to be up-regulated under lipid accumulating conditions [12]. Moreover, a marked increase in the level of acetyl-CoA together with a moderate augmentation of malonyl-CoA and CoA was detected in the green microalgae *Chlorella desiccata*, *Dunaliella tertiolecta*, and *Chlamydomonas reinhardtii* under stress conditions, denoting increased activity of ACCase in these strains [13].
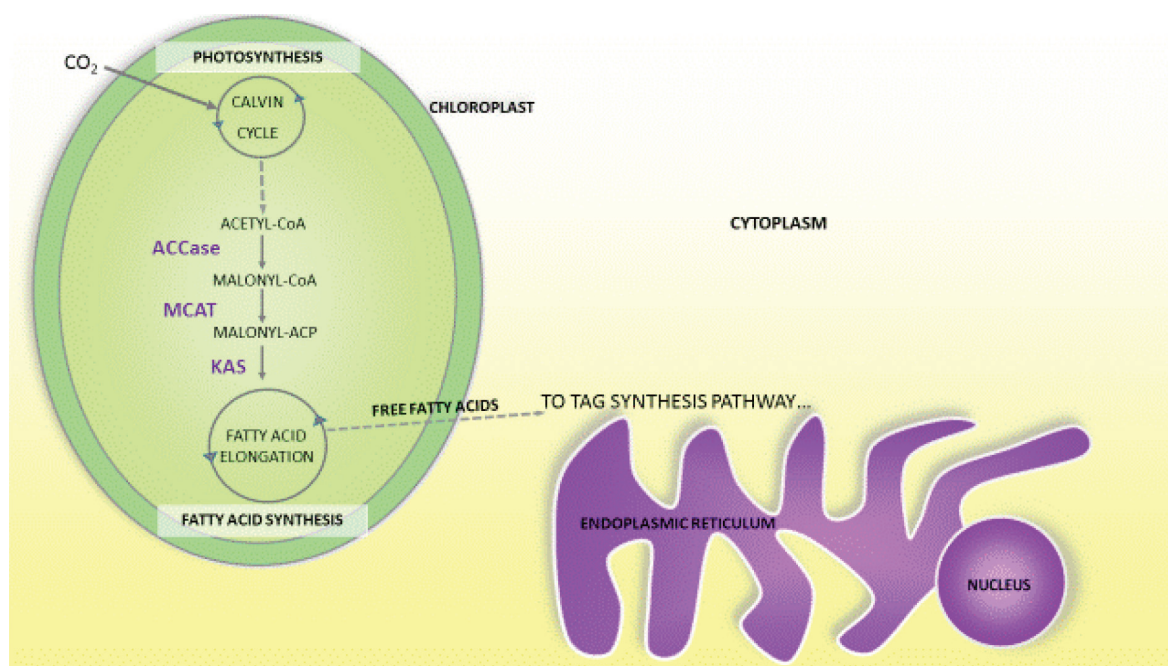
Figure 1. Simplified overview of the pathways involved in FA synthesis in plants. Enzyme abbreviations: ACCase, acetyl-CoA carboxylase; MCAT, malonyl-CoA:Acyl Carrier Protein (ACP) transacylase; KAS, ketoacyl-ACP synthases.

The next step in the FA synthesis is mediated by the malonyl-CoA:Acyl Carrier Protein (ACP) transacylase (MCAT) which transfers the malonyl group from malonyl-CoA to malonyl-ACP [11]. A putative MCAT was identified as a part of the FA biosynthetic pathway in *Nannochloropsis oceanica* [14]. In *Haematococcus pluvialis*, the genes encoding ACP were up-regulated under TAG accumulating conditions (high temperature, high salinity, and nitrogen deficiency) together with other genes involved in FA biosynthesis [15]. In addition, proteomic studies on *Neochloris oleoabundans* revealed an augmented expression of ACP, among other enzymes of the lipid synthesis, under nitrogen starvation [16].

Acyl-ACP is the carbon source or substrate for the elongation of FA. This reaction is catalyzed by enzymes known as ketoacyl-ACP synthases (KASIII, KASI, and KASII). After each condensation, a reduction, dehydration, and second reduction occur. These steps are catalyzed by enzymes known as the FAS complex: beta-ketoacyl-ACP reductase (KAR), hydroxyacyl-ACP dehydrase (HAD), and enoyl-ACP reductase (EAR), respectively [11]. Transcriptome analysis of the diatom *Chaetoceros* sp. GSL56 helped to identify putative enzymes of the FA synthesis pathway. In addition, replacement of ketoacyl-ACP synthase of *Synechococcus* 7002 with *Chaetoceros* ketoacyl-ACP synthase III induced FA synthesis [17]. In line with this, TAG accumulating conditions increased the levels of transcripts for KAS in *H. pluvialis* [15].

The *de novo* resulting FA often with 16 or 18 carbon atoms can undergo the action of elongases and desaturases that add carbon or double bonds, respectively [11]. Particularly, desaturases and elongases are being intensively studied to achieve transgenic long-chain PUFA production [18, 19].

Some reports suggest the presence of both enzyme types in microalgae. In the marine microalgae *Pavlova* sp. and *Isochrysis sp.*, two genes encoding elongases that catalyze the elongation of eicosapentaenoic acid (EPA) to docosahexaenoic acid (DHA) have been reported [20]. In the diatom *Thalassiosira pseudonana*, the genes encoding elongases that mediate the formation of DHA from EPA were successfully overexpressed, thus inducing an increase in DHA content [19]. A delta 5 desaturase was also identified, characterized and overexpressed in the diatom *Phaeodactylum tricornutum* inducing a significant increase in the unsaturated fatty acids [21] .

Upon completion of elongation, FAs are transported to the cytoplasm to act as substrates of the acyl transferases involved in the TAG synthesis. TAG are neutral lipids formed by the esterification of one molecule of glycerol with three FAs. Because of their energy-rich acyl chains, they are the dominant form of stored energy in microalgae. Cellular stresses, such as nutrient deprivation (carbon dioxide, nitrogen, silica, and phosphorous), temperature fluctuation, or high light exposure trigger their formation [22–28]. It has been demonstrated that lipid biosynthetic pathways are induced under these conditions to potentiate the lipid storage (30–60% of dry cell weight), and this mechanism is thought to play a role in microalgae adaptation and survival [24, 29–39]. It has further been reported that multiple stressors have no additive effect on lipid accumulation [24, 40].

Data relating to plant FA and TAG metabolism provided the key to identifying possible molecular targets involved in lipid synthesis and accumulation in microalgae [41]. As shown in **Figure 2**, in plants, the first step of the conventional Kennedy pathway involves the acylation of the glycerol-3-phosphate (G-3-P), catalyzed by the glycerol-3-phosphate acyltransferase (GPAT) to yield lysophosphatidic acid (LPA). GPAT is the rate-limiting step subject to many regulatory controls at the transcriptional and post-transcriptional level and to allosteric mechanisms [42, 43]. Recent studies have revealed the presence of this enzyme in microalgae. In the marine diatom *T. pseudonana*, a membrane-bound GPAT designated TpGPAT was cloned and characterized. The authors observed that G-3-P was the preferred substrate of TpGPAT [44]. A sequence for GPAT with high homology to that of plants was found in *C. reinhardtii*, *Volvox carteri*, *Ostreococcus lucimarinus*, *Ostreococcus tauri*, *Cyanidioschyzon merolae*, and *P. tricornutum*. As in *T. pseudonana*, G-3-P and fatty acyl molecules are likely to be the enzyme substrates, as suggested by the residues present in their active sites [45].

As described in **Figure 2**, lysophosphatidic acid acyltransferase (LPAAT) participates in the second step of the Kennedy pathway. This enzyme catalyzes the acylation of the LPA to yield phosphatidic acid (PA) [46]. Candidate LPAATs have been found in some algal genomes including that of *H. pluvialis* [47, 48], where it has been shown that LPAAT mRNA is induced under high irradiance stress [47]. In addition, it was recently reported that the expression of *C. reinhardtii* LPAAT (CrLPAAT1) is associated with an increase in lipid synthesis and accumulation under nitrogen starvation [48].

Phosphatidic acid phosphohydrolase (PAP) uses PA as substrate to form diacylglycerol (DAG), a precursor of TAG (**Figure 2**) [49]. In eukaryotes, PAP enzymes are the members of the evolutionarily conserved lipin protein family whose activity is related to TAG storage [50]. In the green microalga *C. reinhardtii*, PAP transcripts (named CrPAP2) are induced under stress conditions. In addition, CrPAP2 silencing slightly lowers the lipid content. Thus, in *C. reinhardtii*, as in other eukaryotes, PAP expression is related to lipid synthesis and accumulation [49].
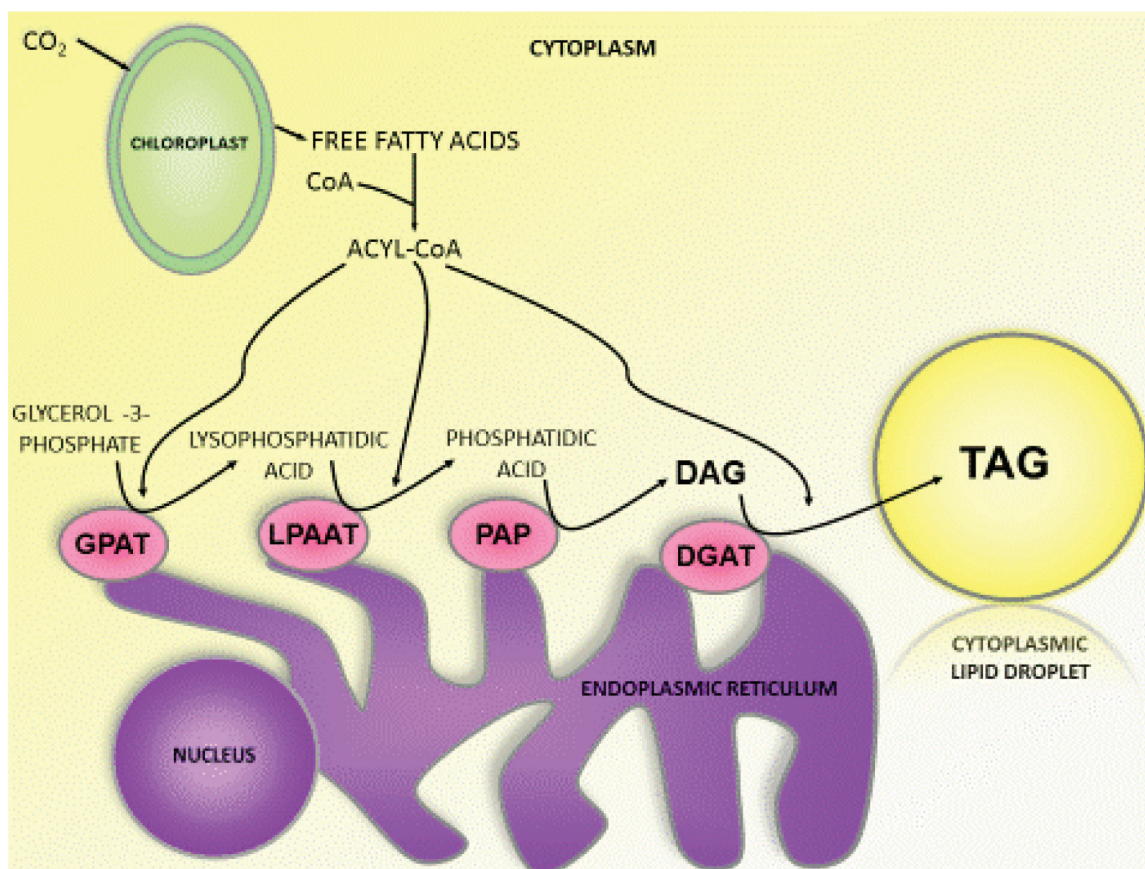
Figure 2. Simplified overview of the pathways involved in TAG synthesis in plants. Enzymes of the conventional Kennedy pathway involved in TAG synthesis and their subcellular localization in plants. Enzyme abbreviations: glycerol-3-phosphate acyltransferase (GPAT); lysophosphatidic acid acyltransferase (LPAAT); phosphatidic acid phosphohydrolase (PAP); diacylglycerol acyltransferase (DAGAT or DGAT). The same enzymes are involved in TAG synthesis in microalgae, but their intracellular localization has not yet been determined.

The last enzyme of the *de novo* TAG synthesis is acyl-CoA:diacylglycerol acyltransferase (DGAT), which catalyzes the acylation of DAG to yield TAG (**Figure 2**) [51]. This enzyme employs DAG and acyl-CoA as substrates, so the resulting TAG is formed through an acyl-CoA-dependent pathway [46] and is a key target to increase TAG synthesis and storage through genetic manipulation [52, 53]. In higher plants, three different types of DGATs participate in the formation of TAG: DGAT1, DGAT2, or DGAT3 [54]. Sequences for DGAT1 and DGAT2 isoforms were found in several algal strains [55]. Sequences for DGAT2, but not DAGT1, or DGAT3, were identified in the green microalga *O. tauri* [56]. DGAT2 was also found in *T. pseudonana* (TpDGAT2). In addition, the expression of DGAT in a TAG-null yeast mutant restored the synthesis of these neutral lipids [57]. In the oleaginous microalga *C. pyrenoidosa* grown under stress conditions, a high correlation was found between DGAT and TAG accumulation [58]. Also in *N. oceanica* IMET1, another oleaginous microalga, seven putative DGAT genes were up-regulated under nitrogen-deficient conditions, when the synthesis of TAG-neutral lipids was significantly increased [59]. In *C. reinhardtii* dgat1 and dgtt1 to dgtt5 genes encode for DGAT1 and DGAT2, respectively [60, 61]. Increased transcript expression of the genes dgat1 and dgtt1 was detected under stress conditions (less sulfur, phosphorous, iron, zinc, or nitrogen). Once more, the evidence suggests that both DGAT1 and DGAT2 could play a role in TAG synthesis as their expression is induced

under TAG-accumulating conditions [62, 63]. In support of this hypothesis, overexpression of a DGAT2 isoform in the marine diatom *P. tricornutum* stimulated the synthesis of neutral lipids and their accumulation in lipid droplets [64].

As can be observed, much research has focused on the acyl-CoA-dependent reaction catalyzed by DGAT. However, the relative contribution of DGAT1 and DGAT2 isoenzymes to TAG accumulation appears to be species-dependent, so further studies should be performed to gain insight into this aspect.

TAG can be formed by the acyl-CoA-dependent pathway, detailed previously, or through acyl-CoA-independent reactions. Acyl-CoA-independent formation of TAG is mediated by the activities of two types of enzyme: the phospholipid:diacylglycerol acyltransferases (PDAT), which catalyze the formation of TAG using DAG and phosphatidylcholine (PC); and the DAG:DAG transacylases (DGTA) which utilize two molecules of DAG to form TAG and MAG [54, 65].

In fact, in *N. oceanica* IMET1, it was reported that membrane polar lipids were converted into TAG when the microalgae were grown under nitrogen deficiency [59]. In agreement with this, the gene encoding the acyltransferase PDAT1 was induced under nitrogen starvation in *C. reinhardtii*. Moreover, TAG content in the *C. reinhardtii* PDAT-null mutant was 25% lower than in the parent strain. It would thus appear that PDAT has a relevant role in TAG accumulation, stimulating the transacylation pathway in both strains [62]. Furthermore, in *C. reinhardtii* it was suggested that PDAT functions as a DGTA with acyl hydrolase activity. PDAT might, therefore, mediate membrane polar lipid turnover in a favorable environment whereas under stress conditions it may participate in phospholipid degradation contributing to TAG synthesis [66].

As already mentioned, many aspects of *C. reinhartii* lipid metabolism have already been characterized, making it the microalga of choice for current purposes [23, 67–73]. Nevertheless, *Chlamydomonas* is a non-oleaginous strain [23]. Other microalgal species with greater potential to yield biodiesel and other high-value products should therefore be more thoroughly investigated.

3. Transcriptomics, proteomics, and metabolomics

A better understanding of the mechanisms involved in TAG enrichment under stress conditions will help to maximize microalgae productivity. However, many biochemical approaches for elucidating molecular pathways depend on the availability of genomic sequence data [29]. Transcriptomics, proteomics, and metabolomics, however, are able to provide a detailed description of cell transcripts (RNA), proteins and metabolites, respectively while completely bypassing the requirement of genomic information [74, 75].

Transcriptome analysis helped to identify sequences of the enzymes involved in the biosynthesis and catabolism of FA, TAG, and starch in *D. tertiolecta*, revealing that this strain shares genetic information, at least in terms of the mentioned pathways, with closely related microalgae species such as *V. carteri* and *C. reinhardtii* [76]. The transcriptome of *N. oleoabundans* was also determined. In this case, the authors quantified the differences between nitrogen-replete and nitrogen-limiting

culture conditions. Under nitrogen deficiency, *N. oleoabundans* showed higher levels of transcripts of FA and TAG synthesis pathways and inhibition of the FA β-oxidation pathway, compared to nitrogen-replete culture conditions [29]. In agreement with this finding, in *C. vulgaris*, transcriptomic [31] and proteomic [77] studies revealed an induction of the enzymes of the FA and TAG synthesis machinery under lipid enrichment conditions. Also, transcription factors associated with these metabolic pathways were augmented under the stress condition [77].

The transcriptome of *C. reinhardtii* showed that genes involved in FA and TAG metabolic pathways and in membrane remodeling were highly induced under neutral lipid accumulation conditions [78]. In this microalga, proteomic studies revealed an augmented rate of lipid synthesis machinery with a concomitant enhancement in FA and TAG; higher levels of starch than under non-stress conditions were also detected by metabolomic analyses. Metabolic pathways such as nitrogen assimilation, amino acid metabolism, oxidative phosphorylation, glycolysis, TCA cycle, and the Calvin cycle suffered adjustments during *C. reinhardtii* [79, 80].

As in *C. vulgaris*, nutrient-deprivation stress in *C. reinhardtii*, *D. tertiolecta*, and *N. oleoabundans* induced the expression of genes involved in FA and TAG synthesis pathways in *P. tricornutum* [81], *Chlorella protothecoides* [82], and *Tisochrysis lutea* [83].

In conclusion, these assembled transcriptomes, proteomes, and metabolomes offer valuable approaches for improving microalgal productivity, providing possible targets for molecular engineering that could enhance microalgae-derived products.

4. Molecular targets for enhancing lipid biosynthesis

Genetic strain modification to improve microalgal productivity and accelerate the industrialization of algal-derived products is a major challenge [84]. Reflecting the fact that enhancement of the FA synthesis pathway had little effect on total lipid content in some plants [85, 86], a growing body of research now focuses on overexpression of the enzymes or heterologous expression of genes involved in the TAG biosynthetic pathway. **Table 1** provides an outline of some of the genetic manipulations performed on several microalgal strains, leading to an improvement in their TAG content.

Enzymes overexpressed or heterologously expressed	Organism	Effect on lipid production (changes over control condition)	References
ME of *Phaeodactylum tricornutum*	*Chlorella pyrenoidosa*	18.7%	[87]
GPAT of *Thalassiosira pseudonana*	Yeast GPAT-deficient mutant	Restored TAG formation	[44]
GPAT of *Lobosphaera incisa*	*Chlamydomonas reinhardtii*	50%	[88]
GPAT of *Phaeodactylum tricornutum*	*Phaeodactylum tricornutum*	2-fold	[89]

Enzymes overexpressed or heterologously expressed	Organism	Effect on lipid production (changes over control condition)	References
G3PDH, GPAT, DGAT, LPAAT and PAP of *Saccharomyces cerevisiae* and *Yarrowia lipolytica*	*Chlorella minutissima*	2-fold	[90]
LPAAT of *Chlamydomonas reinhardtii*	*Chlamydomonas reinhardtii*	20%	[91]
DGAT2 of *Nannochloropsis oceanica*	*Nannochloropsis oceanica*	3.5-fold	[92]
DGAT1 and DGAT2 of *Myrmecia incisa*	*S. cerevisiae* lipid deficient mutant	Re-stored TAG formation	[93]
DGAT2 of *Neochloris oleoabundans*	*S. cerevisiae* DGAT deficient mutant	Re-stored TAG formation	[94]
DGAT 1 of *Phaeodactylum tricornutum*	*S. cerevisiae* DGAT deficient mutant	Re-stored TAG synthesis and lipid body formation	[63]
DGAT 2 of *Phaeodactylum tricornutum*	*Phaeodactylum tricornutum*	35%	[64]
Various DGAT type 2	*Chlamydomonas reinhardtii*	20–44%	[95]

Enzyme abbreviations: ME, malic enzyme; DGAT, diacylglycerol acyltransferase; G3PDH, glycerol-3-phosphate-dehydrogenase; GPAT, glycerol-3-phosphate acyltransferase; LPAAT, lysophosphatidic acid acyltransferase; PAP, phosphatidic acid phosphatase.

Table 1. Some of the genetic modifications performed on metabolic pathways related to lipid synthesis in microalgae and their effect on lipid enrichment.

5. TAG-accumulation in lipid droplets

Lipid droplets (LDs) are cell organelles that are currently the subject of in-depth study in various organisms. These lipid globules not only act as a reservoir of cell carbon and energy, they may also have a role in lipid homeostasis, signaling, trafficking, and interorganelle communications [96, 97]. As previously mentioned, under stress conditions microalgae synthesize TAG and store them as cytoplasmic LDs [22–28], which can vary in size, shape, and function depending on the cell type and the environmental conditions (**Figure 3**) [98]. In eukaryotic cells, LD structure consists of a TAG-rich hydrophobic core surrounded by surface polar glycerolipids into which proteins of the perilipin (Plin) (animal cells) or oleosin and caleosin (plants) families are embedded [99–102]. In microalgae, LD structure is conserved from eukaryotes but different LD proteins have been identified. The analysis of *C. reinhardtii* LDs recognized 16 proteins related to lipid metabolism and a major lipid droplet protein (named MLDP) was identified. MLDP silencing increased the size of the LD, without modifying LD TAG content [68]. In the green microalga, *Nannochloropsis* sp., a hydrophobic lipid droplet surface protein, named LDSP, was identified. The expression of LDSP increased concomitantly with TAG content under oil-accumulating conditions [99]. In *H. pluvialis*, seven proteins were found to be
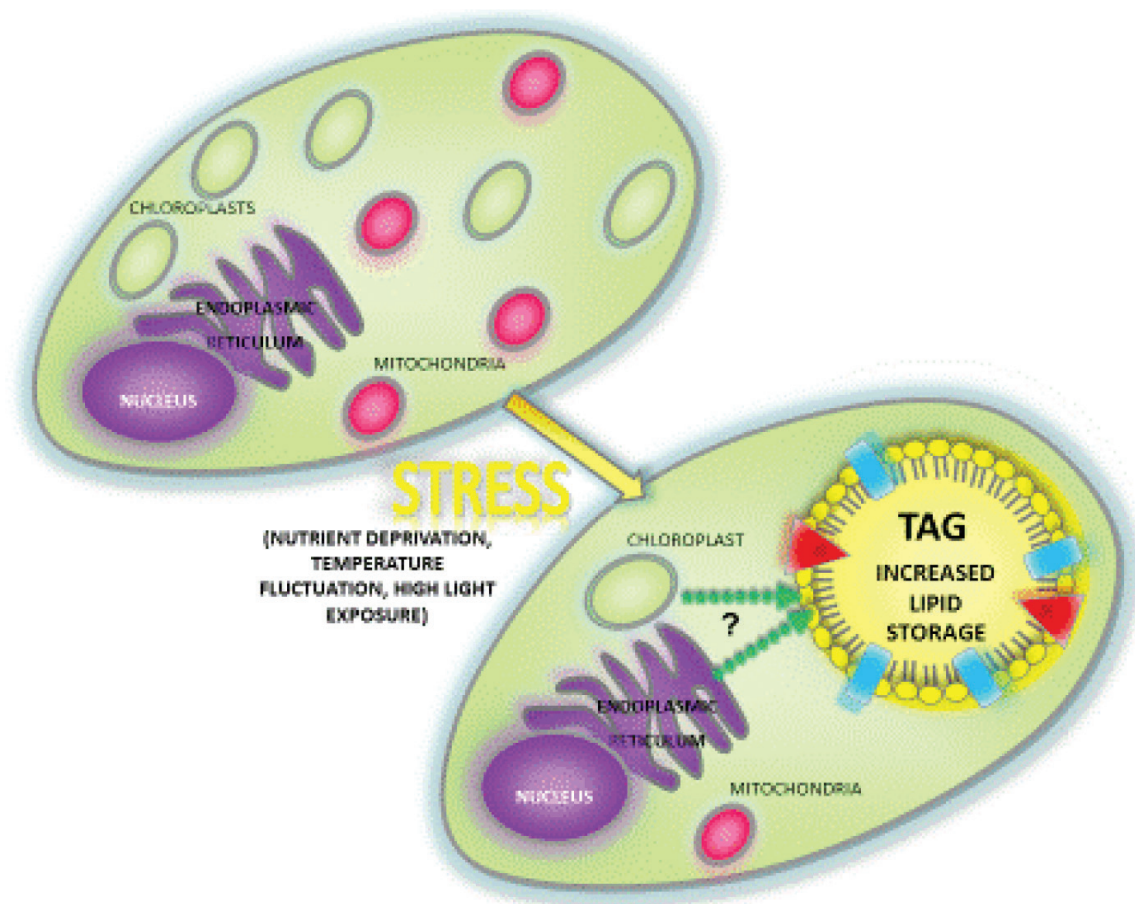
Figure 3. Schematic representation of a cytoplasmic lipid droplet (LD) from microalgae.

associated with LDs. The most abundant of these, *Haematococcus* Oil Globule Protein (HOGP), was homologous to the MLDP of *C. reinhardtii* and its expression was induced under TAG accumulating conditions [103]. LD-associated proteins may also help in the accumulation of TAG in the green microalga *Myrmecia incisa* [104]. Moreover, LDs from *C. reinhardtii* showed the presence of enzymes involved in TAG synthesis (GPAT, and PDAT) and in sterol synthesis, lipid signaling, and trafficking [69]. Further in-depth research should be able to determine the proteins associated with LDs and their role in TAG metabolism in microalgae.

In the oleaginous diatom *Fistulifera* sp., two proteins located in the oil bodies were also detected in the endoplasmic reticulum (ER), suggesting that oil bodies might originate in the ER [105]. The same authors found a signal sequence typical of ER localization in an LD protein called diatom-oleosome-associated-protein 1 (DOAP1) in *Fistulifera solaris* JPCC DA0580 [106]. Related to these findings, the induction of ER stress leads to LD formation in *C. reinhardtii* and *C. vulgaris* [107]. In addition, LDs from *C. reinhardtii* were associated not only with the ER membrane but also with the outer membrane of the chloroplasts [108]. Available data therefore suggest that in microalgae, cytoplasmic LDs are produced in the ER. However, additional studies are required to arrive at a better understanding of the mechanism of LD formation in the ER, and to determine whether chloroplasts play a role in this process.

6. TAG degradation pathways in microalgae

As previously mentioned, the economic feasibility of using microalgae as a source of FA for biodiesel depends to a great extent on improvements in the production process, one of the most significant challenges being to increase lipid yields. The selection of oleaginous strains and the search for different culture strategies to increase lipid biosynthesis constitute viable approaches; blocking the competing pathways of carbohydrate formation may be another. However, both the approaches give rise to a decrease in strain growth [22]. Lipid catabolism has largely been ignored as a relevant pathway for engineering, despite being a competing pathway to lipid biogenesis [109]. However, lipases were identified in *C. reinhardtii* [66, 72, 73] and *T. pseudonana* [110]. In the case of *C. reinhardtii*, CrLIP1 could restore the lipase activity in a *Saccharomyces cerevisiae* lipase-null strain. In addition, *C. reinhardtii* TAG content decreased with increasing expression of CrLIP1 under stress conditions, hydrolyzing mainly DAG and polar lipids [72]. In agreement with this, a galactoglycerolipid lipase was found in *C. reinhardtii*. The main substrates of the enzyme are galactoglycerolipids and the main products are FAs employed for TAG synthesis [74]. In *C. reinhardtii*, phospholipid:diacylglycerol acyltransferase (PDAT) demonstrated both transacylation and acyl hydrolase activities, and could mediate membrane lipid turnover and TAG synthesis [66]. The activity of a multifunctional lipase/phospholipase/acyltransferase of *T. pseudonana* lowered lipid content under both normal and stress conditions [110]. A single gene for PDAT was identified in *H. pluvialis*, though no functional analysis was performed for the gene in this strain [47]. Further studies are required to gain insight into the molecular mechanisms involved in TAG degradation, which could be the key to increased lipid yields in microalgae.

7. Microalgae-based biorefineries

In the context of improving the economic feasibility of microalgae-based biodiesel, a closer look should be taken at the large amounts of TAG produced in some oleaginous microalgae alongside high-value products such as carbohydrates (cellulose and starch); proteins and other high-value compounds like pigments, antioxidants (i.e., β-carotene, astaxanthin), and vitamins [2, 3, 8, 9], all of which may have commercial application in different industrial sectors. Some potentially high-value products found in microalgae are described in **Table 2**.

Product	Microalgal strain	Applications	References
Carbohydrates			
Exopolysaccharides	*Navicula cincta*	Pharmaceutics and agronomics	[111]
Starch, glucose, cellullose	*Chlorella vulgaris FSP-E*	Bioethanol production	[112, 113]
Sulfated extracellular polisaccharides	*Graesiella* sp.	Pharmaceutics	[114]
Lipids			

Product	Microalgal strain	Applications	References
Phytosterols; linoleic (C18:2n6) and alpha linolenic (C18:3n3) fatty acids	*Haematococcus pluvialis*	Human dietary supplement, nutraceutics	[115]
Phytosterols	*Dunaliella tertiolecta, D. salina*	Nutraceutics	[116]
Omega-3 long chain-PUFA	*Isochrysis, Nannochloropsis, Phaeodactylum, Pavlova,* and *Thalassiosira*	Functional food	[117]
Proteins			
Proteins	*Chlorella pyrenoidosa*	Nutraceutics	[118]
Proteins	*Chlorella vulgaris, Nannochloris bacillaris, Tetracystis* sp., *Micractinium reisseri*	Animal feed	[119]
Vitamins			
Tocopherol	*Nannochloropsis oculata, Tetraselmis suecica*	Human dietary supplement, nutraceutics	[120]
Tocopherol, pigments, phenolic compounds	*Desmodesmus* sp.	Human dietary supplement, nutraceutics	[121]
Pigments			
Astaxanthin	*Haematococcus pluvialis*	Antioxidant, cosmetics, pharmaceutics	[122]
Lutein	*Dunaliella salina*	Functional food, animal feed	[123]
Carotenoids	*Phaeodactylum tricornutum*	Cosmetics, pharmaceutics, animal feed	[124]
Carotenoids	*Dunaliella salina*	Cosmetics, pharmaceutics, animal feed	[125]
Others			
Silica	*Navicula cincta*	Abrasive products, insecticides	[111]

Table 2. Recent advances in microalgal-derived high-value products.

8. Conclusion

Oleaginous microalgae grown under stress conditions can synthesize and accumulate large quantities of FA, mainly in the form of TAG, which can then be converted into biodiesel. Although microalgae constitute a promising source of clean energy, knowledge gaps continue to abound in almost all aspects of FA and TAG metabolism for these microorganisms, including the precise identity of enzymatic machinery, the relative contributions of each

enzyme and their precise regulation. Further studies are therefore required to establish the exact metabolic pathways involved in FA and TAG synthesis, accumulation, and degradation in order to develop genetic engineering strategies to obtain microalgal strains with improved capacity to convert their biomass into TAG and other valuable co-products.

Acknowledgements

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