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Research review paper

Current advances in molecular, biochemical, and computational modeling analysis of microalgal triacylglycerol biosynthesis

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ABSTRACT

Triacylglycerols (TAGs) are highly reduced energy storage molecules ideal for biodiesel production. Microalgal TAG biosynthesis has been studied extensively in recent years, both at the molecular level and systems level through experimental studies and computational modeling. However, discussions of the strategies and products of the experimental and modeling approaches are rarely integrated and summarized together in a way that promotes collaboration among modelers and biologists in this field. In this review, we outline advances toward understanding the cellular and molecular factors regulating TAG biosynthesis in unicellular microalgae with an emphasis on recent studies on rate-limiting steps in fatty acid and TAG synthesis, while also highlighting new insights obtained from the integration of multi-omics datasets with mathematical models. Computational methodologies such as kinetic modeling, metabolic flux analysis, and new variants of flux balance analysis are explained in detail. We discuss how these methods have been used to simulate algae growth and lipid metabolism in response to changing culture conditions and how they have been used in conjunction with experimental validations. Since emerging evidence indicates that TAG synthesis in microalgae operates through coordinated crosstalk between multiple pathways in diverse subcellular destinations including the endoplasmic reticulum and plastids, we discuss new experimental studies and models that incorporate these findings for discovering key regulatory checkpoints. Finally, we describe tools for genetic manipulation of microalgae and their potential for future rational algal strain design. This comprehensive review explores the potential synergistic impact of pathway analysis, computational approaches, and molecular genetic manipulation strategies on improving TAG production in microalgae.

Contents

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1. Introduction

Climate change, human population growth, and depletion of natural resources impose a serious burden on the planet. Addressing these challenges while concurrently expanding the global economy will require new technologies that generate renewable resources for food, health products, chemicals, and fuels (Georgianna and Mayfield, 2012; Harvey and Pilgrim, 2011). Oleaginous (oil-producing) microalgae hold promise as a sustainable feedstock for each of these needs, as they can accumulate large amounts of triacylglycerol (TAG) under proper conditions (Benemann, 2013; Hu et al., 2008). TAG is a major storage lipid that plays an important role in energy and carbon storage in many microalgae (Chisti, 2007). It is known that TAG synthesis and storage occur in distinct subcellular compartments within microalgae, and recent evidence indicates that TAG is not only a storage molecule but also plays a crucial role in maintaining intracellular lipid homeostasis, proper membrane structure, and cellular function in response to environmental and developmental cues (Holthuis and Menon, 2014; Kohlwein, 2010). Understanding how these functions and processes are coordinated and regulated will arguably be critical for any sort of rational re-design of TAG biosynthesis in microalgae.

Engineering algae to accumulate high levels of TAG while growing rapidly is an ongoing challenge and only limited success has been reached (Li et al., 2010a; Li et al., 2010b; Tsai et al., 2014). Such efforts benefit from knowledge of the biochemical and molecular mechanisms regulating fatty acid (FA) and TAG biosynthesis, accumulation, and turnover in other organisms, much of it inferred from studies of higher plants. Interestingly, TAG biosynthesis is relatively complicated in species such as *Arabidopsis*, for which acyl-lipid metabolism and its regulation involve over 600 genes and at least 120 enzymatic reactions in different organs of the plant (Li-Beisson et al., 2013). In comparison, in microalgae, TAG biosynthesis and storage occur within a single cell and involve fewer proteins. For instance, based on the JGI v5.5 of the *Chlamydomonas reinhardtii* genome, there are just 113 genes predicted to be involved in major steps of acyl-lipid metabolism (Li-Beisson et al., 2015) in that organism, and the number of genes involved in FA metabolism plus TCA cycle is inferred to be just 106 in the diatom *Phaeodactylum tricornutum* (Mühlroth et al., 2013). Clearly there are other interesting and important metabolic and cell biological differences between plants, green algae, and chromophyte algae, including differences in carbohydrate synthesis and glycolytic pathways, and in the arrangements of their thylakoid membranes (Wilhelm et al., 2006). As our focus is on TAG metabolism, these topics are not discussed in detail in this review.

Indeed, whole genome sequencing, *de novo* transcriptomics, and proteomics analysis of algae have accelerated the basic understanding of FA and TAG biosynthesis for strain improvement (Guarnieri et al., 2011; Merchant et al., 2007; Nguyen et al., 2011; Radakovits et al., 2012). With analytical advancements in lipidomics, it is now possible to quantitatively identify hundreds of natural TAGs and their regioisomers from algae (Rezanka et al., 2014). Using such omics and biochemical information from the model alga *C. reinhardtii*, attempts have been made to derive a draft metabolic network using computational modeling and bioinformatics methods (Boyle and Morgan, 2009; May et al., 2009; May et al., 2008). Mathematical and kinetic models have also been developed to study growth parameters and lipid production in green algae (Packer et al., 2010; Tevatia et al., 2012; Yang et al., 2010). Taken together, current developments in molecular genetics and biochemistry, omics (including genomics, transcriptomics, proteomics, metabolomics, and fluxomics), integrative modeling, and genome engineering tools have the potential to enable precise predictive capabilities and rational engineering of algae for high lipid content, see Fig. 1 (Jiang et al., 2014; Recht et al., 2014; Reijnders et al., 2014). The above technological advances offer a unique opportunity to understand algal lipid metabolism in a holistic manner.

This review begins with an overview of the current knowledge of TAG production in microalgae, based on biochemical, physiological, multi-omics, and pathway modification studies, with a focus on the role of major enzymes involved in TAG metabolism. Then we discuss computational methods and their use in data analysis and pathway prediction, with an emphasis on the application of these methodologies to algal TAG production. Finally, we offer a perspective on recently developed genetic engineering tools that may be used to rationally engineer algal strains for favorable biofuel traits (Fig. 1) and summarize reported genetic modifications outcomes, highlighting some strategies that modelers and experimenters could collaboratively investigate.

2. Fatty acid and triacylglycerol biosynthesis in microalgae

FAs are amphipathic molecules consisting of a carboxylic acid group at one end and a hydrocarbon group at the other, with length and degree of saturation of the hydrocarbon moiety variable among different FA species. Acyl-lipids derived from FAs, primarily glycerolipids, form the basic structural components of cell membranes (Li-Beisson et al., 2013). The physiologically inert storage lipid TAG has three fatty acyl groups esterified with the glycerol backbone. Many important mechanistic insights into FA biosynthesis in algae were inferred using the basic framework of *Arabidopsis* lipid metabolism. However, microalgal lipid metabolism differs from plants in at least two important ways (Liu and Benning, 2012). First, in microalgae (but not plants) the plastidic prokaryotic pathway of TAG synthesis and storage plays a more direct role, and second, algae synthesize glycerolipid with distinct acyl groups not present in plants.