Chapter 8
Triacylglycerol Accumulation in Photosynthetic Cells in Plants and Algae

Zhi-Yan Du and Christoph Benning

Abstract Plant and algal oils are some of the most energy-dense renewable compounds provided by nature. Triacylglycerols (TAGs) are the major constituent of plant oils, which can be converted into fatty acid methyl esters commonly known as biodiesel. As one of the most efficient producers of TAGs, photosynthetic microalgae have attracted substantial interest for renewable fuel production. Currently, the big challenge of microalgae based TAGs for biofuels is their high cost compared to fossil fuels. A conundrum is that microalgae accumulate large amounts of TAGs only during stress conditions such as nutrient deprivation and temperature stress, which inevitably will inhibit growth. Thus, a better understanding of why and how microalgae induce TAG biosynthesis under stress conditions would allow the development of engineered microalgae with increased TAG production during conditions optimal for growth. Land plants also synthesize TAGs during stresses and we will compare new findings on environmental stress-induced TAG accumulation in plants and microalgae especially in the well-characterized model alga *Chlamydomonas reinhardtii* and a biotechnologically relevant genus Nannochloropsis.

Keywords Nutrient deprivation • Photosynthesis • Lipid droplet • Lipid remodeling • Lipid metabolism

Introduction

During the past decade, basic research on lipid metabolism in microalgae and plants has greatly benefitted from funding available for the exploration of sustainable, domestic production of liquid fuels. As fossil carbon-derived fuels are diminishing and will eventually be depleted by the increasing demand of modern societies, the search for sustainable sources of energy has become more urgent. As a result of the burning of fossil fuels, carbon dioxide (CO_2) is released to the atmosphere, which is

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a greenhouse gas and a likely contributor to global warming as its atmospheric concentration has steadily increased since the onset of the industrial revolution (Cheah et al. 2014; Martinez-Boti et al. 2015). Therefore, alternative energy sources should not only be reliable and renewable, but also not further contribute to the increase of atmospheric CO_2. Biofuel products from photosynthetic organisms can potentially meet this challenge. By converting sunlight into chemical energy, photosynthetic organisms such as plants and algae produce biomass and storage compounds, i.e. carbohydrates and TAGs, which can be converted to liquid transportation fuels equivalent to fossil fuels. A big advantage of biofuel is that photosynthetic organisms consume CO_2 from the atmosphere or directly from anthropogenic sources along with the conversion of solar energy, resulting in greenhouse gas reduction as they displace fossil fuels (Barber 2009; Merchant et al. 2012; Ohlrogge and Chapman 2011). Currently, two major forms of biofuels, ethanol and biodiesel, are available in the market and substitute a small portion of global fossil fuels consumed annually (Ohlrogge and Chapman 2011).

The focus here is on biodiesel, one of the commonly used biofuels which is currently primarily produced from edible plant vegetable oils obtained from agricultural crops such as soybean and oil palm (Durrett et al. 2008). Compared with bioethanol, biodiesel has several advantages. First, plant oils as fuel feedstocks have a higher energy density than carbohydrates; biodiesel has a 25 % higher energy content per volume than ethanol (Durrett et al. 2008). Secondly, the net positive energy balance for biodiesel production is much higher than that for ethanol. Although the exact numbers are still debated, by one estimation, biodiesel from soy oil yields 93 % more energy over the total energy input into its production, whereas ethanol from corn starch yields only 25 % more, including extra energy credits from co-products, e.g. for animal feed (Hill et al. 2006). Thirdly, greenhouse gas emissions are reduced 41 % by the production and combustion of biodiesel compared to the fossil fuels it replaced, whereas the number for ethanol is only 12 % (Hill et al. 2006). Biodiesel also generates less air and chemical pollutants derived from pesticides and fertilizers per net energy gain than ethanol (Hill et al. 2006). Fourthly, with respect to transportation fuel, biodiesel can be used directly for diesel engines which are 30 % more efficient than gasoline engines, whereas ethanol has to be blended with conventional petrol/gasoline before it is used. In addition, ethanol needs to be stored separately before use because it can lead to corrosion of pipelines, and it is not practical for certain applications, such as jet fuel or heavy vehicles (Dismukes et al. 2008; Durrett et al. 2008). All these benefits have motivated research into the biosynthesis of TAGs and its regulation in algae and plants, and substantial effort has been spent on the engineering of TAG quality.

Fatty Acid and Triacylglycerol Production in Non-seed Tissues of Plants

Plant oils, primarily TAGs, are abundantly stored in cytosolic lipid droplets of oil seeds, and have been traditionally a common source for edible vegetable oil and biodiesel feedstocks. For example, in 2005, 1.5 % of the soybean harvest in the USA produced 256 million liters of biodiesel, providing 0.09 % of the total USA diesel consumption (US Department of Energy 2007). During the same year, biodiesel contributed ~1.6 % of the EU diesel usage (Commission of the European Communities 2007) and ~0.21 % of that in the USA (US Department of Energy 2007). By 2030, worldwide demand for edible vegetable oils is expected to double due to an increasing population (Bruinsma 2003; Chapman and Ohlrogge 2012). One approach to avoid a direct competition with food supplies has been the introduction of dedicated oleaginous biofuel crops producing non-edible oils such as *Sapindus mukorossi* (soap-nut tree) and *Jatropha curcas* (physic-nut tree) which also can tolerate marginal agricultural land less suitable for the common agricultural crops (Abdulla et al. 2011; Chhetri et al. 2008).

Genetic Engineering of Oil Accumulation in Non-seed Plant Tissues

Another new strategy for non-food oil feedstocks is to produce oil in non-seed plant tissues such as leaves and stems in high biomass crops (Chapman et al. 2013; Durrett et al. 2008). Plant oils can be easily extracted from vegetative tissues, and the residual lignocellulosic biomass can be converted to biofuel feedstocks by deconstruction followed by fermentation or can be burned directly to produce bioelectricity for electric vehicles (Ohlrogge et al. 2009; Vanhercke et al. 2014). If harvestable vegetative tissues accumulate 10 % TAGs on a dry weight basis, the energy yield from the crop would be increased by at least 30 % (Ohlrogge and Chapman 2011).

Most plant leaves already contain ~5 % fatty acids by dry weight in the form of polar membrane lipids that are not easy to use (Yang and Ohlrogge 2009). Neutral lipids such as TAGs can in principle be synthesized in most plant cells, although they primarily accumulate in plant seeds, and only minor amounts are found in leaves, stems, and roots (Yang and Ohlrogge 2009). However, there are intriguing exceptions. Examples include copious amounts of oil in the fruit mesocarp of olive (*Olea europaea*), avocado (*Persea americana*), and oil palm (*Elaeis guineensis*) (Ross et al. 1993; Tranbarger et al. 2011), in tubers of nutsedge (*Cyperus esculentus*) (Stoller and Weber 1975; Zhang et al. 1996), and in stem tissues of Mongolian oilwood (*Tetraena mongolica*) (Wang et al. 2007), suggesting that vegetative-tissue based oil could be a realistic approach for oil production.

In fact, numerous attempts towards the engineering TAG accumulation in vegetative tissues have been carried out over the past decade with various strategies that are generally trying to optimize the flux of carbon into TAG by overexpressing seed transcription factors, increasing TAG/fatty acid synthesis or blocking TAG turnover. For example, transcription factors that normally control plant oil biosynthesis in developing embryos as summarized in recent reviews (Bates et al. 2013; Baud and Lepiniec 2010; Santos-Mendoza et al. 2008) have been explored to produce oil in non-seed tissues by their ectopic production, including *WRINKLED1* (*WRI1*) (Cernac and Benning 2004; Sanjaya et al. 2011), *ABSCISIC ACID INSENSITIVE4* (*ABI4*) (Yang et al. 2011), *LEAFY COTYLEDON1* (*LEC1*) or *LEC2* (Mu et al. 2008; Santos-Mendoza et al. 2005; Stone et al. 2008). In developing embryos of plants, a fraction of TAG is synthesized through acylation of diacylglycerol (DAG) by diacylglycerol acyltransferase (DGAT) (Cases et al. 1998; Lardizabal et al. 2001), and ectopic expression of DGAT or monoacylglycerol acyltransferase (MGAT) increases TAG content in leaves (Andrianov et al. 2010; Bouvier-Nave et al. 2000; Petrie et al. 2012; Sanjaya et al. 2013). In potato tubers, overexpression of the Arabidopsis acetyl-CoA carboxylase leads to increases in TAG and fatty acid synthesis (Klaus et al. 2004). By reducing TAG turnover in a *cgi-58* knockout mutant, mature Arabidopsis leaves show increases in TAG accumulation, while seed storage, germination and plant growth are not affected (James et al. 2010). TAG accumulation has also been observed in leaves and roots in an Arabidopsis *trigalactosyldiacylglycerol1* (*tgd1*) mutant which is disrupted in lipid transfer between the endoplasmic reticulum (ER) and the chloroplast (Xu et al. 2005). Another intriguing Arabidopsis mutant designated *pickle* has embryo-like roots that produce seed storage compounds including oil (Ogas et al. 1997, 1999).

These studies have achieved the goal of engineering oil in non-seed tissues. However, the levels of oil production were initially still far below the 10 % dry weight benchmark most likely because of their single-gene strategies. More recent studies have attempted to further increase TAG content in non-seed tissues by manipulating the expression of multiple genes from different pathways. Various gene combinations have been tried and examined in transgenic plants such as Arabidopsis and tobacco (*Nicotiana tabacum*). Examples include the overexpression of *LEC2* in the fatty acid breakdown mutant *COMATOSE* (*cts2*) (Slocombe et al. 2009), the overexpression of *WRI1* in an Arabidopsis RNAi line designated *AGP*RNAi that reduces the expression of *APS1*, a gene encoding the ADP-glucose pyrophosphorylase (Sanjaya et al. 2011), the coexpression of DGAT1 and oil-body protein oleosin that increases both TAG and leaf biomass (Winichayakul et al. 2013), the disruption of a TAG lipase gene *SUGAR-DEPENDENT1* (*SDP1*) or *PEROXISOMAL TRANSPORTER1* (*PXA1*) in the *tgd1* mutant (Fan et al. 2014), and the coexpression of *WRI1* and *DGAT1* in tobacco (Vanhercke et al. 2013). Compared to these two-gene attempts, three-gene combinations were even more successful in enhancing TAG accumulation. In Arabidopsis, the coexpression of *OLEOSIN1* and the gene encoding phospholipid:diacylglycerol acyltransferase 1 (*PDAT1*) in the *tgd1* mutant boosted leaf TAG content to ~9 % of the dry weight (Fan et al. 2013). In contrast, the coexpression of *WRI1* and *DGAT1* in the lipase mutant

sugar-dependent1 (*sdp1*) resulted in TAG accumulation in leaves, stems and roots ranging from 5 to 8 % of dry weight but along with a ~20 % reduction in leaf biomass (Kelly et al. 2013). By the provision of 3 % (w/v) sucrose, TAG content in the roots of these transgenic plants could be further increased up to 17 % of dry weight (Kelly et al. 2013). Another report showed over 10 % TAG by dry weight in mature tobacco leaves and ~15 % TAG in stems and roots by the coexpression of *WRI1*, *DGAT1* and *OLEOSIN*, three genes involved in different aspects of TAG synthesis (Vanhercke et al. 2014).

Environmental Stresses Induce Oil Accumulation in Plant Vegetative Tissues

Besides manipulation of gene expression, various environmental stresses, such as ozone fumigation, freezing and desiccation, appear to stimulate plant oil accumulation in vegetative tissues (Fig. 8.1) (El-Hafid et al. 1989; Moellering et al. 2010; Navari-Izzo and Rascio 1999; Sakaki et al. 1985). For example, after approximately 6 h fumigation with ozone (0.5 μL/L), TAG accumulation in spinach leaves reached a maximum, whereas three polar lipids, phosphatidylcholine (PtdCho), mono- and digalactosyldiacylglycerol (MGDG and DGDG) decreased strongly during the same period (Sakaki et al. 1985, 1990c). Further analysis such as fatty acid profiling and pulse-chase acetate labeling has revealed that these TAGs are derived from diacylglycerol (DAG) and free fatty acids (FFA) from MGDG due to the activity of a galactolipid:galactolipid galactosyltransferase (GGGT) (Sakaki et al. 1990b, c). Subsequent *in vitro* enzyme activity assays of spinach GGGT suggested its activation by FFA and divalent cations such as Mg^{2+}, Mn^{2+} and Ca^{2+} (Sakaki et al. 1990a). However, the significance of the TAG accumulation in relation to GGGT activation in spinach leaves upon ozone treatment is still not clear.

Stress-induced TAG accumulation has also been observed in Arabidopsis during freezing (Moellering and Benning 2011; Moellering et al. 2010). Studies on Arabidopsis *SENSITIVE TO FREEZING 2* (*SFR2*) (Moellering et al. 2010), a gene encoding a protein annotated as a glycosyl hydrolase family 1 protein at the outer chloroplast envelope membrane (Fourrier et al. 2008; Thorlby et al. 2004), suggested that SFR2 likely is the GGGT in plants and participates in the protection of chloroplast under freezing stress due to its activity causing the formation of oligogalactolipids and DAG by processive transfer of galactosyl moieties from MGDG onto an galactolipid acceptor (Moellering et al. 2010). A more recent study confirmed that SFR2 acts solely as a glycosyltransferase rather than a glycosyl hydrolase and provided insights into its reaction mechanism through structure function studies assisted by a structural homology model (Roston et al. 2014). Under freezing stress, Arabidopsis SFR2 converts nonbilayer-MGDG to bilayer-forming membrane lipids such as tri- and tetra-galactosyldiacylglycerol (TGDG and TeGDG, respectively), as well as DAG that is further acylated to TAG (Moellering et al. 2010). In case of ozone fumigation of spinach leaves, the acyl groups are most

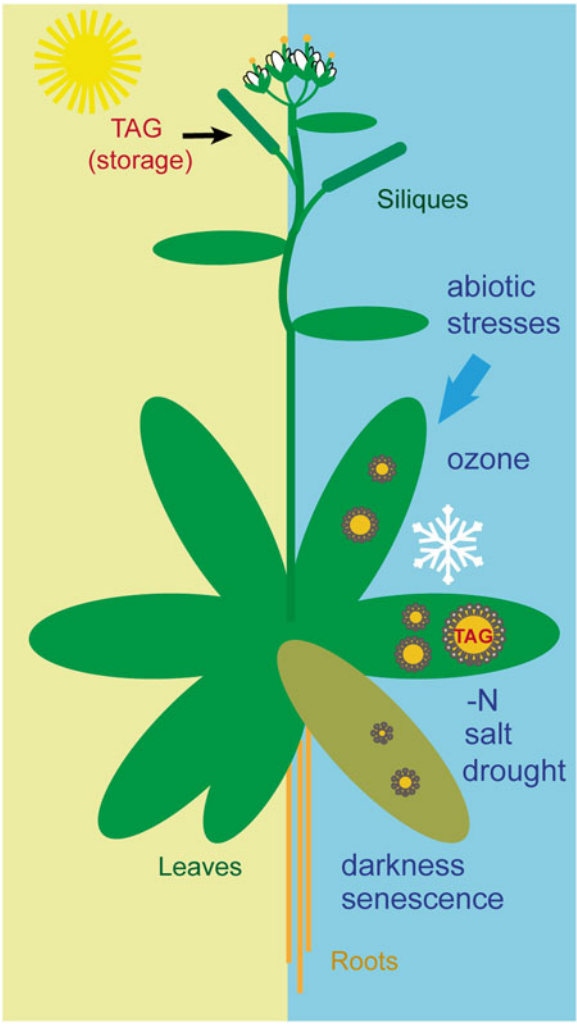

Fig. 8.1 Triacylglycerol accumulation occurs in plant vegetative tissues under environmental stresses. The plant model shown in the figure represents plants described in section "Environmental stresses induce oil accumulation in plant vegetative tissues", e.g. *Arabidopsis thaliana*, *Spinacia oleracea* L. (spinach) and *Gossypium hirsutum* (cotton) plants. The *white* snow flake and the *yellow* leaf indicate freezing condition and leaf senescence, respectively. *TAG* triacylglycerol, *-N* nitrogen deprived, *salt* high salt

likely derived from MGDG hydrolysis (Moellering et al. 2010; Sakaki et al. 1990b, c). The remodeling of membrane lipids by SFR2 upon freezing stress, which leads to severe dehydration of the cell as ice forms first in the apoplast, reduces the tendency of the formation of inter-bilayer hexagonal II phase, increases stabilization of the envelope membranes and, therefore, enhances freezing tolerance (Moellering and Benning 2011; Moellering et al. 2010; Roston et al. 2014). Furthermore, TAG accumulation in Arabidopsis leaves can contribute to the removal of excess membrane lipids like MGDG from the envelope membranes as the organelle shrinks in response to dehydration by the combined activity of TAG-biosynthetic enzymes and SFR2 during freezing treatment or general osmotic stress (Moellering and Benning 2011; Moellering et al. 2010).

Under water-deficit stress conditions, a decrease in MGDG and increase in TAG levels has been observed in a variety of plants such as desiccation-tolerant plants *Craterostigma plantagineum* (blue carpet), *Lindernia brevidens* and *Ramonda serbica* (Serbian-phoenix flower), desiccation-sensitive plants Arabidopsis and *Lindernia subracemosa*, as well as the crops *Gossypium hirsutum* (cotton), *Triticum aestivum* (wheat) and *Zea mays* (maize), indicating an important role of lipid remodeling in plant adaptation to desiccation stress (El-Hafid et al. 1989; Gasulla et al. 2013; Navari-Izzo and Rascio 1999). For instance, a recent study has shown that TAGs in *C. plantagineum* increase from 0.146 to 3.11 nmol mg^{-1} of dry cell weight in desiccated leaves and decrease again following rehydration (Gasulla et al. 2013). Analysis of molecular species has revealed that these TAGs are synthesized from DAG derived either directly from MGDG hydrolysis or due to the activity of SFR2 that converts multiple MGDGs into TGDG/TeGDG and DAG. Simultaneously, a fraction of MGDG is converted into DGDG by UDP-Gal-dependent DGDG synthases DGD1/DGD2 (Gasulla et al. 2013). This conversion of MGDG to DGDG/TGDG/TeGDG and TAG is believed to contribute to the stabilization of membranes during desiccation stress. In addition, TAG accumulation can be induced (or TAG degradation delayed) in Arabidopsis seedlings by the treatment with abscisic acid (Yang et al. 2011), an important plant stress hormone also involved in responses to freezing and desiccation.

TAG accumulation is also observed following senescence/dark treatment in Arabidopsis leaves (Slocombe et al. 2009), which is interpreted as the sequestration of FFA derived from galactolipids (Kaup et al. 2002). During leaf senescence or environmental stresses, disintegration of thylakoid membranes, as well as degradation of chlorophyll and galactolipids, results in the accumulation of toxic intermediates such as free phytol and FFA. In Arabidopsis, *PHYTYL ESTER SYNTHASE1* (*PES1*) and *PES2* encode two acyltransferases (of the esterase/lipase/thioesterase family) performing both phytyl ester synthesis and diacylglycerol acyltransferase activities with broad substrate specificities (Lippold et al. 2012). During developmental senescence or nitrogen deprivation induced senescence, TAG and phytyl esters accumulate in the wild type, whereas the *pes1 pes2* double mutant contains ~30 % less TAG than the wild type but much higher free phytol. This observation indicates that PES1 and PES2 help avoid the accumulation of toxic products of thylakoid membrane degradation during leaf senescence and nitrogen deprivation, by the removal of free phytol and FFA in the form of phytyl esters and eventually sequestration of FFA into TAG (Lippold et al. 2012). TAG accumulation has also been reported by another group using nitrogen-deprived Arabidopsis seedlings (Yang et al. 2011). These findings are particularly intriguing because nitrogen deficiency is currently widely used in laboratory and aquaculture settings to induce TAG accumulation in microalgae, another attractive source of sustainable feedstocks for bioenergy.

Microalgae Accumulate Triacylglycerol upon Different Stresses

Microalgae are eukaryotic photosynthetic microorganisms that are masters at using sunlight, CO_2 and water to produce biomass (Georgianna and Mayfield 2012; Hu et al. 2008). Under optimal growth conditions, microalgae are very efficient in the utilization of solar energy for the production biochemical compounds such as starch, cellulose and other carbohydrates which can readily be used as feedstocks for bioethanol production due to the absence, or low content of lignin from algal biomass compared to biomass derived from land plants (John et al. 2011; Jones and Mayfield 2012). When placed under stress conditions, many species of microalgae produce large amounts of neutral lipids typically in the form of TAGs as storage products for carbon and energy, and they are therefore referred to as oleaginous microalgae (Chisti 2007; Hu et al. 2008). As this typically happens in photosynthetically active cells, TAG accumulation in microalgae is conceptually more similar to stress-induced TAG accumulation in vegetative tissues of plants than the related process in developing plant seeds. Therefore, gaining an understanding of TAG accumulation in microalgae may also provide important insights for the engineering of TAG accumulation in vegetative tissues of plants.

Algal TAGs usually have acyl chains with 16 or 18 carbons esterified to the glycerol (Fig. 8.2) (Liu et al. 2013). These fatty acyl chains are chemically similar to diesel fuel components that typically have 10–15 carbons per molecule, and alga-derived biodiesel is directly compatible with diesel engines (ASTM 2002; Durrett et al. 2008). Thus, oleaginous microalgae can theoretically be used for producing both biodiesel derived from their oil and bioethanol/bioelectricity derived from the biomass left after oil extraction maximizing the overall energy yield (Jones and Mayfield 2012; Ohlrogge et al. 2009).

Moreover, using microalgae as feedstocks for biofuel products has potential advantages as compared to current biofuel crops such as corn and oil palm. Firstly, microalgae accumulate a substantial amount of TAG, commonly 20–50 % of dry weight (Chisti 2007; Hu et al. 2008). Secondly, microalgae grow fast (usually 1–3 doublings per day) and they are more efficient than terrestrial plants in the conversion of solar energy to biomass (Chisti 2013; Stephenson et al. 2011). Thirdly, in principle microalgae require less land area for the same yield of biofuel compared with terrestrial crops and their cultivation can utilize marginal lands such as deserts and saline lake beds that cannot be used for conventional agriculture (Georgianna and Mayfield 2012; Stephenson et al. 2011). Microalgae can be incubated in enclosed photobioreactors throughout the year independent of seasons (Georgianna and Mayfield 2012; John et al. 2011), and can use nutrient-rich wastewater to meet the relatively high demand for water (Venkata Mohan et al. 2015). Marine microalgae cultivation can utilize abundant sea water, but requires a costal location of the production site (Stephenson et al. 2011). Thus, cultivation of microalgae has the potential to minimize or avoid the competition with food crops for arable land and other crop-based agricultural production schemes for biofuels. Fourthly, microalgae produce a variety of valuable co-products or by-products such as biopolymers, pro-

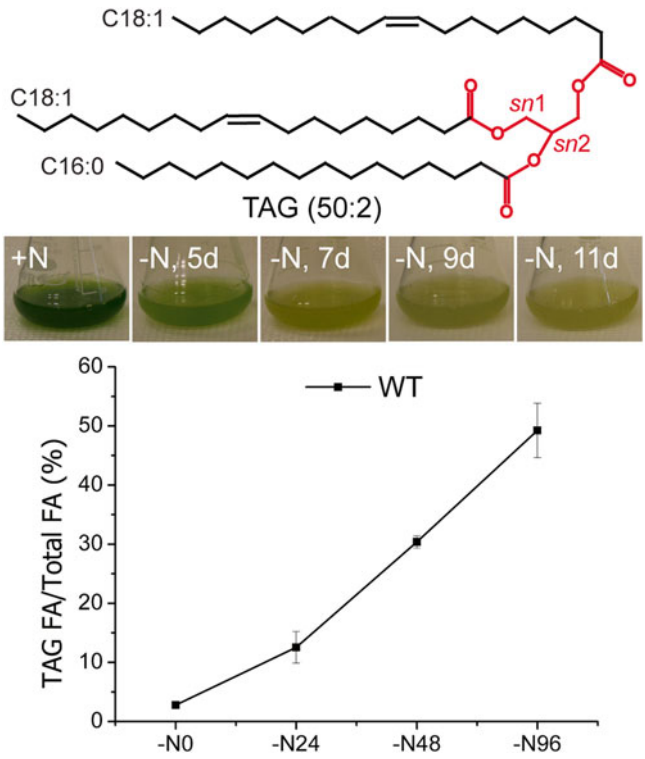

Fig. 8.2 Triacylglycerol is accumulated in nutrient-limited cultures of *Chlamydomonas reinhardtii*. Triacylglycerol (TAG) consists of three acyl chains (in *black*) that are esterified to a glycerol (in *red*). Cells of wild-type Chlamydomonas strain dw 15-1 were incubated in Tris-acetate-phosphate medium supplemented with or without 10 mM NH_4Cl. The ratio of fatty acids (FA) from TAG over total-lipid fatty acids was calculated in Chlamydomonas samples following indicated nitrogen deprivation. *+N* nitrogen replete, *−N* nitrogen deprived. Averages of three independent measurements are given. *Error bars* indicate SD

teins, animal feed and fertilizer (Brennan and Owende 2010; Hu et al. 2008). All these advantages promise a great potential for microalgae-based production of fuels and other products, a promise that, however, still needs to be realized in cost effective ways. But the promise of algae as sustainable feed stocks certainly has globally "fueled" research to explore mechanisms of TAG accumulation in microalgae and to develop algae-based production schemes (Liu and Benning 2013).

Factors Affecting Triacylglycerol Accumulation in Microalgae

Oleaginous microalgae produce only small amounts of TAG during optimal growth or under favorable environmental conditions, under which polar membrane lipids (5–20 % of dry weight) are generally the major lipid compounds (Hu et al. 2008).

As already mentioned, synthesis and accumulation of TAG in microalgae is induced by stress conditions, accompanied by complicated changes in overall fatty acid and lipid composition. Stresses can be chemical or non-chemical in nature and the major chemical-based inducers of TAG accumulation are various nutrient limitations, whereas the major non-chemical stress inducers are temperature and light intensity. Growth phase of a culture and aging of microalgal cultures also affect TAG content and fatty acid composition, likely because nutrients become limited and toxins accumulate as the cultures enter stationary phase.

Nitrogen deficiency is the most frequently studied condition inducing TAG accumulation in different algae (Hu et al. 2008). Microalgae including green algae and diatoms accumulate TAG ~20–50 % upon nitrogen deficiency (Hu et al. 2008). Silicon is another important nutrient that affects cellular lipid metabolism especially in diatoms. In the brackish-water diatom *Cyclotella cryptica*, silicon deficiency induces TAG accumulation in lipid droplets with higher proportions of saturated and mono-unsaturated fatty acids over silicon-replete cells (Roessler 1988; Traller and Hildebrand 2013). Other macro nutrients affecting cellular lipid metabolism include sulfur and phosphorus. For example, sulfur starvation increases the neutral lipids in the green algae *Chlorella ellipsoidea* (Otsuka 1961) and *Chlamydomonas reinhardtii* (Matthew et al. 2009). Phosphorus starvation also promotes TAG accumulation in the fresh water alga *Monodus subterraneus* (Khozin-Goldberg and Cohen 2006) and various marine microalgae such as the diatom *Phaeodactylum tricornutum* and the haptophyte alga *Isochrysis galbana* (Abida et al. 2015; Reitan et al. 1994). Besides macro nutrients, studies on the green alga Chlamydomonas have shown that deficiency in micro nutrients such as zinc and iron also induce TAG accumulation (Kropat et al. 2011; Urzica et al. 2013). In addition, drugs such as Brefeldin A (an ER-stress inducer) have been found to rapidly stimulate TAG accumulation in Chlamydomonas and the freshwater alga *Chlorella vulgaris* (Kim et al. 2013).

Many studies have reported that non-chemical based stresses lead to the formation of TAG in microalgae including unfavorable temperature, light intensity, high salinity and dehydration. For example, increased temperature results in the elevation of lipid content in the freshwater phytoflagellate *Ochromonas danica* (Aaronson 1973), the marine alga *Nannochloropsis salina* (Boussiba et al. 1987) and Chlamydomonas (Hemme et al. 2014). High light intensity increases neutral storage lipid content, mainly TAGs, accompanied by a decrease in total polar lipids (Brown et al. 1996; Khotimchenko and Yakovleva 2005; Napolitano 1994). Furthermore, TAG accumulates in Chlamydomonas during hypoxia in darkness or during extended (24 h) darkness alone (Hemschemeier et al. 2013). Synthesis of TAG can also be induced by high salinity as seen in the green algae *Dunaliella salina* (Takagi et al. 2006) and Chlamydomonas (Siaut et al. 2011), or by dehydration during illumination in the green alga *Chlorella kessleri* (Shiratake et al. 2013).

When reaching the stationary phase, some microalgae such as the green alga *Parietochloris incisa* and the marine dinoflagellate *Gymnodinium* sp. have shown an increase in TAG content (Bigogno et al. 2002; Mansour et al. 2003), which is probably a consequence of nutrient depletion or accumulation of toxic metabolic products.

Culture aging or senescence during prolonged stationary phase also affects lipid metabolism in microalgae. For example, the total lipid content increases along with culture aging in the green alga *Chlorococcum macrostigma* (Collins and Kalnins 1969), and diatoms such as *Thalassiosira fluviatillis* (Conover 1975) and *Coscinodiscus eccentricus* (Pugh 1971). These findings indicate possible roles of TAG accumulation in response to various stimuli, and a better understanding of the mechanism of TAG induction during stress conditions will provide strategies for the engineering of TAG accumulation and TAG quality under conditions favorable for biomass accumulation of microalgae.

Chlamydomonas as a Reference Microalgae to Answer Questions about TAG Accumulation

The unicellular green alga Chlamydomonas traditionally has been used as a model for studies of photosynthesis (Rochaix 1995) or flagella biogenesis (Harris 2001), and has recently been widely adopted as a reference organism for algal TAG metabolism research. Reasons are its simple life cycle and well-developed genetic tools and techniques (Liu and Benning 2013; Merchant et al. 2012). As summarized above, Chlamydomonas cells accumulate TAGs upon various unfavorable conditions such as nutrient deficiency and non-chemical stresses (Liu and Benning 2013; Merchant et al. 2012). Nitrogen deprivation is frequently used to induce TAG accumulation in Chlamydomonas (Fig. 8.2), and will lead to a cessation of cell division and eventually to a cellular state known as cellular quiescence (Tsai et al. 2014).

To understand the mechanism of TAG accumulation in Chlamydomonas, several studies have been carried out to identify key genes by reverse and forward genetic approaches (Liu and Benning 2013; Merchant et al. 2012). For instance, forward genetic screening using insertional mutagenesis (Khozin-Goldberg and Cohen 2011; Li et al. 2012; Merchant et al. 2012; Zhang et al. 2014), deep transcriptome analysis by RNA sequencing (Blaby et al. 2013; Hemschemeier et al. 2013; Juergens et al. 2015; Miller et al. 2010; Park et al. 2015; Schmollinger et al. 2014; Tsai et al. 2014), and proteomics by mass spectrometry (Hemme et al. 2014; Schmollinger et al. 2014; Wang et al. 2009) using wild-type Chlamydomonas or mutant strains have identified genes involved in TAG accumulation and its regulation. Several lipid droplet-focused proteomic studies have reported the presence of a major lipid droplet protein MLDP, which is considered a functional equivalent of plant oleosins (James et al. 2011; Moellering and Benning 2010; Nguyen et al. 2011). Reverse genetic screening based on the analysis of orthologs of characterized genes from yeast, animals and plants have provided candidates for genes involved in TAG biosynthesis (Khozin-Goldberg and Cohen 2011; Merchant et al. 2012; Riekhof and Benning 2009). Previous studies on other eukaryotes such as yeast and Arabidopsis identified two key enzyme families in TAG synthesis, DGATs and PDATs, which are also found in Chlamydomonas (Merchant et al. 2012). Currently, six genes encoding DGATs from two families, type one DGAT

and type two DGTT, are known in Chlamydomonas including *DGAT1* and *DGTT1*-to-*DGTT5* (Boyle et al. 2012; La Russa et al. 2012; Miller et al. 2010). In contrast, only one *PDAT* has been described for Chlamydomonas, which participates in membrane lipid turnover and TAG synthesis (Boyle et al. 2012; Yoon et al. 2012).

A recent mutant screening for Chlamydomonas low TAG mutants led to the discovery of a galactoglycerolipid lipase designated PGD1 (PLASTID GALACTOLIPID DEGRADATION 1), and the respective *pgd1* null-mutant provides a tool to experimentally test the role of TAG accumulation following nitrogen deprivation (Fig. 8.3) (Li et al. 2012). A classic hypothesis is that *de novo* TAG synthesis serves as an electron sink to sequester excess electrons from the photosynthetic electron transport chain, thereby counteracting its possible overreduction which can lead to the formation of harmful reactive oxygen species (ROS) at photosystem I through the Mehler reaction (Hu et al. 2008). The study of the *pdg1* mutant supports the hypothesis that

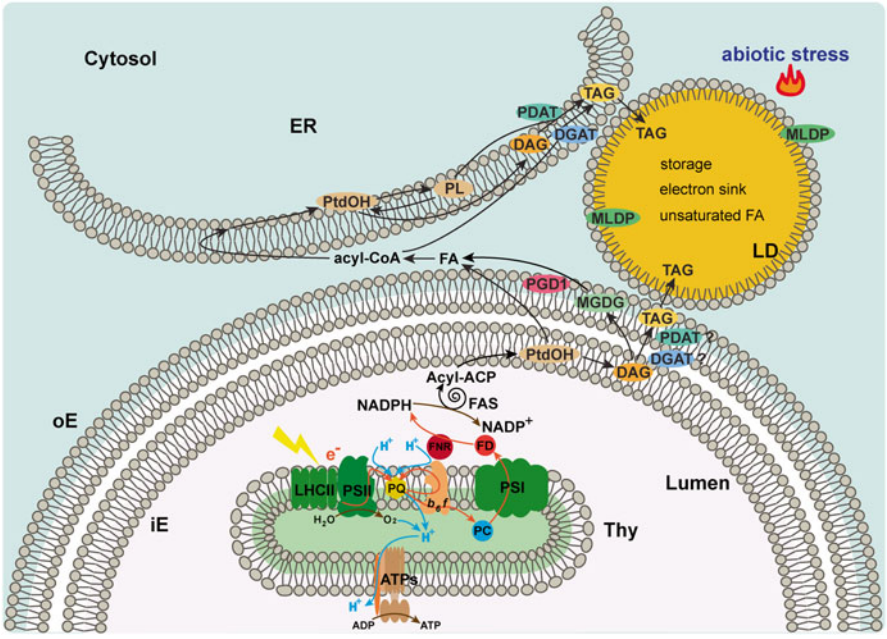

Fig. 8.3 Proposed roles of triacylglycerol accumulation in Chlamydomonas in response to abiotic stresses. *ER* endoplasmic reticulum, *PtdOH* phosphatidic acid, *PL* phospholipid, *DAG* diacylglycerol, *TAG* triacylglycerol, *DGAT* diacylglycerol acyltransferase, *PDAT* phospholipid:diacylglycerol acyltransferase, *LD* lipid droplet, *MLDP* Chlamydomonas major lipid droplet protein, *acyl-CoA* acyl-coenzyme A, *FA* fatty acid, *oE* outer envelope, *iE* inner envelope, *PGD1* PLASTID GALACTOLIPID DEGRADATION 1, *MGDG* monogalactosyldiacylglycerol, *acyl-ACP* acyl-acyl carrier protein, *FAS* fatty acid synthase complex, e^- electron, *FD* ferredoxin, *FNR* ferredoxin:NADP$^+$ reductase, *PQ* plastoquinone and plastoquinol, b_6f, cytochrome b_6f complex, *PC* plastocyanin; H^+, proton, *LHCII* light-harvesting complex II, *PSI and II* photosystems I and II, *ATPs* ATP synthase, *ADP/ATP* adenosine di-/triphosphate, *Thy* thylakoid. Different *color arrows* indicate different fluxes: *black*, fatty acid and lipid pathway; *orange*, linear electron flow; *blue*, proton; *brown*, others

TAG accumulation is essential for Chlamydomonas cells to survive under nitrogen starvation (Li et al. 2012). Results of activity assay, *in vivo* pulse-chase and lipid analysis have shown that Chlamydomonas PGD1 hydrolyzes newly incorporated acyl groups at the *sn-1* position of MGDG that can be converted to acyl-CoA for *de novo* TAG synthesis in the cytosol. During nitrogen deprivation, the *pgd1* mutant produces only about 50 % of TAG compared to the wild type, and becomes chlorotic after ~7 days of treatment. Because of the large TAG decrease in the *pgd1* mutant, presumably reduction pressure of the electron transport chain is increased and molecular oxygen at photosystem I (PSI) could serve under these conditions as an alternative electron acceptor to form ROS that will cause damage to thylakoid membranes and chloroplasts and eventually lead to cell death. Indeed, formation of ROS indicated by thiobarbituric acid reactive substances (TBARS) (Baroli et al. 2003) has been observed in the *pgd1* mutant by day 7 of nitrogen deprivation concomitant with chlorosis (Li et al. 2012). Moreover, blocking of electron transfer at the acceptor side of PSII by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) can reverse the chlorosis of the *pgd1* mutant, but not TAG accumulation, supporting a possible role of TAG as a sink for electrons (Li et al. 2012). However, it should be noted that comparative transcriptome analysis has shown that following nitrogen deprivation, protein biosynthesis in general and the expression of genes encoding many photosynthesis components is down-regulated (Blaby et al. 2013; Juergens et al. 2015; Miller et al. 2010; Park et al. 2015; Schmollinger et al. 2014). Therefore other explanations for the role of TAG accumulation, such as the transient and safe storage of acyl chains for latter resynthesis of membranes, when conditions improve, need to be considered. Furthermore, these experiments were conducted in acetate medium and hence under photoheterotrophic conditions that may promote TAG formation from acetate. In fact, nitrogen deprivation also leads to a redirection of carbon metabolism such that acetate in the medium is no longer converted to cell building blocks by the glyoxylate cycle and gluconeogenesis, but channeled directly into fatty acid biosynthesis (Miller et al. 2010). Recently, it has been suggested that TAG accumulates to particularly high levels when carbon supply exceeds the capacity of starch synthesis in Chlamydomonas, which is usually the case for microalgae under stress conditions such as nutrient deficiency (Fan et al. 2012). Hence, Chlamydomonas starchless mutants are capable to produce more TAG compared to the wild type (Li et al. 2010; Wang et al. 2009; Work et al. 2010). In some microalgae, TAG synthesis is coordinated with the synthesis of carotenoids such as β-carotene, lutein and astaxanthin that can be sequestered in cytosolic carotenoid-rich lipid droplets. Their peripheral distribution in cells has been proposed to serve as a sun screen to prevent excessive photons from striking the chloroplast and photosynthetic membrane under stress conditions (Rabbani et al. 1998; Zhekisheva et al. 2002). An increase in TAG synthesis has also been observed in the marine alga *Desmodesmus* sp. during high light growth following nitrogen deprivation, which is believed to prevent at least in part photo-oxidative damage under these stress conditions (Gorelova et al. 2015).

Even though Chlamydomonas PGD1 is a galactolipid lipase, it is proposed to not contribute to degradation of membranes but aide in the *de novo* synthesis of TAGs

from newly formed fatty acids (Li et al. 2012). However, loss of PGD1 only leads to 50 % reduction in TAG content and other mechanisms to supply precursors of TAG biosynthesis have to be considered. Global transcript analysis indicates that during nitrogen deprivation, genes encoding putative lipases are among those displaying the strongest variations in transcript abundance in Chlamydomonas, which could be a sign for degradation of structural membrane lipids, such as mature MGDG containing 18:3 and 16:4 acyl chains. In fact, a small fraction of TAG contains 16:4 acyl chains, otherwise only found in MDGD of the thylakoid membrane (Liu et al. 2013). These findings are further supported by microscopic observation and lipid analyses indicating that nitrogen deprivation-induced TAG accumulation in lipid droplets occurs concomitantly with the breakdown of thylakoid membranes (Boyle et al. 2012; Iwai et al. 2014). Thus lipid droplet formation is at least in part accompanied by the conversion of polar membrane lipids such as mature MGDG and phosphatidylglycerol (PG) to TAG (Yoon et al. 2012).

Aside from the mechanisms discussed thus far, TAG synthesis involving a PDAT, designated PDAT1, was observed in Chlamydomonas (Fig. 8.3) (Boyle et al. 2012; Yoon et al. 2012). Based on mutants obtained by insertional mutagenesis or artificial microRNA silencing, Chlamydomonas PDAT1 was estimated to contribute up to ~25 % of the total TAG accumulating following nitrogen deprivation due to the turnover of chloroplast membrane lipids, particularly MGDG, sulfoquinovosyldiacylglycerol (SQDG) and PG (Boyle et al. 2012; Yoon et al. 2012). Besides nitrogen deprivation, limitation of iron and zinc in Chlamydomonas cells can also lead to chloroplast/chlorophyll degradation and lipid remodeling (Kropat et al. 2011; Urzica et al. 2013). Under nitrogen deprivation, similar lipid remodeling events that occur in Chlamydomonas have been observed for other algal species such as the model diatom *P. tricornutum* (Abida et al. 2015; Yang et al. 2013), the freshwater alga *M. subterraneus* (Khozin-Goldberg and Cohen 2006), the marine algae *Nannochloropsis gaditana* (Simionato et al. 2013) and *Nannochloropsis oceanica* IMET1 (Jia et al. 2015), and even land plants such as Arabidopsis (Lippold et al. 2012). Thus, TAG accumulation from precursors derived from lipids of the photosynthetic membrane could serve in part as a mechanism to sequester acyl groups for later use, when membranes have to be resynthesized.

Other than nutrient deprivation, stresses such as heat and dark anoxia also trigger TAG accumulation in Chlamydomonas cells (Hemme et al. 2014; Hemschemeier et al. 2013). These stresses lead to the conversion of membrane lipids to TAG which is similar to the observations under nutrient deprivation. However, heat- and dark anoxia-induced TAGs tend to accumulate unsaturated fatty acids, particularly polyunsaturated ones such as linolenic acid (C18:3), compared with TAG produced during nutrient deprivation (Hemme et al. 2014; Hemschemeier et al. 2013). Factors such as temperature and light intensity strongly affect the fatty acid composition in microalgae. For example, it has been observed in many microalgae that increasing temperature leads to more saturated fatty acids whereas decreasing temperature promotes unsaturation of fatty acids (Lynch and Thompson 1982; Renaud et al. 2002; Sato and Murata 1980). High light intensity can also increase the saturation of fatty acids in *Nannochloropsis* sp. cells (Fabregas et al. 2004). It has been reported that

TAG serves as a reservoir of polyunsaturated fatty acids for the rapid formation of membrane lipids upon changes in environmental conditions (e.g. sudden decreases in temperature) in the red alga *Porphyridium cruentum* (Cohen et al. 2000). Thus, in Chlamydomonas polyunsaturated fatty acids derived from the degradation of membrane lipids are likely stored in TAG during non-nutrient deprivation-induced environmental stresses (e.g. heat and dark anoxia) for future utilization. Interestingly, ER stress by Brefeldin A can induce a similar membrane lipid turnover and increase in unsaturation of TAG in Chlamydomonas and *Chlroella vulgaris* (Kim et al. 2013).

Several attempts have been carried out to engineer TAG production in Chlamydomonas (La Russa et al. 2012). For example, three type-two *DGATs*, *DGTT1* to *DGTT3* (also referred to as *DGAT2a*, *b* and *c*) have been independently overexpressed in Chlamydomonas cells but did not increase the intracellular TAG accumulation or significantly alter the composition of the fatty acids compared to the wild type during regular growth condition or under nitrogen or sulfur deprivation (La Russa et al. 2012). In contrast, another research group overexpressed *DGTT4* using a sulphoquinovosyldiacylglycerol 2 (SQD2) promoter that is upregulated during phosphorus starvation, resulting in strongly increased TAG accumulation over the wild type. However, the respective TAG production (~15 mg L^{-1}) was still far below the levels needed for commercial use (Iwai et al. 2014).

A recent study has identified a gene encoding a *SQUAMOSA* promoter-binding-protein-domain containing protein designated *NRR1* (Boyle et al. 2012). It is considered to be an important regulator of TAG synthesis following nitrogen starvation because the *nrr1* mutant produces only ~50 % TAG over the wild type (Boyle et al. 2012). Considering that the single-gene strategies in plant engineering were not very successful whereas triple-gene coexpression of *OLEOSIN*, *WRI1* and *DGAT1* (genes encoding lipid droplet protein, transcription factor and type-one DGAT, respectively) substantially increased TAG production in tobacco vegetative tissues (Vanhercke et al. 2014), multiple-gene coexpression in starchless mutants could be employed to enhance TAG production in Chlamydomonas using its endogenous genes such as *MLDP*, *NRR1* and DGATs in the future. While Chlamydomonas is usually not considered an oleaginous alga for biodiesel production, but a reference organism for the research on lipid metabolism in microalgae, the findings on Chlamydomonas could direct studies on more biotechnologically relevant species such as Nannochloropsis.

Nannochloropsis, an Emerging Model to Study Lipid Metabolism

The oleaginous microalga Nannochloropsis sp., belonging to a genus of unicellular photosynthetic microalgae of the heterokonts, accumulates TAG as the major carbon and energy storage compound under regular or stress conditions (Liu et al. 2013; Meng et al. 2015; Simionato et al. 2013). Nannochloropsis does not

accumulate starch (Vieler et al. 2012b; Wang et al. 2014), which is very similar to the starch-less Chlamydomonas mutants that accumulate substantially more TAG than the starch-containing wild type under nitrogen deprivation (Li et al. 2010; Wang et al. 2009; Work et al. 2010). Thus, TAG may serve as an essential sink for photosynthate and as a primary storage compound during stresses in Nannochloropsis and Chlamydomonas. However, unlike Chlamydomonas, Nannochloropsis already synthesizes substantial amounts of TAG (~10 % of dry weight) under nutrient-rich conditions (Taleb et al. 2015). Even though Nannochloropsis produces up to 20 % of dry weight of carbohydrates such as mono- and polysaccharides under nutrient deprivation (Jia et al. 2015; Vieler et al. 2012b; Wang et al. 2014), TAG is the major reserve compound under those conditions (Jia et al. 2015; Simionato et al. 2013; Taleb et al. 2015; Vieler et al. 2012b), making Nannochloropsis an interesting model genus for lipid metabolism research. In terms of lipid metabolism, another big difference between the green alga Chlamydomonas and the oleaginous alga Nannochloropsis is that Chlamydomonas only contains the betaine lipid diacylglyceryl-N,N,N-trimethyl-homoserine (DGTS) that is believed to be a substitution for PtdCho, a common structural lipid in plants and alga with a similar structure as DGTS (Klug and Benning 2001), whereas Nannochloropsis possesses both DGTS and PtdCho (Jia et al. 2015). In addition, Nannochloropsis has been considered one of the more suitable algal species for feedstocks for biofuel production due to its rapid growth and high oil content (Taleb et al. 2015). It also produces large amounts of high-value polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) that can be used for nutritional supplements (Vieler et al. 2012b). Previous studies have focused on six species of Nannochloropsis, and five of them are marine algae that are widely distributed in the marine ecosystems especially in coastal regions (Andersen et al. 1998; Vieler et al. 2012b). In recent years, the genomes of several Nannochloropsis species have been sequenced, e.g. *N. oceanica* CCMP1779 (Vieler et al. 2012b), *N. gaditana* (Radakovits et al. 2012) and *N. oceanica* IMET1 (Wang et al. 2014), and genetic tools and techniques such as nuclear transformation have been developed to facilitate investigations on gene functions and engineering of metabolic pathways (Li et al. 2014a; Radakovits et al. 2012; Vieler et al. 2012b). For example, *N. oceanica* CCMP1779, a small marine alga of ~3 μm in diameter (Fig. 8.4a), has a relative small genome (28.7 Mb) with ~12,000 genes (Vieler et al. 2012b). It can accumulate considerable amounts of TAG in lipid droplets following nitrogen deprivation, and remetabolizes TAG for growth during nitrogen resupply (Fig. 8.4b, c). RNA sequencing analyses of nitrogen-replete or -deprived cells have identified 19 putative genes that are probably directly involved in TAG synthesis, including 13 *DGAT* and 2 *PDAT* genes (Table 8.1) (Vieler et al. 2012b). Most of the genes are up-regulated in response to nitrogen deprivation with the exception of a phosphatidate phosphatase (*PAP*) and *DGAT1* (Table 8.1). Another proteomic study of the lipid droplet of CCMP1779 cells has identified a predominant lipid droplet surface protein, designated as LDSP. Expression of the respective cDNA in the embryo of an Arabidopsis oleosin mutant, *oleo1*, could partially rescue the function of Arabidopsis OLEOSIN1 (Vieler et al. 2012a).

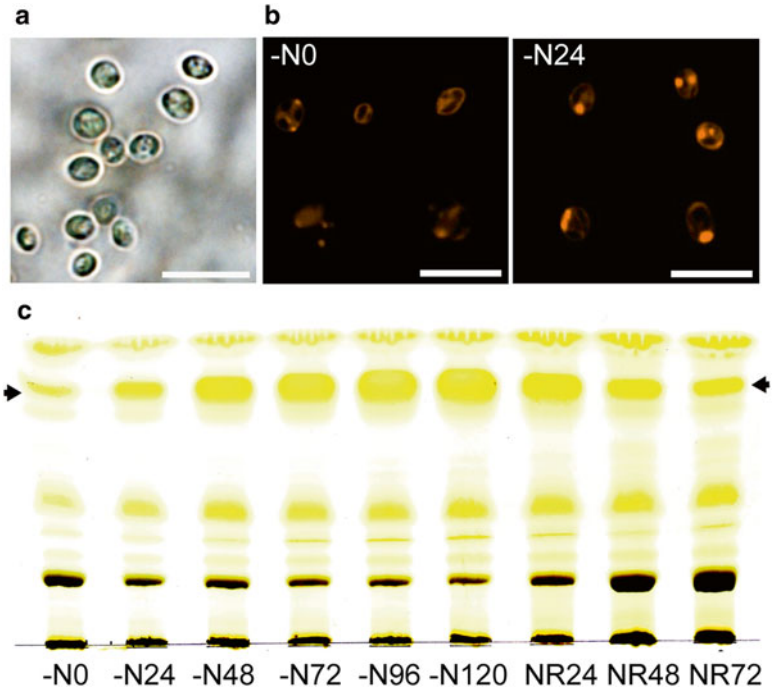

Fig. 8.4 Accumulation of triacylglycerol in *Nannochloropsis oceanica* CCMP1779 following nitrogen deprivation. (**a**) Wild-type *N. oceanica* grown in nitrogen-replete medium. Bar = 10 μm. (**b**) Confocal microscopy images of Nile red-stained wild-type *N. oceanica* cells grown in nitrogen-replete (−N0) or -deprived (−N24) media. Nile red fluorescence (*orange*) indicates lipid droplets (TAG, triacylglycerol). Bars = 10 μm. (**c**) Thin-layer chromatogram of lipid extracts stained for neutral lipids from nitrogen-replete (−N0), -deprived (−N24 to –N120) and -resupplied (NR24–NR72) cultures of wild-type *N. oceanica* at times (hours) indicated. Stained TAG is marked by *black arrows*

Besides *N. oceanica* CCMP1779, genome sequencing and comparative analysis of transcriptomes and lipidomes of other Nannochloropsis species such as *N. gaditana* (Corteggiani Carpinelli et al. 2014) and *N. oceanica* IMET1 (Li et al. 2014b; Wang et al. 2014) have shown putative TAG synthesis pathways and genes involved. Transcriptomic analyses of *N. oceanica* IMET1 cells under nitrogen-replete and -deprived conditions have revealed that many TAG synthesis genes of the Kennedy pathway (acyl-CoA dependent), especially genes encoding seven putative DGATs, are up-regulated upon nitrogen deprivation (Li et al. 2014b). Simultaneously, many genes involved in carbohydrate and protein degradation, as well as genes supplying carbon precursors and energy for *de novo* fatty acid biosynthesis, are increased in their expression and eventually contribute to TAG accumulation. Furthermore, lipidomic analyses using the same Nannochloropsis strain have shown recycling of fatty acids from membrane glycerolipids for TAG biosynthesis following nitrogen deprivation (Li et al. 2014b). Thus, similar lipid remodeling events as shown for

Table 8.1 Putative genes involved in triacylglycerol biosynthesis in *Nannochloropsis oceanica* CCMP1779

Gene	ID	Function	−N/+N
GPAT1	CCMP1779_4533	Glycerol-3-phosphate acyltransferase	1.3
LPAT1	CCMP1779_2512	1-sn-acyl-glycerol-3-phosphate acyltransferase	2.8
LIPIN	CCMP1779_161	Lipin like/ Phosphatidate phosphatase	1.7
PAP	CCMP1779_4742	Phosphatidate phosphatase	1.0
DGAT1	CCMP1779_4340	Diacylglycerol acyltransferase, DGAT Type2	0.6
DGAT2	CCMP1779_3705	Mono/diacylglycerol acyltransferase, Type2	1.2
DGAT3	CCMP1779_7206	Mono/diacylglycerol acyltransferase, Type2	1.7
DGAT4	CCMP1779_9929	Mono/diacylglycerol acyltransferase, Type2	2.8
DGAT5	CCMP1779_3915	Mono/diacylglycerol acyltransferase, Type2	1.4
DGAT6	CCMP1779_9590	Mono/diacylglycerol acyltransferase, Type2	2.5
DGAT7	CCMP1779_3159	Mono/diacylglycerol acyltransferase, Type2	1.5
DGAT8	CCMP1779_358	Mono/diacylglycerol acyltransferase, Type2	1.5
DGAT9	CCMP1779_10272	Mono/diacylglycerol acyltransferase, Type2	2.1
DGAT10	CCMP1779_3159	Mono/diacylglycerol acyltransferase, Type2	1.5
DGAT11	CCMP1779_5368	Mono/diacylglycerol acyltransferase, Type2	3.2
DGAT12	CCMP1779_3592	Mono/diacylglycerol acyltransferase, Type2	3.8
DGAT13	CCMP1779_3520	Diacylglycerol acyltransferase, DGAT Type1	1.8
PDAT1	CCMP1779_2212	Phospholipid/diacylglycerol acyltranferase	1.3
PDAT2	CCMP1779_8602	Phospholipid/diacylglycerol acyltranferase	1.7

−*N* N-deprived for 30 h, +*N* N-replete

Chlamydomonas have been found in Nannochloropsis, indicating that the findings in Chlamydomonas are invaluable for further research on Nannochloropsis (Jia et al. 2015; Martin et al. 2014; Simionato et al. 2013). Taken together, Nannochloropsis information and tool development is rapidly establishing this alga as a potential new reference organism for lipid metabolism research in a biotechnologically relevant microalga.

Conclusions and Perspectives

Plants and algae are highly efficient photosynthetic organisms providing sustainable and clean feedstocks for the production of liquid transportation fuels. Since photosynthetic cells of plants and algae tend to accumulate oils under stress conditions, understanding the mechanism of lipid biosynthesis and metabolism under these condition may provide novel avenues towards the genetic engineering and breeding of stress-tolerant crops and algae. Furthermore, more complete mechanistic insights into stress-induced TAG accumulation will enable the engineering of plants and algae with higher oil content in vegetative cells, but without growth inhibition or yield penalty. Current findings demonstrate similar pathways of TAG biosynthesis

in response to stresses for plants and algae, indicating that the discoveries made during plant or algal studies, especially the knowledge gained for reference organisms such as Arabidopsis and Chlamydomonas, may be widely applicable. By broadly screening of naturally occurring species, high oil algae such as Nannochloropsis were selected as candidates for industrial production. The recent genome sequencing of several Nannochloropsis species, as well as establishment of transcriptome, proteome, lipidome, transformation and cultivation, have provided resources and tools for the engineering of oil content and algal biomass quality to eventually overcome the barriers for the commercialization of algal oil. Likewise, findings about stress-induced accumulation of TAGs in algae have the potential to inspire new strategies for the engineering of oil content in vegetative tissues of plants. Taking a multipronged approach learning from algal and plant system is expected to create synergy towards efforts to meet the challenge of green-sustainable biofuel production in the future.

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