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REVIEW – DOSSIER

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MICRO-ORGANISMES PRODUCTEURS DE LIPIDES

Third-generation biofuels: current and future research on microalgal lipid biotechnology

Yonghua Li-Beisson[a] and Gilles Peltier

CEA - CNRS - Aix Marseille Université (UMR7265), Direction des Sciences du Vivant, Institut de Biologie Environnementale et de Biotechnologie, CEA Cadarache, 13108 Saint-Paul-lez-Durance, France

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Abstract – One pressing issue faced by modern societies is to develop renewable energy for transportation. Microalgal biomass offers an attractive solution due to its high (annual) surface biomass productivity, efficient conversion of solar energy into chemical energy and the ability to grow on non-agricultural land. Despite these considerable advantages, microalgal biofuels are not yet commercially sustainable. Major challenges lie in improving both cultivation technologies and microalgal strains. A microalgal crop species is yet to emerge. In this review, we focus on researches aiming at understanding and harnessing lipid metabolism in microalgae in view of producing lipid-based biofuels such as biodiesel. Current biotechnological challenges and key progresses made in the development of algal models, genetic tools and lipid metabolic engineering strategies are reviewed. Possible future research directions to increase oil yields in microalgae are also highlighted.

Keywords: Microalgae / biodiesel / model alga / lipid metabolism / metabolic engineering

Résumé – Les biocarburants de troisième génération : recherches actuelles et futures sur les lipides de microalgues. Le développement de sources d'énergie renouvelables pour le domaine des transports constitue un défi à venir majeur de nos sociétés. La biomasse issue de microalgues présente une alternative prometteuse en raison de l'efficacité de ces organismes à convertir l'énergie solaire en composés riches en énergie comme les lipides, de leur forte productivité surfacique annuelle et de la possibilité de les produire sur des terres non cultivables. En dépit de ces avantages, les algocarburants ne sont pour l'heure pas économiquement viables. Les défis principaux concernent l'amélioration des technologies de culture ainsi que l'amélioration des souches de microalgues, dont la domestication est un objectif majeur pour la recherche. Nous décrivons dans cette revue les recherches en cours ayant pour but la compréhension et la domestication du métabolisme des lipides en vue de produire des biocarburants dérivés tels que le biodiesel. Les défis biotechnologiques, l'émergence de nouveaux modèles de microalgues ainsi que les développements récents d'outils de génétique et d'ingénierie métabolique sont abordés. Des directions de recherche futures visant à accroître les rendements en huile sont aussi discutées.

Mots clés : Microalgue / biodiesel / modèles / métabolisme lipidique / ingénierie métabolique

1 Introduction

1.1 Biofuel

With the rapid decrease in fossil fuel reserves, the increasing demand in energy, particularly for transportation and the rising concerns about global warming and other related social-economic issues, there is a world-wide urge to develop renewable platforms for fuel production. The solutions to meet the energy demand could be many-fold, ranging from solar, water, wind, nuclear energy to bioenergy. Studies of energy production based on biomass have gained increasing attention because the knowledge generated will not only allow production of fuels but also other high-value chemical molecules essential to the oleo-chemical industries which at the moment are still heavily dependent on fossil fuel. Three generations of biofuels have emerged. The 1st generation biofuel is based on edible plant parts (oilseeds, grains, *etc.*); the 2nd refers to energy production from non-edible plants or non-edible parts of plants; and the 3rd is based on energy production from photosynthetic microorganisms such as microalgae.

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[a] Correspondence: `yonghua.li@cea.fr`

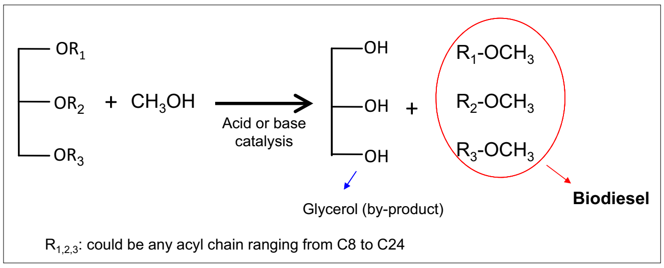

Fig. 1. Structure of a triacylglycerol and its conversion to biodiesel via acid- or based-catalyzed transmethylation reaction.

1.2 Advantages and limits of the 3rd generation biofuel

Microalgae have very high surface productivity, and can be cultivated on non-arable land (therefore not competing with food production). Because microalgae are photosynthetic (*i.e.* CO_2-fixing) organisms, their use provides greenhouse gas mitigation benefits. Depending on the species, microalgae can grow on fresh, brackish, sea, or even waste water and can accumulate up to 60% oil per dry weight under stress conditions (Chisti, 2007). Therefore, microalgae have attracted increasing attention for their potential as producers of biodiesel or other lipid-based biofuels (Beer, *et al.*, 2009; Scott, *et al.*, 2010; Wijffels and Barbosa, 2010). Costs of cultivation in photobioreactors or open ponds, biomass harvest and oil extraction from algal biomass remain very high however (Grima, *et al.*, 2003). In addition, many microalgal species are not suitable for industrial cultures, fatty acid composition of microalgal lipids may not be optimal for use as biofuel, and stress conditions needed to accumulate lipids result in arrest of cell growth and division, causing a strong limitation of biomass productivity (Siaut, *et al.*, 2011). Therefore, biofuel production from microalgae is not yet sustainable, and we have not yet come up with one algal strain which can be called “an algal crop” for biofuel production. Intensive research efforts are needed in both strain development and technology innovations (Delrue, *et al.*, 2012). Here we review key advances on the biotechnological aspects of microalgal oil research.

2 From microalgal lipids to biodiesel

Microalgae have been found to synthesize a large variety of fatty acids and lipids (Hu, *et al.*, 2008; Harwood and Guschina, 2009), the composition of which often reflects adaptation to environmental conditions (Harwood and Guschina, 2009). In photosynthetic organisms, chloroplast is the central compartment of fatty acid synthesis. Fatty acids produced in the plastid are building blocks of all membrane and storage lipids. Upon stresses, almost all microalgae species can produce oil, *i.e.* triacylglycerols (TAGs), the major form of storage lipids in eukaryotic cells. One triacylglycerol molecule is made of three often different fatty acids which are esterified to the 3 hydroxyl groups of a glycerol backbone. Biological oils are precursors to diesel, and can be converted to biodiesel *via* a simple transesterification procedure (Fig. 1). This chemical process liberates fatty acid methyl esters, which are the component of biodiesel. Acyl chains determine the structural thus physical properties of the oil and the biodiesel. Modification of fatty acids (*e.g.* degree of unsaturation, chain length, introduction of functional groups such as hydroxyl or epoxy, *etc.*) has been one of the major targets of genetic engineering in plants and microalgae (Shanklin and Cahoon, 1998). Fatty acids produced by microalgae, thus, represent a potential alternative not only to fossil fuels but also to other petroleum derivatives (synthons for green chemistry).

3 Major biotechnological challenges

3.1 Decoupling oil synthesis from arrest of cell division

The amount of oil produced by a microalga is dependent on species and cultivation conditions (Sheehan, 1998; Hu, *et al.*, 2008). Substantial oil accumulation almost always requires stress conditions. One of the most potent stresses to trigger oil accumulation is nitrogen deprivation. Under such culture conditions, some microalgae such as *Chlorella* species can accumulate up to 60% of oils in its biomass, explaining the considerable regain for microalgae as a biofuel (biodiesel) feedstock (Chisti, 2007). However, nitrogen-starvation limits the overall productivity of the system (Hu, *et al.*, 2008). Maximal lipid yields obtained so far in large scale cultivation systems are 10 to 20 times lower than the theoretical maximum (5000–15 000 gallons per acre per year) (Sheehan, 1998). To circumvent the dependence on stress, dissecting the cellular processes of response to nutrient status, cell division and carbon storage through the study of mutant strains is required.

3.2 Harnessing the complexity of lipid metabolism

Our current understanding of oil biosynthesis in microalgae is still rather limited, although rapid progress has been made lately (Merchant, *et al.*, 2012; Liu and Benning, 2013). The current pathway involves three major spatially separated biochemical steps, *i.e.* plastidial *de novo* fatty acid synthesis, acylation of fatty acids to glycerol, and deposition as oil bodies, the sub-cellular compartment destined for oil storage (Fig. 2). In plants, this seemingly simple route requires the coordinated actions of several hundred proteins, and regulation across three sub-cellular compartments (Li-Beisson, 2010). For oil synthesis alone in micro algae, three distinct pathway have been proposed (Fig. 2). In plans, the best known is an acyl-CoA-dependent pathway, catalyzed by ER membrane bound enzymes (Riekhof, *et al.*, 2005), similarly to what occurs in plants. An alternative route to oil synthesis is present in both plants and yeast, and is catalyzed by phospholipid:diacylglycerol acyltransferase (PDAT) contributing to the synthesis of triacylglycerol using phosphatidylcholine as an acyl donor and *sn*-1,2-diacylglycerol as an acyl acceptor. A homolog of this enzyme is present in some sequenced algal genomes including *C. reinhardtii* where a mutant of PDAT is found to accumulate 30% less oil than its wild-type (Yoon, *et al.*, 2012), thus establishing the contribution of this pathway to oil synthesis in the green algal lineage. A third pathway has recently been proposed by Fan and Xu (Fan, *et al.*, 2011) who

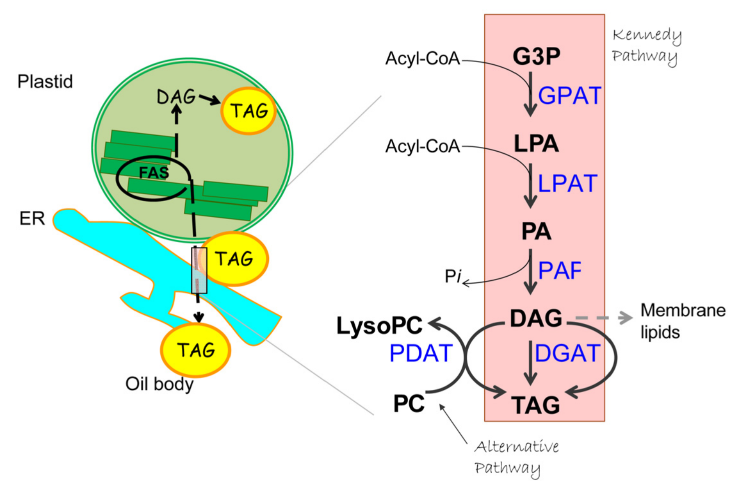

Fig. 2. Lipid biosynthesis in an algal cell, major pathways and cellular locations. CoA, coenzyme A; DAG, diacylglycerol; DGAT, diacylglycerol acyltransferase; ER, endoplasmic reticulum; GPAT, glycerol-3-phosphate acyltransferase; FAS, fatty acid synthesis; G3P, glycerol-3-phosphate; LPAT, lysophosphatidic acid acyltransferase; LPA, lysophosphatidic acid; MAG, monoacylglycerol; PA, phosphatidic acid; PAP, phosphatidic acid phosphatise; PL, phospholipids; PDAT, phospholipid:diacylglycerol acyltransferase.

has demonstrated that at least part of the triacylglycerol synthetic pathway is present in the plastid of *C. reinhardtii*. They have further observed under transmission electron microscope (TEM) that some of the oil droplets formed in the plastid can be secreted and re-located to the cytosol. This was later confirmed by another study (Goodson, *et al.*, 2011). These observations point to the fact that, in *C. reinhardtii* at least, some triacylglycerols are made in the plastid. Thus, a deeper knowledge of the underlying biochemistry, cell biology and genetics of lipid metabolism in this group of organisms is needed.

The champions of current agriculture, for example modern maize, wheat, *etc.*, have been obtained through millennium years of domestication (*i.e.* selective breeding). None of microalgae species so far have yet been subjected to domestication (selection or breeding) for oil production. The goals of microalgae lipid biologists include construction of industrially robust hyper oil-accumulators *via* genetic engineering, thus providing the society and industry with designer algal "crops" (Fig. 3). These goals can only be met through combining comprehensive knowledge on lipid metabolic pathways with development of novel and sophisticated genetic and genomic tools.

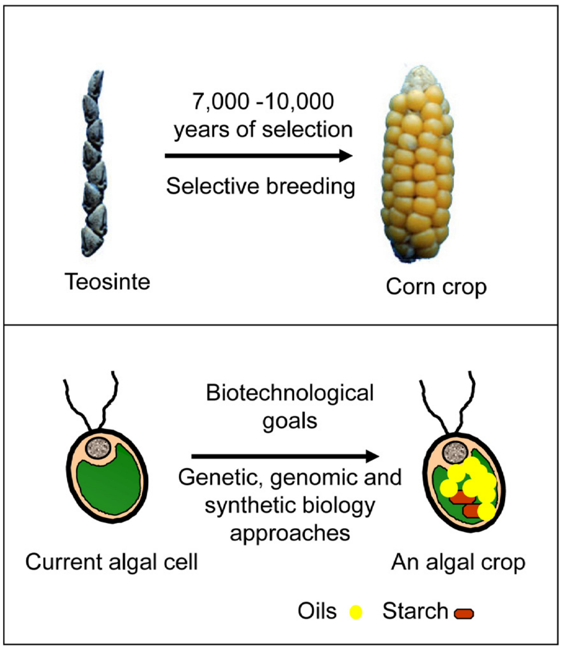

Fig. 3. Domestication of plant crops, and the possible future of an algal crop.

4 Current and emerging algal models for research on lipid-based biofuels

Microalgae are microscopic algae present in freshwater as well as marine systems. It encompasses the largest biodiversity with 200 000–800 000 species exist of which about 40 000 species are described (Andersen, 1992), thus constituting one of the least explored biological resources. Several high-value compounds [for example carotenoids, ω-3 polyunsaturated fatty acids (PUFAs), antioxidants, *etc.*] are currently commercially produced from industrially cultivated species (Wijffels and Barbosa, 2010). An algal crop destined for biofuel production is yet to emerge. Understanding of the function of biological systems in any given group of organism has largely depended on the development of models. Lately, due to the easy access to genome sequencing technologies, more than 10 microalgal species have been sequenced, and many more are in the pipeline. Intensive efforts have been put in place for the development of molecular genetic tools, thus allowing genetic manipulation of any given algal lineage. This development together with our knowledge gained through examining model systems should aid in the master design of an ideal algal cell factory for production of industrially desirable molecules (including diesel). The algae field is still lacking essential models to accommodate its great diversity. Lately, several models for different phyla are emerging and are used for fundamental research in many laboratories. A few of these models are described briefly below.

4.1 *Chlamydomonas reinhardtii*

Among the diversity of microalgal species, the Chlorophyceae *Chlamydomonas reinhardtii* is the best studied microalga at the physiological, as well as genetic and genomic level. The success of *C. reinhardtti* as a model is largely due to the possibility to perform genetic analysis through sexual crosses. It has been used to study various fundamental biological processes including photosynthesis, chloroplast biogenesis, flagella function and assembly, starch accumulation, photobiological hydrogen production, and more recently on lipid accumulation (Harris, 2001; Merchant, *et al.*, 2012).

C. reinhardtii can be grown either photoautotrophically, mixotrophically or heterotrophically (Harris, 2001). Photosynthetic function in *C. reinhardtii* is thus dispensable, a feature which has originally been used to isolate mutants defected in photosynthetic apparatus (Bennoun and Levine, 1967). During its vegetative phase, *Chlamydomonas* nuclear genome is haploid thus allowing identification of mutant phenotypes at the first generation, which facilitated forward genetic approaches. All three genomes (nuclear, plastid and mitochondrion) have been sequenced, and genetic transformation is possible for all three genomes (Rochaix, 2002). Substantial literature and infrastructure is in place, thus opens doors to new discoveries.

Many laboratories have adopted *C. reinhardtii* as a reference organism for studying TAG accumulation (Merchant, *et al.*, 2012), although there has been some debates regarding if *Chlamydomonas* is or not an oleaginous alga. Stressed *Chlamydomonas* cells accumulate oils in oil bodies (Wang, *et al.*, 2009; Moellering and Benning, 2010; Siaut, *et al.*, 2011). Depending on the strains used and stress conditions, the oil content can reach 50% of dry biomass in starch-less mutants in response to N starvation (Li, *et al.*, 2010). With the versatile molecular genetic tools available (Harris, 2001), *Chlamydomonas* thus serves as an excellent model organism for addressing fundamental biological questions related to oil synthesis and degradation.

If *Chlamydomonas* has been widely and successfully used to develop forward genetic approaches to study different processes (photosynthesis, flagellar movement, starch metabolism...) reverse genetic approaches are more limited in this model species. Major drawbacks working with *Chlamydomonas* are the fact that transgene expression is at low efficieng and that no efficient transformation of the nuclear genome based on homologous recombination has been demonstrated. Isolation of mutants affected in a given gene employing PCR-based techniques is feasible but tedious (Gonzalez-Ballester, *et al.*, 2011). With the development of relatively cheap high-throughput genome sequencing (Dutcher, *et al.*, 2012), tagged mutant collections start to emerge (personal communication S. Merchant). Another major concern over a model organism like *C. reinhardtii* is often the fact that it is not an industrial alga and has not historically been cultivated at large scale. But a possible solution to this drawback has lately been provided by Microphyt, a French company located in Montpellier, who has developed technologies for industrial cultivation of *C. reinhardtii* in tubular photobioreactors.

4.2 *Chlorella* sp.

Chlorella is a genus of single-cell green microalgae, belonging to the same phylum Chlorophyta as *Chlamydomonas.* It is spherical in shape, about 2–10 μm in diameter, and can be distinguished from *Chlamydomonas* because it does not have flagella. *Chlorella* consists of over 80 species, isolated from either freshwater or marine environment. It is an attractive food producer because it is high in protein and other essential nutrients; several species are known sources of ω-3 PUFAs (Tokusoglu and Unal, 2003) and astaxanthin (Campenni *et al.* 2013). It is robust and widely cultivated commercially. One species has been sequenced (Blanc, *et al.*, 2010), and genetic manipulation is demonstrated for three species (*C. vulgaris*, *C. ellipsoidea*, and *C. kessleri*) (Leon and Fernandez, 2007; Gong, *et al.*, 2011; Niu, *et al.*, 2011; Chia, *et al.*, 2013). *Chlorella* is an emerging model for study of photosynthetic carbon fixation in the green lineage of eukaryotic microalgae.

4.3 *Ostreococcus tauri*

The marine pico-eukaryote *Ostreococcus* belongs to Chlorophyta, and is the smallest photosynthetic unicellular eukaryote (Palenik, *et al.*, 2007). It has an extremely small genome *i.e.* 12 Mb which is 10 times less than *C. reinhardtii*. *Ostreococcus* offers great advantages for functional genetic studies because it is thought to represent the minimal genes required to carry out essential biological functions. *Ostreoccocus* has emerged as a powerful "ready-to-use" algal model because a set of genetic tools have been developed including high efficiency gene overexpression and gene targeting by homologous recombination (personal communication F.Y Bouget), which makes it more amenable to many functional genetic studies than *Chlamydomonas*. *Ostreococcus* as a model for marine species is, thus, highly complementary to the freshwater green alga *Chlamydomonas*. Finally, substantial amount of literature is present which allows us minimal understanding of its physiology.

4.4 *Phaeodactylum tricornutum*

Diatoms are responsible for a significant proportion of primary marine biomass production. *Phaeodactylum tricornutum* has been developed as a model for diatom research. It is the only species in the genus *Phaeodactylum*. Unlike most other diatoms *P. tricornutum* can grow in the absence of silicon, and the biogenesis of silicified frustules is facultative. *P. tricornutum* can accumulate significant amount of lipids under silicon absence (Sheehan, 1998), and the fact that it can synthesis high amount of ω-3 PUFAs have brought considerable interests in research on lipid metabolism in this diatom. These features together with its high biomass productivity, high lipid content, a fully sequenced genome (Bowler, *et al.*, 2008), and the versatile molecule toolboxes developed (Siaut, *et al.*, 2007), made it a prominent model system for both biodiesel and ω-3 PUFA production. Nonetheless, one of the major issues associated with *P. tricornutum* as a model is that there is no easy way to knock out genes, knock down being mostly available (De Riso, *et al.*, 2009).

4.5 *Nannochloropsis* sp.

Nannochloropsis is a heterokont microalga comprising six species, of which five are marine and one freshwater. Due to its rigorous growth, and high content of ω-3 PUFAs and astaxanthin, it is commonly used in aquaculture applications. Upon stress, it can also accumulate significant amount of neutral lipids thus attracting interests on its development for biofuel production. Draft genome sequences are available for two of the marine species (*N. gaditana* and *N. oceanica*) (Radakovits, *et al.*, 2012; Vieler, *et al.*, 2012). Genetic transformation is available, and more importantly homologous recombination has been demonstrated with high efficiencies for *N. gaditana* (Kilian, *et al.*, 2011). Thus, *Nannochloropsis* represents an emerging model for lipid research on photosynthetic protists. Substantial collaborative effort at international level is required for this organism to become as the premier model for algal research.

5 Looking through current research approaches into future directions

5.1 Bio-prospecting for high performing algal strains suitable for oil production

Only a handful of the tens of thousands of microalgal species known to exist in nature are currently cultured at large scale and used for commercial applications, among which only a few hundreds have been investigated for potential biotechnological applications. Bio-prospecting for new species from local or extreme environments for oil content or other high value product has been an on-going international effort. One of the largest conducted so far has been the survey carried out by the U.S. Department of Energy (Sheehan, 1998). They studied around 300 species selected from 3000 isolated strains. More recently, several research studies for strain selection and induction of lipid biosynthesis have been carried out (de la Vega, *et al.*, 2011; Do Nascimento *et al.* 2012). These studies revealed that the intrinsic ability to produce large quantity of oil and lipid is generally species/strain specific, rather than genus specific. Nonetheless, a survey of the literature by Hu *et al.* (Hu *et al.* 2008) revealed that species belonging to the group of green microalgae (Chlorophyta) generally accumulate oil to a higher level than species of other algal taxa such as cyanobacteria, brown algae, or red algae. In addition to the ability to produce biomass and accumulate oil, other criteria related to cultivation constraints (strain robustness) or to downstream processes (harvesting, oil extraction...) have to be considered together and has been proposed to impact the final productivity of the system and the economy of microalgae-based biodiesel production (Delrue, *et al.*, 2012). Continuing efforts at bio-prospecting of strains isolated from local environment is a key to isolate strains suitable for one particular geological niche.

5.2 Genetic improvement of algal strains

Current crop plants have gone through thousands of years of selective breeding which is a common agricultural practice. However conventional breeding is not suitable for algae strains since sexual cycles are lacking or poorly defined for most species. Metabolic engineering thus represents one of the most promising strategies toward production of suitable algal strains (Rosenberg, *et al.*, 2008). Comparing to classical breeding, genetic engineering is a time-saving technology. Indeed, metabolic engineering has revolutionized the traditional breeding programme and made high contributions to meet our societal needs. One of the most notable examples in successful genetic engineering is probably the creation of "golden rice" *via* genetic engineering of the rice plant to synthesize beta-carotene *via* introduction of two enzymes (Ye *et al.* 2000). Given the latest development in genomics, genetics, and molecular biology, synthetic biology is coming of age technology for addressing basic as well as applied questions in the bio-production of desirable molecules and fuels. For example, genetic engineering has already allowed the isolation of *Chlamydomonas* mutants with higher ability for the photobiological production of hydrogen (Tolleter, *et al.*, 2011); and mutants with reduced antenna sizes showing improved biomass productivity in photobioreactors (Ort, *et al.*, 2011). Heterologous gene expression has been used successfully to redirect microalgal metabolism. For example, heterologous expression of two thioesterases in *Phaeodactylum tricornutum* caused accumulation of novel medium chain fatty acids (Radakovits, *et al.*, 2011). Regulatory proteins controlling oil synthesis have also been isolated in the model *Chlamydomonas*, and overexpression of these proteins led to altered cellular oil content (Yohn, 2011; Boyle, *et al.*, 2012).

Despite the promising achievements, all routine work on metabolic engineering through genetic manipulation remains limited to a few model species where efficient and stable transformation is possible. Common issues associated with other transformation experiments reported in the literature are low efficiency and instability of transgenes introduced. Therefore, there is substantial need for development of molecular tools for other non-model microalgal species. One such achievement is the recent demonstration of high efficiency homologous recombination for an industrially relevant alga *N. gaditana* (Kilian, *et al.*, 2011). Such genetic tools would allow the metabolic engineering of pathways or physiological processes and therefore allow the production of transgenic microalgae that are genetically tailored for the production of biodiesel.

In addition, successful genetic engineering is also dependent on the development of high-throughput screening procedures which allow the rapid and easy screening of a high number of cell lines for desirable phenotypes (high growth rate, high oil content, better fatty acid profile, *etc.*). For example, in screening of high oil accumulators, the semi-quantitative lipophilic dye Nile red coupled with Flow

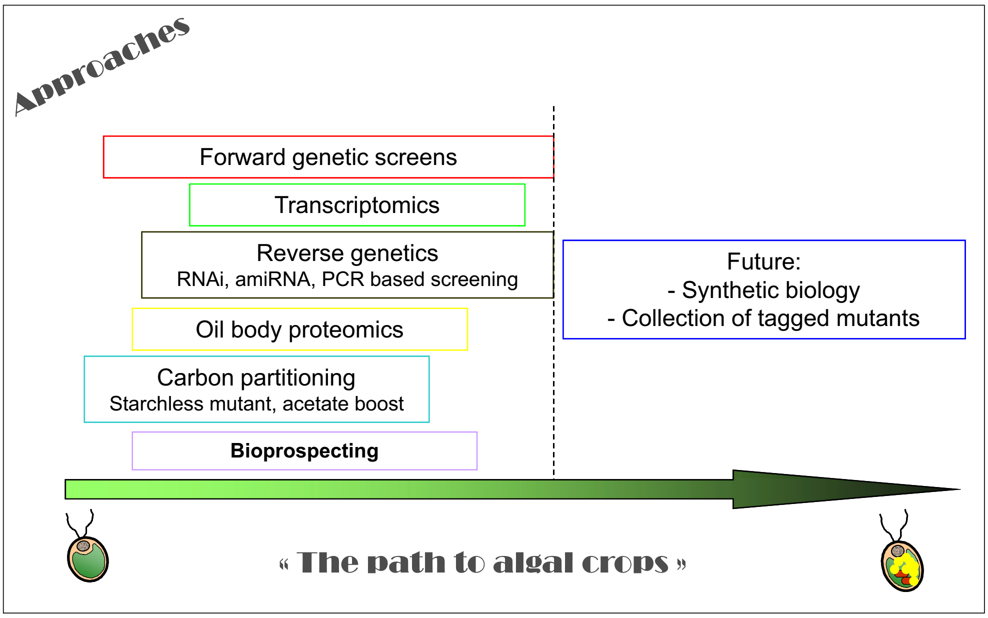

Fig. 4. Current major approaches applied and future directions.

cytometry has been demonstrated as a powerful tool to isolate mutant with altered oil content (ongoing work in the authors' laboratory).

5.3 Advancing knowledge on lipid metabolism in microalgae

A comprehensive view of lipid metabolism in microalgae is still in its infancy. Most of the components of lipid biosynthesis are deduced from homologies to proteins of either known plant or yeast lipid synthetic pathways. To aid in genetic engineering and allow intelligent design of strains, a thorough knowledge of lipid metabolism in microalgae is urgently needed. Recent studies dedicated to advance our understanding of oil metabolism in this alga (summarized in Fig. 4) include: (i) detailed characterization of the oil accumulation kinetics in response to nitrogen depletion (Wang, *et al.*, 2009; Siaut, *et al.*, 2011); (ii) testing the potential impact of diverting carbon precursors from starch synthesis to oil accumulation using starchless mutants (Li, *et al.*, 2010; Siaut, *et al.*, 2011); (iii) identification of proteins associated with oil droplets – the major cellular compartment for oil storage (Moellering and Benning, 2010; Nguyen, *et al.*, 2011); (iv) comparative transcriptomic studies on cells before and after nitrogen removal (Miller, *et al.*, 2010); (v) microscopic and biochemical analyses of the possible sub-cellular locations of oil droplets in *Chlamydomonas* and other algae (Fan, *et al.*, 2011; Goodson, *et al.*, 2011; Fan, *et al.*, 2012); (vi) Forward genetic screening of mutants affected in oil synthesis (Li, *et al.*, 2012). Please note that these are just examples, and are not meant for an exhaustive list. These pioneering works have revealed the potential complexity and divergence of oil metabolism from that of higher plants, and highlights interesting candidate genes for genetic engineering studies. Now it is the right time for verification of gene function using the latest tools developed for *Chlamydomonas* such artificial microRNA techniques to knock-down specific genes (Molnar, *et al.*, 2009), or isolation of specific mutants using PCR-based screening (Gonzalez-Ballester, *et al.*, 2011). In parallel, the development of forward genetic approaches based on efficient and high-throughput screening of mutant showing interesting properties (such as the ability to accumulate oil in optimal growth conditions), will be important to identify regulatory genes controlling oil accumulation.

6 Conclusion

Photosynthetic microalgae have provided us with food and feed, and now possibly also fuel. Research on microalgae lipids have come a long way starting from the 40's during the Second World War in Germany. Given the high impact of research on energy, algal fuel development is tightly related to economic, social and world development. Technologies developed today have progressed from manipulation of one or two genes to more system-based approaches. These technologies include high-throughput genome sequencing, and a suit of –omics technologies (genomics, transcriptomics, proteomics, metabolomics, and lipidomics). These powerful tools if used together with transformation and molecular genetic toolboxes developed for particular algal strains provide ample opportunities for lipid scientists to redesign algal metabolism toward production of oils or other chemical molecules useful for industrial applications.

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References

Andersen RA. 1992. Diversity of eukaryotic algae. *Biodivers. Conserv.* 1: 267–292.

Beer LL, Boyd ES, Peters JW, Posewitz MC. 2009. Engineering algae for biohydrogen and biofuel production. *Curr. Opin. Biotechnol.* 20: 264–271.

Bennoun P, Levine RP. 1967. Detecting Mutants That Have Impaired Photosynthesis by Their Increased Level of Fluorescence. *Plant Physiol.* 42: 1284–1287.

Blanc G, Duncan G, Agarkova I, Borodovski M, Gurnon J, Kuo A, *et al.* 2010. The *Chlorella variabilis* NC64A Genome Reveals Adaptation to Photosymbiosis, Coevolution with Viruses, and Cryptic Sex. *Plant Cell* 22: 2943–2955.

Bowler C, Allen AE, Badger JH, Grimwood J, Jabbari K, Kuo A, *et al.* 2008. The Phaeodactylum genome reveals the evolutionary history of diatom genomes. *Nature* 456: 239–244.

Boyle NR, Page MD, Liu B, Blaby IK, Casero D, Kropat J, *et al.* 2012. Three Acyltransferases and Nitrogen-responsive Regulator Are Implicated in Nitrogen Starvation-induced Triacylglycerol Accumulation in Chlamydomonas. *J. Biol. Chem.* 287: 15811–15825.

Campenni L, Nobre BP, Santos CA, Oliveira AC, Aires-Barros MR, Palavra AM, *et al.* 2013. Carotenoid and lipid production by the autotrophic microalga *Chlorella protothecoides* under nutritional, salinity, and luminosity stress conditions. *Appl. Microbiol. Biotechnol.* 97: 1383–1393.

Chia MA, Lombardi AT, Melão MdGG, Parrish CC. 2013. Lipid composition of *Chlorella vulgaris* (Trebouxiophyceae) as a function of different cadmium and phosphate concentrations. *Aquat. Toxicol.* 128–129: 171–182.

Chisti Y. 2007. Biodiesel from microalgae. *Biotechnol. Adv.* 25: 294–306.

de la Vega M, Diaz E, Vila M, Leon R. 2011. Isolation of a new strain of Picochlorum sp and characterization of its potential biotechnological applications. *Biotechnol. Progress* 27: 1535–1543.

De Riso V, Raniello R, Maumus F, Rogato A, Bowler C, Falciatore A. 2009. Gene silencing in the marine diatom *Phaeodactylum tricornutum*. *Nucleic Acids Res.* 37: e96

Delrue F, Setier PA, Sahut C, Cournac L, Roubaud A, Peltier G, *et al.* 2012. An economic, sustainability, and energetic model of biodiesel production from microalgae. *Bioresour. Technol.* 111: 191–200.

Do Nascimento M, Ortiz-Marquez JCF, Sanchez-Rizza L, Echarte MM, Curatti L. 2012. Bioprospecting for fast growing and biomass characterization of oleaginous microalgae from South-Eastern Buenos Aires, Argentina. *Bioresour. Technol.* 125: 283–290.

Dutcher SK, Li L, Lin H, Meyer L, Giddings TH, Kwan AL, *et al.* 2012. Whole-genome sequencing to identify mutants and polymorphisms in *Chlamydomonas reinhardtii*. *G3 (Bethesda)* 2: 15–22.

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

Fan JL, Andre C, Xu CC. 2011. A chloroplast pathway for the de novo biosynthesis of triacylglycerol in *Chlamydomonas reinhardtii*. *Febs Lett.* 585: 1985–1991.

Gong YM, Hu HH, Gao Y, Xu XD, Gao H. 2011. Microalgae as platforms for production of recombinant proteins and valuable compounds: progress and prospects. *J. Ind. Microbiol. Biotechnol.* 38: 1879–1890.

Gonzalez-Ballester D, Pootakham W, Mus F, Catalanotti C, Magneschi L, *et al.* 2011. Reverse genetics in Chlamydomonas: a platform for isolating insertional mutants. *Plant Methods* **7**: 24.

Goodson C, Roth R, Wang ZT, Goodenough U. 2011. Structural Correlates of Cytoplasmic and Chloroplast Lipid Body Synthesis in *Chlamydomonas reinhardtii* and Stimulation of Lipid Body Production with Acetate Boost. *Eukaryotic Cell* 10: 1592–1606.

Grima EM, Belarbi EH, Fernandez FGA, Medina AR, Chisti Y. 2003. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnol. Adv.* 20: 491–515.

Harris E. 2001. Chlamydomonas as a model organism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52: 363–406.

Harwood JL, Guschina IA. 2009. The versatility of algae and their lipid metabolism. *Biochimie* 91: 679–684.

Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, Darzins A. 2008. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *Plant J.* 54: 621–639.

Kilian O, Benemann CSE, Niyogi KK, Vick B. 2011. High-efficiency homologous recombination in the oil-producing alga *Nannochloropsis* sp. *Proc. Natl. Acad. Sci. USA* 108: 21265–21269.

Leon R, Fernandez E. 2007. Nuclear transformation of eukaryotic microalgae - Historical overview, achievements and problems. *Ado. Exp. Med. Biol.* 616: 1–11.

Li-Beisson Y, Shorrosh B, Beisson F, *et al.* 2010. Acyl lipid metabolism In R Last, ed, The *Arabidopsis* book. American Society of Plant Biologists Rockville, MD.

Li X, Moellering ER, Liu B, Johnny C, Fedewa M, Sears BB, Kuo M-H, 2012. A Galactoglycerolipid Lipase Is Required for Triacylglycerol Accumulation and Survival Following Nitrogen Deprivation in *Chlamydomonas reinhardtii*. *Plant Cell* 24: 4670–4686.

Li Y, Han D, Hu G, Sommerfeld M, Hu Q. 2010. Inhibition of starch synthesis results in overproduction of lipids in *Chlamydomonas reinhardtii*. *Biotechnol. Bioeng.* 107: 258–268

Liu B, Benning C. 2013. Lipid metabolism in microalgae distinguishes itself. *Curr. Opin. Biotechnol.* 24: 300–309.

Merchant SS, Kropat J, Liu B, Shaw J, Warakanont J. 2012. TAG, You're it! Chlamydomonas as a reference organism for understanding algal triacylglycerol accumulation. *Curr. Opin. Biotechnol.* 23: 352–363.

Miller R, Wu GX, Deshpande RR, Vieler A, Gartner K, Li XB, *et al.* 2010. Changes in transcript abundance in *Chlamydomonas reinhardtii* following nitrogen deprivation predict diversion of metabolism. *Plant Physiol.* 154: 1737–1752.

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

Molnar A, Bassett A, Thuenemann E, Schwach F, Karkare S, *et al.* 2009. Highly specific gene silencing by artificial microRNAs in the unicellular alga *Chlamydomonas reinhardtii*. *Plant J.* 58: 165–174.

Nguyen HM, Baudet M, Cuiné S, Adriano J-M, Barthe D, Billon E, *et al.* 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.

Niu YF, Zhang MH, Xie WH, Li JN, Gao YF, Yang WD, *et al.* 2011. A new inducible expression system in a transformed green alga, *Chlorella ulgaris*. *Genet. Mol. Res.* 10: 3427–3434.

Ort DR, Zhu XG, Melis A. 2011. Optimizing Antenna Size to Maximize Photosynthetic Efficiency. *Plant Physiol.* 155: 79–85.

Palenik B, Grimwood J, Aerts A, Rouzé P, Salamov A, Putnam N, *et al.* 2007. The tiny eukaryote Ostreococcus provides genomic insights into the paradox of plankton speciation. *Proc. Natl. Acad. Sci. USA* 104: 7705–7710.

Radakovits R, Eduafo PM, Posewitz MC. 2011. Genetic engineering of fatty acid chain length in *Phaeodactylum tricornutum*. *Metab. Eng.* 13: 89–95.

Radakovits R, Jinkerson RE, Fuerstenberg SI *et al.* 2012. Draft genome sequence and genetic transformation of the oleaginous alga *Nannochloropis gaditana*. *Nat. Commun.* 3: 686

Riekhof WR, Sears BB, Benning C. 2005. Annotation of genes involved in glycerolipid biosynthesis in *Chlamydomonas reinhardtii*: Discovery of the betaine lipid synthase BTA1(Cr). *Eukaryotic Cell* 4: 242–252.

Rochaix JD. 2002. The three genomes of Chlamydomonas. *Photosynth. Res.* 73: 285–293.

Rosenberg JN, Oyler GA, Wilkinson L, Betenbaugh MJ. 2008. A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. *Curr. Opin. Biotechnol.* 19: 430–436.

Scott SA, Davey MP, Dennis JS *et al.* 2010. Biodiesel from algae: challenges and prospects. *Curr. Opin. Biotechnol.* 21: 277–286.

Shanklin J, Cahoon EB. 1998. Desaturation and related modifications of fatty acids. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 49: 611–641.

Sheehan J, Dunahay T, Benemann J, Roessler PG. 1998. A look back at the US Department of Energy's aquatic species program – biodiesel from algae. In US Department of Energy's Office of Fuels Development, Golden, CO: National Renewable Energy Laboratory.

Siaut M, Cuine S, Cagnon C *et al.* 2011. Oil accumulation in the model green alga *Chlamydomonas reinhardtii*: characterization, variability between common laboratory strains and relationship with starch reserves. *BMC Biotechnology* 11: 7.

Siaut M, Heijde M, Mangogna M *et al.* 2007. Molecular toolbox for studying diatom biology in *Phaeodactylum tricornutum. Gene* 406: 23–35.

Tokusoglu O, Unal MK. 2003. Biomass nutrient profiles of three microalgae: Spirulina platensis, *Chlorella vulgaris*, and *Isochrisis galbana*. *J. Food Sci.* 68: 1144–1148.

Tolleter D, Ghysels B, Alric J *et al.* 2011. Control of Hydrogen Photoproduction by the Proton Gradient Generated by Cyclic Electron Flow in *Chlamydomonas reinhardtii*. *Plant Cell* 23: 2619–2630.

Vieler A, Wu G, Tsai C-H *et al.* 2012. Genome, Functional Gene Annotation, and Nuclear Transformation of the Heterokont Oleaginous Alga *Nannochloropsis oceanica* CCMP1779. *PLoS Genet.* 8: e1003064.

Wang ZT, Ullrich N, Joo S, Waffenschmidt S, Goodenough U. 2009. Algal lipid bodies: stress induction, purification, and biochemical characterization in wild-type and starchless *Chlamydomonas reinhardtii*. *Eukaryot Cell* 8: 1856–1868.

Wijffels RH, Barbosa MJ. 2010. An Outlook on Microalgal Biofuels. *Science* 329: 796–799.

Ye X, Al-Babili S, Klöti A, Zhang J, Lucca P, Beyer P, *et al.* 2000. Engineering the Provitamin A (β-Carotene) Biosynthetic Pathway into (Carotenoid-Free) Rice Endosperm. *Science* 287: 303–305.

Yohn C MM, Behnke C, Brand A. 2011. Stress-induced lipid trigger. US Patent.

Yoon K, Han D, Li Y, Sommerfeld M, Hu Q. 2012. Phospholipid: Diacylglycerol Acyltransferase Is a Multifunctional Enzyme Involved in Membrane Lipid Turnover and Degradation While Synthesizing Triacylglycerol in the Unicellular Green Microalga *Chlamydomonas reinhardtii*. *Plant Cell* 24: 3708–3724.

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