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REVIEW

The role of pyruvate hub enzymes in supplying carbon precursors for fatty acid synthesis in photosynthetic microalgae

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Abstract Photosynthetic microalgae are currently the focus of basic and applied research due to an ever-growing interest in renewable energy resources. This review discusses the role of carbon-unit supply for the production of acetyl-CoA, a direct precursor of fatty acid biosynthesis and the primary building block of the growing acyl chains for the purpose of triacylglycerol (TAG) production in photosynthetic microalgae under stressful conditions. It underscores the importance of intraplastidic acetyl-CoA generation for storage lipid accumulation. The main focus is placed on two enzymatic steps linking the central carbon metabolism and fatty acid synthesis, namely the reactions catalyzed by the plastidic isoform of pyruvate kinase and the chloroplastic pyruvate dehydrogenase complex. Alternative routes for plastidic acetyl-CoA synthesis are also reviewed. A separate section is devoted to recent advances in functional genomics studies related to fatty acid and TAG biosynthesis.

Keywords Microalgae · Lipid biosynthesis · Chloroplast pyruvate dehydrogenase complex · Chloroplast pyruvate kinase

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Introduction

Microalgal oils are considered an important feedstock for biodiesel production (Brennan and Owende 2010; Scott et al. 2010) and a source of nutritionally valuable fatty acids (FA) (Cohen and Khozin-Goldberg 2010; Guihéneuf and Stengel 2013; Hu et al. 2008; Klok et al. 2014). Photosynthetic microalgae can accumulate high amounts of energy-rich storage lipids, predominantly triacylglycerols (TAG), and this lipids' biosynthesis can be induced by cultivating the cells under stressful conditions. In photoautotrophic oleaginous species, conditions unfavorable for growth, such as nutrient depletion, e.g., nitrogen starvation, and high irradiance (Bigogno et al. 2002b; Ho et al. 2011; Hu et al. 2008; Pal et al. 2011; Solovchenko 2012), intensify FA-synthesis machinery in the chloroplast, ultimately leading to the deposition of acyl groups in the form of TAG in oil (lipid) droplets. This ability allows microalgae to survive adverse environmental conditions and to mobilize energy-rich resources when growth conditions are restored. Understanding the mechanisms underlying this phenomenon would greatly assist in manipulating FA synthesis and TAG production in photosynthetic microalgae for basic research and biotechnological applications.

In oleaginous species, whose storage lipid biosynthesis is swiftly induced upon sudden changes in nutrient availability, de novo synthesis seems to be the main source of FA for TAG assembly under conditions of photoautotrophy. Compartmentalization of the reactions of the de novo TAG assembly in microalgae may differ from other well-studied photosynthetic organisms and may comprise the chloroplast, as shown, for example, in the green microalga *Chlamydomonas reinhardtii* (Fan et al. 2011; Li et al. 2012). Membrane lipid recycling and turnover play an auxiliary role in providing acyl groups for storage lipid

synthesis under conditions conducive to TAG accumulation (Liu and Benning 2013; Sakurai et al. 2013). As photosynthetic organisms, microalgae accommodate the reactions of de novo FA synthesis catalyzed by the type II fatty acid synthase (FAS) in plastids. However, given the diversity and the distinctive evolutionary history of microalgae, heterotrophic-type FA synthesis by type I FAS in the cytoplasm cannot be excluded (Vieler et al. 2012), in particular, in those groups that are derived from secondary or tertiary endosymbiosis and retain metabolic features of the ancestor heterotrophic host. FA biosynthesis and TAG assembly are complex processes that comprise many enzymes and regulatory factors that are potentially involved in the global regulation of the pathway. These enzymes and factors remain insufficiently studied in microalgae.

The first committed reaction of FA production is the formation of malonyl-CoA from acetyl-CoA by acetyl-CoA carboxylase (ACC). This is considered a critical step in the FA-biosynthetic pathway in plants (Sasaki and Nagano 2004) and algae (Huerlimann and Heimann 2013). Malonyl-CoA is converted to malonyl-ACP (acyl carrier protein) and enters the reactions of FA synthesis, catalyzed by a FAS complex localized in stroma. Aside from being a direct precursor of malonyl-ACP, which serves as the primary building block of the growing acyl chain and the substrate of the initial condensation reaction, acetyl-CoA is also a central cellular metabolite involved in a variety of biochemical reactions that link anabolism and catabolism. It takes part in the synthesis of isoprenoids, certain amino acids, flavonoids, and other important metabolites and is a product of the degradation pathways of FA and some amino acids (Harwood 2005; Oliver et al. 2009). In addition, acetyl-CoA participates in the post-translational modifications, namely acetylation, of differentially localized proteins with diverse functions, including histones and transcription factors, that are implicated in transcriptional regulation, thus serving as a link between metabolic reactions and the global epigenetic regulation of cell behavior (Finkemeier et al. 2011; Nallamilli et al. 2014). Accordingly, due to the diversity of the biochemical pathways in which acetyl-CoA participates and the plausible impermeability of biological membranes to this molecule, its synthesis is suggested to occur in different compartments of the photosynthetic cells, with several enzymes involved in this process. Although some recent publications have postulated acetyl-CoA transport across the chloroplast membrane in microalgal cells (Perrineau et al. 2014; Wase et al. 2014), to our knowledge, no experimental evidence for this postulated transport has been provided so far. Accumulated knowledge, corroborated by recent metabolic and other "omic' studies, reinforces the idea that the supply of carbon units from photosynthesis for intraplastidic acetyl-CoA production, as well as the crosstalk between cellular compartments in providing the carbon skeletons from central metabolism, plays a crucial role in regulating the magnitude of subsequent reactions in de novo FA biosynthesis and TAG assembly in microalgae under nitrogen starvation.

Acetyl-CoA production in plant cells has been intensively studied (Bao et al. 2000; Fatland et al. 2000; Ke et al. 2000; Nikolau et al. 2000; Oliver et al. 2009; Rawsthorne 2002). In microalgae, much still remains unknown, and there is only fragmented information about carbon flux toward the generation of acetyl-CoA for the purposes of de novo FA synthesis in the evolutionarily distinct clades of microalgae and, in particular, about the primary source and coordinated action of different acetyl-CoA-generating routes. It should be noted that a vast majority of large-scale systems biology studies were performed on the model microalga *C. reinhardtii* whose growth was driven by an exogenous organic carbon source. However, under such conditions (namely, in the presence of acetate), respiratory metabolism is prioritized over photosynthesis (Schmollinger et al. 2014).

There are several potential routes for acetyl-CoA production in chloroplasts, which employ different plastid-localized enzymes (Fig. 1). One of the main enzymes is the chloroplast isoform of the pyruvate dehydrogenase complex (cpPDC) that utilizes pyruvate as a substrate. Another potential source of acetyl-CoA in plastids is acetyl-CoA synthetase (ACS), which converts acetate into acetyl-CoA (Ke et al. 2000). In addition, the existence of the so-called "pyruvate dehydrogenase (PDH) bypass," which produces acetyl-CoA by the sequential action of aldehyde dehydrogenase (ALDH) and ACS, has been reported in plants (Wei et al. 2009) and algae (Li et al. 2014). Acetate kinase, another potential source of acetyl-CoA that utilizes acetate, has been identified in *C. reinhardtii* (Wase et al. 2014). In animals and some oleaginous yeasts, the production of acetyl-CoA via another cytoplasmic enzyme, ATP-citrate lyase (ACL), has been reported as a rate-limiting step in the FA-biosynthesis pathway (Ohlrogge and Jaworski 1997). In several earlier biochemical works on isolated chloroplasts, the existence of the plastid isoform of ACL in castor bean, rape, spinach, pea, and tobacco was established (Fritsch and Beevers 1979; Rangasamy and Ratledge 2000; Ratledge et al. 1997). However, later studies in Arabidopsis did not reveal the presence of ACL in chloroplasts by either biochemical or bioinformatics means (Fatland et al. 2000, 2002). Thus, the sparse knowledge on acetyl-CoA production in photosynthetic microalgae is based primarily on the data inferred from studies on higher plants and from recent metabolomics and transcriptomics studies in some microalgal species. In this review, we will summarize the

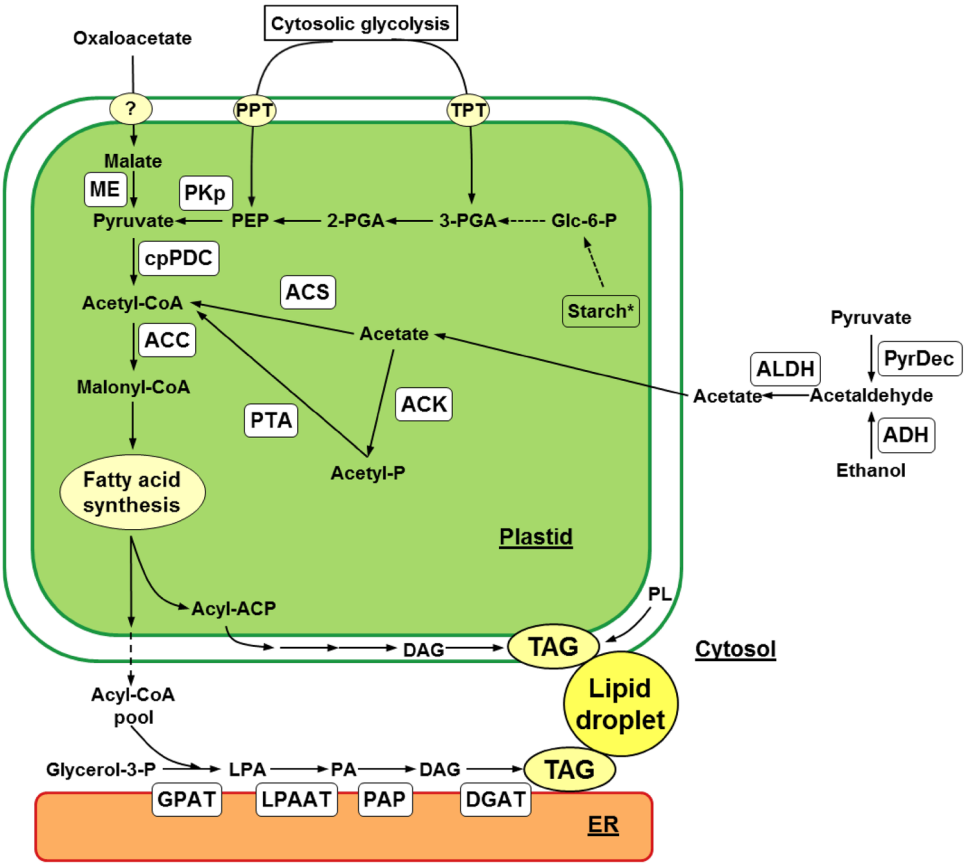

Fig. 1 Simplified representation of the carbon flux toward storage lipid accumulation in photosynthetic microalgae with an emphasis on plastidic acetyl-CoA production. The compartmentalization of glycolytic reactions, and hence their products, depends on the microalgal species. However, due to the transfer of intermediates across the chloroplast membranes, both plastidic and cytosolic glycolysis can supply PEP for PKp activity, which produces pyruvate. Pyruvate can also be produced by chloroplastic ME from malate. This pyruvate is utilized by cpPDC, which is deemed to be the main source of acetyl-CoA during autotrophic growth. When PDC activity is decreased, the PDH bypass involving pyruvate decarboxylase, ADH, ALDH, and ACS, can contribute to acetyl-CoA production in plastids. Other routes, via acetate kinase and phosphate acetyltransferase, which synthesize acetyl-CoA from acetate, have been found in *C. reinhardtii*. Acetyl-CoA is converted to malonyl-CoA, which is used in FA-synthesizing reactions. De novo-synthesized FA are exported out of the chloroplast and used for TAG assembly in the ER. An alternative pathway for de novo TAG generation on the plastid envelope membranes has been revealed in Chlamydomonas. More detailed explanations are given in the text. *ACC* acetyl-CoA carboxylase, *ACK* acetate kinase, *ACS* acetyl-CoA synthetase, *ADH* alcohol dehydrogenase, *ALDH* aldehyde dehydrogenase, *DAG* diacylglycerol, *DGAT* diacylglycerolacyltransferase, *ER* endoplasmic reticulum, *Glc-6-P* glucose-6-phosphate, *GPAT* glycerol-3-phosphate acyltransferase, *LPA* lysophosphatidic acid, *LPAAT* lysophosphatidic acid acyltransferase, *ME* malic enzyme, *PA* phosphatidic acid, *2-PGA* 2-phosphoglycerate, *3-PGA* 3-phosphoglycerate, *PEP* phosphoenolpyruvate, *cpPDC* chloroplastic pyruvate dehydrogenase complex, *PAP* phosphatidic acid phosphatase, *PyrDec* pyruvate decarboxylase, *PKp* plastid pyruvate kinase, *PPT* PEP/phosphate translocator, *PTA* phosphate acetyltransferase, *TAG* triacylglycerol, *TPT* triose phosphate/phosphate translocator

recent data on the enzymes involved in plastidic acetyl-CoA synthesis in photosynthetic organisms and their role in de novo FA and TAG biosynthesis. Although this review concerns mainly microalgae, we cannot avoid mentioning the seminal works on higher plants in which acetyl-CoA production has been much better studied, in comparison to microalgae. The recent advances in the study of carbon flux toward TAG production, using a functional genomic approach, will also be reviewed, with a focus on microalgae.

Enzymes providing acetyl-CoA for de novo FA biosynthesis

Reactions catalyzed by cpPDC and ACS are considered the most important and well-studied routes for acetyl-CoA biosynthesis in plastids (Fig. 1). PDC catalyzes the oxidative decarboxylation of pyruvate to form acetyl-CoA. It consists of three main components: E1 (PDH, EC 1.2.4.1), E2 (dihydrolipoamide acetyltransferase, EC 2.3.1.12), and E3 (dihydrolipoamide dehydrogenase, EC 1.6.4.3). E1 is

composed of alpha (E1α) and beta (E1β) subunits. The pyruvate decarboxylation reaction, followed by the reductive acetylation of E2 cofactor (lipoic acid), catalyzed by PDH, is considered to be a rate-limiting step in the reaction sequence mediating PDC activity (Reid et al. 1977).

In non-plant eukaryotic cells, PDC is exclusively localized within the mitochondria and supplies acetyl-CoA for the Krebs cycle. The cells of higher plants and microalgae possess a second, plastid isoform of the enzyme (Camp and Randall 1985; Reid et al. 1977; Williams and Randall 1979), and its activity serves as a source of acetyl-CoA in these organelles for various biosynthetic reactions, including de novo FA synthesis (Kang and Rawsthorne 1994; Ke et al. 2000; Miernyk and Dennis 1983). Subunits of chloroplastic and mitochondrial PDC (cpPDC and mtPDC, respectively) are encoded by independent nuclear genes in higher plant cells (Tovar-Méndez et al. 2003) and in many photosynthetic eukaryotic microalgae, as inferred from their genomic data (Jinkerson et al. 2013). Every subunit of cpPDC has a specific domain structure and a target plastid presequence (Johnston et al. 1997; Lutziger and Oliver 2000; Mooney et al. 1999).

Plastid and mitochondrial isoforms of PDC have different biochemical features and are regulated by diverse factors (Camp and Randall 1985; Qi et al. 1996; Reid et al. 1977; Tovar-Méndez et al. 2003; Williams and Randall 1979). Remarkably, it was recently discovered that an E2 subunit of cpPDC [dihydrolipoamide acetyltransferase (DLA2)] from *C. reinhardtii* has additional RNA-binding activity; it was found to affect the translation of *psbA* mRNA into the D1 protein—required for the de novo assembly of photosystem II (PSII) (Bohne et al. 2013). Moreover, this reciprocal regulation was shown to rely on the presence of acetate. *C. reinhardtii* is a facultative autotrophic species, requiring a relatively high CO_2 supply (up to 5 %) for photoautotrophic growth, while in the presence of acetate, an organic carbon source, the growth rate is not restricted by carbon supply. Importantly, under mixotrophic conditions, when acetyl-CoA could be synthesized from acetate, cpPDC was partially disassembled, and DLA2 could bind *psbA* mRNA, favoring D1 protein synthesis. The authors proposed that RNA-binding activity might be an intrinsic and ancient feature of all DLA proteins, and suggested that the regulatory function of DLA2 might coordinate lipid and protein biosynthesis for chloroplast membrane biogenesis under nutrient-replete conditions (Bohne et al. 2013).

Acetyl-CoA can also be formed via the so-called PDH bypass, when acetaldehyde is synthesized by either pyruvate decarboxylase from pyruvate or alcohol dehydrogenase from ethanol and then converted to acetate by extraplastidic ALDH (Bucher et al. 1995). Produced by this means, acetate can potentially enter plastids and be used for acetyl-CoA synthesis by plastidic ACS (Fig. 1). Although the existence of this route and its involvement in the process of FA biosynthesis have been proven in plant pollen (Bucher et al. 1995) and sporophytic tissue (Wei et al. 2009), it is unlikely that this pathway plays a major role in providing plastidic acetyl-CoA. Thus, Arabidopsis triple ALDH mutants do not exhibit any visible phenotype (Wei et al. 2009). PDH bypass is perhaps important during certain stages of plant development or under specific growth conditions.

The roles of different enzymes, such as cpPDC and ACS, and the substrates, such as acetate, malate, pyruvate, and acetyl carnitine, have been previously evaluated for acetyl-CoA production in higher plant plastids (Harwood 2005). In vitro studies on isolated spinach and pea chloroplasts have shown that acetate is preferable to pyruvate as a substrate for the synthesis of C16 and C18 FA (Masterson et al. 1990; Roughan et al. 1979), suggesting the involvement of ACS in acetyl-CoA production in photosynthetic cells. However, alterations in the expression of the gene encoding plastid ACS did not lead to significant changes in lipid content in Arabidopsis seeds or leaves (Behal et al. 2002; Lin and Oliver 2008). In vivo studies in Arabidopsis also did not show any correlation between elevated intracellular levels of acetate and FA synthesis (Bao et al. 2000). These findings led to the conclusion that ACS activity is not the main source of acetyl-CoA in plant plastids, at least under the conditions tested.

At the same time, much evidence suggests that cpPDC plays a dominant role in providing acetyl-CoA for FA biosynthesis in the cells of oleaginous plant tissues (Kang and Rawsthorne 1994; Ke et al. 2000; Miernyk and Dennis 1983). For example, the rate of [^{14}C]pyruvate incorporation into the products of plastid lipid biosynthesis in oil seeds was significantly higher than that of [^{14}C]acetate (Kang and Rawsthorne 1994; Miernyk and Dennis 1983). A strong correlation between the accumulation pattern of PDH E1β mRNA and the rate of lipid biosynthesis was observed in developing *Arabidopsis thaliana* seeds (Ke et al. 2000). Similarly, a survey of Arabidopsis expressed sequence tag (EST) data revealed the upregulation of a transcript for the subunit E1α of the plastid PDH in seeds during TAG biosynthesis (Beisson et al. 2003). Furthermore, recent comparative transcriptome and metabolite studies of oil and date palms have confirmed the importance of plastid carbon metabolism and acetyl-CoA supply via cpPDC for FA synthesis and, ultimately, for TAG biosynthesis in oil-accumulating tissues (Bourgis et al. 2011); an over 20-fold increase in the abundance of cpPDC-encoding transcripts was found in oil palm fruit compared with date palm fruit during ripening, suggesting a crucial role for cpPDC in storage lipid production.

In contrast to higher plants, little information is available on the source of acetyl-CoA in the chloroplasts of oleaginous microalgae. Some data on the abundance and expression of putative cpPDC- and ACS-encoding genes are available from genomic and transcriptomic studies. The abundance or upregulation of cpPDC genes has been proposed as one of the molecular mechanisms regulating carbon flux toward the biosynthesis of FA and TAG under nitrogen limitation or depletion in microalgae under conditions of photoautotrophy (Jinkerson et al. 2013; Lv et al. 2013; Rismani-Yazdi et al. 2012). Furthermore, enzymes involved in the direct conversion of pyruvate originating from glycolysis and some coupled reactions utilizing pyruvate and phosphoenolpyruvate (PEP)—the so-called pyruvate hub enzymes—have been suggested as targets for metabolic engineering in microalgae to direct carbon flux to TAG biosynthesis (Jinkerson et al. 2013; Smith et al. 2012). The direct involvement of cpPDC in providing acetyl-CoA for FA synthesis and subsequent TAG production under photoautotrophic conditions was recently shown in *C. reinhardtii* (Shtaida et al. 2014). This study documented a critical role for cpPDC in supplying precursors for FA synthesis in the photoautotrophic cells (grown solely on CO_2) under conditions of nitrogen starvation. Artificial microRNA (amiRNA) *pdh* mutants with decreased expression of a PDH subunit of cpPDC showed an impaired growth phenotype, significantly reduced TAG content, and an altered FA composition under conditions of photoautotrophy and nitrogen deprivation. In microalgae, an elevated FA biosynthesis and the corresponding enhancement in TAG production serve a sink for excessive photosynthetically fixed carbon and NADPH under conditions of nitrogen deficiency (Hu et al., 2008; Li et al. 2012; Solovchenko 2012). An impairment in cpPDC function in the *pdh* mutants under photoautotrophic conditions led to a sustained over-reduction of electron carriers in the photosynthetic electron transport chain stemming likely from the declined demand of the photosynthates by the reactions of FA synthesis (Shtaida et al. 2014).

At the same time, no considerable differences were observed between mutant and control lines cultivated in the presence of acetate, indicating that the switch from one route of acetyl-CoA production to another depended on the carbon regime (Shtaida et al. 2014).

When provided to microalgal cells as a carbon source, acetate is readily utilized for the reactions of lipid synthesis. Indeed, labeling with [^{14}C]acetate has been used extensively in studies on algal lipid biosynthesis, demonstrating active label acquisition by microalgal cells and incorporation into FA and complex lipids (Bigogno et al. 2002a; Guschina and Harwood 2006; Sato et al. 2003), implying ACS activity. Similarly, the label from [^{14}C]acetate was rapidly incorporated into TAG under both nitrogen-replete and nitrogen-depleted conditions in *C. reinhardtii* cells grown mixotrophically; moreover, the cellular content of TAG was positively correlated with an increasing acetate concentration in the nutrient medium (Fan et al. 2012). Furthermore, acetate administration boosted lipid droplet formation in a starchless mutant of *C. reinhardtii* (Goodson et al. 2011). However, the downregulation of ACS at the protein level during cultivation on acetate and nitrogen starvation was observed in the nitrogen-starved Chlamydomonas, while two enzymes—acetate kinase and phosphate acetyltransferase—which can generate acetyl-CoA from acetate in two subsequent reactions, showed no change in protein level (Wase et al. 2014). Congruently, two ACS-coding genes were downregulated at the transcript level in *C. reinhardtii* grown mixotrophically, as reported by Miller et al. (2010). Based on the patterns of protein expression, Wase et al. (2014) suggested that in *C. reinhardtii* cultivated under mixotrophic conditions and nitrogen deficiency, acetate kinase and phosphate acetyltransferase activities are the main sources of acetyl-CoA. On the other hand, ACS candidates have been identified in the lipid droplet proteomes of the green algae *C. reinhardtii* (Moellering and Benning 2010; Nguyen et al. 2011) and *Dunaliella bardawil* (Davidi et al. 2015), implying their possible role in providing acetyl-CoA at the early stages of FA synthesis. Involvement of the TCA cycle in providing carbon skeletons for FAS is elaborated in further sections.

Along with the acetyl-CoA-generating enzymes described above, microalgae possess alternative routes for the production of acetyl-CoA from pyruvate, in particular under anoxic conditions. Currently, most information about fermentation metabolism in microalgae is derived from the studies on *C. reinhardtii* (Gfeller and Gibbs 1984, 1985; Gibbs et al. 1986; Atteia et al. 2006; Mus et al. 2007; Dubini et al. 2009; Philipps et al. 2011; Catalanotti et al. 2012; Magneschi et al. 2012). In *Chlamydomonas*, two enzymes involved in the production of acetyl-CoA from pyruvate under anoxic conditions, pyruvate formate lyase (PFL1) and pyruvate ferredoxin oxidoreductase (PFR, often designated PFOR). PFL1 deemed to be dually localized to chloroplast and mitochondria, and its activity leads to generation of acetyl-CoA and formate. While the chloroplast-localized PFR1 mediates conversion of pyruvate to acetyl-CoA, reduced ferredoxin and CO_2 [reviewed in (Atteia et al. 2013; Catalanotti et al. 2013; Yang et al. 2015)]. Both reactions can occur simultaneously, but the fate of acetyl-CoA produced by PFL1 and PFR may be different. It can be converted to acetate by phosphate acetyltransferase and acetate kinase. Acetyl-CoA can also be converted to ethanol by alcohol dehydrogenase (Gfeller and Gibbs 1984; Gibbs et al. 1986; Mus et al. 2007). PFR1 activity is coupled to formation of H_2 under anaerobic

conditions via generation of reduced ferredoxins and their consequent utilization by the hydrogenases (Dubini et al. 2009). The PFR activity contributes to acetyl-CoA formation for the anaerobic wax ester synthesis in *Euglena gracilis* which proceeds via a malonyl-CoA independent synthesis of FA in mitochondria and directly utilizes acetyl-CoA (Hoffmeister et al. 2005).

Thus, multiple enzymatic reactions can lead to acetyl-CoA synthesis in microalgal cells. The preferred pathway seems to be dependent on the algal species, the growth conditions, and the duration of the stress conditions, and it apparently relies on the carbon regime. CpPDC seems to be the main source of acetyl-CoA under conditions of photoautotrophy, while the roles of the PDH bypass, ACS, and acetate kinase increase under mixotrophy, when PDC activity is less important and reduced. However, more data on different species of microalgae should be accumulated in order to attain a better understanding of plastidic acetyl-CoA production in algal cells. These investigations should be conducted in parallel with cellular localization studies in order to delineate the origin of the reaction.

The role of plastidic pyruvate kinase (PKp) and malic enzyme (ME) in FA biosynthesis

One of the reactions that provides pyruvate for the PDC is catalyzed by pyruvate kinase (PK, or ATP:pyruvate 2-O-phosphotransferase, EC 2.7.1.40), the final enzyme of glycolysis, which irreversibly converts PEP into pyruvate with the concurrent generation of ATP. This enzyme is ubiquitous in both prokaryotes and eukaryotes. In plant cells, including microalgae, two types of PK have been detected: cytosolic (PKc) and plastidic (PKp) (Blakeley et al. 1995; Blakeley et al. 1990; Blakeley et al. 1991; Knowles et al. 1989). However, according to biochemical (Klein 1986) and proteomic (Terashima et al. 2011) studies, *C. reinhardtii* cells lack the payoff, or lower, phase of glycolysis in chloroplasts and, as a result, plastid-localized PK. In the non-plant organism *Toxoplasma gondii*, containing a plastid-like organelle termed apicoplast, PK with dual targeting to both the apicoplast and the mitochondrion was found. In this case, the enzyme's localization depended on the length of its N-terminal fragment, which was determined by the position of the first Met residue in the open reading frame (Saito et al. 2008).

At least two different types of PKp have been identified in higher plants, considered to be components of one enzymatic complex consisting of several subunits (Baud et al. 2007). However, the molecular structure of the complex depends on the species. Most plant PKp are heterotetrameric proteins composed of two subunit types, but heterohexameric structures, as well as others, have also been identified (Ambasht and Kayastha 2002). PKp purified from the green alga *Selenastrum minutum* has been reported as a monomer (Knowles et al. 1989).

The differently localized PKs possess distinct biochemical characteristics. In general, the enzyme is regulated by the substrate of the catalyzed reaction, PEP, as well as by the cations K^+, Mn^{2+}, Mg^{2+}, and H^+, which are essential for its catalytic activity. PK is inactivated by phosphorylation (Muñoz and Ponce 2003).

The importance of PKp in providing pyruvate for de novo FA synthesis has been shown in higher plants. Disruption of the gene encoding the PKp-b1 subunit of the plastid-localized PK of Arabidopsis negatively affected seed germination, and resulted in a 60 % reduction in seed oil content and in significant alterations in the FA profile relative to the wild type (Andre and Benning 2007). Further investigations revealed that mutations in the different subunits of PKp–PKp1 and PKp2—had an additive effect, and an even stronger reduction in seed oil content (up to 70 %) was observed in the double *pkp1pkp2* mutants. Moreover, the two subunits could substitute for each other: the homomeric PKp1 protein efficiently catalyzed pyruvate synthesis in *pkp2* mutants and vice versa (Baud et al. 2007). Gene expression analyses of *A. thaliana* seeds (Ruuska et al. 2002) and *Brassica napus* embryos (Sangwan et al. 1992) revealed a positive correlation between increased PKp gene expression and intensive FA biosynthesis, while cytosolic PK was downregulated at the onset of oil synthesis. The key role of PKp in oil production in plants was shown in a comparative transcriptome study of oil palm versus date palm fruits (Bourgis et al. 2011). The upregulation of PKp, along with another glycolytic enzyme, ATP-dependent phosphofructokinase, was observed in oil palm but not in date palm. Even stronger differences between the two species were determined during oil palm ripening.

In algae, one plastidic (Knowles et al. 1989) and two cytosolic (Davison 1987; Wu and Turpin 1992) PK enzymes have been purified and characterized from green (*Selenastrum minutum*) and brown (*Ascophyllum nodosum*) algae, but their involvement in the pyruvate supply for acetyl-CoA production by cpPDC has not been studied. Today, only limited information, mainly derived from transcriptomic studies, is available on the importance of PKp for de novo FA and TAG biosynthesis in photosynthetic microalgae. Pyruvate cellular metabolism is positioned at the central branch point between protein and lipid metabolism, and plays a major role in shuttling carbon toward storage lipid synthesis in microalgae under nitrogen starvation conditions (Levitan et al. 2015). Transcriptomic studies revealed the upregulation of the PKp gene in the oleaginous chlorophyte *Neochloris oleoabundans*

(Rismani-Yazdi et al. 2012) and the eustigmatophyte *Nannochloropsis oceanica* IMET1 (Li et al. 2014) induced by transfer to nitrogen starvation conditions, which trigger TAG production in microalgae. Moreover, in *N. oceanica*, PKp seemed to be the only enzyme upregulated at the transcript level, while other enzymes involved in plastid glycolysis showed decreased expression under nitrogen deprivation (Li et al. 2014). In our study with the oleaginous green microalga *Parietochloris incisa*, analysis of gene expression by real-time PCR also showed upregulation of PKp under nitrogen deprivation, but the extent and pattern of the transcriptional activation depended on the light intensity and carbon regime (Shtaida et al., unpublished). We conclude that the data obtained for one species can hardly be extrapolated to others due to intrinsic differences in their cellular networks of carbon fluxes, cultivation conditions, carbon regime, and time scale; thus, the need for experimental observation in each particular case is clear.

Aside from PKp, an alternative reaction is known in plastids that generates pyruvate from malate via the photosynthetic form of NADP-ME. ME supplies both carbon and reducing equivalents in the form of NADPH for de novo FA production. Cytosolic and plastid isoforms of the enzyme have been described, and their number in the cells depends on the species (Drincovich et al. 2001). In fungi, ME seems to be indispensable for storage lipid accumulation, providing NADPH for FA biosynthesis. Overexpression of fungal ME led to a significant increase in oil content in the cells (Wynn et al. 1999; Zhang et al. 2007), while an *Aspergillus nidulans* mutant lacking ME synthesized only half of the lipid content relative to a parental strain (Wynn and Ratledge 1997). ME activity was also found in plastids from higher plants (Kang and Rawsthorne 1994; Smith et al. 1992b). In non-photosynthetic plastids, termed leucoplasts, from developing castor endosperm, the rate of incorporation of [^{14}]C label from malate into FA was much higher than that from [^{14}C]pyruvate (Smith et al. 1992a). In the Arabidopsis *wri1* mutant with severely reduced oil content, metabolic flux analysis revealed that reduced carbon flow through PKp in this mutant is partially compensated for by plastidic ME (Lonien and Schwender 2009).

So far, little is known about the role of ME in FA and TAG biosynthesis in microalgae. Analysis of the expression of lipid-metabolism-related genes in *Chlorella pyrenoidosa* revealed upregulation of two out of three ME-encoding genes under conditions of nutrient depletion in the stationary phase, when storage lipids are intensively synthesized (Fan et al. 2014). Data obtained by RNA sequencing in a study with the diatom microalga *P. tricornutum* cultivated under nitrogen deprivation indicated a dramatic increase in the expression of the gene encoding the putative isoform of ME with predicted chloroplast localization, and the observed increase in expression was associated with intensive TAG biosynthesis (Yang et al. 2013). It is likely that ME might play an important role as an additional source of pyruvate, as well as of NADPH, for de novo FA biosynthesis, especially under specific conditions. This statement is corroborated by a recent study of the physiological and metabolic responses of the green microalga *Haematococcus pluvialis* to high irradiance and nitrogen starvation using integration of the data obtained from metabolomic profiling, protein activity assays, and data-driven integrative modeling (Recht et al. 2014). Elevated levels of some TCA-cycle metabolites and increased activity of the TCA-cycle enzymes were observed under experimental conditions. The integrative data analysis allowed authors to assign to the TCA cycle a central role in supplying carbon precursors for de novo FA biosynthesis by providing excessive malate, which can be transported to the chloroplast and utilized by ME-producing pyruvate (Fig. 1). This assumption was also supported by a computational approach, although the necessity of direct experimental validation was underscored (Recht et al. 2014).

Functional genomics approach to studying carbon flux toward FA synthesis

TAG is not the only storage product in plant and algal cells. In plants, a substantial amount of starch is accumulated under different growth conditions, dependent upon the plant organ and developmental stage (Gibon et al. 2009; Zeeman and Rees 1999). Green microalgae can store relatively high amounts of both starch and TAG under stress conditions (Merzlyak et al. 2007; Siaut et al. 2011; Takeshita et al. 2014). The factors determining carbon partitioning between lipids and carbohydrates and affecting carbon flux toward lipid production are addressed in the following section as corroborated by functional genomics and metabolomics studies with *C. reinhardtii*, as well as some other species.

Gene expression analyses of developing oil seeds and maize embryos demonstrated the coordinated expression of the enzymes involved in the reactions of de novo FA biosynthesis (Lee et al. 2002; O'Hara et al. 2002; Ruuska et al. 2002). Nuclear-encoded genes for plastid-localized enzymes, such as ACC subunits, ketoacyl synthetase (KAS), cpPDC, PKp, and FAD2 (an oleate desaturase), displayed similar patterns of changes in gene expression. These genes demonstrated coordinated bell-shaped expression patterns with maximum values preceding active oil synthesis. In contrast, FA-modifying enzymes (desaturases and elongases) and oleosin transcripts had a

different expression profile and were induced at later stages of oil accumulation. Comparative transcriptomic and metabolomic analyses of fruits of the closely related oil palm (which stores up to 90 % oil) and date palm (which accumulates starch almost exclusively) revealed, surprisingly, that genes encoding TAG-assembly enzymes are expressed at similar levels in both species, but the carbon flux to lipids is more than 100 times higher in oil palm (Bourgis et al. 2011). Notably, genes of the plastidic enzymes of the central carbon metabolism, including PKp and cpPDC, and de novo FA biosynthesis were also significantly upregulated in oil palm (Bourgis et al. 2011). These results confirmed the carbon flux toward plastid acetyl-CoA and FA biosynthesis, rather than TAG assembly in the endoplasmic reticulum, as the main control points for the regulation of TAG accumulation in oily fruits. Furthermore, an over 50-fold increase was observed in the expression of the ortholog of Arabidopsis WRINKLED involved in the global regulation of lipid biosynthesis (To et al. 2012).

The contemporary functional genomics approach to studying cell metabolic pathways lies in collecting and summarizing data arrays from different "-omics" analyses, such as transcriptomics, proteomics, and metabolomics. This allows researchers to look at the broad picture of cell metabolism and to better understand the complex relationships between the genome and the genome-determined phenotype. Global analysis of Arabidopsis co-expression networks revealed co-expression of all of the genes encoding the complete set of 18 enzymes required for the synthesis of C18 FA from acetyl-CoA (Mentzen et al. 2008). Remarkably, the FA-biosynthesis subgraph of the network also contained all of the genes encoding the four cpPDC subunits, genes for the final reactions of biotin and lipoic acid biosynthesis, which are obligate co-factors of ACC and PDC, respectively, and one of the 14 Arabidopsis genes encoding different PK isoforms, annotated as PKp (At5g52920). At the same time, there was no coordination between the gene expression of ACS and FA-biosynthesis enzymes. This coordinated expression led the authors to suggest that "fatty acid synthesis" begins not from acetyl-CoA, but from PEP, the substrate of PK. We believe that the data obtained from plant studies could be relevant to studies on microalgal lipid synthesis, since they can reveal the general regulatory mechanisms of lipid accumulation, which might be common to both plants and green microalgae (Chlorophyta) due to their evolutionary relationship.

Regarding microalgae, the functional genomics approach is now widely employed in a growing number of species to study cell metabolism under conditions that induce TAG biosynthesis. This type of research has attracted special attention, as it places strong emphasis on the commercial application of microalgal lipids as precursors for biofuel, and provides mechanistic insight into oil synthesis by photosynthetic microalgae. Among the different algal species, the model green microalga *C. reinhardtii* has been one of the most intensively studied. Miller et al. (Miller et al. 2010) were among the first to analyze the global changes in Chlamydomonas gene expression in response to nitrogen deprivation, favoring TAG accumulation. The transfer of Chlamydomonas to a nitrogen-depleted Tris–acetate–phosphate (TAP) medium (mixotrophic conditions) resulted in an overall decrease in the abundance of most of the genes of protein biosynthesis, the photosynthetic apparatus, and FA β-oxidation. A substantial increase was observed in the expression of one of the diacylglycerol acyltransferase (DGAT) genes (catalyzing the committed step in TAG assembly); however, its total number of transcripts was low relative to others. The expressions of other FA- and TAG-biosynthesis-related genes were either unchanged or just slightly upregulated [KASI, acyl–acyl carrier protein (ACP) thioesterase], along with the genes encoding TCA-cycle enzymes. Several lipases (putative TAG and glycerolipid lipases) also showed increased gene expression, supporting the significant impact of chloroplast membrane remodeling on Chlamydomonas TAG assembly (Miller et al. 2010). Later, an RNA-sequencing analysis, which was more detailed in terms of time span, revealed two DGAT enzymes in Chlamydomonas (DGAT1 and DGTT1) that were highly upregulated at the transcriptional level shortly after the transfer to nitrogen deprivation (Boyle et al. 2012). The transcriptional activation of the phospholipid diacylglycerolacyltransferase (PDAT1), which catalyzes the reaction of acyl-CoA-independent TAG production by transferring acyl groups from phospholipids to diacylglycerol, was less pronounced, but an insertional *pdat1* mutant accumulated 25 % less TAG than the parental strain. A potential regulator of nitrogen-starvation-induced TAG biosynthesis, a so-called nitrogen-responsive regulator (NRR), was also identified. The loss-of-function *nrr1* mutant showed a dramatic (50 %) reduction in TAG content, pointing to the essential role of this protein in lipid biosynthesis.

Since both TAG and starch are accumulated to high levels in Chlamydomonas under stress conditions, they can compete for the carbon units and energy required for their synthesis. It seems logical that by blocking carbon flow toward one pathway (e.g., starch biosynthesis), higher amounts of the other (TAG) can be obtained. This was indeed observed in ADP-glucose pyrophosphorylase mutants, impaired in starch biosynthesis. Due to the existence of a number of wild-type strains and their starchless mutant progenitors, some studies have compared the two (Siaut et al. 2011). The experimental approach of using mutant and complemented strains enables a better assessment of the mutation's role in oil accumulation. Thus, a comparison

of the *sta6* starchless Chlamydomonas mutant with the complemented *STA6* strain, grown under nitrogen-replete and depleted conditions in TAP, for transcription, protein activities, and metabolites, revealed relatively few differences between the two, mostly relating to central carbon metabolism (Blaby et al. 2013). Genes of isocitrate lyase (*ICL1*), malate synthase (*MS1*), transaldolase (*TAL1*), fructose biphosphatase (*FBP1*), and PEP carboxykinase (*PCK1*), encoding key reactions of the central carbon metabolism such as the glyoxylate cycle and gluconeogenesis, were upregulated in the starchless *sta6* than in the *STA6* strain. Two out of seven acyltransferase genes implicated in TAG assembly (*DGTT2* and the putative homolog of type-3 acyltransferase) were also found to be differentially expressed in the *sta6* mutant as compared with *STA6*, with an early response in the former to the transfer to nitrogen starvation (0.5–2 h), while most of the other genes were activated later. Several possible models for enhanced TAG production by *sta6* were proposed (Goodenough et al. 2014). According to one of the models, the upregulation of *TAL1*, *FBP1,* and *PCK1* might enhance flux through the pentose phosphate pathway (PPP), leading to an increase in the pool of reduced nucleotides for de novo TAG synthesis. Another possibility arose from the assumption that in the absence of ADP-glucose pyrophosphorylase activity in the *sta6* mutant, glucose-6-phosphate is converted to trehalose, a signaling molecule found in Chlamydomonas. However, trehalose levels still need to be analyzed in both strains to test this possibility (Blaby et al. 2013). Upregulation of *TAL1*, *FBP1*, *PCK1*, as well as of citrate synthase, genes encoding the glyoxylate cycle enzymes ICL and MS, and two isoforms of ACS, was observed in both the *sta6* mutant and *cw15* strains under nitrogen depletion boosted with acetate after two days of cultivation (Goodenough et al. 2014). These genes, along with candidate acetate transporters, were suggested to have upstream regulatory elements that were able to sense the cell's acetate status. Glycerol phosphate dehydrogenase, along with predicted DGAT isoforms (DGTT2) and putative TAG lipase, were upregulated in *sta6*, indicating their essential role in the *sta6* mutant's enhanced accumulation of TAG.

Recent quantitative proteomics studies have also revealed that under nitrogen starvation in TAP, glycolytic and TCA-cycle enzymes were upregulated in *C. reinhardtii* cells (Longworth et al. 2012). This was accompanied by an increase in some proteolytic enzymes, along with the enzymes of PSII, indicating increased cyclic phosphorylation, while the amounts of enzymes related to carbon fixation and PSI decreased. These data again showed that TAG accumulation under nitrogen deprivation occurs at the expense of cell growth and the production of major cell components, such as protein and nucleic acids.

The most recent functional genomics studies generally confirm the accumulated data concerning TAG biosynthesis in Chlamydomonas under nitrogen-replete and depleted conditions. Enrichment of the proteins governing lipid metabolism on the background of a total decline in cell protein content was found in Chlamydomonas by comparative proteomics of cells grown in nitrogen-supplemented and nitrogen-free TAP media (Schmollinger et al. 2014). At the same time, a transcriptome analysis revealed a decrease in the mRNA abundance of the specific enzymes of de novo FA biosynthesis under nitrogen deprivation (such as ACC, FAS, enoyl reductase, and ACP), again pinpointing the significance of recycling existing thylakoid membranes for TAG production in Chlamydomonas. In agreement with previous works, *DGAT1* and *DGTT1* (*DGAT2*) genes were upregulated under nitrogen starvation, while the expression of other genes encoding lipid-metabolism-related enzymes was stable or decreased (Schmollinger et al. 2014). An integrated analysis of the Chlamydomonas stress response, using metabolite and protein profiling, revealed only two lipid-metabolism enzymes that increased under nitrogen starvation in TAP: long-chain acyl-CoA synthetase and glycerol-3-phosphate dehydrogenase (Wase et al. 2014). Acetate kinase and phosphate acetyltransferase activities were proposed as a potential source of acetyl-CoA for TAG biosynthesis, since ACS was downregulated at the protein level. Enzymes of the glyoxylate cycle, along with those of gluconeogenesis and the PPP, decreased under the experimental conditions. Again, as expected for nitrogen starvation, loss of photosynthetic pigments and a decrease in the proteins of the photosynthetic apparatus were observed in nitrogen-deprived Chlamydomonas cells. The authors also found an increase in the citrate level in coordination with the elevated levels of pyruvate carboxylase, citrate synthase and ACL as nitrogen starvation and TAG biosynthesis progressed, and the addition of external citrate resulted in increased TAG accumulation in the cells. An increase in cytosolic glycolysis and a concomitant decrease in chloroplastic glycolysis, along with the reduction in the enzymes of the PPP, glyoxylate cycle and gluconeogenesis, were observed (Wase et al. 2014).

Briefly summarizing the functional genomics data on Chlamydomonas cell metabolism under nitrogen deprivation, it is important to mention the general decrease in photosynthetic activity concomitant with the loss of photosynthetic pigments, and the decreased activity of the glyoxylate cycle, gluconeogenesis, and the PPP accompanied by increased proteolytic activity in the cells. The TCA cycle seems to serve as a hub controlling the balance between different cell metabolic processes by redirecting carbon flux due to the increased activities of citric acid cycle enzymes. The activation of lipases during intensive

TAG accumulation indicates the importance of membrane lipid remodeling in nitrogen-deprived cells. Remarkably, either no or only slight changes were observed in the activities of the enzymes of lipid metabolism, indicating the existence of other control points for the process of TAG accumulation.

In addition to the relatively well-studied model, *C. reinhardtii* cells, other oleaginous microalgae have been recently extensively analyzed for their systemic response to a stressful environment. For example, a transcriptomic analysis of the green oleaginous microalga *N. oleoabundans* showed the upregulation of genes encoding PK, cpPDC, FAS, and the enzymes of the PPP. Both homomeric ACC and heteromeric ACC genes were downregulated, but an increase in biotin carboxylase gene expression was found (Rismani-Yazdi et al. 2012). In contrast to Chlamydomonas, no change was detected in the expression of DGAT, while oleoyl-ACP hydrolase and acyl-ACP thioesterase displayed increased mRNA abundance, most likely to reduce the pathway's feedback inhibition. The gene homologous to plant *SnRK1*, the global regulator of carbon partitioning, was also slightly overexpressed under nitrogen deprivation, suggesting its role in carbon metabolism in algae as well.

A transcriptomic analysis of an oleaginous heterokont microalga, *Nannochloropsis gaditana*, under nitrogen starvation revealed no significant changes in the expression of the genes of lipid metabolism, even during the most intensive TAG accumulation, while the mitochondrial genes encoding respiration enzymes were substantially inhibited (Corteggiani Carpinelli et al. 2013). The authors suggested that acetyl-CoA and NAD(P)H, normally used for mitochondrial respiration, might have been directed to the TAG-biosynthetic pathway, promoting its increased accumulation. Comparative transcriptomic and lipidomic studies of *N. oceanica* IMET1, under nitrogen-replete and depleted conditions, revealed major differences in gene expression between the oleaginous IMET1 and the non-oleaginous Chlamydomonas (Li et al. 2014). The gene encoding type I FAS was not identified in Chlamydomonas, and 11 genes of DGAT2, as compared with five genes in Chlamydomonas, were reported for IMET1. It was also shown that the overall transcript level of genes encoding the Kennedy-pathway enzymes and DGAT was significantly higher in IMET1. In contrast to *C. reinhardtii*, the upregulation of PDH bypass genes was observed in *Nannochloropsis* under nitrogen starvation. This study reported the transcriptional activation of TCA-cycle enzymes (excluding citrate synthase) in *N. oceanica* IMET1, along with β-oxidation enzymes, indicating the important role of carbon shuffled from membrane lipid recycling in TAG biosynthesis in IMET1 (Li et al. 2014). During long-term nitrogen deprivation in Nannochloropsis, three out of the five PDH genes showed progressive upregulation over 14 days, illuminating the key role for PDC transcriptional activation in TAG accumulation (Jia et al. 2015).

Another group of microalgae, the diatoms, is of interest to researchers due to their important role in nature as one of the most successful and abundant groups of unicellular photosynthetic organisms in the marine environment, their unique cell organization, and their potential ability to serve as a future source of biofuel. Diatoms belong to the Chromista group, possessing four chloroplast membranes that were formed during evolution in the process of secondary endosymbiosis. Among the diatoms, *P. tricornutum* and *Thalassiosira pseudonana* have been most intensively studied, and their genomes have been sequenced (Armbrust et al. 2004; Bowler et al. 2008). Analysis of the diatoms' genomes revealed the presence of unique features in the organization of the diatoms' central carbon metabolism and its versatility inside the diatom group (Kroth et al. 2008; Smith et al. 2012; Valenzuela et al. 2012). Among the conserved features found was the existence of the lower payoff phase of glycolysis in the mitochondria (Kroth et al. 2008; Smith et al. 2012), which was proposed to serve as a primary pathway for precursor supply to the TCA cycle since cytosolic glycolysis consists of only the upper half of the reactions. The presence of glycolytic reactions in the chloroplast depends on the species. This complex compartmentalization of the central carbon metabolism's reactions makes metabolite transport across intracellular membranes of particular importance, and mitochondria may play the role of carbon-flux regulator between different cell parts (Smith et al. 2012). The authors also proposed referring to the enzymes involved in the direct pyruvate conversion and interconversion of C3 and C4 intermediates, namely PK, PDC, pyruvate carboxylase, pyruvate phosphate dikinase regulatory protein, PEP carboxylase, pyruvate phosphate dikinase, and some others, as well as the related malate dehydrogenase, as "pyruvate hub enzymes." The intracellular localization of these enzymes depends on the species of diatom. Researchers have assigned the role of a metabolite network subjected to modifications during diatom evolution to the pyruvate hub enzymes (Smith et al. 2012).

The changes in the transcriptome, proteome, and metabolome under conditions of nutrient deprivation, which favors TAG production in diatoms, were also analyzed (Bromke et al. 2013; Guerra et al. 2013; Levitan et al. 2015; Valenzuela et al. 2012). Similar to green algae, an increase in TCA-cycle genes and enzymes was observed under nitrogen deprivation in both *P. tricornutum* (Valenzuela et al. 2012) and *T. pseudonana* (Bromke et al. 2013), while the expression of lipid-metabolism enzymes barely changed. The existence of C4-like photosynthesis was

suggested in *P. tricornutum* (Kroth et al. 2008), and the genes encoding enzymes involved in this process, along with carbonic anhydrase genes, were upregulated during lipid accumulation in nitrogen-deprived cells, which was proposed as an additional mechanism to increase carbon concentration around the chloroplasts (Valenzuela et al. 2012).

Recently, a comparison of data obtained from the quantitative proteomics of *P. tricornutum* cells grown in nitrogen-supplemented and nitrogen-free media revealed major changes in the proteins involved in the branched-chain amino acids degradation during TAG accumulation, linking amino acid catabolism and TAG synthesis via acetyl-CoA production and the malate shunt (Ge et al. 2014). Real-time PCR and gel blot analyses revealed four enzymes of the branched-chain amino-acid-degradation pathway that were markedly upregulated during lipid biosynthesis. Among them, the most pronounced increase in expression was observed for the β-subunit of the mitochondrion-localized methylcrotonyl-CoA carboxylase (MCC2). Silencing of *MCC2* genes resulted in a decrease in TAG content in two *mcc* lines by ca. 30–40 % in comparison to wild-type cells. It seems that the product of the MCC reaction (acetyl-CoA) increases flux through the TCA cycle in the mitochondrion, which produces malate exported via the malate shunt to the plastids, where it is converted to pyruvate by ME, followed by PDC-catalyzed acetyl-CoA synthesis and, finally, by its incorporation into FA. Accordingly, branched-chain amino acid degradation serves as a potential source of carbon and energy for TAG accumulation in diatoms (Ge et al. 2014) and should be investigated in detail in other groups of algae, along with the action of the glyoxylate shunt, in providing carbon skeletons for the enhanced storage lipid synthesis under nitrogen starvation. Novel important insights into the fate of cellular carbon and nitrogen under conditions favoring the synthesis of storage lipids have recently been obtained in *P. tricornutum* (Guerra et al. 2013; Levitan et al. 2015). The authors emphasized that the cells of the diatom under nitrogen starvation remodel the intermediate metabolism by redirecting the photosynthetically fixed carbon toward storage lipids, while, concurrently, shunting the internal pools of nitrogen toward the nitrogen assimilation machinery (Levitan et al. 2015). Among lipid-biosynthesis-related genes, ACCase and one DGAT isoform showed increased expression (Guerra et al. 2013).

Besides nitrogen starvation, other nutrient deficiencies can cause storage lipid accumulation. Sulfur deficiency is also studied for its ability to induce TAG production in Chlamydomonas (González-Ballester et al. 2010; Boyle et al. 2012; Cakmak et al. 2012; Matthew et al. 2009; Cakmak et al. 2014). Some changes in *C. reinhardtii* cell metabolism appeared to be common for two types of nutrient stresses, nitrogen and sulfur starvation, such as cell cycle arrest, upregulation of PPP enzymes, activation of the mechanisms of reactive oxygen species scavenging, chloroplast degradation, and decrease in photosynthetic activity (Cakmak et al. 2012, 2014); however, physiological and metabolic consequences of nutrient depletion were less severe under sulfur deprivation. TAG was produced within 24 h following the transfer of *C. reinhardtii* cells to the sulfur-free medium and remained to be elevated in the H_2-producing stage induced by the onset of anaerobic conditions (Matthew et al. 2009). In correlation to the amount of TAG produced, nitrogen starvation elicited more profound increases in *DGTT1* and *DGAT1* mRNA as compared to sulfur deprivation. Although sulfur deprivation significantly impacted expression of *DGTT1* and *DGAT1*, the expression of *PDAT* remained unaltered (Boyle et al. 2012), indicating that stress-specific mechanisms implicated in the regulation of acyl-CoA dependent and independent pathways of TAG biosynthesis, possibly at the post-transcriptional level.

Thus, functional genomics studies of microalgal cells grown under the stress conditions that induce TAG accumulation revealed, on the one hand, some uniform responses to nutrient deprivation, such as total protein loss, a decrease in photosynthetic activity and membrane lipid components, changes in carbon flux partitioning, and TAG accumulation; on the other hand, they showed the diversity of regulatory mechanisms among different species and the need for experimental observation in each particular case. However, despite the variety of mechanisms shown in both plants and algae by various methods, FA and TAG accumulation is connected to changes in the expression of the central carbon metabolism's core enzymes, which can be considered potential targets for bioengineering to improve lipid content or composition.

Conclusions

As a potential source for biodiesel and nutritionally valuable FA, photosynthetic microalgae, which are capable of synthesizing and storing high amounts of energy-rich TAG in response to environmental cues, are of increasing interest to researchers worldwide. Progress in algal lipid studies in the last few years and the increasing availability of genomic information for oleaginous and non-oleaginous algal species provide powerful tools for manipulating TAG production in microalgae, although much still remains unknown.

Despite the variability in the cell structure and metabolism of algal cells, it is possible to define certain commonalities in the process of lipid biosynthesis and find uniform factors regulating storage lipid accumulation in

different oleaginous microalgal species: (i) oleaginous microalgae can accumulate high TAG contents under unfavorable conditions, such as nutrient deficiency and high irradiance; (ii) in many microalgae, accumulation of storage lipids either occurs in parallel or lags behind the accumulation of storage carbohydrates. Lipids and storage carbohydrates likely compete with each other for carbon units. Impairment of storage carbohydrate biosynthesis can serve as a powerful tool to raise TAG amounts in both microalgal and plant species; (iii) functional genomics studies in microalgae and higher plants have revealed that TAG accumulation is largely dependent on the carbon flux toward lipid biosynthesis, rather than on the regulation of some specific enzymes of the FA- or TAG-biosynthetic pathways. It has been consistently found that there are factors regulating the whole process of lipid accumulation that are not directly involved in the biosynthetic pathways, such as NRR in Chlamydomonas (Boyle et al. 2012) or WRINKLED in Arabidopsis (Focks and Benning 1998); and (iv) different groups of microalgae reveal unique and shared responses of their central carbon and lipid metabolism.

However, deeper investigations into the complex processes of FA and TAG biosynthesis in diverse microalgae are required for the informed manipulation of storage lipid content by both cultivation strategies and genetic engineering. Redirecting the carbon flux toward FA synthesis by changing the expression of the central carbon metabolism's core enzymes or by manipulating the activity of known transcription factors, such as NRR, or combining these approaches, seems to be a more successful method by which to increase TAG content in microalgal cells, rather than enhancing the activity of individual enzymes directly involved in FA biosynthesis and TAG assembly.

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References

Ambasht PK, Kayastha A (2002) Plant pyruvate kinase. Biol Plant 45:1–10. doi:10.1023/A:1015173724712

Andre C, Benning C (2007) Arabidopsis seedlings deficient in a plastidic pyruvate kinase are unable to utilize seed storage compounds for germination and establishment. Plant Physiol 145:1670–1680. doi:10.1104/pp.107.108340

Armbrust EV, Berges JA, Bowler C et al (2004) The genome of the diatom *Thalassiosira pseudonana*: ecology, evolution, and metabolism. Science 306:79–86. doi:10.1126/science.1101156

Atteia A, van Lis R, Gelius-Dietrich G, Adrait A, Garin J, Joyard J, Rolland N, Martin W (2006) Pyruvate formate-lyase and a novel route of eukaryotic ATP synthesis in *Chlamydomonas mitochondria*. J Biol Chem 281:9909–9918

Atteia A, van Lis R, Tielens AG, Martin WF (2013) Anaerobic energy metabolism in unicellular photosynthetic eukaryotes. Biochim Biophys Acta 1827:210–223. doi:10.1016/j.bbabio.2012.08.002

Bao X, Focke M, Pollard M, Ohlrogge J (2000) Understanding in vivo carbon precursor supply for fatty acid synthesis in leaf tissue. Plant J 22:39–50. doi:10.1046/j.1365-313x.2000.00712.x

Baud S, Wuillème S, Dubreucq B, de Almeida A, Vuagnat C, Lepiniec L, Miquel M, Rochat C (2007) Function of plastidial pyruvate kinases in seeds of *Arabidopsis thaliana*. Plant J 52:405–419. doi:10.1111/j.1365-313X.2007.03232.x

Behal R, Lin M, Back S, Oliver D (2002) Role of acetyl-coenzyme A synthetase in leaves of *Arabidopsis thaliana*. Arch Biochem Biophys 402:259–267. doi:10.1016/S0003-9861(02)00086-3

Beisson F, Koo AJ, Ruuska S, Schwender J, Pollard M, Thelen JJ, Paddock T, Salas JJ, Savage L, Milcamps A, Mhaske VB, Cho Y, Ohlrogge JB (2003) Arabidopsis genes involved in acyl lipid metabolism. A 2003 census of the candidates, a study of the distribution of expressed sequence tags in organs, and a web-based database. Plant Physiol 132:681–697. doi:10.1104/pp.103.022988

Bigogno C, Khozin-Goldberg I, Adlerstein D, Cohen Z (2002a) Biosynthesis of arachidonic acid in the oleaginous microalga *Parietochloris incisa* (Chlorophyceae): radiolabeling studies. Lipids 37:209–216

Bigogno C, Khozin-Goldberg I, Cohen Z (2002b) Accumulation of arachidonic acid-rich triacylglycerols in the microalga *Parietochloris incisa* (Trebouxiophyceae, Chlorophyta). Phytochemistry 60:135–143. doi:10.1016/S0031-9422(02)00037-7

Blaby IK, Glaesener AG, Mettler T, Fitz-Gibbon ST, Gallaher SD, Liu B, Boyle NR, Kropat J, Stitt M, Johnson S, Benning C, Pellegrini M, Casero D, Merchant SS (2013) Systems-level analysis of nitrogen starvation-induced modifications of carbon metabolism in a *Chlamydomonas reinhardtii* starchless mutant. Plant Cell Online 25:4305–4323. doi:10.1105/tpc.113.117580

Blakeley S, Plaxton W, Dennis D (1990) Cloning and characterization of a cDNA for the cytosolic isozyme of plant pyruvate kinase: the relationship between the plant and non-plant enzyme. Plant Mol Biol 15:665–669. doi:10.1007/BF00017842

Blakeley SD, Plaxton WC, Dennis DT (1991) Relationship between the subunits of leucoplast pyruvate kinase from *Ricinus communis* and a comparison with the enzyme from other sources. Plant Physiol 96:1283–1288

Blakeley S, Gottlob-McHugh S, Wan J, Crews L, Miki B, Ko K, Dennis DT (1995) Molecular characterization of plastid pyruvate kinase from castor and tobacco. Plant Mol Biol 27:79–89

Bohne A-V, Schwarz C, Schottkowsk M, Lidschreiber M, Piotrowski M, Zerges W, Jr Nickelsen (2013) Reciprocal regulation of protein synthesis and carbon metabolism for thylakoid membrane biogenesis. PLoS Biol 11:1–18

Bourgis F, Kilaru A, Cao X, Ngando-Ebongue GF, Drira N, Ohlrogge JB, Arondel V (2011) Comparative transcriptome and metabolite analysis of oil palm and date palm mesocarp that differ dramatically in carbon partitioning. Proc Natl Acad Sci U S A 108:12527–12532. doi:10.1073/pnas.1115243108

Bowler C, Allen AE, Badger JH et al (2008) The Phaeodactylum genome reveals the evolutionary history of diatom genomes. Nature 456:239–244

Boyle NR, Page MD, Liu B, Blaby IK, Casero D, Kropat J, Cokus SJ, Hong-Hermesdorf A, Shaw J, Karpowicz SJ, Gallaher SD, Johnson S, Benning C, Pellegrini M, Grossman A, Merchant SS (2012) Three acyltransferases and nitrogen-responsive regulator are implicated in nitrogen starvation-induced triacylglycerol accumulation in *Chlamydomonas*. J Biol Chem 287:15811–15825. doi:10.1074/jbc.M111.334052

Brennan L, Owende P (2010) Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sustain Energy Rev 14:557–577. doi:10.1016/j.rser.2009.10.009

Bromke MA, Giavalisco P, Willmitzer L, Hesse H (2013) Metabolic analysis of adaptation to short-term changes in culture conditions of the marine diatom *Thalassiosira pseudonana*. PLoS One 8:e67340. doi:10.1371/journal.pone.0067340

Bucher M, Brander K, Sbicego S, Mandel T, Kuhlemeier C (1995) Aerobic fermentation in tobacco pollen. Plant Mol Biol 28:739–750. doi:10.1007/BF00021197

Cakmak T, Angun P, Ozkan AD, Cakmak Z, Olmez TT, Tekinay T (2012) Nitrogen and sulfur deprivation differentiate lipid accumulation targets of *Chlamydomonas reinhardtii*. Bioengineered 3:343–346. doi:10.4161/bioe.21427

Cakmak ZE, Ölmez TT, Çakmak T, Menemen Y, Tekinay T (2014) Induction of triacylglycerol production in *Chlamydomonas reinhardtii*: comparative analysis of different element regimes. Bioresour Technol 155:379-387. doi:http://dx.doi.org/10.1016/j.biortech.2013.12.093

Camp PJ, Randall DD (1985) Purification and characterization of the pea chloroplast pyruvate dehydrogenase complex: a source of acetyl-CoA and NADH for fatty acid biosynthesis. Plant Physiol 77:571–577

Catalanotti C, Dubini A, Subramanian V, Yang W, Magneschi L, Mus F, Seibert M, Posewitz MC, Grossman AR (2012) Altered fermentative metabolism in *Chlamydomonas reinhardtii* mutants lacking pyruvate formate lyase and both pyruvate formate lyase and alcohol dehydrogenase. Plant Cell Online 24:692–707. doi:10.1105/tpc.111.093146

Catalanotti C, Yang W, Posewitz MC, Grossman AR (2013) Fermentation metabolism and its evolution in algae. Front Plant Sci 4:RAJRAJAEAJ. doi:10.3389/fpls.2013.00150

Cohen Z, Khozin-Goldberg I, Ratledge C (2010) Searching for PUFA-rich microalgae. In: Cohen Z (ed) Single cell oils, 2nd edn. AOCS Press, Champaign, pp 201–224

Corteggiani Carpinelli E, Telatin A, Vitulo N, Forcato C, D'Angelo M, Schiavon R, Vezzi A, Giacometti GM, Morosinotto T, Valle G (2013) Chromosome scale genome assembly and transcriptome profiling of *Nannochloropsis gaditana* in nitrogen depletion. Mol Plant 7:323–335. doi:10.1093/mp/sst120

Davidi L, Levin Y, Ben-Dor S, Pick U (2015) Proteome analysis of cytoplasmatic and plastidic β-carotene lipid droplets in *Dunaliella bardawil*. Plant Physiol 167:60–79. doi:10.1104/pp.114.248450

Davison IR (1987) Partial purification and preliminary characterization of pyruvate kinase from the brown alga *Ascophyllum nodosum*. Br Phycol J 22:401–409

Drincovich MF, Casati P, Andreo CS (2001) NADP-malic enzyme from plants: a ubiquitous enzyme involved in different metabolic pathways. FEBS Lett 490:1–6. doi:10.1016/S0014-5793(00)02331-0

Dubini A, Mus F, Seibert M, Grossman AR, Posewitz MC (2009) Flexibility in anaerobic metabolism as revealed in a mutant of *Chlamydomonas reinhardtii* lacking hydrogenase activity. J Biol Chem 284:7201–7213. doi:10.1074/jbc.M803917200

Fan J, Andre C, Xu C (2011) A chloroplast pathway for the de novo biosynthesis of triacylglycerol in *Chlamydomonas reinhardtii*. FEBS Lett 585:1985–1991. doi:10.1016/j.febslet.2011.05.018

Fan J, Yan C, Andre C, Shanklin J, Schwender J, Xu C (2012) Oil accumulation is controlled by carbon precursor supply for fatty acid synthesis in *Chlamydomonas reinhardtii*. Plant Cell Physiol 53:1380–1390. doi:10.1093/pcp/pcs082

Fan J, Cui Y, Wan M, Wang W, Li Y (2014) Lipid accumulation and biosynthesis genes response of the oleaginous *Chlorella pyrenoidosa* under three nutrition stressors. Biotechnol Biofuels 7:17

Fatland B, Anderson M, Nikolau BJ, Wurtele ES (2000) Molecular biology of cytosolic acetyl-CoA generation. Biochem Soc Trans 28:593–595

Fatland BL, Ke J, Anderson MD, Mentzen WI, Cui LW, Allred CC, Johnston JL, Nikolau BJ, Wurtele ES (2002) Molecular characterization of a heteromeric ATP-citrate lyase that generates cytosolic acetyl-coenzyme A in Arabidopsis. Plant Physiol 130:740–756. doi:10.1104/pp.008110

Finkemeier I, Laxa M, Miguet L, Howden AJ, Sweetlove LJ (2011) Proteins of diverse function and subcellular location are lysine acetylated in Arabidopsis. Plant Physiol 155:1779–1790. doi:10.1104/pp.110.171595

Focks N, Benning C (1998) wrinkled1: a novel, low-seed-oil mutant of Arabidopsis with a deficiency in the seed-specific regulation of carbohydrate metabolism. Plant Physiol 118:91–101

Fritsch H, Beevers H (1979) ATP citrate lyase from germinating castor bean endosperm. Plant Physiol 63:687–691

Ge F, Huang W, Chen Z, Zhang C, Xiong Q, Bowler C, Yang J, Xu J, Hu H (2014) Methylcrotonyl-CoA carboxylase regulates triacylglycerol accumulation in the model diatom *Phaeodactylum tricornutum*. Plant Cell Online 26:1681–1697

Gfeller RP, Gibbs M (1984) Fermentative metabolism of *Chlamydomonas reinhardtii*: I. Analysis of fermentative products from starch in dark and light. Plant Physiol 75:212–218

Gfeller RP, Gibbs M (1985) Fermentative metabolism of *Chlamydomonas reinhardtii*: II. Role of plastoquinone. Plant Physiol 77:509–511

Gibbs M, Gfeller RP, Chen C (1986) Fermentative metabolism of *Chlamydomonas reinhardtii*: III. Photoassimilation of acetate. Plant Physiol 82:160–166

Gibon Y, Pyl E-T, Sulpice R, Lunn JE, Höhne M, Günther M, Stitt M (2009) Adjustment of growth, starch turnover, protein content and central metabolism to a decrease of the carbon supply when Arabidopsis is grown in very short photoperiods. Plant Cell Environ 32:859–874. doi:10.1111/j.1365-3040.2009.01965.x

González-Ballester D, Casero D, Cokus S, Pellegrini M, Merchant SS, Grossman AR (2010) RNA-seq analysis of sulfur-deprived *Chlamydomonas* cells reveals aspects of acclimation critical for cell survival. Plant Cell Online 22:2058–2084

Goodenough U et al (2014) The path to triacylglyceride obesity in the sta6 strain of *Chlamydomonas reinhardtii*. Eukaryot Cell 13:591–613. doi:10.1128/ec.00013-14

Goodson C, Roth R, Wang ZT, Goodenough U (2011) Structural correlates of cytoplasmic and chloroplast lipid body synthesis in *Chlamydomonas reinhardtii* and stimulation of lipid body production with acetate boost. Eukaryot Cell 10:1592–1606. doi:10.1128/EC.05242-11

Guerra LT, Levitan O, Frada MJ, Sun JS, Falkowski PG, Dismukes GC (2013) Regulatory branch points affecting protein and lipid biosynthesis in the diatom *Phaeodactylum tricornutum*. Biomass Bioenergy 59:306–315. doi:10.1016/j.biombioe.2013.10.007

Guihéneuf F, Stengel D (2013) LC-PUFA-enriched oil production by microalgae: accumulation of lipid and triacylglycerols containing n-3 LC-PUFA is triggered by nitrogen limitation and inorganic carbon availability in the marine haptophyte *Pavlova lutheri*. Mar Drugs 11:4246–4266

Guschina IA, Harwood JL (2006) Lead and copper effects on lipid metabolism in cultured lichen photobionts with different phosphorus status. Phytochemistry 67:1731–1739. doi:10.1016/j.phytochem.2006.01.023

Harwood JL (ed) (2005) Fatty acid biosynthesis plant lipids: biology, utilisation, and manipulation. Blackwell Publishing, Oxford, pp 27–66

Ho SH, Chen CY, Chang JS (2011) Effect of light intensity and nitrogen starvation on CO_2 fixation and lipid/carbohydrate production of an indigenous microalga *Scenedesmus obliquus* CNW-N. Bioresour Technol 113:244–252. doi:10.1016/j.biortech.2011.11.133

Hoffmeister M, Piotrowski M, Nowitzki U, Martin W (2005) Mitochondrial trans-2-enoyl-CoA reductase of wax ester fermentation from *Euglena gracilis* defines a new family of enzymes involved in lipid synthesis. J Biol Chem 280:4329–4338. doi:10.1074/jbc.M411010200

Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, Darzins A (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J 54:621–639. doi:10.1111/j.1365-313X.2008.03492.x

Huerlimann R, Heimann K (2013) Comprehensive guide to acetyl-carboxylases in algae. Crit Rev Biotechnol 33:49–65. doi:10.3109/07388551.2012.668671

Jia J, Han D, Gerken HG, Li Y, Sommerfeld M, Hu Q, Xu J (2015) Molecular mechanisms for photosynthetic carbon partitioning into storage neutral lipids in *Nannochloropsis oceanica* under nitrogen-depletion conditions. Algal Res 7:66–77. doi:10.1016/j.algal.2014.11.005

Jinkerson RE, Radakovits R, Posewitz MC (2013) Genomic insights from the oleaginous model alga *Nannochloropsis gaditana*. Bioengineered 4:37–43. doi:10.4161/bioe.21880

Johnston ML, Luethy MH, Miernyk JA, Randall DD (1997) Cloning and molecular analyses of the Arabidopsis thaliana plastid pyruvate dehydrogenase subunits. Biochim Biophys Acta 1321:200–206. doi:10.1016/S0005-2728(97)00059-5

Kang F, Rawsthorne S (1994) Starch and fatty acid synthesis in plastids from developing embryos of oilseed rape (*Brassica napus* L.). Plant J 6:795–805. doi:10.1046/j.1365-313X.1994.6060795.x

Ke J, Behal RH, Back SL, Nikolau BJ, Wurtele ES, Oliver DJ (2000) The role of pyruvate dehydrogenase and acetyl-coenzyme A synthetase in fatty acid synthesis in developing Arabidopsis seeds. Plant Physiol 123:497–508

Klein U (1986) Compartmentation of glycolysis and of the oxidative pentose-phosphate pathway in *Chlamydomonas reinhardii*. Planta 167:81–86. doi:10.1007/BF00446372

Klok AJ, Lamers PP, Martens DE, Draaisma RB, Wijffels RH (2014) Edible oils from microalgae: insights in TAG accumulation. Trends Biotechnol 32:521–528. doi:10.1016/j.tibtech.2014.07.004

Knowles VL, Dennis DT, Plaxton WC (1989) Purification of a novel pyruvate kinase from a green alga. FEBS Lett 259:130–132. doi:10.1016/0014-5793(89)81511-X

Kroth PG, Chiovitti A, Gruber A, Martin-Jezequel V, Mock T, Parker MS, Stanley MS, Kaplan A, Caron L, Weber T, Maheswari U, Armbrust EV, Bowler C (2008) A model for carbohydrate metabolism in the diatom *Phaeodactylum tricornutum* deduced from comparative whole genome analysis. PLoS One 3:e1426. doi:10.1371/journal.pone.0001426

Lee JM, Williams ME, Tingey SV, Rafalski JA (2002) DNA array profiling of gene expression changes during maize embryo development. Funct Integr Genomics 2:13–27. doi:10.1007/s10142-002-0046-6

Levitan O, Dinamarca J, Zelzion E, Lun DS, Guerra LT, Kim MK, Kim J, Van Mooy BA, Bhattacharya D, Falkowski PG (2015) Remodeling of intermediate metabolism in the diatom *Phaeodactylum tricornutum* under nitrogen stress. Proc Natl Acad Sci USA 112:412–417. doi:10.1073/pnas.1419818112

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. doi:10.1105/tpc.113.121418

Li X, Moellering ER, Liu B, Johnny C, Fedewa M, Sears BB, Kuo MH, Benning C (2012) A galactoglycerolipid lipase is required for triacylglycerol accumulation and survival following nitrogen deprivation in *Chlamydomonas reinhardtii*. Plant Cell 24:4670–4686. doi:10.1105/tpc.112.105106

Lin M, Oliver D (2008) The role of acetyl-coenzyme a synthetase in Arabidopsis. Plant Physiol 147:1822–1829. doi:10.1105/tpc.112.105106

Liu B, Benning C (2013) Lipid metabolism in microalgae distinguishes itself. Curr Opin Biotechnol. doi:10.1016/j.copbio.2012.08.008

Longworth J, Noirel J, Pandhal J, Wright PC, Vaidyanathan S (2012) HILIC- and SCX-based quantitative proteomics of *Chlamydomonas reinhardtii* during nitrogen starvation induced lipid and carbohydrate accumulation. J Proteome Res 11:5959–5971. doi:10.1021/pr300692t

Lonien J, Schwender J (2009) Analysis of metabolic flux phenotypes for two Arabidopsis mutants with severe impairment in seed storage lipid synthesis. Plant Physiol 151:1617–1634. doi:10.1104/pp.109.144121

Lutziger I, Oliver DJ (2000) Molecular evidence of a unique lipoamide dehydrogenase in plastids: analysis of plastidic lipoamide dehydrogenase from *Arabidopsis thaliana*. FEBS Lett 484:12–16. doi:10.1016/S0014-5793(00)02116-5

Lv H, Qu G, Qi X, Lu L, Tian C, Ma Y (2013) Transcriptome analysis of *Chlamydomonas reinhardtii* during the process of lipid accumulation. Genomics 101:229–237. doi:10.1016/j.ygeno.2013.01.004

Magneschi L, Catalanotti C, Subramanian V, Dubini A, Yang W, Mus F, Posewitz MC, Seibert M, Perata P, Grossman AR (2012) A mutant in the ADH1 gene of *Chlamydomonas reinhardtii* elicits metabolic restructuring during anaerobiosis. Plant Physiol 158:1293–1305. doi:10.1104/pp.111.191569

Masterson C, Wood C, Thomas DR (1990) L-acetylcarnitine, a substrate for chloroplast fatty acid synthesis. Plant Cell Environ 13:755–765. doi:10.1111/j.1365-3040.1990.tb01091.x

Matthew T, Zhou W, Rupprecht J, Lim L, Thomas-Hall SR, Doebbe A, Kruse O, Hankamer B, Marx UC, Smith SM, Schenk PM (2009) The metabolome of *Chlamydomonas reinhardtii* following induction of anaerobic H2 production by sulfur depletion. J Biol Chem 284:23415–23425

Mentzen WI, Peng J, Ransom N, Nikolau BJ, Wurtele ES (2008) Articulation of three core metabolic processes in Arabidopsis: fatty acid biosynthesis, leucine catabolism and starch metabolism. BMC Plant Biol 8:76. doi:10.1186/1471-2229-8-76

Merzlyak MN, Chivkunova OB, Gorelova OA, Reshetnikova IV, Solovchenko AE, Khozin-Goldberg I, Cohen Z (2007) Effect of nitrogen starvation on optical properties, pigments, and arachidonic acid content of the unicellular green alga *Parietochloris incisa* (Trebouxiophyceae, Chlorophyta). J Phycol 43:833–843. doi:10.1111/j.1529-8817.2007.00375.x

Miernyk JA, Dennis DT (1983) The incorporation of glycolytic intermediates into lipids by plastids isolated from the developing endosperm of castor oil seeds (*Ricinus communis* L.). J Exp Bot 34:712–718. doi:10.1093/jxb/34.6.712

Miller R, Wu G, Deshpande RR, Vieler A, Gärtner K, Li X, Moellering ER, Zäuner S, Cornish AJ, Liu B, Bullard B, Sears BB, Kuo MH, Hegg EL, Shachar-Hill Y, Shiu SH, Benning C (2010) Changes in transcript abundance in *Chlamydomonas reinhardtii* following nitrogen deprivation predict diversion of metabolism. Plant Physiol 154:1737–1752

Moellering ER, Benning C (2010) RNA interference silencing of a major lipid droplet protein affects lipid droplet size in *Chlamydomonas reinhardtii*. Eukaryot Cell 9:97–106

Mooney BP, Miernyk JA, Randall DD (1999) Cloning and characterization of the dihydrolipoamide S-acetyltransferase subunit of

the plastid pyruvate dehydrogenase complex (E2) from Arabidopsis. Plant Physiol 120:443–452
Muñoz M, Ponce E (2003) Pyruvate kinase: current status of regulatory and functional properties. Comp Biochem Physiol B Biochem Mol Biol 135:197–218. doi:10.1016/S1096-4959(03)00081-2
Mus F, Dubini A, Seibert M, Posewitz MC, Grossman AR (2007) Anaerobic acclimation in *Chlamydomonas reinhardtii*: anoxic gene expression, hydrogenase induction, and metabolic pathways. J Biol Chem 282:25475–25486
Nallamilli BR, Edelmann MJ, Zhong X, Tan F, Mujahid H, Zhang J, Nanduri B, Peng Z (2014) Global analysis of lysine acetylation suggests the involvement of protein acetylation in diverse biological processes in rice *Oryza sativa*. PLoS One 9:e89283. doi:10.1371/journal.pone.0089283
Nguyen HM, Baudet M, Cuiné S, Adriano JM, Barthe D, Billon E, Bruley C, Beisson F, Peltier G, Ferro M, Li- Beisson Y (2011) Proteomic profiling of oil bodies isolated from the unicellular green microalga *Chlamydomonas reinhardtii*: with focus on proteins involved in lipid metabolism. Proteomics 11:4266–4273
Nikolau BJ, Oliver DJ, Schnable PS, Wurtele ES (2000) Molecular biology of acetyl-CoA metabolism. Biochem Soc Trans 28:591–593
O'Hara P, Slabas AR, Fawcett T (2002) Fatty acid and lipid biosynthetic genes are expressed at constant molar ratios but different absolute levels during embryogenesis. Plant Physiol 129:310–320. doi:10.1104/pp.010956
Ohlrogge JB, Jaworski JG (1997) Regulation of fatty acid synthesis. Annu Rev Plant Physiol Plant Mol Biol 48:109–136. doi:10.1146/annurev.arplant.48.1.109
Oliver DJ, Nikolau BJ, Wurtele ES (2009) Acetyl-CoA—Life at the metabolic nexus. Plant Sci 176:597–601. doi:10.1016/j.plantsci.2009.02.005
Pal D, Khozin-Goldberg I, Cohen Z, Boussiba S (2011) The effect of light, salinity, and nitrogen availability on lipid production by Nannochloropsis sp. Appl Microbiol Biotechnol 90:1429–1441. doi:10.1007/s00253-011-3170-1
Perrineau M-M, Gross J, Zelzion E, Price DC, Levitan O, Boyd J, Bhattacharya D (2014) Using natural selection to explore the adaptive potential *of Chlamydomonas reinhardtii*. PLoS One 9:e92533. doi:10.1371/journal.pone.0092533
Philipps G, Krawietz D, Hemschemeier A, Happe T (2011) A pyruvate formate lyase-deficient *Chlamydomonas reinhardtii* strain provides evidence for a link between fermentation and hydrogen production in green algae. Plant J 66:330–340. doi:10.1111/j.1365-313X.2011.04494.x
Qi Q, Trimming BA, Kleppinger-Sparace KF, Emes MJ, Sparace SA (1996) Pyruvate dehydrogenase complex and acetyl-CoA carboxylase in pea root plastids: their characterization and role in modulating glycolytic carbon flow to fatty acid biosynthesis. J Exp Bot 47:1889–1896. doi:10.1093/jxb/47.12.1889
Rangasamy D, Ratledge C (2000) Compartmentation of ATP:citrate lyase in plants. Plant Physiol 122:1225–1230
Ratledge C, Bowater MD, Taylor PN (1997) Correlation of ATP/citrate lyase activity with lipid accumulation in developing seeds of *Brassica napus* L. Lipids 32:7–12
Rawsthorne S (2002) Carbon flux and fatty acid synthesis in plants. Prog Lipid Res 41:182–196. doi:10.1016/S0163-7827(01)00023-6
Recht L, Töpfer N, Batushansky A, Sikron N, Gibon Y, Fait A, Nikoloski Z, Boussiba S, Zarka A (2014) Metabolite profiling and integrative modeling reveal metabolic constraints for carbon partitioning under nitrogen starvation in the green algae *Haematococcus pluvialis*. J Biol Chem 289:30387–30403. doi:10.1074/jbc.M114.555144
Reid EE, Thompson P, Lyttle CR, Dennis DT (1977) Pyruvate dehydrogenase complex from higher plant mitochondria and proplastids. Plant Physiol 59:842–848. doi:10.1104/pp.59.5.842
Rismani-Yazdi H, Haznedaroglu BZ, Hsin C, Peccia J (2012) Transcriptomic analysis of the oleaginous microalga *Neochloris oleoabundans* reveals metabolic insights into triacylglyceride accumulation. Biotechnol Biofuels 5:74. doi:10.1186/1754-6834-5-74
Roughan PG, Holland R, Slack CR (1979) Acetate is the preferred substrate for long-chain fatty acid synthesis in isolated spinach chloroplasts. Biochem J 184:565–569
Ruuska SA, Girke T, Benning C, Ohlrogge JB (2002) Contrapuntal networks of gene expression during Arabidopsis seed filling. Plant Cell Online 14:1191–1206
Saito T, Nishi M, Lim MI, Wu B, Maeda T, Hashimoto H, Takeuchi T, Roos DS, Asai T (2008) A novel GDP-dependent pyruvate kinase isozyme from Toxoplasma gondii localizes to both the apicoplast and the mitochondrion. J Biol Chem 283:14041–14052. doi:10.1074/jbc.M709015200
Sakurai K, Moriyama T, Sato N (2013) Detailed identification of fatty acid isomers sheds light on the probable precursors of triacylglycerol accumulation in photoautotrophically grown *Chlamydomonas reinhardtii*. Eukaryot Cell 13:256–266. doi:10.1128/EC.00280-13
Sangwan R, Gauthier D, Turpin D, Pomeroy MK, Plaxton W (1992) Pyruvate-kinase isoenzymes from zygotic and microspore-derived embryos of *Brassica napus*. Planta 187:198–202. doi:10.1007/BF00201938
Sasaki Y, Nagano Y (2004) Plant acetyl-CoA carboxylase: structure, biosynthesis, regulation, and gene manipulation for plant breeding. Biosci Biotechnol Biochem 68:1175–1184
Sato N, Tsuzuki M, Kawaguchi A (2003) Glycerolipid synthesis in Chlorella kessleri 11 h. I. Existence of a eukaryotic pathway. Biochim Biophys Acta 1633:27–34. doi:10.1016/S1388-1981(03)00069-6
Schmollinger S, Mühlhaus T, Boyle NR et al (2014) Nitrogen-sparing mechanisms in Chlamydomonas affect the transcriptome, the proteome, and photosynthetic metabolism. Plant Cell Online 26:1410–1435. doi:10.1105/tpc.113.122523
Scott S, Davey M, Dennis J, Horst I, Howe C, Lea-Smith D, Smith A (2010) Biodiesel from algae: challenges and prospects. Curr Opin Biotechnol 21:277–286. doi:10.1016/j.copbio.2010.03.005
Shtaida N, Khozin-Goldberg I, Solovchenko A, Chekanov K, Didi-Cohen S, Leu S, Cohen Z, Boussiba S (2014) Downregulation of a putative plastid PDC E1α subunit impairs photosynthetic activity and triacylglycerol accumulation in nitrogen-starved photoautotrophic *Chlamydomonas reinhardtii*. J Exp Bot 65:6563–6576. doi:10.1093/jxb/eru374
Siaut M, Cuiné S, Cagnon C, Fessler B, Nguyen M, Carrier P, Beyly A, Beisson F, Triantaphylidès C, Li-Beisson Y, Peltier G (2011) Oil accumulation in the model green alga *Chlamydomonas reinhardtii*: characterization, variability between common laboratory strains and relationship with starch reserves. BMC Biotechnol 11:7. doi:10.1186/1472-6750-11-7
Smith RG, Gauthier DA, Dennis DT, Turpin DH (1992a) Malate- and pyruvate-dependent fatty acid synthesis in leucoplasts from developing castor endosperm. Plant Physiol 98:1233–1238
Smith RG, Gauthier DA, Dennis DT, Turpin DH (1992b) Malate- and pyruvate-dependent fatty acid synthesis in leucoplasts from developing castor endosperm. Plant Physiol 98:1233–1238. doi:10.1104/pp.98.4.1233
Smith SR, Abbriano RM, Hildebrand M (2012) Comparative analysis of diatom genomes reveals substantial differences in the organization of carbon partitioning pathways. Algal Res 1:2–16. doi:10.1016/j.algal.2012.04.003

Solovchenko AE (2012) Physiological role of neutral lipid accumulation in eukaryotic microalgae under stresses. Russ J Plant Physiol 59:167–176. doi:10.1134/s1021443712020161

Takeshita T, Ota S, Yamazaki T, Hirata A, Zachleder V, Kawano S (2014) Starch and lipid accumulation in eight strains of six Chlorella species under comparatively high light intensity and aeration culture conditions. Bioresour Technol 158:127–134. doi:10.1016/j.biortech.2014.01.135

Terashima M, Specht M, Hippler M (2011) The chloroplast proteome: a survey from the *Chlamydomonas reinhardtii* perspective with a focus on distinctive features. Curr Genet 57:151–168. doi:10.1007/s00294-011-0339-1

To A, Joubès J, Barthole G, Lécureuil A, Scagnelli A, Jasinski S, Lepiniec L, Baud S (2012) WRINKLED transcription factors orchestrate tissue-specific regulation of fatty acid biosynthesis in Arabidopsis. Plant Cell Online 24:5007–5023. doi:10.1105/tpc.112.106120

Tovar-Méndez A, Miernyk JA, Randall DD (2003) Regulation of pyruvate dehydrogenase complex activity in plant cells. Eur J Biochem 270:1043–1049. doi:10.1046/j.1432-1033.2003.03469.x

Valenzuela J, Mazurie A, Carlson R, Gerlach R, Cooksey K, Peyton B, Fields M (2012) Potential role of multiple carbon fixation pathways during lipid accumulation in *Phaeodactylum tricornutum*. Biotechnol Biofuels 5:1–17. doi:10.1186/1754-6834-5-40

Vieler A et al (2012) Genome, functional gene annotation, and nuclear transformation of the heterokont oleaginous alga *Nannochloropsis oceanica* CCMP1779. PLoS Genet 8:e1003064. doi:10.1371/journal.pgen.1003064

Wase N, Black PN, Stanley BA, DiRusso CC (2014) Integrated quantitative analysis of nitrogen stress response in *Chlamydomonas reinhardtii* using metabolite and protein profiling. J Proteome Res 13:1373–1396. doi:10.1021/pr400952z

Wei Y, Lin M, Oliver D, Schnable P (2009) The roles of aldehyde dehydrogenases (ALDHs) in the PDH bypass of Arabidopsis. BMC Biochem 10:7

Williams M, Randall DD (1979) Pyruvate dehydrogenase complex from chloroplasts of *Pisum sativum* L. Plant Physiol 64:1099–1103

Wu HB, Turpin DH (1992) Purification and characterization of pyruvate kinase from the green alga *Chlamydomonas reinhardtii*. J Phycol 28:472–481. doi:10.1111/j.0022-3646.1992.00472.x

Wynn JP, Ratledge C (1997) Malic enzyme is a major source of NADPH for lipid accumulation by *Aspergillus nidulans*. Microbiology 143:253–257. doi:10.1099/00221287-143-1-253

Wynn JP, AbA Hamid, Ratledge C (1999) The role of malic enzyme in the regulation of lipid accumulation in filamentous fungi. Microbiology 145:1911–1917. doi:10.1099/13500872-145-8-1911

Yang ZK, Niu YF, Ma YH, Xue J, Zhang MH, Yang WD, Liu JS, Lu SH, Guan Y, Li HY (2013) Molecular and cellular mechanisms of neutral lipid accumulation in diatom following nitrogen deprivation. Biotechnol Biofuels 6:67. doi:10.1186/1754-6834-6-67

Yang W, Catalanotti C, Wittkopp TM, Posewitz MC, Grossman AR (2015) Algae after dark: mechanisms to cope with anoxic/hypoxic conditions. Plant J. doi:10.1111/tpj.12823

Zeeman SC, Rees TA (1999) Changes in carbohydrate metabolism and assimilate export in starch-excess mutants of Arabidopsis. Plant Cell Environ 22:1445–1453. doi:10.1046/j.1365-3040.1999.00503.x

Zhang Y, Adams IP, Ratledge C (2007) Malic enzyme: the controlling activity for lipid production? Overexpression of malic enzyme in *Mucor circinelloides* leads to a 2.5-fold increase in lipid accumulation. Microbiology 153:2013–2025. doi:10.1099/mic.0.2006/002683-0