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Comparative analysis of diatom genomes reveals substantial differences in the organization of carbon partitioning pathways

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ABSTRACT

A major challenge in the development of microalgal strains for large-scale production is the optimization of biomass accumulation and production of fuel-relevant molecules such as triacylglycerol. Selecting targets for genetic manipulation approaches will require a fundamental understanding of the organization and regulation of carbon metabolic pathways in these organisms. Functional genomic and metabolomics data is becoming easier to obtain and process, however interpreting the significance of these data in a physiological context is challenging since the metabolic framework of all microalgae remains poorly understood. Owing to a complex evolutionary history, diatoms differ substantially from many other photosynthetic organisms in their intracellular compartmentation and the organization of their carbon partitioning pathways. A comparative analysis of the genes involved in carbon partitioning metabolism from *Thalassiosira pseudonana*, *Phaeodactylum tricornutum*, and *Fragilariopsis cylindrus* revealed that diatoms have conserved the lower half of glycolysis in the mitochondria, the upper half of glycolysis (including key regulatory enzymes) in the cytosol, and several mitochondrial carbon partitioning enzymes. However, some substantial differences exist between the three diatoms investigated, including the translocation of metabolic pathways to different compartments, selective maintenance and horizontal acquisition of genes, and differential gene family expansions. A key finding is that metabolite transport between intracellular compartments is likely to play a substantial role in the regulation of carbon flux. Analysis of the carbon partitioning components in the mitochondria suggests an important role of this organelle as a carbon flux regulator in diatoms. Differences between the analyzed species are specific examples of how diatoms may have modified their carbon partitioning pathways to adapt to environmental niches during the diversification of the group. This comparative analysis highlights how even core central pathways can be modified considerably within a single algal group, and enables the identification of suitable targets for genetic engineering to enhance biofuel precursor production.

1. Introduction

Algal biofuels research is gaining momentum, fueled in part by the ease of obtaining genomic and transcriptomic information. A goal of many researchers is to mine this data to identify gene targets for manipulation that will improve growth and lipid, or more specifically, triacylglycerol (TAG), accumulation characteristics that will drive down the cost of production. There are several approaches that can be taken to enhance the TAG content in microalgae including over-expressing fatty acid or TAG biosynthesis genes, inhibiting lipid catabolism, and inhibiting metabolic pathways that compete with lipid biosynthesis for carbon intermediates such as the synthesis of storage carbohydrates [1]. Many carbon metabolic pathways are extensively studied and consequently it may be assumed that these pathways and their genes are well understood in microalgae. However, algae have an evolutionary history that is quite divergent from many model organisms, and the biochemistry of these pathways in algae is in general poorly characterized. Characterization of these pathways and their regulation in microalgae is

Abbreviations: ACCase, acetyl-CoA carboxylase; ATP-PFK, ATP-dependent phosphofructokinase; CCM, carbon concentrating mechanism; DNRA, dissimilatory nitrate reduction to ammonia; ED, Entner–Dourdoff glycolysis; EGT, endosymbiotic gene transfer; EMP, Embden–Meyerhof–Parnas glycolysis; ENO, enolase; FA, fatty acid; FBA, fructose-bisphosphate aldolase; FBP, fructose 1,6 bisphosphatase; Fru 2,6 bisP, fructose 2,6 bisphosphate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GLK, glucokinase; GPI, glucose-6-phosphate isomerase; HGT, horizontal gene transfer; MDH, malate dehydrogenase; ME, malic enzyme; OAA, oxaloacetate; PC, pyruvate carboxylase; PDRP, pyruvate phosphate dikinase regulatory protein; PEP, phosphoenolpyruvate; PEPC, phosphoenolpyruvate carboxylase; PEPCK, phosphoenolpyruvate carboxykinase; PEPS, phosphoenolpyruvate synthase; PFK, phosphofructokinase; PF2K/F26BP, bifunctional 6-phosphofructo-2-kinase/fructose 2,6 bisphosphatase; PGAM, phosphoglycerate mutase; PGK, phosphoglycerate kinase; PK, pyruvate kinase; PPDK, pyruvate phosphate dikinase; PP_i-PFK, pyrophosphate-dependent phophofructokinase; TAG, triacylglycerol; TCA, tricarboxylic acid; TPI, triose phosphate isomerase.

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essential to identify the appropriate gene targets to modify for increased productivity.

In early work to modify algal strains for improved fuel precursor molecule production, acetyl-CoA carboxylase (ACCase), the first committed step of fatty acid biosynthesis, was successfully overexpressed in the diatom *Cyclotella cryptica* [2]. Despite an increased enzyme activity, there was no resulting increase in TAG content, suggesting that there are other factors that regulate the flux of carbon into fatty acid biosynthesis. Generally, microalgae do not accumulate TAG during growth, and only develop lipid bodies during the stationary growth phase or when nutrient-limited [3,4]. Under silicon-limited TAG accumulation conditions in *C. cryptica*, it was also demonstrated that the flux of carbon was repartitioned from storage carbohydrates into lipid over the course of TAG induction [5,6]. Blocking storage carbohydrate synthesis, as demonstrated in starchless mutants of the green alga *Chlamydomonas reinhardtii*, also enhances TAG accumulation [7]. From these data, it is reasonable to expect that enhancing carbon flux towards TAG by reducing flux to competing pathways is a viable approach to improve de novo TAG biosynthesis and accumulation in algae.

Central carbon metabolic pathways are reasonable targets for the modification of intracellular carbon flux in algae. In all known photosynthetic organisms, the primary pathways involved in the partitioning of fixed carbon into either storage carbohydrates or TAG are glycolysis, gluconeogenesis, and pyruvate metabolism (Fig. 1). Glycolysis, or the catabolism of hexoses to produce pyruvate and ATP, provides the cell with energy and metabolic intermediates required to supply either the TCA cycle or fatty acid biosynthesis. Gluconeogenesis is essentially the reverse of the glycolysis pathway in that pyruvate is converted to hexoses that supply storage carbohydrate biosynthetic pathways. In this way, these pathways and their subsequent branch points act as partitioning regulators of intracellular carbon flux.

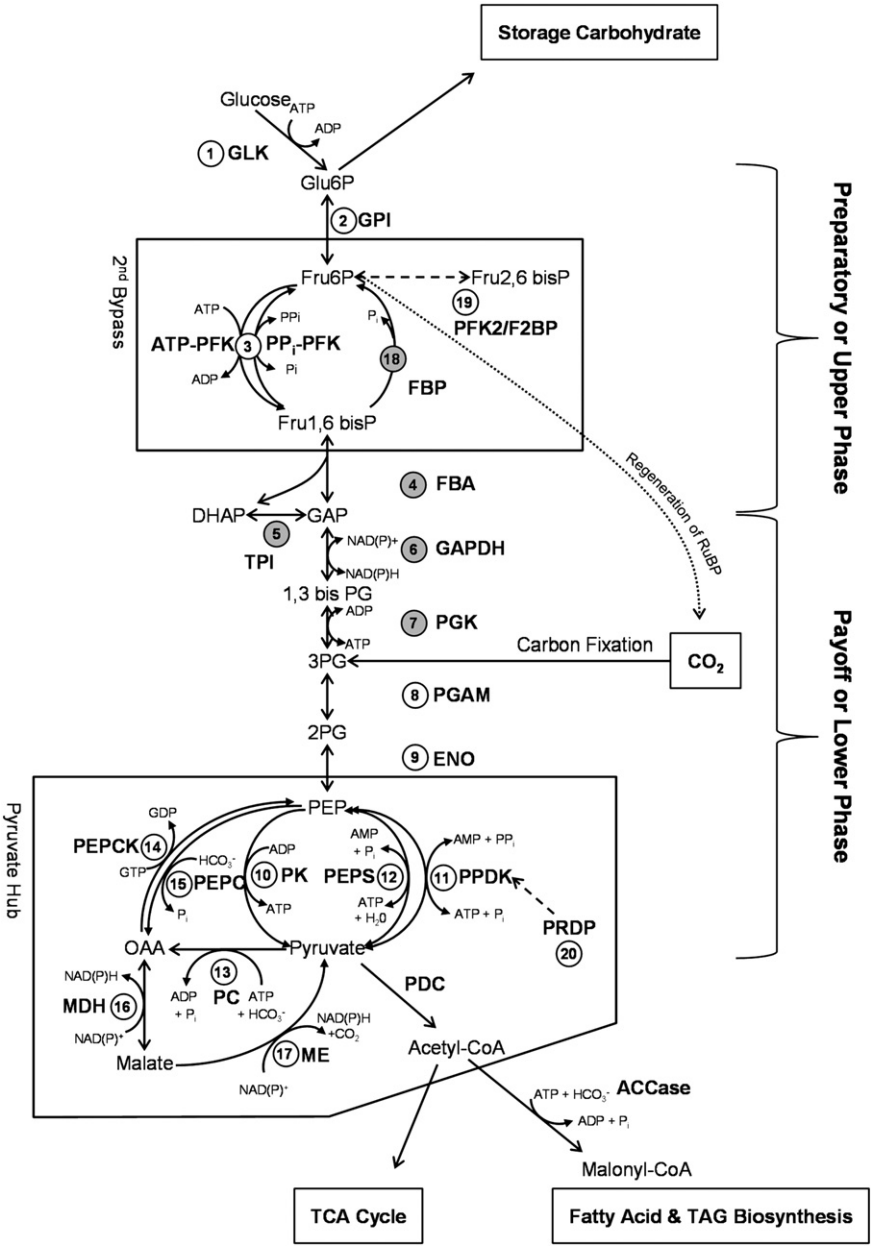

Fig. 1. Schematic of carbon partitioning enzymes involved in glycolysis, gluconeogenesis and pyruvate metabolism. Circled numbers adjacent to enzyme abbreviations correspond to those in Table 1. Shaded circles denote enzymes that are also involved in the Calvin–Benson cycle. Arrows indicate the directionality of the reaction catalyzed by each enzyme. Brackets indicate the two phases of glycolysis; the upper phase (GLK to TPI) requires an initial energy investment, while the lower phase (GAPDH to PFK) produces ATP and reducing equivalents.