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Research review paper

Leveraging algal omics to reveal potential targets for augmenting TAG accumulation

Neha Arora[a], Philip T. Pienkos[b], Vikas Pruthi[a], Krishna Mohan Poluri[a], Michael T. Guarnieri[b,*]

[a] Department of Biotechnology, Indian Institute of Technology Roorkee, Uttarakhand 247667, India
[b] National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO 80401, USA

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ABSTRACT

Ongoing global efforts to commercialize microalgal biofuels have expedited the use of multi-omics techniques to gain insights into lipid biosynthetic pathways. Functional genomics analyses have recently been employed to complement existing sequence-level omics studies, shedding light on the dynamics of lipid synthesis and its interplay with other cellular metabolic pathways, thus revealing possible targets for metabolic engineering. Here, we review the current status of algal omics studies to reveal potential targets to augment TAG accumulation in various microalgae. This review specifically aims to examine and catalog systems level data related to stress-induced TAG accumulation in oleaginous microalgae and inform future metabolic engineering strategies to develop strains with enhanced bioproductivity, which could pave a path for sustainable green energy.

List of abbreviations

AAD	Acyl-ACP destaurase
ACCase	Acetyl CoA carboxylase
ACP	Acyl carrier protein
AMPK	adenosine monophosphate activated kinase
ATP	Adenosine triphosphate
BC	Biotin carboxylase
BCCP	Biotin carboxyl carrier protein
BODIPY	boron-dipyrromethene
CA	carbonic anhydrase
CCM	CO_2 concentrating mechanism
cDNA	Complementary dideoxyribonucleic acid
CE-MS	capillary electrophoresis mass spectroscopy
DAG	Diacylglycerol
DGAT	Diglyceride acyltransferase
DGD	digalactosyl diacylglycerol
EMS	ethyl methane sulfonate
ENR	enoyl ACP reductase
ER	Endoplasmic reticulum
EST	Expression sequence tags
FAEEs	Fatty acid ethyl esters
FAMEs	Fatty acid methyl esters
FAS	fatty acid synthase
FkBP	FK-506 binding protein
GADPH	glyceraldehyde 3 phosphate dehydrogenase
GC-MS	gas chromatography- mass spectroscopy
GS	Glutamine synthase
HAD	3-hydroxybutyrl- Acyl-Carrier Protein
KAS	3-Ketoacyl-Acyl-Carrier Protein
LC-MS	liquid chromatography- mass spectroscopy
LDSP	lipid droplet surface protein
LHC	Light harvesting center
MCAT	malonyl-CoA acyl transferase
MDH	malate dehydrogenase
MGD	mono galactosyl diacylglycerol
mRNA	messenger ribonucleic acid
NADPH	Nicotinamide adenine dinucleotide phosphate
NGS	Next generation sequencing
NMR	Nuclear magnetic resonance spectroscopy
PAP	Phosphatidate phosphate
PC	Pyruvate carboxylase
PDAT	Phospholipid:diacylglycerol acyltransferase
PDH	Pyruvate dehydrogenase
PEPC	Phosphoenol pyruvate carboxylase
PGAM	Phosphoglycerate mutase
PGK	Phosphoglycerate kinase
PK	Pyruvate kinase
PMM	Phosphomannomutase
PPH	Phosphopyruvate hydratase
RT-PCR	Reverse transcriptase- Polymerase chain reaction

* Corresponding author.
E-mail address: michael.guarnieri@nrel.gov (M.T. Guarnieri).

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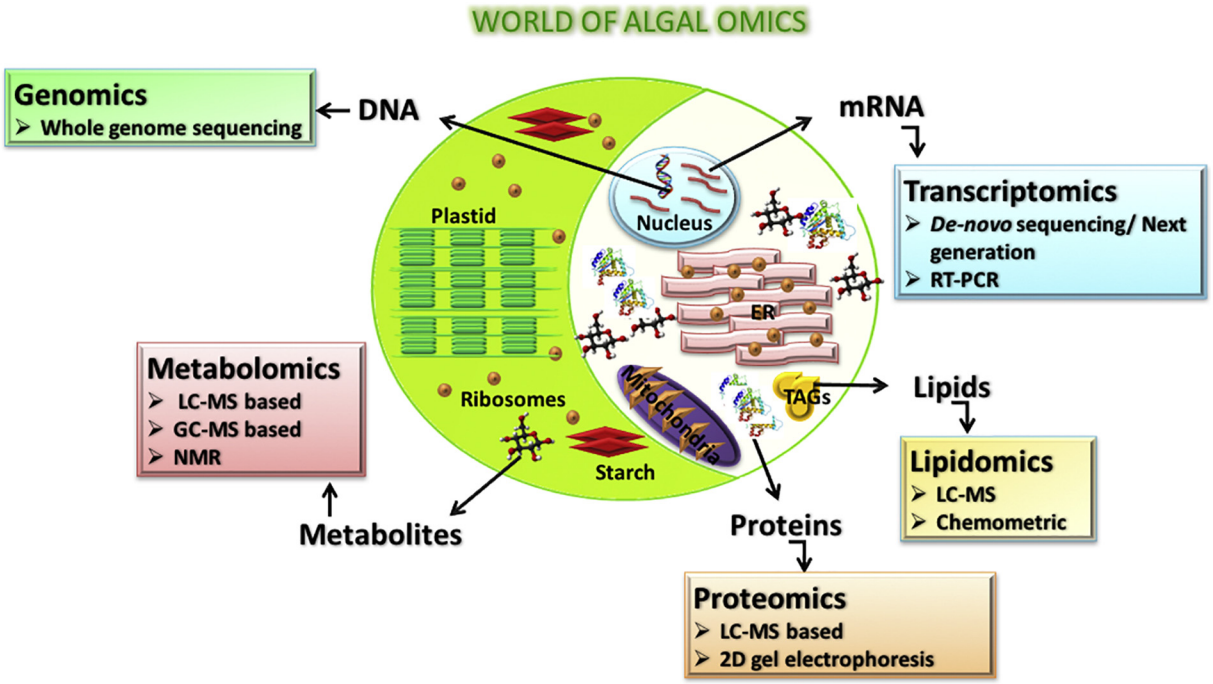

Fig. 1. Omics techniques for identifying potential targets for increasing TAG accumulation in microalgae.

RuBisCo	Ribulose-1,5-bisphosphate carboxylase/oxygenase
RuBP	Ribulose-1,5-bisphosphate
SAGE	Serial analysis of gene expression
SQD2	Sulfoquinovosyl diacylglycerol
TAG	Triacylglycerol
TCA	Tri carboxylic acid
TLA1	Truncated light harvesting antenna 1
αCT	α carboxytransferases
βCT	β carboxytransferases

1. Introduction

In light of current environmental and energy supply concerns, growing attention is being devoted to developing renewable energy as a means to reduce global warming and strengthen energy security. The utilization of non-food-based feedstocks for renewable fuels will be helpful in overcoming the food versus fuel dilemma. The transportation sector is one of the leading users of fossil fuels, calling for replacement with bio-based fuels such as biodiesel (made from fatty acid methyl/ethyl esters or FAMEs/FAEES), renewable diesel and renewable jet fuel (made from hydrodeoxygenation of fatty acids), and bioethanol (made from carbohydrates) (Chen and Smith, 2012). Among the renewable feedstocks, microalgae offer an edge in comparison to other conventional plant based feedstocks owing to their relatively rapid growth rate, higher yields of bioenergy per hectare, and their ability to thrive without a need for arable land along with reduction of freshwater, thus mitigating potential impact on food supplies (Lee et al., 2014a, 2014b). Furthermore, microalgae can capture CO_2 not only from the environment but from different industrial waste streams, which might provide a positive sustainability impact (Hasunuma et al., 2016). However, various economic barriers exist towards economical and sustainable deployment of microalgae-based biodiesel on large scale, including fresh water and nutrient availability, process stability and downstream processing (harvesting, extraction and production). One approach to enable favorable process economics is via enhancement of TAG accumulation, which will require a more complete understanding of lipogenesis induction and biosynthetic mechanisms, and bottlenecks related thereto.

Microalgae (and cyanobacteria) harness sunlight and CO_2 from the environment to synthesize lipids, carbohydrates, proteins and various value-added products (e.g. carotenoids, phycobiliproteins, sterols and vitamins). Among these, neutral lipids (triacylglycerols) and carbohydrates can serve as efficient raw materials for biodiesel and bioethanol and biochemical production, respectively. Triacylglycerols (TAGs) are composed of fatty acids with a glycerol backbone, which can be transesterified to form fatty acid methyl esters (FAMEs) (Lohman et al., 2015). The transesterification process involves reaction of 1 mole of TAGs with 3 moles of methanol at moderate temperatures, catalyzed by acid, alkali, or enzyme, to form 1 mole of glycerol and 3 moles of FAMEs, which can be utilized for biodiesel (Ramos et al., 2009). They can accumulate large quantities of TAGs (20-60 % of dry cell weight) under adverse conditions in specialized lipid bodies (Klok et al., 2014). The adverse conditions can be categorized as physical (e.g. shifts in temperature, light intensity, wavelength of light) and chemical (e.g. nutrient depletion, salinity, CO_2, heavy metal exposure) (Arora et al., 2017a, 2017b; Singh et al., 2016; Ho et al., 2014a, 2014b). TAGs help microalgae withstand the imposed stress by maintaining intracellular lipid homeostasis, cellular function and energy supply (Lenka et al., 2016). However, prolonged stress can result in breakdown of the photosynthetic apparatus, resulting in chlorophyll degradation (manifesting as chlorosis), limiting cell division, and reducing the overall TAG productivity (Vonlanthen et al., 2015). Additionally, there is an inverse relation between active growth and lipogenesis. Therefore, an optimum balance between growth rate and TAG accumulation is essential for commercial algal biofuel production (Davis et al., 2011). Microalgal omics can provide insights for improved cultivation practices or guidance for strain improvement strategies to achieve maximal lipid and biomass accumulation, and potential decoupling of these processes.

The term “omics” has come to be used in any biological study that aims to characterize and quantify large datasets of an organism’s structure, function and dynamics requiring informatics (bioinformatics tools for dataset assembly, annotations and integration) to archive (creation and storage of databases) and interpret high resolution molecular maps. In this context, we use the term to reflect four major components: genomics (sequencing of complete genomes),

transcriptomics (mRNA-transcription), proteomics (protein translation and post-translation regulation) and metabolomics (metabolites) as shown in Fig. 1 (Guarnieri and Pienkos, 2015). Omics studies strive to analyze entire classes of molecules which can play a vital role in underpinning key regulatory elements (genes, proteins, metabolites, and interactions thereof) and mechanisms reflecting the physiological details of microalgal growth, adaptation to cultivation changes, resistance to predators, and characteristics involved in harvesting (Hannon et al., 2011). Understanding the aforementioned traits can accelerate the basic understanding of TAG synthesis in microalgae in response to stress by identifying regulatory pathways and key genes/proteins/metabolites leading to strategic genetic engineering and strain improvement, thereby expediting the pursuit of economically feasible algal oil production.

Extensive transcriptomics, proteomics and metabolomics studies on various microalgae exposed to environmental stresses have reported differential expression and/or abundance of specific genes, proteins, or metabolites that contribute directly or indirectly towards augmentation of intracellular TAG accumulation. However, the available data have not been systematically integrated, thus hindering advancement of algal biofuel omics. Hence, the present review focuses on the state of the art methodologies involved in algal omics for identifying key components and governing mechanisms surrounding TAG productivity in an effort to index and synthesize these data, and ultimately inform strategies targeting economically viable algal fuel production in the near future. We examine the technological advances in the field of algal omics, emerging techniques for analysis, and computational methods required to evaluate the vast omics data. The compiled data ultimately aims to both catalog and catalyze the basic understanding of algal biology and lipid synthesis to enable hypothesis-driven bioengineering of microalgae for lipid-derived fuel production.

2. Overview of TAG accumulation in microalgae

In-depth knowledge of lipid metabolism could lead to successful modifications in the expression and/or abundance of key lipid biosynthetic and regulatory elements, resulting in overall improved TAG accumulation in microalgae (Bellou et al., 2014; Radakovits et al., 2010). Extensive biochemical characterization coupled with whole genome sequencing, transcriptomics, proteomics and metabolomics of different microalgae have revealed a detailed mechanism of lipid catabolism and anabolism under various growth modes (autotrophic, heterotrophic, mixotrophic) and stress (physiological, chemical and operational) conditions (For detailed review, please refer to, Lenka et al., 2016; Radakovits et al., 2010). It is only through detailed study and comparative analysis that we will be able to determine if regulatory mechanisms for lipid metabolism are common to multiple species or if they vary widely across the broad algal phylogenic space. As this review notes, there are already indications that both commonality and diversity in regulatory mechanisms are in play, but it is clear that the best likelihood for success at manipulating lipid metabolic regulation will involve omic data arising from the specific strains of interest.

Briefly, neutral lipid (TAG) synthesis in microalgae can be subdivided into two steps: *de-novo* fatty acid synthesis occurring in the plastid, and acyl-lipid assembly in endoplasmic reticulum (Kennedy pathway) (Fig. 2). There also exists an alternative to the Kennedy pathway, the acyl-CoA independent pathway for TAG accumulation in microalgae involving phospholipid:diacylglycerol acyl transferase (PDAT). It is postulated that PDATs transfer a fatty acyl moiety from phospholipid to DAG (diacylglycerol). PDAT utilizes the chloroplast membrane lipids including monogalactosyl diacylglycerol (MGDG), sulfoquinovosyl diacylglycerol (SQDG) and phosphatidylglycerol (PG) as substrate for the synthesis of TAGs. Thus, both *de-novo* and acyl-CoA independent pathways contribute to overall TAG accumulation in microalgae.

3. Mechanistic insights into algal omics and their role in augmenting TAG accumulation

Over the past few years, utilization of omic technologies to understand algal lipid metabolism under different stress conditions has revealed potential targets in various microalgae which can pave the path for targeted genetic engineering (Guarnieri and Pienkos, 2015). The detailed literature of the main omic components: genomics, transcriptomics, proteomics, and metabolomics along with related technologies are discussed below individually.

3.1. Genomics

Next generation sequencing (NGS) has facilitated an increase in the availability of whole genome sequences of a number of microalgal species in public databases (Table 1). The first microalgal genome was published in 2004 for *Cyanidioschyzon merolae*, an inhabitant of sulfur rich acid hot springs (Sasso et al., 2011). *C. merolae* has the smallest genome of all the photosynthetic eukaryotes reported to date, containing single nucleus, mitochondria and plastid (Matsuzaki et al., 2004). The sequencing of this microalga shed light into the evolutionary origin and fundamental differentiation and multiplication traits of photosynthetic eukaryotes. Later, the genome sequence of the first model organism of green algae, *Chlamydomonas reinhardtii,* was published. The complete acylglycerol pathway of *C. reinhardtii* was reconstructed *in-silico* by Riekhof et al., providing a strong foundation for examination of lipid metabolism in green microalgae (Schuhmann et al., 2012). Currently, there are around 30 whole genome sequences available for different green algae and diatoms, as listed in Table 1. These green microalgae were chosen based on their ecological role, phylogenetic distribution or potential as biofuel sources.

Similarly, diatoms also represent valuable sources for many value added bio-products and biofuels. The first diatom to be fully sequenced was *Thalassiosira pseudonana* followed by the pennate diatom *Phaeodactylum tricornutum* which is also a model organism for diatoms (Sasso et al., 2011). These two diatoms provided important information regarding the evolution and metabolic pathways. For example, Kermarrec et al. were successful in identifying diatom taxa in bulk samples of a mock community using pyrosequencing, which was beneficial in evaluating the impact of environmental stressors and stability of the ecosystems (Kermarrec et al., 2013). The whole genome sequences of three other diatoms (*Fragilariopsis cylindrus, T. oceanica, Cyclotella cryptica)* are also available in the public databases (Table 1).

In addition to nuclear genome sequences of these microalgae, mitochondrial and plastid genomes are accessible for still more algal species and these have provided considerable information regarding the genes/ enzymes encoded by these organelles (for a complete list, please refer to Lü et al., 2011). Whole genome sequencing (chromosomal and organelle) has provided an extensive knowledge on the evolution and adaptation in extreme environments, revealing both conserved and unique metabolic pathways involved in various processes such as photosynthesis, carbon acquisition, lipid synthesis, and value-added product synthesis.

3.2. Transcriptomics

Global transcriptional profiling of microalgal cells using NGS can provide valuable insights into pathways and regulatory genes associated with the enhanced production of neutral lipids in microalgae by identification of key transcriptional regulators altering lipid biosynthesis (Rismani-Yazdi et al., 2012). Moreover, comparative transcriptomic analysis of algal genomes provides deeper understanding of differential gene expression in oleaginous vs. non-oleaginous strains by identifying universal biosynthetic and control elements. To this end, various transcriptomic analyses of different oleaginous microalgae have been carried out under nutrient deprivation (mainly nitrogen), carbon

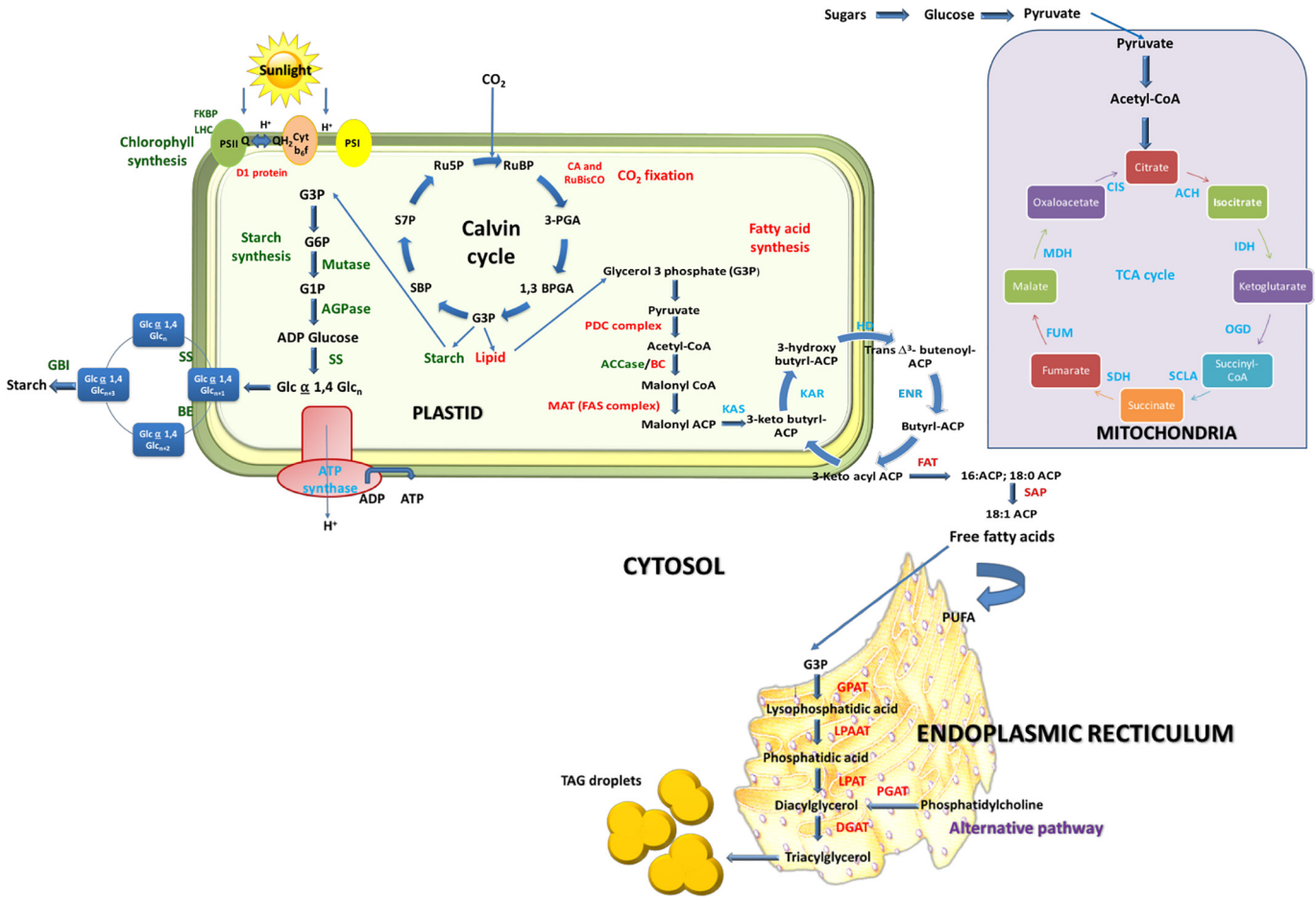

Fig. 2. Metabolic pathways illustrating primary targets that have been identified via omics analyses as upregulating (red), downregulated (green) or displaying inter-species differential regulation (blue) under varied stress conditions in microalgae.

dioxide limitation, etc., to shed light on the mechanisms underlying the augmented lipid accumulation (Table 2).

Nitrogen is an essential nutrient required by microalgae for their normal metabolic activities. Its deficiency causes perturbations in the *de-novo* synthesis of amino acids, nucleic acids and various cellular constituents metabolism including in many cases a drastic increase in lipid content inside cells (Yang et al., 2013). For this reason, the changes in gene expression profiles of various microalgae such as *C. reinharditii*, *Chlorella vulgaris, Botryococcus braunii, Botryosphaerella sudeticus, Neochloris oleoabundans, Phaeodactylum tricornutum, Tetraselmis* M8*, Monoraphidium neglectum* and *Micractinium pusillum* under nitrogen deplete conditions have been extensively reported in the literature as listed in Table 2. These studies have delineated a systematic profile of differential expression of genes belonging to various metabolic pathways such as ribosome biosynthesis, RNA processing, protein metabolism, photosynthesis, TCA (tricarboxylic acid) cycle, nitrogen assimilation, energy generation, carbon fixation, carbohydrate metabolism and pentose phosphate metabolism along with the elevation in lipid accumulation under nitrogen deprived conditions (Fig. 2).

Although many similarities are present in these datasets, variations were observed among the expression profiles of the genes related to these aforementioned pathways, which could be attributed to biological distinctions among the microalgal species or to differences in experimental conditions. A substantial increase in the transcripts of acyl carrier protein (ACP) gene, DGAT isoforms such as DGAT-1, DGAT-2A, DGAT-2B, DGAT-2E (catalyzing the last step of TAG synthesis), biotin carboxylase (which regulates ACCase (acetyl CoA carboxylase) activity through carboxylation of the biotin moiety of the enzyme), thioesterase genes (Fat A and thioesterease oleoyl-ACP hydrolase), acyl-ACP (acyl carrier protein) desaturase (AAD), delta 15 saturase, lipases, and saposin has been commonly reported across an array of analyses during nitrogen starvation (Li et al., 2016; López et al., 2015; Li et al., 2014a, 2014b; Sun et al., 2013; Yang et al., 2013; Msanne et al., 2012; Rismani-Yazdi et al., 2012).

Most of the aforementioned studies have also reported an apparent decline in transcripts of ACCase (plastidial/ chloroplastic) and malonyl-CoA:ACP transacylase (MCAT), but their relative mRNA abundance was higher relative to other genes in the cells. This may be the reason that overexpression of ACCase in microalgae has not resulted in enhanced TAG accumulation (Dunahay et al., 1995; Valenzuela et al., 2012). ACCase in microalgae can occur in two isoforms; heteromeric (prokaryotic) or homomeric (eukaryotic). ACCase is comprised of four domains- biotin carboxylase (BC), biotin carboxyl carrier protein (BCCP) and two carboxytransferases (αCT and βCT) which are located on separate subunits in heteromeric species, whereas homomeric species contain all subunits on a single polypeptide chain (Huerlimann and Heimann, 2013). Isoform differentiation can affect the overall efficiency of strain engineering as it has been reported that overexpression of cytosolic homomeric ACCase coupled with plastidial subcellular localization is more efficient in enhancing fatty acid synthesis as opposed to heteromeric plastidial isoform (Roesler et al., 1997). However, the expression of genes involved in fatty acid chain elongation reactions such as KAR (3-ketoacyl-ACP synthase), KAS (3-Ketoacyl-Acyl-Carrier Protein) II, KAS III, HAD (3-hydroxybutyrl-ACP), ENR (enoyl ACP reductase) decreased during nitrogen stress in microalgae such as *C. reinhardtii* and *Nannochloropsis,* but increased in *Chlorella sorokiniana,*

Table 1
List of green microalgae and diatoms genome sequences which have been completed.

Microalgae	Type	Genome length (Mb)	Publication (year)	Sequencing technology	Reference
Coccomyxa sp. LA000219	Green algae	47.8	2014	Illumina	https://www.ncbi.nlm.nih.gov/assembly/GCA_000812005.1#/st
Coccomyxa sp. SUA001	Green algae	11.75	2015	Ion torrent	https://www.ncbi.nlm.nih.gov/assembly/GCA_001244535.1/
Nannochloropsis oceanica LAMB0001	Green algae	27.63	2011	Illumina	https://www.ncbi.nlm.nih.gov/assembly/GCA_000226695.1
Nannochloropsis oceanica OZ-1	Green algae	28.02	2016	454 GS FLX Titanium, Illumina	https://www.ncbi.nlm.nih.gov/assembly/GCA_001614235.1
Volvox Carteri f. magariensis	Green algae	137.68	2010	Sanger	Prochnik et al., 2010
Thalassiosira pseudomona CCMP1335	Diatom	32.43	2004	Shot gun	Armbrust et al., 2004
Nannochloropsis gaditana CCMP526	Green algae	33.99	2012	454 GS FLX Titanium, Illumina	Jinkerson et al., 2013
Micromonas psuilla CCMP1545	Green algae	21.96	2009	ABI 3730	https://www.ncbi.nlm.nih.gov/assembly/GCF_000151265.2
Phaeodactylum tricornutum CCAP 1055/1	Diatom	27.45	2008	-	Bowler et al., 2008
Coelastrella sp. M60	Green algae	80.22	2015	Illumina	https://www.ncbi.nlm.nih.gov/assembly/GCA_001630525.1/
Picochlorum sp. SENEW3	Green algae	13.39	2014	Illumina-Mi seq	Foflonker et al., 2016
Micromonas sp. ASP10-01a	Green algae	19.58	2014	Illumina-Mi seq	https://www.ncbi.nlm.nih.gov/assembly/GCA_001430725.1/
Ostreococcus lucimarinus	Green algae	13.20	2010	-	https://www.ncbi.nlm.nih.gov/assembly/GCF_000092065.1/
Chlamydomonas sphaeroides	Green algae	122.18	2016	Hi seq Illumina	Hirashima et al., 2016
Chlamydomonas debaryana	Green algae	120.36	2016	Illumina-Mi seq	
Chlamydomonas asymmetrica	Green algae	141.92	2016	Illumina-Mi seq	
Chlamydomonas applanata	Green algae	78.5	2016	Illumina-Mi seq	
Micromonas commoda	Green algae	21.10	2009		https://www.ncbi.nlm.nih.gov/assembly/GCF_000090985.2/
Cymbomonas tetramitiformis	Green algae	281.27	2016	Hi seq Illumina	Satjarak et al., 2016
uncultured Bathycoccus	Green algae	5.18	2011	Sanger, 454 FLX	https://www.ncbi.nlm.nih.gov/assembly/GCA_000259855.1/
Monoraphidium neglectum	Green algae	69.71	2013	-	Bogen et al., 2013
Auxenochlorella protothecoides	Green algae	22.92	2014	454 GS FLX Titanium, Illumina HiSeq 2000	https://www.ncbi.nlm.nih.gov/assembly/GCF_000733215.1
Gonium pectorale	Green algae	148.80	2016	454; Illumina MiSeq; ABI3730	https://www.ncbi.nlm.nih.gov/assembly/GCA_001584585.1/
Chlorella pyrenoidosa	Green algae	56.99	2015	454	https://www.ncbi.nlm.nih.gov/assembly/GCA_001430745.1/
Parachlorella kessleri	Green algae	59.18	2016	454 GS FLX+	Ota et al., 2016
Coccomyxa subellipsoidea C-169	Green algae	48.83	2012	Sanger	Blanc et al., 2012
Chlorella vulgaris	Green algae	37.34	2015	Illumina HiSeq	https://www.ncbi.nlm.nih.gov/assembly/GCA_001021125.1/
Chlorella variabilis NC64A	Green algae	45.16	2010	Sanger	Blanc et al., 2010
Ostreococcus tauri	Green algae	12.56	2006	-	Derelle et al., 2006
Chlamydomonas reinhardtii	Green algae	120.40	2007	-	Merchant et al., 2007
Trebouxia gelatinosa	Green algae	61.73	2015	Illumina HiSeq	https://www.ncbi.nlm.nih.gov/assembly/GCA_000818905.1/
Chromochloris zofingiensis SAG 211-14	Green algae	58	2017	Illumina HiSeq	Roth et al., 2017
Fragilariopsis cylindrus	Diatom	68.97	2016	-	Mock et al., 2017
Thalassiosira oceanica	Diatom	92.18	2012	454 GS FLX Titanium	https://www.ncbi.nlm.nih.gov/assembly/GCA_000296195.2
Cyclotella cryptica	Diatom	161.7	2016	Illumina HiSeq	Traller et al., 2016

Table 2
Overview of the transcriptomic studies carried out in various green microalgae and diatoms under different stress conditions for increasing TAG accumulation.

Microalgae	Cultivation media	Method used for analysis	Genes/pathway up regulated	Genes/pathway down regulated	Reference
Lipid accumulating phase					
Nannochloropsis sp.	f/2 medium	IlluminaHiseq2000	Genes : Sphinganine-1-phosphate aldolase, Acetyl-CoA acyltransferase, Glutaryl-CoA dehydrogenase, 3-Hydroxyacyl-CoA dehydrogenase, Acetyl-CoA acyltransferase, Long-chain acyl-CoA synthetase, Long-chain-fatty-acid—CoA ligase, 1-Acyl-sn-glycerol-3-phosphate acyltransferase	-	Zheng et al. (2013)
Chlamydomonas reinhardtii	Sueoka's high salt medium		Pathway: ribosome biogenesis, peptide metabolic process and RNA processing. Genes: KAR (3-ketoacyl-ACP reductase, and ENR (enoly-ACP reductase, and genes involved in TAG biosynthesis, such as G3PDH (glycerol-3-phosphate dehydrogenase, LPAAT (lyso-phosphatidic acid acyltransferase, and LPCAT (lyso-phosphatidylcholine acyltransferase	Pathway: Photosynthesis (21 genes, nucleoside triphosphate metabolic pathways(6 genes)	Lv et al. (2013)
Fistulifera solaris	f/2	454 Titanium and 454 Titanium XL	Pathway: Glycerol lipid biosynthesis, Fatty acid degradation, Lipid recycling Gene: Monogalactosyl diacylglycerol (MGD), Digalactosyl diacylglycerol (DGD), sulfoquinovosyl diacylglycerol (SQD2)	Pathway: TCA, nitrogen metabolism	Tanaka et al. (2015)
		Illumina	Genes: Malic enzyme, ferrodoxin NADP+ reductase	Genes: G6PD, PGD	Osada et al. (2017)
Nitrogen deprivation					
Botryococcus braunii 779	Bold's modified Bristol (BB)	Next generation sequencing (Illumina HiSEQ 2000)	Pathway: Citrate cycle, glycolysis, gluconeogenesis, pentose phosphate pathway, carbon fixation metabolism Genes: ammonia permease, glutamine synthases, and glutamate synthases	Pathway: Photosynthesis (light harvesting), ribosomes, nitrogen metabolism	Fang et al. (2015)
Botryosphaerella sudeticus			Genes: Lipid metabolism enzymes: monoacylglycerol lipase (EC 3.1.1.23), 3-oxoacyl-[acyl-carrier-protein] reductase (EC 1.1.1.100)	Pathway: Photosynthesis	Sun et al. (2013)
Botryococcus braunii UTEX 572	Chu-13	qRT-PCRs	Genes: Oleic acid synthesis- stearoyl-ACP desaturase gene	-	Choi et al. (2011)
C. reinhardtii	TAP medium	Next generation sequencing (Illumina Genome Analyzer II)	Pathway: Lipid metabolism, Glycolysis Genes: Diacylglycerol acyltransferases (DGTT3), ammonium transporter (AMM4), Protein kinase activity, Protein-Tyr kinase activity, Protein Ser/Thr kinase.	Pathway: Photosynthesis- Cyclophilin, PSI reaction center, DNA replication initiation.	Miller et al. (2010)
	Sueoka's high saltmedium	Semi quantitative RT-PCRs	Genes: Diacylglycerol:acyl-CoA acyltransferases (DGTT1, DGTT3, DGTT4)	Genes: 3-ketoacyl-ACP Synthase I (KASI), 3-ketoacyl-ACP reductase (KAR)	Msanne et al. (2012)
	TAP medium	Next generation sequencing (Illumina HiSEQ 2000)	Gene: Putative palmitoyl-protein thioesterase-encoding gene, TAG lipases, DGAT1, DGAT2 and Phospholipid diacylglycerol acyltransferase (PDAT1)	Pathway: Membrane lipid synthesis Gene: α-Carboxyltransferase, β-Carboxyltransferase, Acetyl-CoA biotin carboxyl carrier, Acyl carrier protein, UDP-sulfoquinovose synthase, Sulfoquinovosyl diacylglycerol synthase, malonyl CoA-acyl carrier protein transferase	Boyle et al. (2012)
			Genes: NADPH/NADP+ ratio upregulating genes, DGAT 1, DGAT2, lipases, saposin, mitochondrial glycerol-3-phosphate dehydrogenease, TAG lipases	Genes: Chaperons, carbon metabolism genes, periplasmic carbonic anhydrase 1, PDC, ACCase, malonyl-coA: ACP, KAR, HAD, chlorophyll genes, isoprenoids genes, porphyrin genes, glycerolipids genes	Garcia de Lomana and Baliga (2010)
Chlorella sorokiniana	Kuhl medium	Illumina Hiseq2000 and RT-PCR	Gene: RBCL, phosphoglycerate kinase (PGK), DGAT, biotin carboxylase, KAS II, KAS III, KAR, DGAT	Gene: Starch synthase, ACCase, MAT, 1,4,α-glucan branching enzyme	Li et al. (2016)
Dunaliella tertriolecta	f/2	Illumina MiSeq	Pathway: Glutamate synthesis, Lipid biosynthesis	Pathway: Photosynthesis,C1 metabolism, TCA cycle Gene: FKBP-peptidyl-propyl cis-trans isomerase, FKBO-methyl transferase, Glutamate-1-semialdehyde amino transferase, methylenetetrahydrofolate reductase (MTHFR), Gamma-glutamyl hydrolase (GGH), met E, Phosphoenol pyruvate carboxylase (PEPC), Malate dehydrogenase (MDH), Pyruvate dehydrogenase (PDH).	Shin et al., 2015

(*continued on next page*)

Table 2 (*continued*)

Microalgae	Cultivation media	Method used for analysis	Genes/pathway up regulated	Genes/pathway down regulated	Reference
Nannochloropsis		SoLiD	Gene: Glutamine synthase, glutamate synthase, glutamine amido transferase, guanine deaminase, cysteine synthase, cullin, Ubiquitin specific proteases, Autophagy-related proteins, ACCase	Gene: NADH dehydrogenase, α-ketoglutarate dehydrogenase	Carpinelli et al. (2014)
		Illumina Hiseq2000	Pathway: Channels and vascular trafficking proteins. Gene: DGAT-2A/2B, PDAT, Galactolipase gene (PSD1), lipases genes, ACP gene, KAS I	Pathway: Photosynthesis, DNA replication, protein folding/ modifications Gene: ACCase, HAD	
Neochloris oleoabundans	Modified Bold-3 N	Illumina Hiseq2000	Pathway: Carboxylic acid, lipid biosynthetic process, NADPH regeneration, pentose-phosphate pathway, phospholipid, metabolic process, lipid transport, nitrate metabolism and nitrate assimilation. Genes: Biotin carboxylase, malonyl-CoA ACP transacylase (MAT), beta-ketoacyl-ACP synthase (KAS), beta-hydroxyacyl-ACP dehydrase (HAD), enoyl-ACP reductase (EAR), thioesterases oleoyl ACP hydrolase (OAH), Acyl-ACP thioesterase A (FatA), acyl-ACP desaturase (AAD), glycerol-3-phosphate acyltransferase (GPAT), acyl-glycerol- 3-phosphate acyltransferase (AGPAT), phospholipases, pyruvate kinase dehydrogenase complex	Pathway: carbon fixation, photosynthesis, protein synthesis, fatty acid degradation, and starch synthase Genes: ACCase, beta-ketoacyl-ACP reductase (KAR), delta-12 desaturase, acyl-CoA oxidase (ACOX1), acetyl-CoA acetyltransferase (ACAT), AGPase,α-amylase	Rismani-Yazdi et al. (2012)
Phaeodactylum tricornutum	ASPII		Genes: conserved cyclin (cyc B1), nitrate/ammonia/urea transporters, glutamate dehydrogenase, glutamate synthetases, malic enzyme,α-carbonic anhydrase, β oxidation genes, fatty acid chain modification genes, acyl-ACP desaturase	Gene: Carbamoyl phosphate synthase, LHC genes, Chlorophyll a genes, TCA genes, Pyruvate kinase-6, ACCase, lipid particle protein	Valenzuela et al. (2012)
	f/2-Si		Pathway: nitrogen fixation, carbon fixation, glycolysis and the TCA cycle Genes: ammonium transporters, glutamine synthase, nitrate reductase, ferredoxin-nitrite reductase, light harvesting complex, fucoxanthin chlorophyll a/c protein, phosphoenolpyruvate carboxylase, malic enzyme, fructose-1,6-bisphosphatase, isocitrate dehydrogenase, citrate synthase, isocitrate dehydrogenase, diacylglycerol acyltransferase, phospholipid:diacylglycerol acyltransferase	Pathway: photosynthesis, gluconeogenesis, glyoxylate cycle, chrysolaminarin synthesis and sucrose metabolism. Genes: Ferredoxin-NADP + reductase, isocitrate lyase, malate synthase, phosphoenolpyruvate carboxykinase, Lipases	Yang et al. (2015a, 2015b)
C. vulgaris var L3	modified Bold's Basal Medium	Real Time-PCR analysis	Genes: Nitrate reductase, malic enzyme	Genes: RuBisCo, ADP-glucose pyrophosphorylase (AGPase), starch phosphorylase, ATP citrate lyase, phosphoenolpyruvate carboxylase (PEPCase), biotin carboxylase subunit of the heteromeric form (accC).	Ikaran et al. (2015)
Micractinium pusillum	High Salt Medium		Genes: glyceraldehyde 3-phosphate dehydrogenase, glycogen or starch phosphorylase, aldose 1-epimerase, strombine dehydrogenase, dihydrolipoamide dehydrogenase, acyltransferase 3, diacylglycerol kinase, nitrate reductase, cytochrome cd1-nitrite reductase-like superfamily, NADH:cytochrome b5 reductase	Genes: 3-isopropylmalate dehydrogenase, nucleoside diphosphate kinase 1, checkpoint 1-like protein, light-harvesting complex I, RNA (uracil-5-) methyltransferase/TrmA	Li et al. (2012)
Tisochrysis lutea (lipid mutant)	Modified Conway medium	Illumina sequencing	Gene: GDLS lipase	Gene: Long chain fatty acid ligase (ACLS)	Carrier et al., 2014
Monoraphidium neglectum	ProF medium	Illumina	Pathway: Glycolysis, TCA Gene: PEP carboxylase	Pathway: Photosynthesis, gluconeogenesis, Protein synthesis	Jaeger et al. (2017)
Tetraselmis M8	F/2	Next generation sequencing (Illumina HiSEQ 2000)	Pathway: Glycolysis, Lipid Genes: HD, KAS, LPAAT, LPAT,	Pathway: Carbon metabolism, Photosynthesis, Protein synthesis, TCA Genes: Fructose 1, 6 bisphosphate, Fructose 1, 6 bisaldolase, ENR, KAR, DGAT	Lin et al. (2012)
Carbon dioxide deprivation (> 5 % CO_2)					
Chlorella pyrenoidosa	BBM	Next generation sequencing (Illumina HiSEQ 2000)	Genes: Phosphoenolpyruvate carboxylase, malate dehydrogenase, malic enzyme, pyruvate phosphate, pyruvate orthophosphate dikinase, acetyl coenzyme A, nitrite transporter, ABC transport protein, carbonic anhydrase	Genes: fructose-2,6-bisphosphatase (FBP), triosephosphate isomerase (TIM), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ribose-5-phosphate isomerase, malate synthase, acyl-ACP desaturase, g518 gene,	Fan et al. (2016)
Salinity Stress (1 M NaCl)					

(*continued on next page*)

Table 2 (*continued*)

Microalgae	Cultivation media	Method used for analysis	Genes/pathway up regulated	Genes/pathway down regulated	Reference
Picochlorum strain SENEW3	Artificial seawater based Guillard's f/2 medium	MiSeq Personal Genome Sequencer (Illumina)	Pathway: Photorespiration, proline synthesis, Nitrate and urea assimilation, Starch synthesis Genes:Glycolate dehydrogenease	Genes:Glycolate oxidase	Foflonker et al. (2016)
Mutant					
Dunaliella tertilolecta	Basal media + 0.5 M NaCl	Illumina	Pathway: Photosynthesis, ATP synthesis, Inositol phosphate metabolism Gene: ACCase,	Gene: FabG, 3-oxoacyl-(ACP) reductase	Yao et al. (2015)
C. vulgaris	BBM	Illumina	Gene: Nitrate reductase, Siroheme synthase, superoxide dismutase, G6PDH, GPI, ADH, E3 ubiquitin	-	Sarayloo et al., (2017)
D. tertriolecta	f/2	Illumina Hiseq2000	Pathways: Photosynthesis, glycolysis Genes: cytochrome b6f, LHCAI, LHCBI, fructose 1, 6 bisphosphae	Pathways: valine, leucine and Isoleucine degradation, Genes: Acetyl CoA transferase,	Yao et al., 2017
Cold stress					
C. reinhardtii (diploids-colcemid treated)	TAP	Illumina Hiseq2000	Genes: Ribosomal proteins, PS I, PS II, LHC, NADH dehydrogenase, ATP synthases, NADPH-ubiquinone oxidoreductase, Triose phosphate isomerase, Sedoheptulose 1, 7 bis phosphatase, Fructose 1, 6 bisaldolase, RuBisCo, SNF related kinase I, cytosolic ribosomal protein L22	Genes: Sucrose synthase, AGPase, Transketolase, aconitase, pyruvate kinase	Kwak et al., (2017)
UV stress					
Chlorella sp. UMACC 237	BBM	Illumina	-	Pathways: Fatty acid degradation, valine, leucine and Isoleucine degradation, sucrose and starch metabolism Genes: Superoxide dismutase, catalase, trehalose 6 phosphate synthase	Poong et al., (2017)
Silicon deficiency					
Thalassiosira pseudonana	Artificial sea water	Microarray and Illumina	Pathway: Photorespiration, Calvin-Benson cycle, glycolysis, pigment biosynthesis Genes: ACCase, DGAT1, FAS II, LPLAT/AGPAT	Pathway: Cell division. Photosynthesis, Translation, Ribosome	Smith et al., (2016)

Tertraselmis M8 *and Neocloris oleoabundans,* highlighting the potentially unique biosynthetic mechanisms employed by diverse algae (Li et al., 2016; Y. Li et al., 2014a, 2014b; López et al., 2015; Msanne et al., 2012; Rismani-Yazdi et al., 2012; Lim et al., 2017). Furthermore, various transcriptomics studies in both diatom and green microalgae have revealed the vital role of lipid recycling (as opposed to *de novo* lipid synthesis) in increasing the overall TAG accumulation during nitrogen deprivation. The genes which showed overexpression during lipid conversion from chloroplast glycerolipids encoded the enzymes PDAT, 3-*sn*-phosphatidate phosphohydrolase (PAP), MGD synthase, digalactosyl diacylglycerol synthase (DGD 1n), SQD2 synthase, galactolipase gene and fatty acid chain modification genes, indicating that membrane remodeling is indeed employed during nitrogen depletion in microalgae (Tanaka et al., 2015; Li et al., 2014a, 2014b; Yang et al., 2013). It must be noted that total fatty acid content can increase as much as threefold during nutrient starvation, indicating that lipid recycling is not the only mechanism at work (Laurens et al., 2014).

Nitrogen deprivation induces a physiological response which increases algal nitrogen assimilation rates by upregulating the transcripts of glutamine synthase, glutamate synthase, glutamine amido transferase, nitrate/ammonia/urea transporters, glutamate dehydrogenase and ammonia permease (Sun et al., 2016; Yao et al., 2015; Carpinelli et al., 2014; Yang et al., 2013; Valenzuela et al., 2012). Glutamine synthase (GS) has a high affinity for ammonia and catalyzes the integration of ammonia into glutamine which in turn may be acted upon by glutamate synthase to transfer the amido group from glutamine to 2-oxoglutarate (Carpinelli et al., 2014). This forms two molecules of glutamic acid which can then re-enter the cycle. Moreover, an increase in the levels of guanine deaminase (catalyzing the conversion of guanine to xanthine and ammonia) and cysteine synthase (catalyzing interconversion of serine and cysteine) increases the intracellular pool of ammonia inside the microalgal cells during nitrogen deprivation (Carpinelli et al., 2014). This increase in nitrogen assimilation genes occurs in parallel to decrease in photosynthetic activity by down regulation of light harvesting complex genes (LHC), chlorophyll-a genes, ribosome genes, and FK-506 (FKBP) binding protein subfamily genes, which are prerequisite for the synthesis of chlorophyll (Sun et al., 2016; Fang et al., 2015; Li et al., 2014a, 2014b; Yang et al., 2013). Apart from the aforementioned pathways, genes involved in protein degradation such as cullin, ubiquitin specific proteases, endopeptidases, aminopeptidases, and autophagy-related proteins have been observed to be upregulated in an array of microalgae under nitrogen deprivation, while chaperon gene expression decreases, indicating the enhancement in protein misfolding rates and endoplasmic reticulum stress, thus linking protein recycling to TAG biosynthesis (López et al., 2015; Li et al., 2014a, 2014b; Rismani-Yazdi et al., 2012). Using various pathways, algal cell recycles intracellular nitrogenous compounds (amino acids, proteins, chlorophyll, nucleic acids) and assimilates small amounts of extracellular nitrogen into critical metabolites, thereby allowing the cell to repartition the dwindling nitrogen supply based on evolution-based survival strategies during growth in nitrogen deprived conditions.

The CO_2 concentrating mechanism (CCM) plays a critical role in maintaining carbon flux in microalgae under stress. CCM can be classified into two categories, biophysical and biochemical. The biophysical mode utilizes direct transport of inorganic carbon across cell membranes using transporters and ribulose-1,5-bisphosphate carboxylase/oxygenase RuBisCO, while the biochemical mode utilizes a C4-CCM mechanism, involving active transport of inorganic carbon across the cell membrane and local enhancement of CO_2 concentration (Moroney and Somanchi, 1999). The biophysical mode involves conversion of carbon dioxide and ribulose bisphosphate into 3- phosphoglycerate. On the other hand, the biochemical mechanism involves the active spatial separation of the fixation of CO_2 by phosphoenolpyruvate (PEP) carboxylase resulting in a C4 dicarboxylic acid with carbonic anhydrase causing the interconversion of CO_2 and bicarbonate (Valenzuela et al., 2012). An increase in the expression levels of the α-carbonic anhydrase gene was reported in *C. reinharditii* and *P. tricornutum* under nitrogen derivation, and in *C. pyrendoidosa* under low CO_2 conditions indicating activation of biochemical CCM during lipid accumulation (Fan et al., 2015; López et al., 2015; Valenzuela et al., 2012).

Due to the decrease in cell growth by the microalgal cells during nitrogen deprivation, an apparent decrease in genes involved in C1 metabolism, TCA cycle, gluconeogenesis, starch synthesis and pentose phosphate pathway have been widely reported (Li et al., 2016; Sun et al., 2016; Tanaka et al., 2015; Valenzuela et al., 2012; Carpinelli et al., 2014; Lim et al., 2017). Major genes involved in these pathways were phosphoenol pyruvate carboxylase (PEPC), malate dehydrogenase (MDH), pyruvate dehydrogenase (PDH), glyceraldehyde phosphate dehydrogenase, transketolase, aconitase, isocitrate dehyrdrogenase, oxoglutarate dehydrogenase, succinyl CoA lyase, succinate dehydrogenase and fructose 1-6- bisphosphatase respectively. PEPC fixes CO_2 into phosphoenol pyruvate to make oxaloacetate (global substrate for gluconeogenesis, urea cycle, and TCA) while MDH catalyzes the reversible reaction of malate to oxaloacetate and PDH converts pyruvate to acetyl CoA (Sun et al., 2016). Furthermore, there are reports of a decrease in transcripts of starch synthase and 1,4, α-glucan branching enzyme indicating reduction of starch synthesis with simultaneous increase in malic enzyme which converts malate to pyruvate with formation of NADPH (nicotinamide adenine dinucleotide phosphate), thereby increasing TAG accumulation in *P. tricornutum* and *C. sorokiniana* (Li et al., 2016; Yang et al., 2013; Valenzuela et al., 2012). In contrast to the above findings, researchers have observed increases in the transcripts levels of genes involved in TCA cycle under nitrogen depletion (Fang et al., 2015; Yang et al., 2013). Further, the transcriptomic studies on the lipid mutant of *Dunaliella tertiolecta* showed an increase in the transcripts of genes related to photosynthesis, ATP (adenosine triphosphate) synthesis, and ACCase, as well as decreases in gene expression for the Fab G gene, encoding 3-oxoacyl-(ACP) reductase, thereby leading to accumulation of short chain/unsaturated fatty acids (Yao et al., 2015).

3.3. Proteomics

Transcriptional profiling of mRNAs represents only transient changes in the expression of genes, but many of the key regulatory pathways differ at the post-transcriptional level. Further, the correlation between transcriptomic and proteomic data has been reported to be less than 50%, which could be partly due to insufficient protein turnover (Choi et al., 2013a). Thus, in-depth understanding of stress-induced TAG accumulation in microalgae ultimately requires integration of transcriptomics and proteomics. Quantitative algal proteomics identifies and quantifies the dynamics of protein abundance and its corresponding function both at translational and post-translational level in response to any environmental stress leading to augmentation of TAG.

As noted above, nitrogen starvation is a well-documented trigger for TAG accumulation in microalgae, thus it has been exploited the most for studying the changes in proteome of various algae and diatoms as listed in Table 3. These studies have reported numerous differentially abundant proteins under nitrogen deplete/replete conditions, resulting in the alteration of various biochemical pathways such as carbohydrate metabolism, photosynthesis, cell division, nitrogen assimilation, catabolic processes, protein degradation/folding, translation, nucleotide metabolism, and energy precursors, concomitantly increasing the intracellular lipid content (Fig. 2). Among these differentially expressed proteins, the common listed upregulated proteins involved in fatty acid metabolism in various algal species were ACP, malonyl-CoA:ACP transacyclase, lipid droplet surface protein (LDSP), ACCase, MAT (malonyl-CoA acyl transferase), enoyl-acyl carrier protein reductase (Fab I), trans-2 enoyl CoA reductase, and the four condensing enzymes involved during fatty acid synthesis (KAS, HD, ENR and DGAT). The

Table 3 Summary of proteomics studies done in various microalgae/diatoms under different stress conditions for understanding differential protein expression.

Microalgae	Cultivation media	Method used for analysis	Limitation/mode	Reference
Chlorella vulgaris	BBM	GeLC/MS	Nitrogen replete/Nitrogen deplete	Guarnieri et al., (2013)
			Nitrogen deplete	Guarnieri et al., (2011)
	Watanabe media + NaCl	2DE/MALDI-TOF	heterotrophic-Na^+induction two-step regime	Li et al., (2015)
C. protothecoides	BCM + 10g/L glucose		Copper stress	Li et al. (2013a, 2013b)
	Basal medium		Two stage- Nitrogen deplete	Li et al. (2014a, 2014b)
	Watanabe medium		Heterotrophic- Nitrogen deplete	Li et al. (2013a, 2013b)
			Heterotrophy- photo induced	Li et al., (2015)
C. sorokiniana	BG11	nanoLC-MS/MS	Light intensity/inoculum size	Ma et al., (2013)
Chlamydomonas reinhardtii	TAP	2DE/MALDI-TOF	Lipid mutants	Choi et al., (2013)
				Lee et al., (2014a, 2014b)
				Velmurugan et al., (2014)
C. reinhardtii cw15 sta1-2				Nguyen et al., 2011
C. reinhardtii strains cw15 and sta6		iTRAQ and nanoLC-MS/MS	Nitrogen deplete	Wang et al., 2012
C. reinhardtii (CCAP 11/32CW15+)		HILIC- and SCX		Longworth et al., (2012)
Chlamydomonas strain CC125		iTRAQ and nanoLC-MS/MS		Wase et al., (2014)
C. reinhardtii CC-503 cw mt+	TAP	GeLC/MSMS	Salinity	Siththisarn et al. (2017)
C. reinhardtii CC 1618 cw15, arg7 mt-		LC MS/MS		Mastrobuoni et al., 2012
Scenedesmus dimorphus	BBM	2DE/MALDI-TOF	Lipid mutants	Choi et al., 2013
Tisochrysis lutea	Walne's medium			Garnier et al., (2014)
Phaeodactylum tricornutum	F/2 + Si medium	LC-MS/MS	Nitrogen deplete	Longworth et al., (2016)
Nannochloropsis oculata	Artificial seawater enriched with f/2 medium			Tran et al., (2016)
Neochloris oleoabundans	BBM		Heterotrophic- Nitrogen deplete	Morales-Sánchez et al., (2016)
Nannochloropsis oceanica IMET1	Artificial seawater medium	2DE/MALDI-TOF	Nitrogen deplete	Dong et al., (2013)
Phaeodactylum tricornutum	F/2 + Si medium			Yang et al., (2014)
		NanoLC-MS/MS		Ge et al., (2014)
Isochrysis galbana		2DE/MALDI-TOF		Song et al., (2013)
Fistulifera sp. Strain JPCC DA0580	10 f medium	nanoLC-MS/MS	Oil body associated proteins	Nojima et al., (2013)
Dunaliella salina	Modified medium with 1M NaCl	LC MS/MS	Salinity stress	Wei et al., (2017)
Dunaliella parva	f/2	iTRAQ	Nitrogen deplete	Shang et al., 2017
Chlorella sp. FC2IITG	BG11	2D and iTRAQ	Two stage nitrogen deprivation	Rai et al., (2017)

LDSP plays a key role in the formation and is a structural component of lipid droplets similar to oleosins in the higher plant (Tran et al., 2016). Such an increase in the above-mentioned protein levels occurred simultaneously with a decline in the levels of adenosine monophosphate activated kinase (AMPK), fatty acid catabolism (acyl CoA dehydrogenase) and stearoyl-ACP desaturase respectively. AMPK inhibits the ACCase activity via phospho-regulation it and so a decrease in its level implicates increased activity of ACCase, which catalyzes the first step of fatty acid biosynthesis (Guarnieri et al., 2013). Furthermore, stearoyl-ACP desaturase catalyzes the formation of oleoyl ACP from stearyl-ACP resulting in a decrease in polyunsaturated fatty acids and an increase in unsaturated and mono saturated fatty acids during nitrogen deprivation (Dong et al., 2013). It should be noted that increase in protein levels under nitrogen deplete growth conditions require the recycling of amino acids from other proteins, indicating the evolutionary and mechanistic importance of these proteins as a means to mitigate nutrient starvation.

Parallel to increased fatty acid biosynthesis during nitrogen stress, researchers have commonly reported declines in photosynthesis related proteins such as RuBisCO subunits, cytochrome c/b6, ribosomal proteins, uroporophyrogen decarboxylase (synthesis of porphyrin), coproporphyrinogen III oxidase (chlorophyll metabolic pathway), geranyl geranyl pyrophosphate synthetize (GGPP; carotenoid synthesis), light harvesting components, violaxanthin/chlorophyll a binding protein, ferredoxin NADP(+) reductase (Fd-$NADP^+$), carbonic anhydrase and oxygen evolving enhancer protein (Tran et al., 2016; Longworth et al., 2016; Garnier et al., 2014; Yang et al., 2014; Dong et al., 2013; Li et al., 2013b; Song et al., 2013; Shang et al., 2017). Fd-$NADP^+$ transfers electrons from ferredoxin to NADPH while carbonic anhydrase catalyzes the conversion of CO_2 to HCO_3^- for active transport into the cell, and RuBisCO catalyzes the fixation of CO_2 into the Calvin Cycle. Thus, decreased levels of these three proteins result in drop-off in the rate of carbon fixation in parallel to photosynthesis and photosynthetic pigments. To compensate for this loss, microalga cells increase the levels of plastid ribulose-3-phosphate epimerase under nitrogen deprivation which catalyzes the reversible epimerization of D ribulose-5-phosphate to D-xylulose-5-phosphate, ultimately increasing the yield of RuBP (Ribulose-1,5-bisphosphate), thus promoting carbon fixation and production of 3-phosphate-glyceraldehyade (precursor for TAG) (Yang et al., 2014).

Nitrogen deprivation also impacted carbohydrate metabolism in *Nannochloropsis oceanica* IMET1 by upregulating glycolysis enzymes including phosphoglycerate kinase (PGK), pyruvate kinase (PK), phosphopyruvate hydratase (PPH), glyceraldehyde 3 phosphate dehydrogenase (GADPH), phosphomannomutase (PMM), thereby increasing pyruvate pools (precursor of acetyl co-A) and redirecting the flux to lipid biosynthesis (Yang et al., 2014; Dong et al., 2013). On the other hand, the enzymes involved in the progression of TCA cycle showed enhanced abundance under nitrogen stress including malate dehydrogenase (catalyzes conversion of malate to oxaloacetate), citrate synthase (conversion of acetyl CoA and oxaloacetate to citric acid and CoA), fumarate hydratase (fumaric acid to malic acid), pyruvate carboxylase (pyruvate to oxaloacetate), while decreased levels were seen with malate synthase, isocitrate lyase, succinate dehydrogenase and isocitrate dehydrogenase, causing accumulation of acetyl CoA (Wase et al., 2014; Song et al., 2013). The overexpression of malic enzyme, which generates NADPH through reduction of $NADP^+$ during conversion of malate to pyruvate, was shown to correlate with increased lipid accumulation in microalgae (Li et al., 2014a, 2014b; Guarnieri et al., 2013). An increase in the levels of transaldolase, phosphoglycerate mutase (PGAM) and pyruvate dehydrogenase E1 component further resulted in redirecting carbon flux to lipid synthesis (Li et al., 2014a, 2014b; Yang et al., 2014).

Microalgae also modulate their nitrate assimilation in response to

Table 4 List of metabolomics studies reported in various green microalgae and diatoms to identify potential lipid triggers.

Microalgae	Cultivation media	Method used for analysis	Limitation/mode	Reference
Chlamydomonas reinhardtii	TAP	LC-MS/MS GC-MS	Nitrogen depletion	Blaby et al., (2013) Wase et al., (2014)
Chlamydomonas sp. JSC4	Modified Bold 3 N medium	LC-MS/MS	Light + nitrogen depletion Salinity + nitrogen depletion	Ho et al., (2015) Ho et al., (2014a, 2014b)
C. reinhardtii CC 1618 cw15, arg7 mt-	TAP	LC MS/MS	Salinity	Mastrobuoni et al., 2012
Chlorella vulgaris	LC Oligo medium	HPLC	Nitrogen depletion + cadmium stress	Chia et al., 2015
C. sorokiniana	BG-11	GC-TOF-MS	Inoculum size	Lu et al., (2012)
Scenedesmus obliquus, *Synechocystis sp.* PCC6803 *Anabaena sp.* PCC7120			-	Cheng et al., 2012
Schizochytrium sp.	-	NMR + GC-MS	-	Mioso et al., (2014)
Pseudochoricystis ellipsoidea	A5	LC-MS/MS	Nitrogen depletion	Ito et al., (2013)
Aurantiochytrium sp.	GPY		6-Benzylaminopurine (6-BAP)	Yu et al., 2016
Crypthecodinium cohnii	Basal medium + 9 g/L glucose + 25 g/L sea salt	GC-MS + LC-MS/MS	Butylated hydroxyanisole (BHA)	Sui et al., (2014)

nitrogen stress by overexpressing nitrate reductase (reduces NO_3^- to NO_2^-) and glutamate dehydrogenase, potentially increasing nitrogen assimilation capacity (Li et al., 2014a, 2014b; Dong et al., 2013). Further, a universal increase in the activity of ATP synthase has been reported indicating increase in the synthesis of ATP providing energy to the cell during adverse conditions. Nitrogen depletion resulted in decrease in the levels of DNA polymerase, RNA polymerase, elongation factor with concurrent increase in various heat shock proteins, which play a vital role in protection of cell's integrity by stabilizing proteins and membrane (Longworth et al., 2016; Li et al., 2014a, 2014b; Yang et al., 2014; Li et al., 2013b). Longworth, et al., reported increases in low CO_2 inducible proteins in *C. reinhardtii* during a switch from autotrophic to heterotrophic mode during nitrogen starvation with subsequent utilization of acetate available in the medium or metabolism of intracellular carbohydrates (Longworth et al., 2012).

Apart from changes in the proteome of microalga in response to nitrogen stress, other effects from environmental stimuli have also been investigated including copper deprivation, light intensity/inoculum size, and heterotrophic cultivation modes (Table 3). Lastly, the effect of salt stress on the proteomic profile of *C. vulgaris* studied by Li et al. reported an increase in Mao-C like protein (modulates fatty acids), Spp30 like protein (transports lipid to Golgi for packaging and profiling to maintain cell structure integrity) and glycine rich-like proteins (which form an independent structure within the extracellular matrix to assist in proper cell wall assembly), resulting in overall increase in lipid accumulation (Li et al., 2015). Interestingly, they also reported an increase in 60S ribosomal protein L5 and 40S ribosomal protein S12 which was contradictory to the nitrogen depletion assisted lipid accumulation in microalgae. The possible reason might be that an increase in these ribosomal proteins could promote functional restructuring of protein synthesis in *C. vulgaris* under salt stress.

Additionally, the availability of *C. reinhardtii* starchless and lipid mutants has been exploited for studying changes in expression of proteins relative to the parent strains (Garnier et al., 2014; Lee et al., 2014a, 2014b; Choi et al., 2013a; Wang et al., 2012). Most of the responses reported in these lipid mutants were similar to the observations obtained under nitrogen starvation, such as activation of glycolysis, TCA, heat shock, ATP synthase, and fatty acid biosynthesis proteins, along with decreases in the abundance of ribosomal, photosynthetic, nucleotide synthesis proteins. However, thiamine metabolic enzymes represented one of the few differences in protein expression observed in in starchless mutant of *C. reinhardtii* (sta 6) as compared to wild type (cw15) (Wang et al., 2012). Elevation in the expression of two main proteins involved in thiamine biosynthesis (hyroxymethyl pyrimidine phosphate synthase and thiazole biosynthetic enzyme) indicate a decrease in carbon fixation and sugar synthesis by the mutant strain, as thiamine plays a vital role in intermediary carbon metabolism (Wang et al., 2012). Further, Choi et al. reported an increase in 3-methyl-2-oxobutanoate hydroxymethyl transferase in ethyl methane sulfonate (EMS) of *Scenedesmus obliquus* indicating an escalation in pantothenate biosynthetic pathway, thereby increasing the synthesis of coenzyme-A and metabolism of proteins and fats, resulting in accumulation of lipids inside the microalga (Choi et al., 2013a).

3.4. Metabolomics

The metabolome is the qualitative and quantitative collection of all low molecular weight compounds such as amino acids, carbohydrates, nucleotides, organic acids, energy compounds, fatty acids, lipids, and cofactors that are responsible for maintaining cell's biological processes (Dunn and Ellis, 2005). These metabolites are products of cellular regulatory processes including those implicated by transcriptomics and proteomics, but also involving regulation of enzyme activities through feedback-mediated mechanisms. Metabolomic analysis can thus shed additional light into a cell's response towards any environment stimuli, and offer additional insight for strain engineering considerations (Ito et al., 2013). Measurement of metabolites does not require prior knowledge of an organism's genomic information and can therefore be an ideal omics tool for evaluating cellular responsiveness of any non-model organism, though a more complete picture can be derived from integrated analysis of multiple omics platforms. However, accurately measuring the metabolite profile of an organism exposed to different environment cues is cumbersome due to wide variation in the chemical properties including polarity, solubility, volatility, ionic charge and molecular weight (Ito et al., 2013). To combat this scenario, various techniques to quantify the metabolome of an organism have been developed such as capillary electrophoresis mass spectroscopy (CE-MS), gas chromatography- mass spectroscopy (GC-MS), liquid chromatography- mass spectroscopy (LC-MS), nuclear magnetic resonance spectroscopy (NMR), and Fourier transform ion cyclotron resonance- mass spectroscopy (FTICR-MS) (Dunn and Ellis, 2005).

Recently, metabolomics has been applied to identify biochemical targets that are differentially synthesized during lipid accumulation in various microalgae and diatoms upon exposure to different conditions (Table 4), complementing the existing knowledge bases generated via transcriptomics and proteomics analyses. In the case of nitrogen deprivation, an increase in the levels of citrate, malate, succinate, fructose 1, 6 bisphosphate, glucose 6 phosphate, isocitrate, and 2-oxoglutarate are observed, with a concurrent decrease in amino acids (phenylalanine, tryptophan, aspartate, arginine, glutamine, proline, ornithine, citrulline and asparagine) indicated activation of glycolysis and amino acid recycling occurring in parallel with intracellular lipid accumulation (Wase et al., 2014; Blaby et al., 2013; Ito et al., 2013; Chen et al., 2017). Further, Wase et al. reported an increase in trehalose, a non-reducing disaccharide which helps in stabilizing cellular membranes and proteins maintaining cell's integrity through osmoregulation (Wase

et al., 2014). Interestingly, the metabolic profile of *C. vulgaris* altered under simultaneous nitrogen deprivation and cadmium stress showing an increase in the free amino acids (proline, valine, isoleucine, sarcosine, phenylalanine, methionine), which could be due to microalga's defense mechanism against cadmium (Chia et al., 2013). Again, it is important to note that regulatory mechanisms to increase levels of specific amino acids relative to others during nitrogen starvation indicates an important evolutionary response by cells. Sarcosine, glycine, valine, thioproline, methionine, phenylalanine, glutamine and ornithine help in the complexation of heavy metal on microalgal cell membrane while glutathione helps in synthesizing phytochelatins which in turn quench the reactive oxygen species (ROS) generated due to heavy metal stress inside the cells (Meharg, 2002). Proline is a well-known osmoregulatory molecule playing a vital role in scavenging free radicals; it also serves as a stabilizer and electron sink alleviating side effects caused by heavy metal on microalgae (Zoghlami et al., 2013). Likewise, an increase in metabolites playing a crucial role in augmenting TAG accumulation (ethanolamine, glycerol, glycerol 3-phosphate, acetyl Co-A, 3 phosphoglyceric acid, 2-ketoglutaric acid) was reported by Ho et al., Sui et al. and Yu et al., while studying effects of salinity, light intensity, 6-benzyl amino purine (phytohormone), and butylated hyroxyanisole (anti-oxidant) on different algal species (Yu et al., 2016; Ho et al., 2015; Ho et al., 2014a, 2014b; Sui et al., 2014). The above discussed studies on the metabolic profiles offer a snapshot of various biomarkers that accompany TAG accumulation, thus improving the basic understanding of the systemic response.

3.5. Lipidomics

Lipidomics is a branch of metabolomics employed to identify and differentiate classes of lipids, as well as the molecules that interact with these lipids (Su et al., 2013). Both LC-MS and chemometric methods combined with multivariate analysis are the most widely used techniques for carrying out lipidomics (Su et al., 2013). Characterizing the dynamics in the lipidomic in response to different environmental cues can help in understanding not only the lipid metabolism of microalgae but may also inform manipulation of the lipid yield and profile to achieve desirable results for favorable biofuel production metrics (Melo et al., 2015). To date, there are limited studies that have characterized changes in the lipidome in response to various stress conditions such as temperature, salt, and nutrient depletion. These studies identified a series of lipid biomarkers including free fatty acids, harderoporphyrin, phosphatidylglycerol, 1,2 diacylglycerol-3-0-4′-(*N, N*-trimethyl)-homoserine, TAG, cholesterol, sulphoquinovosyldiacylglycerol, lysosulphoquinovosyldiacylglycerol, digalactosyldiacylglycerol and lysodigalactosyldiacylglycerol, that are differentially regulated in order to adapt to the given environmental stress in various microalgae (*Nitzschia closterium f. minutissima*; *C. reinhardtii*, *N. oceanica* IMET1, *Dunaliella tertiolecta*, *Chloromonas*, *Chlamydomonas nivalis*, respectively) (Yang et al., 2015a, 2015b; Lee et al., 2014a, 2014b; Li et al., 2014a, 2014b; Lu et al., 2012, 2013; Su et al., 2013).

Additionally, recent studies have characterized integral proteins attached with lipid droplets that aids in oil globule formation and interaction with other organelles (Peled et al., 2011). Among the integral proteins, the presence of oleosin (structural protein) and caleosin (calcium binding lipid body protein) have been reported in various green algae including *C. reinhardtii*, *D. salina*, *H. pluvialis*, *C. variablis*, *Coccomyxa sp. C-169*, *Chlorella*, *N. oceanica*, *N. gaditana*, *N. granulata*, and *N. salina* (Goold et al., 2015; Tsai et al., 2015; Huang et al., 2013; Davidi et al., 2012; Nguyen et al., 2012; Lin et al., 2012; Vieler et al., 2012; Peled et al., 2011; Moellering and Benning, 2010). Most of these studies have identified and functionally characterized Major Lipid droplet protein (MLDP) and Lipid Droplet Surface protein (LDSP). MLDP, a 28 kDa hydrophobic protein was shown to provide integrity to the lipid droplets by interacting with tubulins while its suppression (~60% reduction in MLDP gene expression) resulted in 40 % increase in lipid droplet size with concurrent reduction in lipolysis (Tsai et al., 2015; Moellering and Benning, 2010). Interestingly, increases in lipid droplet size did not increase the overall TAG accumulation inside the microalga. On the other hand, LDSPs are actively involved in formation and stabilization of lipid droplets as its expression was directly proportional to accumulation of TAGs (Vieler et al., 2012), underscoring the potential value in such lipidomic approaches. However, this microalgal field is still in its infancy and further detailed studies are imperative for identifying potential biomarkers that could be exploited for enhancing lipid yield in microalgae for biofuel production.

4. Conventional genetic engineering strategies to improve lipid accumulation in microalgae

Transgenic microalgae are gaining focus due to their potential to provide researchers with the opportunity to reconstruct and remodel the microalga's lipid biosynthetic (and related) pathways to examine and develop high TAG accumulating phenotypes. In recent years, microalgae have been successfully transformed using various genetic tools such as random integrative selection marker and homologous recombination (HR)-mediated DNA incorporation and RNA silencing via electroporation and biolistic transformation (Ghosh et al., 2016). Microalgae can be transformed either in the nuclear, chloroplast or mitochondrial genomes depending on the type of gene/construct to be expressed or deleted (Gimpel et al., 2015). For example, enzymes related to secondary metabolism can be engineered for differential expression in either nuclear or plastid genomes. Transformation in the nuclear genome offers the advantage of post-translational modification along with simple protocols and high flexibility as it allows the use of heterologous promoters and untranslated regions (Gimpel et al., 2015). However, it could result in low expression of genes due to copy number, gene silencing, and positional effects. On the other hand, though plastidial expression has limited post-translation modification potential, and often strict codon bias, it offers multi-copy gene expression along with precise targeting by aiding high(er) efficiency homologous recombination and gene knockout (Gimpel et al., 2015).

Despite technological advancements, only a few microalgal strains have been metabolically engineered for enhanced TAG accumulation; these include *Cyclotella cryptica*, *P. tricornutum*, *T. pseudonana*, *C. reinhardtii*, *Chorella minutissimsa*, *C. ellipsoidea*, and *N.gaditana*. (for detailed list see review by (Banerjee et al., 2016; Gimpel et al., 2015; Ho et al., 2014a, 2014b). Recently, *D. tertiolecta*, *C. reinhardtii* (CC-849) and *Nannochloropsis salina* have been genetically engineered to increase their intracellular lipid content (Fei et al., 2017; Kang et al., 2017; Wang et al., 2017; Wei et al., 2017). There are four major routes to increase lipid production in microalgae proposed to date: (a) enhancing flux and/or biosynthetic rate of fatty acid metabolism, (b) inhibiting competing carbon pathways (e.g. starch synthesis) (c) improving energy efficiency and carbon uptake and (d) reducing TAG catabolism. To date, most of the recombinant studies for increasing lipid accumulation in microalgae have targeted enhanced flux via overexpression of lipid biosynthetic genes; ACCase was the first gene to be overexpressed (3-4 fold) in diatom *C. cryptica* and then in *Navicula saprophila,* though neither case resulted in an increase in TAG accumulation (Dunahay et al., 1995; Schuhmann et al., 2016). Similar efforts have been made in other microalgal strains (*C. reinhardtii*, *P. tricornutum*) by overexpression of other lipid accumulating genes such as DGAT isoforms (DGAT2-1, DGAT 5), KAS III, FAS (fatty acid synthase) enzymes, and ME, while RNA silencing of DGAT2-4 resulted in ~30-50 % increase in lipid content (Schuhmann et al., 2016; Scranton et al., 2015; Gimpel et al., 2015). Apart from these lipid genes, thioesterase has been overexpressed in the nuclear genomes of *P. tricornutum,* and *C. reinhardtii,* which did not result in increase in fatty acid synthesis, but significantly increased the C12-C14 fatty acids (Gimpel et al., 2015). However, recently, the overexpression of fatty acid- ACP thioesterase in *C. reinhardtii* resulted in an increase in the total lipid by 14-15 % (Wei

et al., 2017). Moreover, overexpression of LPAAT (from *Brassica napus*) and GDPI (*S. cerevisiae*) in *C. reinhardtii* resulted in an increase in total fatty acids by 17.4 % and 23.6 % as compared to non-transformed cells (Wang et al., 2017). Further, knockout of a multifunctional lipase gene resulted in 3-fold higher lipid accumulation in *T. pseudonana* (Gimpel et al., 2015).

In a recent patent by Sapphire Energy, Inc, a mRNA encoding protein (SNO3) classified as CREB Binding Protein/P300 and related TAZ zinc finger proteins (JGI Chlre v3 protein ID 147817) was transformed in *C. reinhardtii* wild-type strain CC-1690 21 gr mt + (Yohn et al., 2016). Overexpression of SNO3 resulted in increase in the phospholipid levels, though no increase in TAG, in the absence of nitrogen starvation (Yohn et al., 2016). In addition, knockdown mutants of the SNO3 gene resulted in lower lipid content in N-deplete cultures relative to wild-type. They also proposed that targets such as SNO3 can be used to transform other microalgal strains inducing high lipid accumulation without compromising the growth. Moreover, proteins homologous to SNO3 can be identified by using BLAST to query the published genome or transcriptome of any oleaginous microalgae.

In a few other studies, an attempt was made to block the competing starch synthesis pathway. AGPase or isoamaylase deletion in *C. reinhardtii* resulted in higher TAG content compared to wild type strains under nitrogen deplete conditions (Schuhmann et al., 2016). However, this increase in lipid accumulation was minor compared to starchless mutants of the respective microalgal strain, indicating alternative pathways may be upregulated in engineered strains, thus requiring a thorough knowledge of the regulatory metabolic pathways for successful metabolic manipulation (Work et al., 2010). Further, disrupting the primary carbon pathways can lead to low biomass generation, thus knockdown rather than knockout of lipid catabolism pathways can be a viable alternative. The above approach has been evaluated in model lipid accumulating diatom; *T. pseudonana* in which a predicted hydrolase (Thaps3_264297) responsible for breakdown of lipid was knocked down using antisense and RNAi approaches. The deleted mutant showed 2.4-3.3 fold higher lipid accumulation under silicon deficiency as compared to wild type without compromise in growth (Trentacoste et al., 2013). In a recent study, overexpression of an E2- conjugating enzyme (Cr UBC2), which has homology to a yeast and Arabidopsis MMS2/UEV) in *C. reinhardtii* increased the lipid content by 20 % as compared to wild type. E2-conjugating enzyme is crucial for transferring ubiquitin and ubiquitin like protein to substrates and actively involved in DNA repair and DNA damage tolerance. These results suggested that a protein substrate of CrUBC13–CrUBC2 polyubiquitination is involved in lipid accumulation (Fei et al., 2017).

Although the above-mentioned genes boosted the lipid accumulation in respective microalgal strains, the results vary from species to species i.e. one target may be effective in one microalgal strain but fail to increase fatty acid synthesis in another strain. It is also worth pointing out that it is not unusual for researchers to use lipid extraction and gravimetric analysis or lipophilic dyes such as Nile Red or Bodipy (boron-dipyrromethene) along with fluorometry to measure TAG content. Results from this approach do not always correlate well with actual lipid content. This scenario calls for the identifying and exploiting universal lipid triggers that could substantially boost the TAG production without inhibiting cell growth. This can be achieved by integrating the omics data of all the prospective high lipid accumulating strains under various stress conditions, as discussed below.

5. Identification of multi-omics targets for strain engineering

We evaluated the above summarized multi-omic data and identified potential global targets that were differentially expressed at both transcriptome and proteome level, which could be tested further in microalgal strains for improving TAG accumulation. For example, the first target that was upregulated in all the microalgal strains grown under stress for high lipid accumulation was biotin carboxylase (BC), a subunit of ACCase. BC catalyzes the first half of the reaction for the conversion of acetyl CoA to malonyl CoA (Shintani et al., 2017). Furthermore, the inhibitor of ACCase, AMPK, could be deleted or silenced, which could increase the expression of ACCase. Thus, manipulation of multiple genes in a single pathway may be necessary to achieve the desired results when a single target (i.e. enhancing the expression of ACCase) is ineffective.

Another potential pathway for increasing TAG accumulation can be lipid recycling, as it is crucial for maintaining lipid pool homeostasis inside microalgal cells. For example, overexpression of glycolipid assembly genes such as PDAT, PAP, MGD, DGD and SQD2 could potentially boost the TAG accumulation inside microalgal cells. A study carried out by Zhang et al. showed that RNAi silencing of PDAT1 in *Arabidopsis* resulted in sterile pollen that lacked oil bodies (Zhang et al., 2009). Further, double mutants of DGAT1 and PDAT1 resulted in 70-80 % decrease in oil bodies inside pollen, signifying the synergistic effect of DGAT1 and PGAT1 on TAG biosynthesis. Similar genetic engineering approaches could be applied with simultaneous overexpression of DGAT1 and PGAT1 gene in microalgal strains. Moreover, in another study, the SQD2 deficient mutant of *Rhodobacter sphaeroides* showed accumulation of phosphatidylglycerol in cells as compared to wild type under phosphate deprivation emphasizing its role in lipid recycling during stress conditions and making it a potential candidate for genetic engineering (Benning et al., 1993).

Apart from lipid biosynthesis genes, other pathways that could boost TAG productivity and/or accumulation include targeted improvements to photosynthetic efficiency and modifying carbon assimilation pathways. The efficiency of microalgae to absorb the photons and subsequently energy conversion (ATP, NADPH) affects the overall lipid synthesis productivity (Ghosh et al., 2016). Omic studies showed a universal decrease in LHC and chlorophyll genes in microalgal strains when cultivated under stress conditions. Decrease in the expression profile of these genes result in reduced cell growth and biomass, leading to loss of TAG productivity. Thus, characterization and modulation of LHC genes associated with photosystem I (PSI) and related genes can play a vital role in increasing microalgal lipid production. The first genetic engineering effort in this context was done by Mussgunug, et al., where their team silenced all twenty LHC protein isoforms in *C. reinhardtii* which resulted in a 68% drop in chlorophyll content (Mussgnug et al., 2005). In another study, the TLA1 gene (truncated light harvesting antenna 1) was overexpressed in *C. reinhardtii* which resulted in 13 % increase in chlorophyll content in cells; when the same gene was silenced, the chlorophyll content dropped by 68 % reduction (Mitra et al., 2012). Similarly, knockdown of three major LCHII proteins (LHCNB1, 2, and 3) in *C. reinhardtii* resulted in 50 % reduction in chlorophyll and a growth rate 85% as fast as the wild type (Oey et al., 2013). Further, overexpression of cyanobacterial D1 (low light isoform) in *C. reinhardtii* resulted in 11 % higher dry cell weight as compared to wild type (Vinyard et al., 2013). Although these studies were not directed at lipid content, the insights gained can provide a strong foundation for further manipulation of TAG accumulation in microalgae. Tuning the photosynthetic machinery using genetic engineering approaches to increase the growth rate with adaptation to high light would be a major breakthrough in the large- scale cultivation of microalgae and could increase lipid yields along with biomass productivity even with no changes in lipid accumulation rates.

The carbon assimilation pathway (Calvin–Benson–Bassham cycle) indirectly controls intracellular carbohydrate and lipid production. RuBisCO present in the plastid fixes CO_2 into RuBP, which results in the conversion to 3-phosphoglycerate (Gimpel et al., 2015). Under stress conditions, the reduction of RuBisCO activity hinders efficient carbon flux through Calvin cycle especially when CO_2 is not enriched in the medium (Ho et al., 2014a, 2014b). Thus, increasing the activity of RuBisCO can result in an increase in carbon flux towards lipid and/or carbohydrate biosynthesis. Further, the above integrated omic data showed universal differential expression of carbon assimilation genes

including RuBisCO and carbonic anhydrase. However, the few attempts made to date to engineer RuBisCO subunits (rbcS or rbc L) in *C. reinhardtii* did not result in substantial increase in CO_2/O_2 specificity and also decreased the growth rate (Gimpel et al., 2015). Nonetheless, future engineering approaches focused on simultaneous targeting of both RuBisCO subunits (small and large) in parallel with tuning carbon concentration mechanisms (α carbonic anhydrase 6,7) offers a promising path forward. Additional promising targets that influence intermediary carbon metabolism include overexpression of transaldolase, PGAM and PDE1 complex, which may redirect the carbon flux to lipid synthesis.

Lastly engineering transcriptional factors (TF) is an emerging technology that can potentially be exploited for augmenting TAG accumulation in microalgae. To date, around 147 putative TFs and 87 putative transcriptional regulators (TRs) have been identified in *C. reinhardtii* (Manuelle et al., 2009). Another interesting aspect that was patented by Sapphire Energy was overexpression of transcriptional factor-SN03 in *C. reinhardtii* that resulted in increase in total lipid accumulation from approximately 25% in wild-type to about 36% in the SNO3 strain under nitrogen replete conditions, though no increase in TAG, as described above. Likewise, a lipogenesis TF was overexpressed in *C. ellipsoidea* which resulted in 52 % increased lipid accumulation (Gimpel et al., 2015). Recently, knocking out a transcriptional regulator of lipid accumulation; Zn(II)2Cys6 (ZnCys)homolog of fungal Zn(II) 2Cys6-encoding genes) in *N. gaditana* improved the partitioning of total carbon to lipids from 20% (wild type) to 40–55% (mutant) in nutrient-replete conditions. Further, the lipid productivity was doubled when this transcriptional regulator was attenuated in the 5′ UTR and RNAi using a CRISPR–Cas9 reverse-genetics approach (Ajjawi et al., 2017). In addition to the aforementioned examples, it is also possible to exploit TFs that have been identified and tested in plant systems for increasing TAG content in algae. Candidates for evaluation in algae include growth regulating factor 2 (GRF2) and wrinkled gene 1(WRI1). GRF2 isolated from *Brassica napus* was expressed in *Arabidopsis* led to increase (> 50 %) in seeds while simultaneously increasing the photosynthetic activity (> 40 %) (Liu et al., 2010). Furthermore, overexpression of WRI1 gene in *Arabidopsis* enhanced the oil content to 40 % while synergistic overexpression of WRI1 with DGAT1 increased the lipid content up to 100 fold (59 % TAG content) in the seeds (Liu et al., 2010). This target has been recently verified in *N. salina,* as heterologous expression of AtWRI showed 44.7 % increase in lipid content as compared to untransformed cells (Kang et al., 2017). Further, they showed that the engineered strains showed downregulation of triacylglycerol lipase, diacylglycerol kinase, while upregulation of lysophopholipase, acyl-lysophosphatidylglycerol acyltransferase 1, diacylglycerol acyltransferase family protein and pyruvate phosphate dikinase respectively. However, there is still a long road ahead in terms of determining the biological functions of microalgal TFs and identification of lipid modulating TFs.

6. Leveraging omics with systems biology to augment TAG productivity in microalgae

Currently, algal omics data sets are spread across digital repositories in various formats which makes it difficult to properly access or interpret, restricting the development of a comprehensive, integrative view of algal metabolic networks. Additionally, omics databases are heterogeneous (nucleotide sequences, amino acid sequences and mass spectra, and metabolite profiles) with different formats of data representation. Thus, there is an imperative need for data integration in order to bring to bear the full value of the mechanistic data embedded in these disparate datasets (Akula et al., 2009). The goal of data integration is three-fold: first, generate an accurate picture of the behavior of microalgal species with respect to perturbation in cultivation conditions and subsequent TAG accumulation, ultimately linking omics and metadata; second, develop a holistic overview of the system at different hierarchical levels by concurrently analyzing multiple components; and third, to elucidate (and potentially predict) the interplay between these varied hierarchies. This integration will thus facilitate correlation between genes, proteins and metabolites, giving an overview of the system biology network of microalgal species, which can result in hypothesis driven research.

Systems biology combines combining high throughput experimental omics data with statistically driven mathematical models and computational tools to develop comprehensive models of cellular metabolism (Rodríguez-moyá and Gonzalez, 2017). The use of statistical tools can overcome the poor correlation between transcriptomics and proteomics data by compensating for the biases in the data collection methodologies. For example, a Zero-inflated Poissson repression model addressed the issues of incomplete proteomics data by taking into consideration the undetected proteins (missed due to instrumental limitations) (Zhang et al., 2017). In addition, the predicted expression values of the proteins analyzed by experimental mRNA levels can also be used to correct the experimental protein abundance data. Moreover, co-inertia analysis (CIA) can be used to visualize genes and protein expression in turn forming a relationship between two or more omics data sets. Another mathematical approach, correlative network-Granger causality-time series correlation, facilitates the interpretation of time dependent system responses along with the identification of time dependent variables (Zivy et al., 2015). The use of the above mentioned statistically driven models can provide novel and important advances for understanding lipid metabolism and other regulatory pathways in microalgae. Further, synchronized multivariate analysis can identify the variability between different omic data sets, identifying statistically significant alterations at the cellular level.

There are currently various tools and platforms to integrate omics data, including Bio Warehouse, Pointillist, 3Omics, Paintomics, KaPPA-view, VANTED, MAYDAY and InterOmics (for detailed review refer (Van Assche et al., 2015). These computational tools have been extensively applied for integrating bacterial, yeast, and mammalian data, thus providing a baseline for integrating algal omics data in the near future. Currently, there is an integrated data base for *C. reinhardtii*, ChlamyCys, which consists of genome wide insights into the regulation of metabolic networks with genome annotation (Zhang et al., 2017). Another online tool developed by Las Alamos National laboratory is Greenhouse, which is a centralized website that displays and shares sequence-based and meta-data relevant to the improvement and advancement of algal production feedstocks (LANL's Greenhouse.lanl.gov). For diatoms, there exists a Diatom EST database which enlists more than 200,000 ESTs from two model diatoms, *T. pseudonana* and *P. tricornutum* grown under a diverse range of environmental parameters (Maheswari et al., 2009). Moreover, a user friendly algal proteome database; Alga-PrAS (Algal Protein Annotation Suite) has been developed which contains 510,123 protein sequences from proteome of 31 algal and 3 plant species (Kurotani et al., 2017). This database provides information about physiochemical, secondary structure properties, post translational modifications and subcellular localization of these proteins. Further, recently the first integrated omics database for cyanobacteria was reported-CyanOmics (Yang et al., 2015a, 2015b). The database comprises the complete genome sequence of *Synechococcus* sp. 7002 with functional annotation, transcriptome and proteomes under different growth conditions. Additionally, the database also has built-in browsing, navigating, sequence alignment and data visualization features where users can perform analyses according to their own requirements.

The development of appropriate mathematical models can also describe the microalgal system along with the prediction of the system's response to perturbations enabling dynamic modeling of algal cellular metabolism. This involves construction of an initial framework model for a particular microalgal species by understanding the structure and identifying key elements controlling TAG accumulation such as gene networks, protein-protein interaction and metabolic pathways. The above model can now be provided with the high throughput omics data

of the microalga cultivated under different stress conditions which can then integrated. The model is then adapted by building a memory data base so that it can correlate well with the experimental data and predict the experimental responses. This sort of *in silico* mathematical models of high lipid accumulating microalgal strains can help predict unconventional gene targets for boosting TAG accumulation. One such modeling system is flux balance analysis (FBA) which contains metabolic reactions and all the genes encoding each enzyme of an organism (Orth et al., 2010). It calculates the flow of metabolites by utilizing this metabolic network thereby making it possible to predict the growth rate or the rate of production of any biotechnologically important metabolite of the organism (Orth et al., 2010). Currently there are more than 35 metabolic models of various industrially relevant microorganisms. However, few FBA models have been constructed for algae to date, including *Synechocystis*, *Arthrospira platensis*, *C. reinhardtii* and *Chlorella* sp. FC2 IITG, *P. tricornutum* (Kim et al., 2016; Muthuraj et al., 2013; Chang et al., 2011; Cogne et al., 2011; Boyle and Morgan, 2009). These networks offer a broad knowledgebase of the biochemistry and global networks underlying the metabolic fluxes under autotrophic, heterotrophic and mixotrophic growth conditions. Further, these metabolic maps represent a platform for phenotypic outcomes with system perturbations and pathways for lipid biosynthesis in microalgae thereby expanding the lipid metabolism repertoire and potential targets for genetic engineering (Chang et al., 2011).

Genome scale metabolic network frameworks have been developed for a limited number of algae, including AlgaGEM (for *C. reinhardtii*), DRUM (for *T. lutea* and *C. sorokiniana*), iLB1027_lipid (for *P. tricornutum*), iCZ843 (for *C. vulgaris* UTEX 395), iN934 for *N. salina*, and iRJL321 for *N. gaditana,* which predict the microalgal metabolism in response to perturbations in the growth conditions (Baroukh et al., 2016; Gomes et al., 2011; Levering et al., 2016; Zuñiga et al., 2016; Loira et al., 2017; Shah et al., 2017; Qian et al., 2017). AlgaGEM accounts for 866 unique ORFs, 1862 metabolites, 2249 gene enzyme reactions and 1725 unique reactions. This model was built to predict the changes in the hydrogen production in response to growth conditions and in turn predicting novel targets for high hydrogen production (Gomes et al., 2011). On the other hand, DRUM is a dynamic modelling system that handles the non-balanced growth conditions of any microalga and depicts the accumulation of metabolites. This model provided a basic understanding of interplay of lipids and carbohydrate accumulation in *T. lutea;* while for *C. sorokiniana* it provided new insights into the diauxic heterotrophic growth on acetate and butyrate (Baroukh et al., 2015, 2016). iCZ843 is an highly curated model that can accurately predict phenotypes under photoautotrophic, heterotrophic and mixotrophic conditions (Zuñiga et al., 2016). It predicted 843 genes, 2294 reactions and 1770 metabolites which can guide successful lab experiments for increasing growth performance. iN934 predicted 2345 reactions, 934 genes and proposed 82 different knockout targets for strain optimization to increase the TAG content, while iRJ1321 provided insights into the role of nitrogen limitation in *N. gaditana* by modeling and predicting 1321 genes, 1918 reaction and 1862 metabolites (Loira et al., 2017; Shah et al., 2017). Further, a mathematical model for *Coelastrum* sp. HA1 was developed under different nitrogen conditions which was then successfully applied to *C. sorokiniana* under different light intensities (Yang et al., 2017).

Thus, these data analysis tools may make it possible for all the omics layers (genomics, proteomics and metabolomics) to be combined together with metabolic modeling to provide insights into the flexibility of microalgal metabolic pathways. This multi-dimensional integration can lead to the simplification of metabolic fluxes of any microalgae of interest thereby maximizing efficiency and specificity of genetic engineering approaches. In brief, a multi-omics pipeline comprises of the following steps: (1) stepwise collection of all omic data arising from transcriptomics, proteomics and metabolomics analyses; (2) identification of linkages between different omics (transcriptomics-proteomics-metabolomics) using biological databases such as KEGG, SEED, Metacyc, BKM-react, Brenda, Uniprot, Expasy, PubChem, ChEBI and ChemSpider which provide information about reactions/metabolites and associated enzymes and genes; (3) correlation and reconstruction of a metabolic network by integration of all these data into a single and comprehensive interactome; (4) filling of the gaps in the metabolic model by using homology-based searches; (5) validation of the metabolic model by comparing the experimental data with the metabolic network generated data; (6) using *in-silico* analyses with validated models to query strain manipulation scenarios such as knockouts simulation, environmental challenges, and other cultivation parameters to suggest further wet-lab experiments (Cisek et al., 2016; Fondi and Liò, 2015).

7. Conclusions & future perspectives

Multi-omic analyses have become an established tool for biocatalyst development and offer a powerful path towards hypothesis-driven strain-engineering strategies in microalgae for enhanced TAG biosynthesis. Extensive comparative omics analyses have been conducted in microalgae in an effort to elucidate lipid accumulation mechanisms and strain-engineering targets. Here we have cataloged an array of such omic pursuits across diverse species and under varied environmental cues in order to gain further insight into universal TAG regulatory genes and mechanisms. We note that this review is far from comprehensive, but is intended to be instructive for future strain-engineering pursuits grounded in integrative systems approaches. Additionally, we acknowledge the nature of omic experimental design, wherein varied culture conditions (e.g. light intensity and cycling, temperature, sampling harvest times) prevents facile cross-omics comparative analyses. Notably, a series of conserved regulatory genes and mechanisms have emerged from an array of omic datasets under differential stress and cultivation conditions, despite this disparate data. However, substantial intra- and interspecies discrepancies remain; this further underscores the necessity for comprehensive systems approaches, encompassing the integration of physiological data with multi-omic and genome-wide computational modeling. Indeed, rapidly emerging genome scale modeling capabilities will play an integral role in assimilation of these data, enabling predictive capacity for hypothesis-driven strain engineering and media formulation.

Ultimately, in order to bring to bear the full promise of microalgae, improvements must be realized across the entire value chain. This will undoubtedly require continued efforts in bioprospecting and advances in algal genetic tool development in order to identify and develop novel strains with robust deployment characteristics. To achieve maximum productivity, strain-engineering pursuits in non-model algae will need to focus on enhancing multiple traits beyond TAG accumulation, including enhanced photosynthetic efficiency, CO_2 utilization, and predator and pest tolerance mechanisms, among others. Concurrently, continued efforts targeting outdoor deployment and conversion optimization will also be requisite, encompassing cultivation, harvesting, extraction, and upgrading. However, it is clear that systems integration of omic datasets will play a fundamental role on the path to achieving economically viable algal biofuels.

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References

Ajjawi, I., Verruto, J., Aqui, M., Soriaga, L.B., Coppersmith, J., Kwok, K., Peach, L., Orchard, E., Kalb, R., Xu, W., Carlson, T.J., Francis, K., Konigsfeld, K., Bartalis, J., Schultz, A., Lambert, W., Schwartz, A.S., Brown, R., Moellering, E.R., 2017. Lipid production in Nannochloropsis gaditana is doubled by decreasing expression of a single transcriptional regulator. Nat. Biotechnol. 35, 647–652. http://dx.doi.org/10.1038/nbt.3865.

Akula, S.P., Miriyala, R.N., Thota, H., Rao, A.A., Gedela, S., 2009. Bioinformation techniques for integrating -omics data. Bioinformation 6, 284–286.

Armbrust, E.V., Berges, J.A., Bowler, C., Green, B.R., Martinez, D., Putnam, N.H., Zhou, S., Allen, A.E., Apt, K.E., Bechner, M., Brzezinski, M.A., Chaal, B.K., Chiovitti, A., Davis, A.K., Demarest, M.S., Detter, J.C., Glavina, T., Goodstein, D., Hadi, M.Z., Hellsten, U., Hildebrand, M., Jenkins, B.D., Jurka, J., Kapitonov, V.V., Kröger, N., Lau, W.W.Y., Lane, T.W., Larimer, F.W., Lippmeier, J.C., Lucas, S., Medina, M., Montsant, A., Obornik, M., Parker, M.S., Palenik, B., Pazour, G.J., Richardson, P.M., Rynearson, T.A., Saito, M.A., Schwartz, D.C., Thamatrakoln, K., Valentin, K., Vardi, A., Wilkerson, F.P., Rokhsar, D.S., 2004. The genome of the diatom *Thalassiosira Pseudonana*: ecology, evolution, and metabolism. Science 306, 79–86. (80-). https://doi.org/10.1126/science.110115.

Arora, N., Gulati, K., Patel, A., Pruthi, P.A., Poluri, K.M., Pruthi, V., 2017a. A hybrid approach integrating arsenic detoxification with biodiesel production using oleaginous microalgae. Algal Res. 24, 29–39. http://dx.doi.org/10.1016/j.algal.2017.03.012.

Arora, N., Patel, A., Sharma, M., Mehtani, J., Pruthi, P.A., Pruthi, V., Poluri, K.M., 2017b. Insights into the enhanced lipid production characteristics of a fresh water microalga under high salinity conditions. Ind. Eng. Chem. Res. (25), 7413–7421. http://dx.doi.org/10.1021/acs.iecr.7b00841.

Assche, R., van Broeckx, V., Boonen, K., Maes, E., 2015. Integrating -omics: systems biology as explored through C. elegans Research. J. Mol. Biol. 427, 3441–3451. http://dx.doi.org/10.1016/j.jmb.2015.03.015.

Banerjee, C., Dubey, K.K., Shukla, P., 2016. Metabolic engineering of microalgal based biofuel production: prospects and challenges. Front. Microbiol. 7, 432. http://dx.doi.org/10.3389/fmicb.2016.00432.

Baroukh, C., Munoz-Tamayo, R., Steyer, J.P., Bernard, O., 2015. A state of the art of metabolic networks of unicellular microalgae and cyanobacteria for biofuel production. Metab. Eng. 30, 49–60. http://dx.doi.org/10.1016/j.ymben.2015.03.019.

Baroukh, C., Bernard, O., Baroukh, C., Bernard, O., 2016. Metabolic modeling of C. sorokiniana diauxic heterotrophic growth. IFAC-Papers On Line 49, 330–335. http://dx.doi.org/10.1016/j.ifacol.2016.12.148.

Bellou, S., Baeshen, M.N., Elazzazy, A.M., Aggeli, D., Sayegh, F., Aggelis, G., 2014. Microalgal lipids biochemistry and biotechnological perspectives. Biotechnol. Adv. 32, 1476–1493. http://dx.doi.org/10.1016/j.biotechadv.2014.10.003.

Benning, C., Beattyt, J.T., Prince, R.C., Somerville, C.R., 1993. The sulfolipid sulfoquinovosyldiacylglycerol is not required for photosynthetic electron transport in Rhodobacter sphaeroides but enhances growth under phosphate limitation. Proc. Natl. Acad. Sci. 90, 1561–1565.

Blaby, I.K., Glaesener, A.G., Mettler, T., Fitz-Gibbon, S.T., Gallaher, S.D., Liu, B., Boyle, N.R., Kropat, J., Stitt, M., Johnson, S., Benning, C., Pellegrini, M., Casero, D., Merchant, S.S., 2013. Systems-level analysis of nitrogen starvation-induced modifications of carbon metabolism in a Chlamydomonas reinhardtii starchless mutant. Plant Cell 25, 4305–4323. http://dx.doi.org/10.1105/tpc.113.117580.

Blanc, G., Duncan, G., Agarkova, I., Borodovsky, M., Gurnon, J., Kuo, A., Lindquist, E., Lucas, S., Pangilinan, J., Polle, J., Salamov, A., Terry, A., Yamada, T., Dunigan, D.D., Grigoriev, I.V., Claverie, J.-M., Van Etten, J.L., 2010. The *Chlorella variabilis* NC64A genome reveals adaptation to photosymbiosis, coevolution with viruses, and cryptic sex. Plant Cell 22, 2943–2955. http://dx.doi.org/10.1105/tpc.110.076406.

Blanc, G., Agarkova, I., Grimwood, J., Kuo, A., Brueggeman, A., Dunigan, D.D., Gurnon, J., Ladunga, I., Lindquist, E., Lucas, S., Pangilinan, J., Pröschold, T., Salamov, A., Schmutz, J., Weeks, D., Yamada, T., Lomsadze, A., Borodovsky, M., Claverie, J., Grigoriev, I.V., Van Etten, J.L., 2012. The Genome of the Polar Eukaryotic Microalga Coccomyxa Subellipsoidea Reveals Traits of Cold Adaptation.

Bogen, C., Al-Dilaimi, A., Albersmeier, A., Wichmann, J., Grundmann, M., Rupp, O., Lauersen, K.J., Blifernez-Klassen, O., Kalinowski, J., Goesmann, A., Mussgnug, J.H., Kruse, O., 2013. Reconstruction of the lipid metabolism for the microalga *Monoraphidium* neglectum from its genome sequence reveals characteristics suitable for biofuel production. BMC Genomics 14. http://dx.doi.org/10.1186/1471-2164-14-926.

Bowler, C., Allen, A.E., Badger, J.H., Grimwood, J., Jabbari, K., Kuo, A., Maheswari, U., Martens, C., Maumus, F., Otillar, R.P., Rayko, E., Salamov, A., Vandepoele, K., Beszteri, B., Gruber, A., Heijde, M., Katinka, M., Mock, T., Valentin, K., Verret, F., Berges, J.A., Brownlee, C., Cadoret, J.P., Chiovitti, A., Choi, C.J., Coesel, S., De Martino, A., Detter, J.C., Durkin, C., Falciatore, A., Fournet, J., Haruta, M., Huysman, M.J.J., Jenkins, B.D., Jiroutova, K., Jorgensen, R.E., Joubert, Y., Kaplan, A., Kröger, N., Kroth, P.G., La Roche, J., Lindquist, E., Lommer, M., Martin-Jézéquel, V., Lopez, P.J., Lucas, S., Mangogna, M., McGinnis, K., Medlin, L.K., Montsant, A., Le Secq, M.P.O., Napoli, C., Obornik, M., Parker, M.S., Petit, J.L., Porcel, B.M., Poulsen, N., Robison, M., Rychlewski, L., Rynearson, T.A., Schmutz, J., Shapiro, H., Siaut, M., Stanley, M., Sussman, M.R., Taylor, A.R., Vardi, A., Von Dassow, P., Vyverman, W., Willis, A., Wyrwicz, L.S., Rokhsar, D.S., Weissenbach, J., Armbrust, E.V., Green, B.R., Van De Peer, Y., Grigoriev, I.V., 2008. The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. Nature 456, 239–244. http://dx.doi.org/10.1038/nature07410.

Boyle, N.R., Morgan, J., 2009. Flux balance analysis of primary metabolism in Chlamydomonas reinhardtii. BMC Syst. Biol. 3, 4. http://dx.doi.org/10.1186/1752-0509-3-4.

Boyle, N.R., Page, M.D., Liu, B., Blaby, I.K., Casero, D., Kropat, J., Cokus, S.J., Hong-hermesdorf, A., Shaw, J., Karpowicz, S.J., Gallaher, S.D., Johnson, S., Benning, C., Pellegrini, M., Grossman, A., Merchant, S.S., 2012. Three acyltransferases and nitrogen-responsive regulator are implicated in nitrogen starvation-induced triacylglycerol accumulation in Chlamydomonas. J. Biol. Chem. 287, 15811–15825. http://dx.doi.org/10.1074/jbc.M111.334052.

Carpinelli, C.E., Telatin, A., Vitulo, N., Forcato, C., Angelo, M.D., Schiavon, R., Vezzi, A., Mario, G., Morosinotto, T., 2014. Chromosome scale genome assembly and transcriptome profiling of Nannochloropsis gaditana in nitrogen depletion. Mol. Plant 7, 323–335. http://dx.doi.org/10.1093/mp/sst120.

Carrier, G., Garnier, M., Le Cunff, L., Bougaran, G., Probert, I., De Vargas, C., Corre, E., Cadoret, J.P., Saint-Jean, B., 2014. Comparative transcriptome of wild type and selected strains of the microalgae *Tisochrysis lutea* provides insights into the genetic basis, lipid metabolism and the life cycle. PLoS One 9. http://dx.doi.org/10.1371/journal.pone.0086889.

Chang, R.L., Ghamsari, L., Manichaikul, A., Hom, E.F.Y., Balaji, S., Fu, W., Shen, Y., Hao, T., Palsson, B.Ø., Salehi-ashtiani, K., Papin, J.A., 2011. Metabolic network reconstruction of Chlamydomonas offers insight into light-driven algal metabolism. Mol. Syst. Biol. 7, 1–13. http://dx.doi.org/10.1038/msb.2011.52.

Chen, J.E., Smith, A.G., 2012. A look at diacylglycerol acyltransferases (DGATs) in algae. J. Biotechnol. 162, 28–39. http://dx.doi.org/10.1016/j.jbiotec.2012.05.009.

Chen, H., Zheng, Y., Zhan, J., He, C., Wang, Q., 2017. Comparative metabolic profiling of the lipid-producing green microalga Chlorella reveals that nitrogen and carbon metabolic pathways contribute to lipid metabolism. Biotechnol. Biofuels (1), 1–20. http://dx.doi.org/10.1186/s13068-017-0839-4.

Cheng, J.S., Niu, Y.H., Lu, S.H., Yuan, Y.J., 2012. Metabolome analysis reveals ethanolamine as potential marker for improving lipid accumulation of model photosynthetic organisms. J. Chem. Technol. Biotechnol. 87, 1409–1418. http://dx.doi.org/10.1002/jctb.3759.

Chia, M.A., Lombardi, A.T., Ahii, M., Teresa, A., Grac, M., Melão, G., Parrish, C.C., 2013. Lipid composition of Chlorella vulgaris (Trebouxiophyceae) as a function of different cadmium and phosphate concentrations. Aquat. Toxicol. 129, 171–182. http://dx.doi.org/10.1016/j.aquatox.2012.12.004.

Chia, M.A., Lombardi, A.T., da Graça Gama Melão, M., Parrish, C.C., 2015. Combined nitrogen limitation and cadmium stress stimulate total carbohydrates, lipids, protein and amino acid accumulation in *Chlorella vulgaris* (Trebouxiophyceae). Aquat. Toxicol. 160, 87–95. http://dx.doi.org/10.1016/j.aquatox.2015.01.002.

Choi, G., Kim, B., Ahn, C., 2011. Effect of nitrogen limitation on oleic acid biosynthesis in Botryococcus braunii. J. Appl. Phycol. 23, 1031–1037. http://dx.doi.org/10.1007/s10811-010-9636-1.

Choi, Y.E., Hwang, H., Kim, H.S., Ahn, J.W., Jeong, W.J., Yang, J.W., 2013. Comparative proteomics using lipid over-producing or less-producing mutants unravels lipid metabolisms in *Chlamydomonas reinhardtii*. Bioresour. Technol. 145, 108–115. http://dx.doi.org/10.1016/j.biortech.2013.03.142.

Cisek, K., Krochmal, M., Klein, J., Mischak, H., 2016. The application of multi-omics and systems biology to identify therapeutic targets in chronic kidney disease. Nephrol. Doa. Transplant. 31, 2003–2011. Full reviews. https://doi.org/10.1093/ndt/gfv364.

Cogne, G., Ru, M., Bockmayr, A., Titica, M., Legrand, J., 2011 n.d. A model-based method for investigating bioenergetic processes in autotrophically growing eukaryotic microalgae: application to the green algae Chlamydomonas reinhardtii. Biotechnol. Prog. 27, 631–640. doi:https://doi.org/10.1002/btpr.596

Davidi, L., Katz, A., Pick, U., 2012. Characterization of major lipid droplet proteins from Dunaliella. Planta 236, 19–33. http://dx.doi.org/10.1007/s00425-011-1585-7.

Davis, R., Aden, A., Pienkos, P.T., 2011. Techno-economic analysis of autotrophic microalgae for fuel production. Appl. Energy 88, 3524–3531. http://dx.doi.org/10.1016/j.apenergy.2011.04.018.

Derelle, E., Ferraz, C., Rombauts, S., Rouze, P., Worden, A.Z., Robbens, S., et al., 2006. Genome analysis of the smallest free-living eukaryote *Ostreococcus* tauri unveils many unique features. Proc. Natl. Acad. Sci. U. S. A. 103, 11647–11652.

Dong, H., Williams, E., Wang, D., Xie, Z., Hsia, R., Jenck, A., Halden, R., Li, J., Chen, F., Place, A.R., 2013. Responses of Nannochloropsis oceanica IMET1 to long-term nitrogen starvation and recovery. Plant Physiol. 162, 1110–1126. http://dx.doi.org/10.1104/pp.113.214320.

Dunahay, T.G., Jaruis, E.E., Roessler, P.G., 1995. Genetic transformation of the diatoms Cyclotella cryptzca. J. Appl. Phycol. 1012, 1004–1012.

Dunn, W.B., Ellis, D.I., 2005. Metabolomics: Current Analytical Platforms and Methodologies. 24http://dx.doi.org/10.1016/j.trac.2004.11.021.

Fan, J., Xu, H., Luo, Y., Wan, M., 2015. Impacts of CO_2 concentration on growth, lipid accumulation, and carbon-concentrating-mechanism-related gene expression in oleaginous Chlorella. Appl. Microbiol. Biotechnol. 9, 2451–2462. http://dx.doi.org/10.1007/s00253-015-6397-4.

Fan, J., Xu, H., Li, Y., 2016. Transcriptome-based global analysis of gene expression in response to carbon dioxide deprivation in the green algae Chlorella pyrenoidosa. Algal Res. 16, 12–19. http://dx.doi.org/10.1016/j.algal.2016.02.032.

Fang, L., Sun, D., Xu, Z., He, J., Qi, S., Chen, X., Chew, W., Liu, J., 2015. Transcriptomic analysis of a moderately growing subisolate Botryococcus braunii 779 (Chlorophyta) in response to nitrogen deprivation. Biotechnol. Biofuels 1–21. http://dx.doi.org/10.1186/s13068-015-0307-y.

Fei, X., Li, X., Li, P., Deng, X., 2017. Involvement of Chlamydomonas DNA damage tolerence gene UBC2 in lipid accumulation. Algal Res. 22, 148–159. http://dx.doi.org/10.1016/j.algal.2016.12.019.

Foflonker, F., Ananyev, G., Qiu, H., Morrison, A., Palenik, B., Dismukes, G.C., Bhattacharya, D., 2016. The unexpected extremophile: tolerance to fluctuating salinity in the green alga Picochlorum. Algal Res. 16, 465–472. http://dx.doi.org/10.1016/j.algal.2016.04.003.

Fondi, M., Liò, P., 2015. Multi -omics and metabolic modelling pipelines: challenges and tools for systems microbiology. Microbiol. Res. 171, 52–64. http://dx.doi.org/10.1016/j.micres.2015.01.003.

Garcia de Lomana, A.L., Baliga, N., 2010. Transcriptional program for nitrogen starvation-induced lipid accumulation in Chlamydomonas reinhardtii. Biotechnol. Biofuels 8, 796–799. http://dx.doi.org/10.1126/science.1189003.
Garnier, M., Carrier, G., Rogniaux, H., Nicolau, E., Bougaran, G., 2014. Comparative proteomics reveals proteins impacted by nitrogen deprivation in wild-type and high lipid-accumulating mutant strains of Tisochrysis lutea. J. Proteome 105, 107–120. http://dx.doi.org/10.1016/j.jprot.2014.02.022.
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 26, 1681–1697. http://dx.doi.org/10.1105/tpc.114.124982.
Ghosh, A., Khanra, S., Mondal, M., Halder, G., Tiwari, O.N., Saini, S., Kumar, T., Gayen, K., 2016. Progress toward isolation of strains and genetically engineered strains of microalgae for production of biofuel and other value added chemicals: a review. Energy Convers. Manag. 113, 104–118. http://dx.doi.org/10.1016/j.enconman.2016.01.050.
Gimpel, J.A., Henríquez, V., Mayfield, S.P., 2015. In metabolic engineering of eukaryotic microalgae: potential and challenges come with great diversity. Front. Microbiol. 6, 1–14. http://dx.doi.org/10.3389/fmicb.2015.01376.
Gomes, C., Dal, D.O., Quek, L., Palfreyman, R.W., Nielsen, L.K., 2011. AlgaGEM – a genome-scale metabolic reconstruction of algae based on the Chlamydomonas reinhardtii genome. BMC Genomics 12, 1–10.
Goold, H., Beisson, F., Peltier, G., Li-Beisson, Y., 2015. Microalgal lipid droplets: composition, diversity, biogenesis and functions. Plant Cell Rep. 34, 545–555. http://dx.doi.org/10.1007/s00299-014-1711-7.
Guarnieri, M.T., Pienkos, P.T., 2015. Algal omics: unlocking bioproduct diversity in algae cell factories. Photosynth. Res. 123, 255–263. http://dx.doi.org/10.1007/s11120-014-9989-4.
Guarnieri, M.T., Nag, A., Smolinski, S.L., Darzins, A., Seibert, M., Pienkos, P.T., 2011. Examination of triacylglycerol biosynthetic pathways via de novo transcriptomic and proteomic analyses in an unsequenced microalga. PLoS One 6. http://dx.doi.org/10.1371/journal.pone.0025851.
Guarnieri, M.T., Nag, A., Yang, S., Pienkos, P.T., 2013. Proteomic analysis of Chlorella vulgaris: potential targets for enhanced lipid accumulation. J. Proteome 93, 245–253. http://dx.doi.org/10.1016/j.jprot.2013.05.025.
Hannon, M., Gimpel, J., Tran, M., Rasala, B., Mayfield, S., 2011. Biofuels from algae: challenges and potential. Biofuels 1, 763–784.
Hasunuma, T., Matsuda, M., Kondo, A., 2016. Improved Sugar-Free Succinate Production by Synechocystis sp. Pcc 6803 Following Identification Of The Limiting Steps In Glycogen Catabolism. 3. pp. 130–141. http://dx.doi.org/10.1016/j.meteno.2016.04.003.
Hirashima, T., Tajima, N., Sato, N., 2016. Draft Genome Sequences of Four Species of Chlamydomonas. http://dx.doi.org/10.1128/genomeA.01070-16.
Ho, S.-H., Nakanishi, A., Ye, X., Chang, J.-S., Hara, K., Hasunuma, T., Kondo, A., 2014a. Optimizing biodiesel production in marine Chlamydomonas sp. JSC4 through metabolic profiling and an innovative salinity-gradient strategy. Biotechnol. Biofuels 7, 97. http://dx.doi.org/10.1186/1754-6834-7-97.
Ho, S., Ye, X., Hasunuma, T., Chang, J., Kondo, A., 2014b. Perspectives on engineering strategies for improving biofuel production from microalgae — a critical review. Biotechnol. Adv. 32, 1448–1459. http://dx.doi.org/10.1016/j.biotechadv.2014.09.002.
Ho, S., Nakanishi, A., Ye, X., Chang, J., Chen, C., Hasunuma, T., Kondo, A., 2015. Dynamic metabolic profiling of the marine microalga Chlamydomonas sp. JSC4 and enhancing its oil production by optimizing light intensity. Biotechnol. Biofuels 1–17. http://dx.doi.org/10.1186/s13068-015-0226-y.
Huang, N.-L., Huang, M.-D., Chen, T.-L.L., Huang, A.H.C., 2013. Oleosin of subcellular lipid droplets evolved in green algae. Plant Physiol. 161, 1862–1874. http://dx.doi.org/10.1104/pp.112.212514.
Huerlimann, R., Heimann, K., 2013. Comprehensive guide to acetyl-carboxylases in algae. Crit. Rev. Biotechnol. 33, 49–65. http://dx.doi.org/10.3109/07388551.2012.668671.
Ikaran, Z., Urreta, I., Castañón, S., 2015. The effect of nitrogen limitation on the physiology and metabolism of Chlorella vulgaris var L3. Algal Res. 10, 134–144. http://dx.doi.org/10.1016/j.algal.2015.04.023.
Ito, T., Tanaka, M., Shinkawa, H., Nakada, T., Ano, Y., Kurano, N., Soga, T., Tomita, M., 2013. Metabolic and morphological changes of an oil accumulating treboux-iophycean alga in nitrogen-deficient conditions. Metabolomics 9, 178–187. http://dx.doi.org/10.1007/s11306-012-0463-z.
Jaeger, D., Winkler, A., Mussgnug, J.H., Kalinowski, J., Goesmann, A., Kruse, O., 2017. Time-resolved transcriptome analysis and lipid pathway reconstruction of the oleaginous green microalga Monoraphidium neglectum reveal a model for triacylglycerol and lipid hyperaccumulation. Biotechnol. Biofuels 10, 1–34. http://dx.doi.org/10.1186/s13068-017-0882-1.
Jinkerson, R.E., Radakovits, R., Posewitz, M.C., 2013. Genomic insights from the oleaginous model alga *Nannochloropsis* gaditana. Bioengineered 4. http://dx.doi.org/10.4161/bioe.21880.
Kang, N.K., Kim, E.K., Kim, Y.U., Lee, B., Jeong, W.J., Jeong, B.R., Chang, Y.K., 2017. Increased lipid production by heterologous expression of AtWRI1 transcription factor in Nannochloropsis salina. Biotechnol. Biofuels 10, 1–14. http://dx.doi.org/10.1186/s13068-017-0919-5.
Kermarrec, L., Franc, A., Rimet, F., Chaumeil, P., Humbert, J.F., Bouchez, A., 2013. Next-generation sequencing to inventory taxonomic diversity in eukaryotic communities : a test for freshwater diatoms. Mol. Ecol. Resour. 13, 607–619. http://dx.doi.org/10.1111/1755-0998.12105.
Kim, J., Fabris, M., Baart, G., Kim, M.K., Goossens, A., Vyverman, W., Falkowski, P.G., Lun, D.S., 2016. Flux balance analysis of primary metabolism in the diatom Phaeodactylum tricornutum. Plant J. 85, 161–176. http://dx.doi.org/10.1111/tpj.13081.
Klok, A.J., Lamers, P.P., Martens, D.E., Draaisma, R.B., Wijffels, R.H., 2014. Edible oils from microalgae : insights in TAG accumulation. Trends Biotechnol. 32, 521–528. http://dx.doi.org/10.1016/j.tibtech.2014.07.004.
Kurotani, A., Yamada, Y., Sakurai, T., 2017. Alga-PrAS (Algal Protein Annotation Suite): a database of comprehensive annotation in algal proteomes. Plant Cell Physiol. 58, 1–7. http://dx.doi.org/10.1093/pcp/pcw212.
Kwak, M., Park, W.K., Shin, S.E., Koh, H.G., Lee, B., Jeong, B. ryool, Chang, Y.K., 2017. Improvement of biomass and lipid yield under stress conditions by using diploid strains of Chlamydomonas reinhardtii. Algal Res. 26, 180–189. http://dx.doi.org/10.1016/j.algal.2017.07.027.
Laurens, L.M.L., Wychen, S., van Mcallister, J.P., Arrowsmith, S., Dempster, T.A., Mcgowen, J., Pienkos, P.T., 2014. Strain, biochemistry, and cultivation-dependent measurement variability of algal biomass composition. Anal. Biochem. 452, 86–95. http://dx.doi.org/10.1016/j.ab.2014.02.009.
Lee, B., Choi, G., Choi, Y., Sung, M., Park, M.S., Yang, J., 2014a. Enhancement of lipid productivity by ethyl methane sulfonate-mediated random mutagenesis and proteomic analysis in Chlamydomonas reinhardtii. Korean J. Chem. Eng. 31, 1036–1042. http://dx.doi.org/10.1007/s11814-014-0007-5.
Lee, S., Kim, S., Hyun, S., Won, H., Hong, S., Cho, B., Lee, C., Lee, H., Choi, H., 2014b. Fatty acids and global metabolites profiling of Dunaliella tertiolecta by shifting culture conditions to nitrate deficiency and high light at different growth phases. Process Biochem. 49, 996–1004. http://dx.doi.org/10.1016/j.procbio.2014.02.022.
Lenka, S.K., Carbonaro, N., Park, R., Miller, S.M., Thorpe, I., Li, Y., 2016. Current advances in molecular, biochemical, and computational modeling analysis of microalgal triacylglycerol biosynthesis. Biotechnol. Adv. 34, 1046–1063. http://dx.doi.org/10.1016/j.biotechadv.2016.06.004.
Levering, J., Broddrick, J., Dupont, C.L., Peers, G., Beeri, K., Mayers, J., Gallina, A.A., Allen, A.E., Palsson, O., Zengler, K., 2016. Genome-scale model reveals metabolic basis of biomass partitioning in a model diatom. PLoS One 4, 1–22. http://dx.doi.org/10.1371/journal.pone.0155038.
Li, Y., Fei, X., Deng, X., 2012. Novel molecular insights into nitrogen starvation-induced triacylglycerols accumulation revealed by differential gene expression analysis in green algae Micractinium pusillum. Biomass Bioenergy 42, 199–211. http://dx.doi.org/10.1016/j.biombioe.2012.03.010.
Li, Y., Mu, J., Chen, D., Han, F., Xu, H., Kong, F., Xie, F., Feng, B., 2013a. Production of biomass and lipid by the microalgae Chlorella protothecoides with heterotrophic-Cu (II) stressed (HCuS) coupling cultivation. Bioresour. Technol. 148, 283–292. http://dx.doi.org/10.1016/j.biortech.2013.08.153.
Li, Y., Yuan, Z., Mu, J., Chen, D., Feng, B., 2013b. Proteomic analysis of lipid accumulation in Chlorella protothecoides cells by heterotrophic N deprivation coupling cultivation. Energy Fuel 27, 4031–4040.
Li, J., Han, D., Wang, D., Ning, K., Jia, J., Wei, L., Jing, X., Huang, S., Chen, J., Li, Y., Hu, Q., Xu, J., 2014a. Choreography of transcriptomes and lipidomes of nannochloropsis reveals the mechanisms of oil synthesis in microalgae. Plant Cell 26, 1645–1665. http://dx.doi.org/10.1105/tpc.113.121418.
Li, Y., Han, F., Xu, H., Mu, J., Chen, D., Feng, B., Zeng, H., 2014b. Bioresource technology potential lipid accumulation and growth characteristic of the green alga Chlorella with combination cultivation mode of nitrogen (N) and phosphorus (P). Bioresour. Technol. 174, 24–32. http://dx.doi.org/10.1016/j.biortech.2014.09.142.
Li, Y., Mu, J., Chen, D., Xu, H., 2015. Proteomics analysis for enhanced lipid accumulation in oleaginous Chlorella vulgaris under a heterotrophic-Na + induction two-step regime. Biotechnol. Lett. 37, 1021–1030. http://dx.doi.org/10.1007/s10529-014-1758-0.
Li, L., Zhang, G., Wang, Q., 2016. De novo transcriptomic analysis of Chlorella sorokiniana reveals differential genes expression in photosynthetic carbon fixation and lipid production. BMC Microbiol. 1–12. http://dx.doi.org/10.1186/s12866-016-0839-8.
Lim, D.K.Y., Schuhmann, H., Thomas-Hall, S.R., Chan, K.C.K., Wass, T.J., Aguilera, F., Adarme-Vega, T.C., Dal'Molin, C.G.O., Thorpe, G.J., Batley, J., Edwards, D., Schenk, P.M., 2017. RNA-Seq and metabolic flux analysis of Tetraselmis sp. M8 during nitrogen starvation reveals a two-stage lipid accumulation mechanism. Bioresour. Technol. 244, 1281–1293. http://dx.doi.org/10.1016/j.biortech.2017.06.003.
Lin, I.P., Jiang, P.L., Chen, C.S., Tzen, J.T.C., 2012. A unique caleosin serving as the major integral protein in oil bodies isolated from Chlorella sp. cells cultured with limited nitrogen. Plant Physiol. Biochem. 61, 80–87. http://dx.doi.org/10.1016/j.plaphy.2012.09.008.
Liu, J., Hua, W., Zhan, G., Wei, F., Wang, X., Liu, G., Wang, H., 2010. Increasing seed mass and oil content in transgenic Arabidopsis by the overexpression of wri1 -like gene from Brassica napus. Plant Physiol. Biochem. 48, 9–15. http://dx.doi.org/10.1016/j.plaphy.2009.09.007.
Lohman, E.J., Gardner, R.D., Pedersen, T., Peyton, B.M., Cooksey, K.E., Gerlach, R., 2015. Optimized inorganic carbon regime for enhanced growth and lipid accumulation in Chlorella vulgaris. Biotechnol. Biofuels 1–13. http://dx.doi.org/10.1186/s13068-015-0265-4.
Loira, N., Mendoza, S., Paz Cortés, M., Rojas, N., Travisany, D., Di Genova, A., Gajardo, N., Ehrenfeld, N., Maass, A., 2017. Reconstruction of the microalga Nannochloropsis salina genome-scale metabolic model with applications to lipid production. BMC Syst. Biol. 11, 1–17. http://dx.doi.org/10.1186/s12918-017-0441-1.
Longworth, J., Noirel, J., Pandhal, J., Wright, P.C., 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.
Longworth, J., Wu, D., Huete-ortega, M., Wright, P.C., Vaidyanathan, S., 2016. Proteome response of Phaeodactylum tricornutum, during lipid accumulation induced by nitrogen depletion. Algal Res. 18, 213–224. http://dx.doi.org/10.1016/j.algal.2016.06.015.
López, A., Lomana, G., de Schäuble, S., Valenzuela, J., Imam, S., Carter, W., Bilgin, D.D., Yohn, C.B., Turkarslan, S., Reiss, D.J., Orellana, M.V., Price, N.D., Baliga, N.S., 2015. Transcriptional program for nitrogen starvation - induced lipid accumulation in Chlamydomonas reinhardtii. Biotechnol. Biofuels 8, 1–18. http://dx.doi.org/10.1186/s13068-015-0391-z.
Lü, J., Sheahan, C., Fu, P., 2011. Metabolic engineering of algae for fourth generation

biofuels production. Energy Environ. Sci. 4, 2451–2466. http://dx.doi.org/10.1039/c0ee00593b.
Lu, N., Wei, D., Jiang, X.L., Chen, F., Yang, S.T., 2012. Regulation of lipid metabolism in the snow alga Chlamydomonas nivalis in response to NaCl stress: an integrated analysis by cytomic and lipidomic approaches. Process Biochem. 47, 1163–1170. http://dx.doi.org/10.1016/j.procbio.2012.04.011.
Lu, N., Wei, D., Chen, F., Yang, S., 2013. Lipidomic profiling reveals lipid regulation in the snow alga Chlamydomonas nivalis in response to nitrate or phosphate deprivation. Process Biochem. 48, 605–613. http://dx.doi.org/10.1016/j.procbio.2013.02.028.
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. http://dx.doi.org/10.1016/j.ygeno.2013.01.004.
Ma, Q., Wang, J., Lu, S., Lv, Y., Yuan, Y., 2013. Quantitative proteomic profiling reveals photosynthesis responsible for inoculum size dependent variation in Chlorella sorokiniana. Biotechnol. Bioeng. 110, 773–784. http://dx.doi.org/10.1002/bit.24762.
Maheswari, U., Mock, T., Armbrust, E.V., Bowler, C., 2009. Update of the Diatom EST Database : a new tool for digital transcriptomics. Nucleic Acids Res. 37, 1001–1005. http://dx.doi.org/10.1093/nar/gkn905.
Manuelle, N., Courchesne, D., Parisien, A., Wang, B., Lan, C.Q., 2009. Enhancement of lipid production using biochemical, genetic and transcription factor engineering approaches. 141, 31–41. http://dx.doi.org/10.1016/j.jbiotec.2009.02.018.
Mastrobuoni, G., Irgang, S., Pietzke, M., Aßmus, H.E., Wenzel, M., Schulze, W.X., Kempa, S., 2012. Proteome dynamics and early salt stress response of the photosynthetic organism *Chlamydomonas reinhardtii*. BMC Genomics 13, 1–13. http://dx.doi.org/10.1186/1471-2164-13-215.
Matsuzaki, M., Misumi, O., Shin-i, T., 2004. Genome sequence of the ultrasmall unicellular red alga Cyanidioschyzon merolae. Nature 428, 653–657.
Meharg, A.A., 2002. Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. New Phytol. 154, 29–43.
Melo, T., Alves, E., Azevedo, V., So, A., Neves, B., Domingues, P., Calado, R., Abreu, M.H., Rosário, M., 2015. Lipidomics as a new approach for the bioprospecting of marine macroalgae — Unraveling the polar lipid and fatty acid composition of Chondrus crispus. Algal Res. 8, 181–191. http://dx.doi.org/10.1016/j.algal.2015.02.016.
Merchant, S.S., Prochnik, S.E., Vallon, O., Harris, E.H., Karpowicz, S.J., Witman, G.B., Terry, A., Salamov, A., Fritz-laylin, L.K., Maréchal-drouard, L., Marshall, W.F., Qu, L., Nelson, D.R., Sanderfoot, A.A., Spalding, M.H., Kapitonov, V.V., Ren, Q., Ferris, P., Lindquist, E., Shapiro, H., Lucas, S.M., Grimwood, J., 2007. The chlamydomonas genome reveals the evolution of key animal and plant functions. 318.
Miller, R., Wu, G., Deshpande, R.R., Vieler, A., Gärtner, K., Li, X., Moellering, E.R., Zäuner, S., Cornish, A.J., Liu, B., Bullard, B., Sears, B.B., Kuo, M.-H.M.-H., Hegg, E.L., Shachar-Hill, Y., Shiu, S.-H.S.-H., Benning, C., Gartner, K., Li, X., Moellering, E.R., Zauner, S., Cornish, A.J., Liu, B., Bullard, B., Sears, B.B., Kuo, M.-H.M.-H., Hegg, E.L., Shachar-Hill, Y., Shiu, S.-H.S.-H., Benning, C., 2010. Changes in transcript abundance in Chlamydomonas reinhardtii following nitrogen deprivation predict diversion of metabolism. Plant Physiol. 154, 1737–1752. http://dx.doi.org/10.1104/pp.110.165159.
Mioso, R., Marante, F.J.T., Gonz??lez, J.E.G., Rodr??guez, J.J.S., de Laguna, I.H.B., 2014. Metabolite profiling of Schizochytrium sp. by GC-MS, an oleaginous microbial source of biodiesel. Braz. J. Microbiol. 45, 403–409. http://dx.doi.org/10.1590/S1517-83822014000200006.
Mitra, M., Kirst, H., Dewez, D., Melis, A., 2012. Modulation of the light-harvesting chlorophyll antenna size in Chlamydomonas reinhardtii by TLA1 gene over-expression and RNA interference. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 367, 3430–3443. http://dx.doi.org/10.1098/rstb.2012.0229.
Mock, T., Otillar, R.P., Strauss, J., McMullan, M., Paajanen, P., Schmutz, J., Salamov, A., Sanges, R., Toseland, A., Ward, B.J., Allen, A.E., Dupont, C.L., Frickenhaus, S., Maumus, F., Veluchamy, A., Wu, T., Barry, K.W., Falciatore, A., Ferrante, M.I., Fortunato, A.E., Glöckner, G., Gruber, A., Hipkin, R., Janech, M.G., Kroth, P.G., Leese, F., Lindquist, E.A., Lyon, B.R., Martin, J., Mayer, C., Parker, M., Quesneville, H., Raymond, J.A., Uhlig, C., Valas, R.E., Valentin, K.U., Worden, A.Z., Armbrust, E.V., Clark, M.D., Bowler, C., Green, B.R., Moulton, V., Van Oosterhout, C., Grigoriev, I.V., 2017. Evolutionary genomics of the cold-adapted diatom *Fragilariopsis* cylindrus. Nature 541, 536–540. http://dx.doi.org/10.1038/nature20803.
Moellering, E.R., Benning, C., 2010. RNA interference silencing of a major lipid droplet protein affects lipid droplet size in Chlamydomonas reinhardtii. Eukaryot. Cell 9, 97–106. http://dx.doi.org/10.1128/EC.00203-09.
Morales-Sánchez, D., Kyndt, J., Ogden, K., Martinez, A., 2016. Toward an understanding of lipid and starch accumulation in microalgae: a proteomic study of Neochloris oleoabundans cultivated under N-limited heterotrophic conditions. Algal Res. 20, 22–34. http://dx.doi.org/10.1016/j.algal.2016.09.006.
Moroney, J.V., Somanchi, A., 1999. How do algae concentrate co2 to increase the efficiency of photosynthetic carbon fixation? Plant Physiol. 119, 9–16. http://dx.doi.org/10.1104/pp.119.1.9.
Msanne, J., Xu, D., Konda, A.R., Casas-Mollano, J.A., Awada, T., Cahoon, E.B., Cerutti, H., 2012. Metabolic and gene expression changes triggered by nitrogen deprivation in the photoautotrophically grown microalgae Chlamydomonas reinhardtii and Coccomyxa sp. C-169. Phytochemistry 75, 50–59. http://dx.doi.org/10.1016/j.phytochem.2011.12.007.
Mussgnug, J.H., Wobbe, L., Elles, I., Claus, C., Hamilton, M., Fink, A., 2005. NAB1 is an RNA binding protein involved in the light-regulated differential expression of the light-harvesting antenna of Chlamydomonas reinhardtii. Plant Cell 17, 3409–3421. http://dx.doi.org/10.1105/tpc.105.035774.algae.
Muthuraj, M., Palabhanvi, B., Misra, S., 2013. Flux balance analysis of Chlorella sp. FC2 IITG under photoautotrophic and heterotrophic growth conditions. Photosynth. Res. 118, 167–179. http://dx.doi.org/10.1007/s11120-013-9943-x.
Nguyen, H.M., Baudet, M., Cuine, S., Adriano, J.M., 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.
Nguyen, H.M., Baudet, M., Cuiné, S., Adriano, J.M., Barthe, D., Billon, E., Bruley, C., Beisson, F., Peltier, G., Ferro, M., Li-Beisson, Y., 2012. 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. http://dx.doi.org/10.1002/pmic.201100114.
Nojima, D., Yoshino, T., Maeda, Y., Tanaka, M., Nemoto, M., Tanaka, T., 2013. Proteomics analysis of oil body-associated proteins in the oleaginous diatom. J. Proteome Res. 12, 5293–5301. http://dx.doi.org/10.1021/pr4004085.
Oey, M., Ross, I.L., Stephens, E., Steinbeck, J., Wolf, J., Radzun, K.A., Kügler, J., Ringsmuth, A.K., Kruse, O., Hankamer, B., 2013. RNAi knock-down of LHCBM1, 2 and 3 increases photosynthetic H2 production efficiency of the green alga Chlamydomonas reinhardtii. PLoS One 8. http://dx.doi.org/10.1371/journal.pone.0061375.
Orth, J.D., Thiele, I., Palsson, B.Ø., 2010. What is flux balance analysis ? Nat. Publ. Gr. 28, 245–248. http://dx.doi.org/10.1038/nbt.1614.
Osada, K., Maeda, Y., Yoshino, T., Nojima, D., Bowler, C., Tanaka, T., 2017. Enhanced NADPH production in the pentose phosphate pathway accelerates lipid accumulation in the oleaginous diatom Fistulifera solaris. Algal Res. 23, 126–134. http://dx.doi.org/10.1016/j.algal.2017.01.015.
Ota, S., Oshima, K., Yamazaki, T., Kim, S., Yu, Z., Yoshihara, M., Takeda, K., Takeshita, T., Hirata, A., Bišová, K., Zachleder, V., Hattori, M., Kawano, S., 2016. Highly efficient lipid production in the green alga *Parachlorella* kessleri: draft genome and transcriptome endorsed by whole-cell 3D ultrastructure. Biotechnol. Biofuels 9, 1–10. http://dx.doi.org/10.1186/s13068-016-0424-2.
Peled, E., Leu, S., Zarka, A., Weiss, M., Pick, U., Khozin-Goldberg, I., Boussiba, S., 2011. Isolation of a novel oil globule protein from the green alga haematococcus pluvialis (chlorophyceae). Lipids 46, 851–861. http://dx.doi.org/10.1007/s11745-011-3579-4.
Poong, S.W., Lim, P.E., Phang, S.M., Wong, C.Y., Pai, T.W., Chen, C.M., Yang, C.H., Liu, C.C., 2017. Transcriptome sequencing of an Antarctic microalga, Chlorella sp. (Trebouxiophyceae, Chlorophyta) subjected to short-term ultraviolet radiation stress. J. Appl. Phycol. 1–13. http://dx.doi.org/10.1007/s10811-017-1124-4.
Prochnik, S.E., Umen, J., Nedelcu, A.M., Hallmann, A., Miller, S.M., Nishii, I., Ferris, P., Kuo, A., Mitros, T., Fritz-Laylin, L.K., Hellsten, U., Chapman, J., Simakov, O., Rensing, S.A., Terry, A., Pangilinan, J., Kapitonov, V., Jurka, J., Salamov, A., Shapiro, H., Schmutz, J., Grimwood, J., Lindquist, E., Lucas, S., Grigoriev, I.V., Schmitt, R., Kirk, D., Rokhsar, D.S., 2010. Genomic analysis of organismal complexity in the multicellular green alga volvox carteri. Science 329, 223–226. http://dx.doi.org/10.1126/science.1188800.
Qian, X., Kim, M.K., Kumaraswamy, G.K., Agarwal, A., Lun, D.S., Dismukes, G.C., 2017. Flux balance analysis of photoautotrophic metabolism: uncovering new biological details of subsystems involved in cyanobacterial photosynthesis. Biochim. Biophys. Acta Bioenerg. 1858, 276–287. http://dx.doi.org/10.1016/j.bbabio.2016.12.007.
Radakovits, R., Jinkerson, R.E., Darzins, A., Posewitz, M.C., 2010. Genetic engineering of algae for enhanced biofuel production. Eukaryot. Cell 9, 486–501. http://dx.doi.org/10.1128/EC.00364-09.
Rai, V., Muthuraj, M., Gandhi, M.N., Das, D., Srivastava, S., 2017. Real-time iTRAQ-based proteome profiling revealed the central metabolism involved in nitrogen starvation induced lipid accumulation in microalgae. Sci. Rep. 7, 1–16. http://dx.doi.org/10.1038/srep45732.
Ramos, M.J., Fernández, C.M., Casas, A., Rodríguez, L., Pérez, Á., 2009. Influence of fatty acid composition of raw materials on biodiesel properties. Bioresour. Technol. 100, 261–268. http://dx.doi.org/10.1016/j.biortech.2008.06.039.
Rismani-Yazdi, H., Haznedaroglu, B.Z., Hsin, C., Peccia, J., 2012. Transcriptomic analysis of the oleaginous microalga Neochloris oleoabundans reveals metabolic insights into triacylglyceride accumulation. Biotechnol. Biofuels 5, 1–16.
Rodríguez-moyá, M., Gonzalez, R., 2017. Systems biology approaches for the microbial production of biofuels systems biology approaches for the microbial production of biofuels. Biofuels 2, 291–310.
Roesler, K., Shintani, D., Savage, L., Boddupalli, S., Ohlrogge, J., 1997. Targeting of the Arabidopsis homomeric acetyl-coenzyme A carboxylase to plastids of rapeseeds. Plant Physiol. 113, 75–81. http://dx.doi.org/10.1104/pp.113.1.75.
Roth, M.S., Cokus, S.J., Gallaher, S.D., Walter, A., Lopez, D., Erickson, E., Endelman, B., Westcott, D., Larabell, C.A., Merchant, S.S., Pellegrini, M., Niyogi, K.K., 2017. Chromosome-level genome assembly and transcriptome of the green alga Chromochloris zofingiensis illuminates astaxanthin production. Proc. Natl. Acad. Sci. 114, E4296–E4305. http://dx.doi.org/10.1073/pnas.1619928114.
Sarayloo, E., Tardu, M., Unlu, Y.S., Simsek, S., Cevahir, G., Erkey, C., Kavakli, I.H., 2017. Understanding lipid metabolism in high-lipid-producing Chlorella vulgaris mutants at the genome-wide level. Algal Res. 28, 244–252. http://dx.doi.org/10.1016/j.algal.2017.11.009.
Sasso, S., Pohnert, G., Lohr, M., Mittag, M., Hertweck, C., 2011. Microalgae in the post genomic era: a blooming reserviour for new natural products. Micro. Biol. Rev. 36, 761–785. http://dx.doi.org/10.1111/j.1574-6976.2011.00304.x.
Satjarak, A., Paasch, A.E., Graham, L.E., Kim, E., 2016. Complete chloroplast genome sequence of phagomixotrophic green alga *Cymbomonas* tetramitiformis. Genome Announc. 4, e00551-16. http://dx.doi.org/10.1128/genomeA.00551-16.
Schuhmann, H., Lim, D.K., Schenk, P.M., 2012. Perspectives on metabolic engineering for increased lipid contents in microalgae. Biofuels 3, 71–86. http://dx.doi.org/10.4155/bfs.11.147.
Schuhmann, H., Lim, D.K.Y., Schenk, P.M., 2016. Perspectives on metabolic engineering for increased lipid contents in microalgae Perspectives on metabolic engineering for increased lipid contents in microalgae. Biofuels 3, 71–86.
Scranton, M.A., Ostrand, J.T., Fields, F.J., Mayfield, S.P., 2015. Chlamydomonas as a model for biofuels and bio-products production. Plant J. 82, 523–531. http://dx.doi.org/10.1111/tpj.12780.
Shah, A.R., Ahmad, A., Srivastava, S., Jaffar Ali, B.M., 2017. Reconstruction and analysis of a genome-scale metabolic model of Nannochloropsis gaditana. Algal Res. 26, 354–364. http://dx.doi.org/10.1016/j.algal.2017.08.014.
Shang, C., Zhu, S., Wang, Z., Qin, L., Alam, M.A., Xie, J., Yuan, Z., 2017. Proteome response of *Dunaliella parva* induced by nitrogen limitation. Algal Res. 23, 196–202.

http://dx.doi.org/10.1016/j.algal.2017.01.016.
Shin, H., Hong, S., Kim, H., Yoo, C., Lee, H., Choi, H., Lee, C., Cho, B., 2015. Elucidation of the growth delimitation of *Dunaliella tertiolecta* under nitrogen stress by integrating transcriptome and peptidome analysis. Bioresour. Technol. 194, 57–66. http://dx.doi.org/10.1016/j.biortech.2015.07.002.
Shintani, D., Roesler, K., Shorrosh, B., Savage, L., Ohlrogge, J., 2017. Antisense expression and overexpression of biotin carboxylase in tobacco leaves. Plant Physiol. 114, 881–886.
Singh, P., Kumari, S., Guldhe, A., Misra, R., Rawat, I., Bux, F., 2016. Trends and novel strategies for enhancing lipid accumulation and quality in microalgae. Renew. Sust. Energ. Rev. 55, 1–16. http://dx.doi.org/10.1016/j.rser.2015.11.001.
Sithtisarn, S., Yokthongwattana, K., Mahong, B., Roytrakul, S., Paemanee, A., Phaonakrop, N., Yokthongwattana, C., 2017. Comparative proteomic analysis of *Chlamydomonas reinhardtii* control and a salinity-tolerant strain revealed a differential protein expression pattern. Planta 246, 843–856. http://dx.doi.org/10.1007/s00425-017-2734-4.
Smith, S.R., Glé, C., Abbriano, R.M., Traller, J.C., Davis, A., Trentacoste, E., Vernet, M., Allen, A.E., Hildebrand, M., 2016. Transcript level coordination of carbon pathways during silicon starvation-induced lipid accumulation in the diatom Thalassiosira pseudonana. New Phytol. 210, 890–904. http://dx.doi.org/10.1111/nph.13843.
Song, P., Li, L., Liu, J., 2013. Proteomic analysis in nitrogen-deprived Isochrysis galbana during lipid accumulation. PLoS One 8, 1–13. http://dx.doi.org/10.1371/journal.pone.0082188.
Su, X., Xu, J., Yan, X., 2013. Lipidomic changes during different growth stages of Nitzschia closterium f. minutissima. Metabolomics 9, 300–310. http://dx.doi.org/10.1007/s11306-012-0445-1.
Sui, X., Niu, X., Shi, M., Pei, G., Li, J., Chen, L., Wang, J., Zhang, W., 2014. Metabolomic analysis reveals mechanism of antioxidant butylated hydroxyanisole on lipid accumulation in Crypthecodinium cohnii. J. Agric. Food Chem. 62, 12477–12484.
Sun, D., Zhu, J., Fang, L., Zhang, X., Chow, Y., Liu, J., 2013. De novo transcriptome profiling uncovers a drastic downregulation of photosynthesis upon nitrogen deprivation in the nonmodel green alga Botryosphaerella sudeticus. BMC Genomics 14, 1–18.
Sun, X., Shen, J., Bai, F., Xu, N., 2016. Transcriptome profiling of the microalga Chlorella pyrenoidosa in response to different carbon dioxide concentrations. Mar. Genomics 29, 81–87. http://dx.doi.org/10.1016/j.margen.2016.05.002.
Tanaka, T., Maeda, Y., Veluchamy, A., Tanaka, M., Abida, H., Maréchal, E., Bowler, C., Muto, M., Sunaga, Y., Tanaka, M., Yoshino, T., Taniguchi, T., Fukuda, Y., Nemoto, M., Matsumoto, M., Wong, P.S., Aburatani, S., Fujibuchi, W., 2015. Oil accumulation by the oleaginous diatom Fistulifera solaris as revealed by the genome and transcriptome. Plant Cell 27, 162–176. http://dx.doi.org/10.1105/tpc.114.135194.
Traller, J.C., Cokus, S.J., Lopez, D.A., Gaidarenko, O., Smith, S.R., McCrow, J.P., Gallaher, S.D., Podell, S., Thompson, M., Cook, O., Morselli, M., Jaroszewicz, A., Allen, E.E., Allen, A.E., Merchant, S.S., Pellegrini, M., Hildebrand, M., 2016. Genome and methylome of the oleaginous diatom Cyclotella cryptica reveal genetic flexibility toward a high lipid phenotype. Biotechnol. Biofuels 9, 1–20. http://dx.doi.org/10.1186/s13068-016-0670-3.
Tran, N.T., Padula, M.P., Evenhuis, C.R., Commault, A.S., Ralph, P.J., Tamburic, B., 2016. Proteomic and biophysical analyses reveal a metabolic shift in nitrogen deprived Nannochloropsis oculata. Algal Res. 19, 1–11. http://dx.doi.org/10.1016/j.algal.2016.07.009.
Trentacoste, E.M., Shrestha, R.P., Smith, S.R., Glé, C., Hartmann, A.C., Hildebrand, M., 2013. Metabolic engineering of lipid catabolism increases microalgal lipid accumulation without compromising growth. PNAS 110, 19748–19753. http://dx.doi.org/10.1073/pnas.1309299110/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1309299110.
Tsai, C.H., Zienkiewicz, K., Amstutz, C.L., Brink, B.G., Warakanont, J., Roston, R., Benning, C., 2015. Dynamics of protein and polar lipid recruitment during lipid droplet assembly in Chlamydomonas reinhardtii. Plant J. 83, 650–660. http://dx.doi.org/10.1111/tpj.12917.
Valenzuela, J., Mazurie, A., Carlson, R.P., Gerlach, R., Cooksey, K.E., Peyton, B.M., Fields, M.W., 2012. Potential role of multiple carbon fixation pathways during lipid accumulation in Phaeodactylum tricornutum. Biotechnol. Biofuels 5, 1–17.
Velmurugan, N., Sung, M., Yim, S.S., Park, M.S., Yang, J.W., Jeong, K.J., 2014. Systematically programmed adaptive evolution reveals potential role of carbon and nitrogen pathways during lipid accumulation in Chlamydomonas reinhardtii. Biotechnol. Biofuels 7, 117. http://dx.doi.org/10.1186/s13068-014-0117-7.
Vieler, A., Brubaker, S.B., Vick, B., Benning, C., 2012. A lipid droplet protein of Nannochloropsis with functions partially analogous to plant oleosins. Plant Physiol. 158, 1562–1569. http://dx.doi.org/10.1104/pp.111.193029.
Vinyard, D.J., Gimpel, J., Ananyev, G.M., Cornejo, M.A., Golden, S.S., Mayfield, S.P., Dismukes, G.C., 2013. Natural variants of photosystem II subunit D1 tune photochemical fitness to solar intensity. J. Biol. Chem. 288, 5451–5462. http://dx.doi.org/10.1074/jbc.M112.394668.
Vonlanthen, S., Dauvillée, D., Purton, S., 2015. Evaluation of novel starch-deficient mutants of Chlorella sorokiniana for hyper-accumulation of lipids. Algal Res. 12, 109–118. http://dx.doi.org/10.1016/j.algal.2015.08.008.
Wang, H., Alvarez, S., Hicks, L.M., 2012. Comprehensive comparison of iTRAQ and label-free LC-based quantitative proteomics approaches using two Chlamydomonas reinhardtii strains of interest for biofuels engineering. J. Proteome Res. 11, 487–501.
Wang, C., Li, Y., Lu, J., Deng, X., Li, H., Hu, Z., 2017. Effect of overexpression of LPAAT and GPD1 on lipid synthesis and composition in green microalga Chlamydomonas reinhardtii. J. Appl. Phycol. 1–9. http://dx.doi.org/10.1007/s10811-017-1349-2.
Wase, N., Black, P.N., Stanley, B.A., Dirusso, C.C., 2014. Integrated quantitative analysis of nitrogen stress response in Chlamydomonas reinhardtii using metabolite and protein profiling. J. Proteome Res. 13, 1373–1396. http://dx.doi.org/10.1021/pr400952z.
Wei, L., Xin, Y., Wang, Q., Yang, J., Hu, H., Xu, J., 2017. RNAi-based targeted gene knockdown in the model oleaginous microalgae Nannochloropsis oceanica. Plant J. 89, 1236–1250. http://dx.doi.org/10.1111/tpj.13411.
Work, V.H., Radakovits, R., Jinkerson, R.E., Meuser, J.E., Elliott, L.G., Vinyard, D.J., Laurens, L.M.L., Dismukes, G.C., Posewitz, M.C., 2010. Increased lipid accumulation in the Chlamydomonas reinhardtii sta7 - 10 starchless isoamylase mutant and increased carbohydrate synthesis in complemented strains. Eukaryot. Cell 9, 1251–1261. http://dx.doi.org/10.1128/EC.00075-10.
Yang, Z.-K., Niu, Y.-F., Ma, Y.-H., Xue, J., Zhang, M.-H., Yang, W.-D., Liu, J.-S., Lu, S.-H., Guan, Y., Li, H.-Y., 2013. Molecular and cellular mechanisms of neutral lipid accumulation in diatom following nitrogen deprivation. Biotechnol. Biofuels 6, 67. http://dx.doi.org/10.1186/1754-6834-6-67.
Yang, Z., Ma, Y., Zheng, J., 2014. Proteomics to reveal metabolic network shifts towards lipid accumulation following nitrogen deprivation in the diatom Phaeodactylum tricornutum. J. Appl. Phycol. 26, 73–82. http://dx.doi.org/10.1007/s10811-013-0050-3.
Yang, D., Song, D., Kind, T., Ma, Y., Hoefkens, J., Fiehn, O., 2015a. Lipidomic analysis of Chlamydomonas reinhardtii under nitrogen and sulfur deprivation. PLoS One 10, 1–16. http://dx.doi.org/10.1371/journal.pone.0137948.
Yang, Y., Feng, J., Li, T., Ge, F., Zhao, J., 2015b. Original article CyanOmics: an integrated database of omics for the model cyanobacterium Synechococcus sp. PCC 7002. Database (Oxford) 1–9. http://dx.doi.org/10.1093/database/bau127.
Yang, Z., Zhao, Y., Liu, Z., Liu, C., Hu, Z., Hou, Y., 2017. A mathematical model of neutral lipid content in terms of initial nitrogen concentration and validation in Coelastrum sp. ha-1 and application in Chlorella sorokiniana. Biomed. Res. Int. 2017. http://dx.doi.org/10.1155/2017/9253020.
Yao, L., Tan, T.W., Ng, Y.K., Hon, K., Ban, K., Shen, H., Lin, H., Lee, Y.K., 2015. RNA Seq transcriptomic analysis with Bag2D software identifies key pathways enhancing lipid yield in a high lipidproducing mutant of the non - model green alga Dunaliella tertiolecta. Biotechnol. Biofuels 8, 1–16. http://dx.doi.org/10.1186/s13068-015-0382-0.
Yao, L., Shen, H., Wang, N., Tatlay, J., Li, L., Tan, T.W., Lee, Y.K., 2017. Elevated acetyl-CoA by amino acid recycling fuels microalgal neutral lipid accumulation in exponential growth phase for biofuel production. Plant Biotechnol. J. 15, 497–509. http://dx.doi.org/10.1111/pbi.12648.
Yohn, C., Brand, A., Michael, M., Behnke, C.A., 2016. Transformation of algae for increasing lipid production. U.S. patent 9428779 B2, filed February 1, 2011, and issued August 30, 2016.
Yu, X., Sun, J., Zheng, J., Sun, Y., Wang, Z., 2016. Metabolomics analysis reveals 6-benzylaminopurine as a stimulator for improving lipid and DHA accumulation of Aurantiochytrium sp. J. Chem. Technol. Biotechnol. 91, 1199–1207. http://dx.doi.org/10.1002/jctb.4869.
Zhang, M., Fan, J., Taylor, D.C., Ohlrogge, J.B., 2009. DGAT1 and PDAT1 acyltransferases have overlapping functions in Arabidopsis triacylglycerol biosynthesis and are essential for normal pollen and seed development. Plant Cell 21, 3885–3901. http://dx.doi.org/10.1105/tpc.109.071795.
Zhang, W., Li, F., Nie, L., 2017. Integrating multiple “omics” analysis for microbial biology: application and methodologies. Microbiology 156, 287–301. http://dx.doi.org/10.1099/mic.0.034793-0.
Zheng, M., Tian, J., Yang, G., Zheng, L., Chen, G., Chen, J., Wang, B., 2013. Transcriptome sequencing, annotation and expression analysis of Nannochloropsis sp. at different growth phases. Gene 523, 117–121. http://dx.doi.org/10.1016/j.gene.2013.04.005.
Zivy, M., Wienkoop, S., Pinheiro, C., Goulas, E., Carpentier, S., 2015. The quest for tolerant varieties: the importance of integrating “omics” techniques to phenotyping. Front. Plant Sci. 6, 1–11. http://dx.doi.org/10.3389/fpls.2015.00448.
Zoghlami, L., Djebali, W., Abbes, Z., Hediji, H., Maucourt, M., Moing, A., Brouquisse, R., Chaïbi, W., 2013. Metabolite modifications in Solanum lycopersicum roots and leaves under cadmium stress. Afr. J. Biotechnol. 10, 567–579. http://dx.doi.org/10.4314/ajb.v10i4.
Zuñiga, C., Li, C.T., Huelsman, T., Levering, J., Zielinski, D.C., McConnell, B.O., Long, C.P., Knoshaug, E.P., Guarnieri, M.T., Antoniewicz, M.R., Betenbaugh, M.J., Zengler, K., 2016. Genome-scale metabolic model for the green alga Chlorella vulgaris UTEX accurately predicts phenotypes under autotrophic, heterotrophic, and mixotrophic growth conditions. Plant Physiol. 172, 589–802. http://dx.doi.org/10.1104/pp.16.00593.