Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Chemical Biology

Metabolic and cellular organization in evolutionarily diverse microalgae as related to biofuels production

Mark Hildebrand[1], Raffaela M Abbriano[1], Juergen EW Polle[3], Jesse C Traller[1], Emily M Trentacoste[2], Sarah R Smith[1] and Aubrey K Davis[1]

Microalgae are among the most diverse organisms on the planet, and as a result of symbioses and evolutionary selection, the configuration of core metabolic networks is highly varied across distinct algal classes. The differences in photosynthesis, carbon fixation and processing, carbon storage, and the compartmentation of cellular and metabolic processes are substantial and likely to transcend into the efficiency of various steps involved in biofuel molecule production. By highlighting these differences, we hope to provide a framework for comparative analyses to determine the efficiency of the different arrangements or processes. This sets the stage for optimization on the based on information derived from evolutionary selection to diverse algal classes and to synthetic systems.

Addresses
[1] Marine Biology Research Division, Scripps Institution of Oceanography, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0202, USA
[2] Integrated Oceanography Division, Scripps Institution of Oceanography, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0202, USA
[3] Department of Biology, CUNY Brooklyn College, 2900 Bedford Avenue 200 NE, Brooklyn, NY 11210, USA

Corresponding author: Hildebrand, Mark (mhildebrand@ucsd.edu)

Current Opinion in Chemical Biology 2013, **17**:506–514

This review comes from a themed issue on **Energy**

Edited by **Michael D Burkart** and **Stephen P Mayfield**

For a complete overview see the Issue and the Editorial

Available online 26th March 2013

http://dx.doi.org/10.1016/j.cbpa.2013.02.027

Introduction

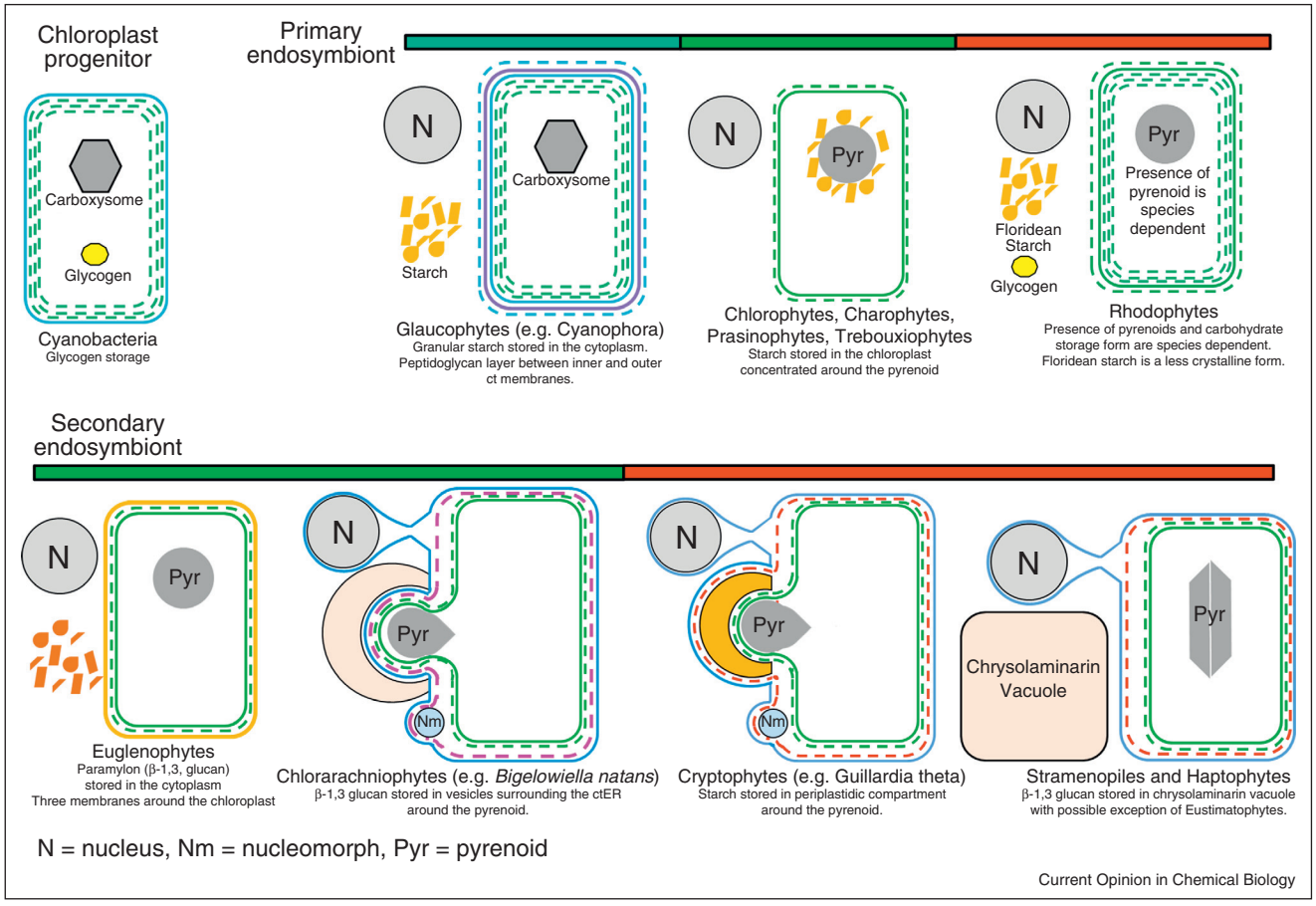
Improving the productivity of strains is a major factor in making algal biofuels economically viable [1••]. Algal productivity is ultimately dependent on the efficiency of carbon fixation and the downstream cellular processes that convert photosynthate into useful fuel precursors. The diversity of contemporary microalgal metabolism has been shaped by multiple endosymbiotic acquisitions, environmental factors, and evolutionary selection. The result has been distinct intracellular compartmentation and unique organizational schemes among different algal classes [2••], especially in relation to the location of carbon fixation enzymes and carbohydrate storage (Figure 1). Organizational differences likely affect processes such as photosynthesis, carbon flux through metabolic networks, and biosynthesis of fuel-relevant compounds. The goal of this review is to highlight the relevance of these aspects of algal diversity to biofuel molecule production.

Light harvesting and photoprotection

The evolution of microalgae has generated a variety of components and organizational schemes of the photosynthetic apparatus (Figure 2). All microalgae have light harvesting antenna complexes, PSII, the cytochrome b_6f complex, and PSI. The use of the bulky phycobilisomes (peripherally associated with the thylakoid membrane) for light harvesting in cyanobacteria, glaucophytes, and rhodophytes results in a relatively large spacing between the photosynthetic membranes (Figure 2a and c), which could affect photosynthetic capacity [3]. Downsizing of the light harvesting complexes is apparent in rhodophytes, which have membrane-integral LHCs, and cryptomonads, which utilize unassembled biliproteins in the lumen of the thylakoids, enabling stacked thylakoid grana (Figure 2). Stacked grana arose independently in both chlorophytes and in derivatives of the red algae, and may serve to enhance light capture and connectivity between PSIIs with large functional antenna size [3,4]. The numbers of grana stacks differ; chromalveolates typically have three, while chlorophytes can have 2–3 times more [5•]. In chlorophytes, PSII is highly enriched in the grana and PSI in the stroma thylakoids, while in chromalveolates, they are nearly equally distributed [6]. Chlorophytes use LHCs specific for either PSI or PSII (Figure 2), and stramenopiles such as diatoms use fucoxanthin chlorophyll binding proteins (FCPs) in a similar capacity [7•]. Stramenopile FCPs have a carotenoid:chlorophyll ratio of 4:4 compared with 14:4 in LHCs for chlorophytes, resulting in a shift of absorbance into the 460–570 nm range, which is not accessible to chlorophytes [8].

Efficient photosynthesis requires balance between light absorbed by PSI and PSII and dissipation of energy from excess absorbed light. The distribution of light between the photosystems in cyanobacteria, cryptomonads, rhodophytes, and chlorophytes (and higher plants) involves state transitions in which a phosphatase/kinase driven

Figure 1



Organization of carbon fixation and carbohydrate storage in evolutionarily-diverse classes of microalgae. Colored bars denote the overall class of algae: blue-green = glaucophyte; green = green algae and endosymbiotic derivatives; red = red algae and endosymbiotic derivatives. Membrane systems associated with the cell (cyanobacteria), cyanelle (glaucophytes), or chloroplasts (others), are color coded to denote cyanobacterial-like (blue-green), inner and outer chloroplast membranes [green, except in glaucophytes, which contain a peptidoglycan layer in between (purple)], and secondary endosymbiosis membranes in Euglenophytes (gold), Chlorarachniophytes (fuschia and blue for periplastid and ER, respectively) and Cryptophytes, Stramenopiles, and Haptophytes (red and blue for periplastid and ER, respectively). Different carbohydrate storage forms are labeled and denoted by different colors. Text highlights the major features in each.

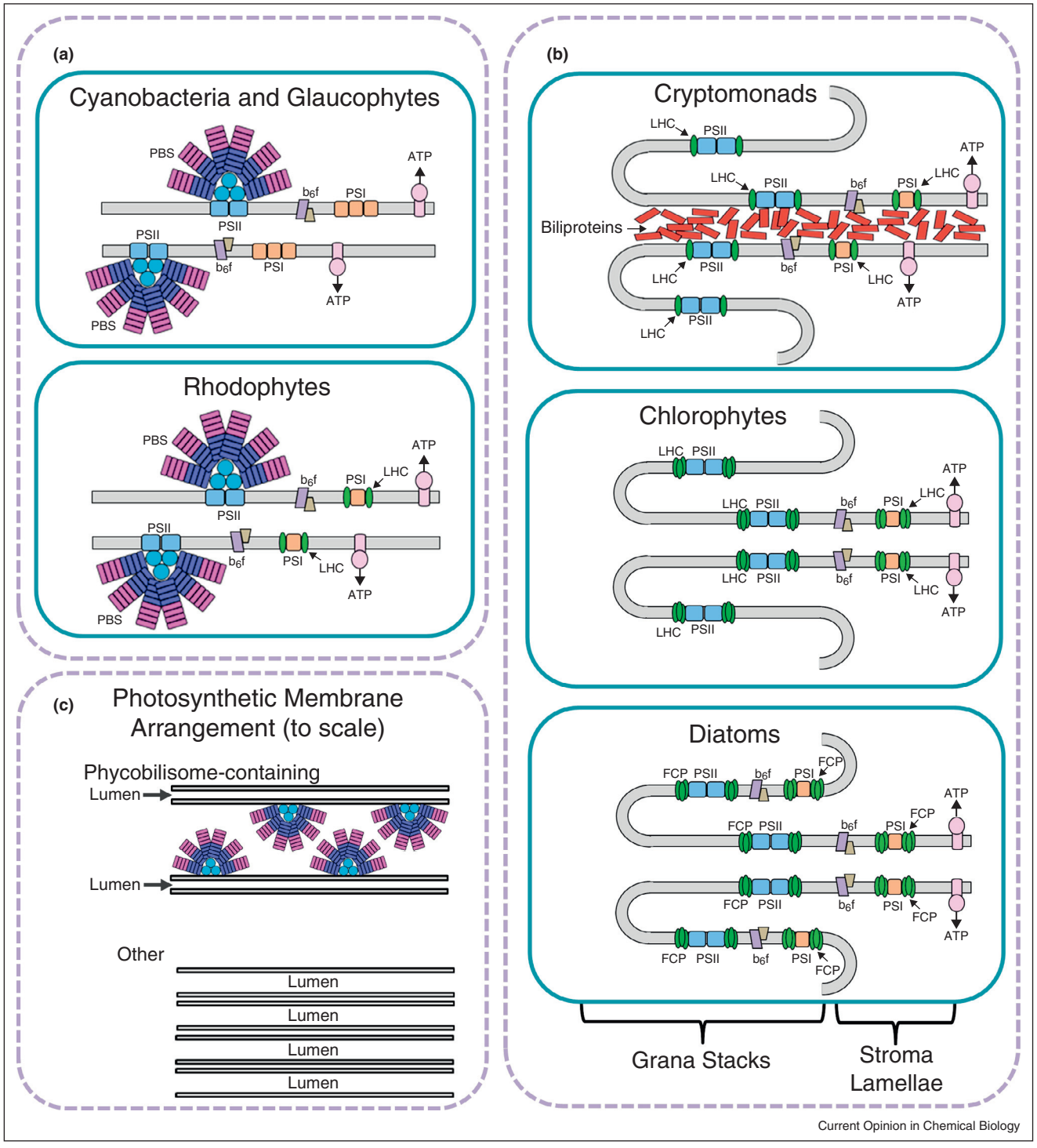
reversible physical movement of the LHC occurs between PSII and PSI [9]. State transitions have not been documented in diatoms [5], and none are reported for the eustigmatophyceae. Instead, diatoms balance photosystem activity by quenching photon absorption by PSII as a result of de-epoxidation of xanthophyll pigments [10]. A direct comparison showed that this process resulted in 2-fold less generation of wasted electrons than state transitions in a chlorophyte [10]. Quenching in the antenna system also reduces damage to the photosystems, which carries a high energetic replacement cost [11•]. Dissipation of excess light in photosynthesis is primarily achieved through non-photochemical quenching (NPQ). Different strategies have developed for NPQ in evolutionarily distinct classes of algae, including rapid rates of synthesis or high accumulation of de-expoidized xanthophylls [12]. Xanthophyll cycling systems are apparently lacking in phycobilisome-containing organisms and the Chlorarachniophyta [11•,13], and NPQ in cryptophytes differs from other chromalveolates [14].

Differences in photosynthetic processes are likely to affect light harvesting efficiency, which ultimately translates into altered growth and product molecule accumulation. There are very few definitive analyses comparing the relative efficiency of the described diverse photosynthetic arrangements. Such information would not only aid in developing strategies for improved light capture in diverse classes of microalgae, but potentially in the development of artificial photosynthesis approaches.

Carbon fixation and concentration

Carbon fixation in the Calvin–Benson cycle is catalyzed by RuBisCO, which has a low CO_2-saturated maximum

Figure 2



Organizational schemes of the photosynthetic apparatus in evolutionarily diverse classes of microalgae. *Key*: PBS = phycobilisomes; PSII = photosystem II; b_6f = cytochrome b_6f complex; PSI = photosystem I; ATP = adenosine triphosphate; LHC = light harvesting complex; FCP = fucoxanthin chlorophyll binding protein complex. **(a)** Clusters the phycobilisome-containing classes. **(b)** Clusters classes containing grana stacks and stroma lamellae. **(c)** Shows the relative spacing of photosynthetic membranes in phycobilisome-containing and other chloroplasts.

catalytic rate and competitive oxygenase activity resulting in photorespiration. To compensate, microalgae have taken advantage of different strategies to maximize carbon fixation efficiency. One involves the use of RuBisCO with improved affinity for CO_2 and selectivity for CO_2 relative to O_2 [15]. Cyanobacterial-type RuBisCO forms IA and IB (found in cyanobacteria and green algae) generally have a low affinity for CO_2 and a low CO_2/O_2 selectivity relative to red algal-derived forms IB and ID, however the latter has a lower turnover rate [15]. Kinetic and regulatory variabilities suggest that different forms of RuBisCO are evolutionarily selected to function optimally in different subcellular environments [16].

Carbon concentrating mechanisms (CCMs) are another way to increase carbon fixation efficiency, and these can be classified as being either biophysical (involving localized enhancement of CO_2) or biochemical (involving specific enzymatic pathways). The biophysical mechanism of concentrating RuBisCO in carboxysomes and pyrenoids allows for regulation of CO_2 delivery [17••,18•]. Cyanobacteria and chlorophytes rely largely on biophysical CCMs by transporting and accumulating bicarbonate and converting it to CO_2 near RuBisCO via carbonic anhydrase [19]. Diatoms have high efficiency CCMs and may utilize both biophysical and biochemical mechanisms, which includes C4 fixation [18•,20,21].

Our literature search did not identify substantial differences in the complement of other Calvin–Benson enzymes across evolutionarily-diverse classes of microalgae, however, there seem to be differences in their regulation [22].

Intracellular organization of carbon fixation and carbohydrate storage

Highly diverse cellular organizational schemes for carbon fixation and carbohydrate storage have developed in microalgae (Figure 1). Cyanobacteria contain carboxysomes, and store carbohydrate as water-soluble glycogen, whereas glaucophytes store starch in the cytoplasm (Figure 1). Data suggest that cytoplasmic starch formation generated from ADP-glucose exported from the chloroplast resulted from the merging of bacterial and eukaryote pathways of storage polysaccharide metabolism [23••]. In the chlorophytes and related algae, gene duplications and enzyme retargeting resulted in starch synthesis being relocated to the chloroplast [24]. Pyrenoid-associated starch in chlorophytes (Figure 1) could play a role in carbon concentration [25], or photoprotection [24]. In rhodophytes, pyrenoids are not present in all species, and carbohydrate is stored in the cytoplasm as either glycogen or Floridean starch, which is a less crystalline starch form lacking amylose. In euglenophytes, carbohydrate is stored cytoplasmically as a highly crystalline fibrillar β-(1,3)-linked glucan called paramylon [26]. Chlorarachniophytes store a β-(1,3)-linked glucan within a cytoplasmic vacuole that surrounds the pyrenoid which projects from the plastid [27]. Cryptophytes have a similarly localized pyrenoid (which arose distinctly from the chlorarachniophyte arrangement), yet store starch between the outer chloroplast and periplastid membranes (Figure 1). Stramenopiles and haptophyes have centrally localized pyrenoids and store a soluble β-(1,3)-linked glucan called chrysolaminarin cytoplasmically in the large chrysolaminarin vacuole [28,29]. There may be exceptions to this; there is no documentation on the location of carbohydrate storage in the eustigmatophyte lineage of the stramenopiles, which includes *Nannochloropsis*. There are substantial differences in the accessibility of different storage forms of carbohydrates; starch granules are less accessible energetically and biophysically than less crystalline forms or than water soluble carbohydrates [28,30], and such differences should affect intracellular energetics.

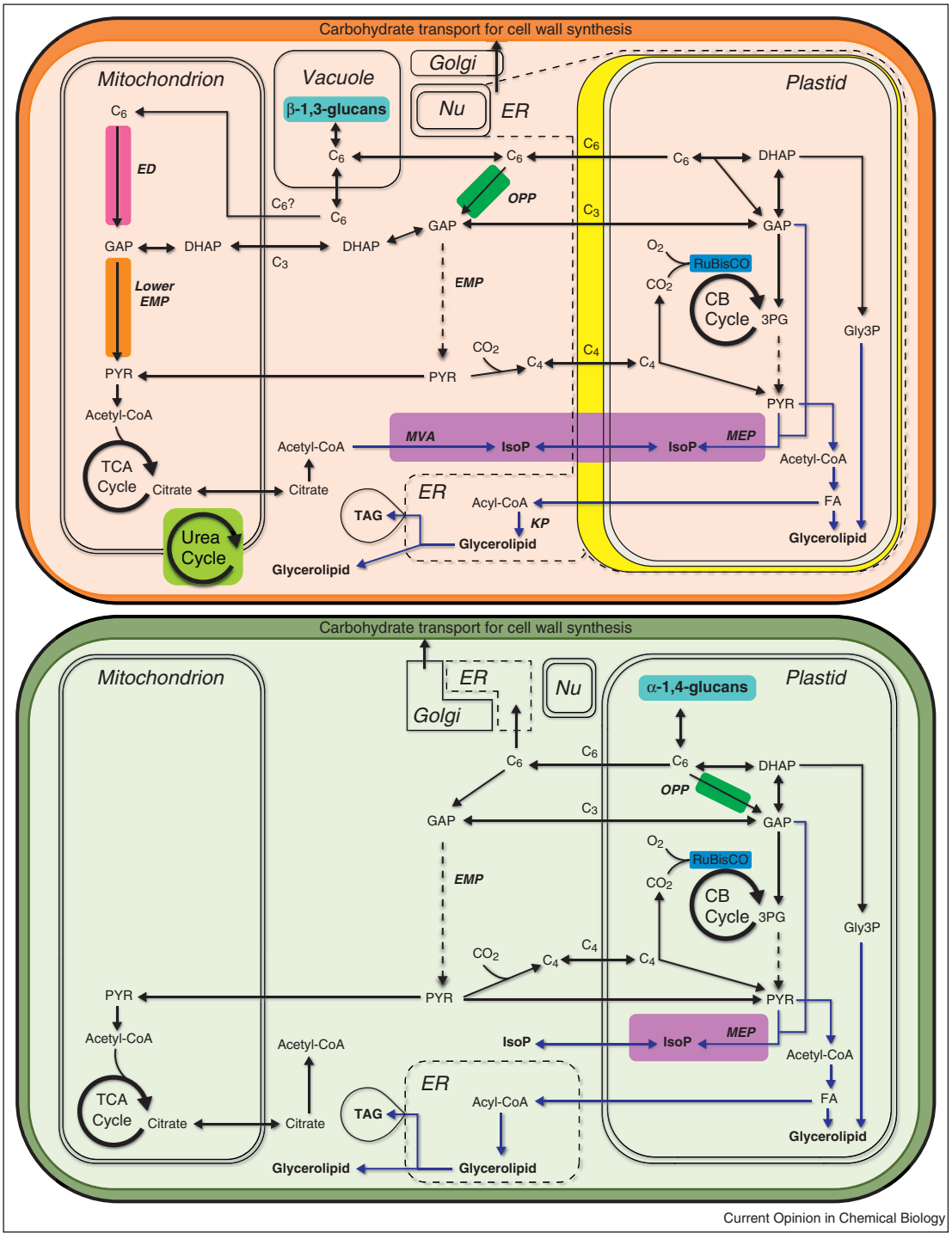
Compartmentation of core metabolic networks

The diverse intracellular compartmentation schemes in microalgae (Figure 1), coupled with evolutionary gene replacement and retargeting, have transformed algal metabolic capabilities [31,32•,33••] and resulted in unconventional routes for intracellular carbon flux. Some metabolic models in green algae do not include a compartmentation component [34,35]; however, it is becoming apparent that compartmentation is an important consideration, and that transport should play essential roles in carbon flux [36,37]. Substantial localization differences have been documented in essential metabolic networks, in this case among species within a single clade [38••]. To illustrate these points, we compared central carbon networks in chlorophytes and diatoms as well-studied primary and secondary endosymbionts, respectively (Figure 3).

In chlorophytes and diatoms the Embden–Meyerhof–Parnas (EMP) pathway of glycolysis is not commonly complete in either the cytosol or chloroplast [38••,39], which necessitates carbon flux across plastid membranes [33••]. Diatoms have additional EMP glycolysis capabilities in the mitochondria (Figure 3; [40,41]), which could potentially produce pyruvate in proximity to the TCA cycle and reducing equivalents to feed oxidative phosphorylation [38]. Recently, the Entner–Doudoroff glycolytic pathway was described in diatom mitochondria (Figure 3; [42]), suggesting that the catabolism of C6 compounds to pyruvate is possible.

The oxidative pentose phosphate pathway (OPP), which supplies ribose-5-phosphate for *de novo* nucleotide biosynthesis in addition to a source of NADPH for fatty acid biosynthesis, is co-localized with the reductive pentose phosphate pathway (Calvin–Benson cycle) in the plastids of green algae and higher plants (Figure 3). The activities of these two pathways are tightly light regulated in these organisms to avoid futile cycling [43]. In diatoms, OPP

Figure 3



Central carbon metabolic network map of a diatom and green alga highlight key differences. Upper; Diatom (Bacillariophyta). Lower, Green Algae (Chlorophyta). *Key*: Organelles: ER = endoplasmic reticulum; Nu = nucleus. *Pathway abbreviations*: CB = Calvin–Benson; ED = Entner–Doudoroff glycolysis; EMP = Emden–Meyerhoff–Parnas glycolysis; KP = Kennedy pathway; MEP = methylerythritolphosphate isoprenoid biosynthesis pathway; MVA = mevalonate isoprenoid biosynthesis pathway; OPP = oxidative pentose phosphate pathway; TCA = citric acid cycle. *Metabolite/enzyme abbreviations*: 3PG = 3-phosphoglycerate; DHAP = dihydroxyacetone phosphate; FA = fatty acid; GAP = glyceraldehyde-3-phosphate; Gly3P = glycerol-3-phosphate; IsoP = isoprenoid; ML = membrane lipids; PYR = pyruvate; RuBisCO = ribulose-bisphosphate carboxylase oxygenase; TAG = triacylglycerol. C3, 4, 6 refer to carbon skeletons containing the specified number of carbon molecules. Dashed arrows between 3PG and PYR denote the presence or absence of a complete pathway in different species of the represented classes. Shaded boxes within each cell indicate features that are distinct comparing diatoms and green algae. (Dark blue) Diatom RuBisCO has a higher CO_2:O_2 selectivity than the chlorophyte form.

and nucleotide biosynthesis occur in the cytosol, implying that coordination between the oxidative and reductive portions of the pentose phosphate pathway differs from Chlorophytes, and there is an alternative mechanism to transport reducing equivalents into diatom plastids for fatty acid biosynthesis [41,44,45].

The cellular location of acetyl-CoA is important for a number of pathways including fatty acid and isoprenoid biosynthesis. The phosphotransacetylase-acetate kinase (PTA-ACK) pathway interconverts acetate and acetyl-CoA through an acetyl-phosphate intermediate [46]. PTA and ACK are differentially localized in chlorophytes and diatoms [42,46] suggesting differences in ability to interconvert acetate and acetyl-CoA in various parts of the cell. This can affect the availability of acetyl-CoA for compartmentalized processes.

Diatoms contain a urea cycle, which other eukaryotic microalgae and land plants lack (Figure 3; [47]). This feature allows for a higher efficiency of nitrogen assimilation from catabolic processes, and may enable diatoms to more effectively recycle intracellular nitrogen [48•]. The urea cycle therefore could play an important role when the cell is accumulating fuel precursors during nitrogen-deprivation.

Stramenopiles, haptophytes, cryptophytes, and chlorarachniophytes have the periplastid compartment (PPC) surrounding the chloroplast which is an additional compartment relative to chlorophytes. The PPC has been proposed to be involved in inorganic carbon acquisition [49] and in diatoms carbonic anhydrase enzymes were localized there [21,50]. Secondary endosymbionts require additional sets of transporters to traverse the additional membranes, which may complicate metabolite exchange.

For reasons of simplicity, the major carbon flux necessary to build algal cell walls was ignored in this review, but there should be differences comparing the silicified cell walls of diatoms compared with organic walls of other microalgae [51].

Product molecule synthesis

Triacylglycerol (TAG) is produced from diacylglycerol (DAG) in microalgae through two major routes: the Kennedy Pathway involving transfer of acyl-CoA units onto DAG, catalyzed by diacylglycerol acyltransferase (DGAT), and an acyl-CoA-independent pathway in which acyl groups are transferred from phospholipids, catalyzed by phospholipid:diacylglycerol acyltransferase (PDAT) [52]. Analysis of DGATs showed differences in the number and types of isoforms present, even within individual algal lineages [53]. Attempts to manipulate DGATs for increased lipid production have had little success [54], suggesting that the acyl-CoA-independent route may deserve more consideration as a contributor than previously thought. Our initial analysis found different numbers of PDAT isoforms between microalgal species, implying that this pathway may be as complex across algal lineages as DGAT and the Kennedy pathway. TAG biosynthesis has long been thought to occur predominantly in the ER, however recently it was shown in *Chlamydomonas reinhardtii* that a plastid-localized process may contribute [55,56].

Isoprenoid molecules are important precursors for generation of biofuels [57,58]. Two major pathways exist for isoprenoid biosynthesis in algae, the cytosolic mevalonate (MVA) pathway using acetyl-CoA, and the plastidic methylerythritolphosphate (MEP) pathway, which is glyeraldehyde-3P and pyruvate dependent [59•]. Chlorophytes have only the MEP pathway, but diatoms additionally have the MVA pathway (Figure 3). The interplay of precursor synthesis and regulation of both pathways is complex with many unknowns [59•]. Specifically important for metabolic engineering of improved and/or novel biofuels may be carbon partitioning between the isoprenoids and fatty acids.

Conclusions and future directions

This review highlights the substantial differences in photosynthesis, metabolic networks, and intracellular organization of evolutionarily-distinct classes of microalgae as related to biofuel precursor molecule production. Given the presented examples, one cannot assume that the core carbon metabolism in diverse algal classes will be similar. To facilitate a broadly-informed development of algal biofuels, it will be necessary to use systems biology approaches coupled with biochemical characterization in detailed metabolic studies of examples from the different major algal lineages. It is likely that particular processes or arrangements are more advantageous for fuel production in some classes of algae than others, and determining relative efficiencies could enable optimization of productivity in current strains, and ultimately

Figure 3 Legend (Continued) (Purple) Diatom isoprenoid biosynthesis combines a cytosolic MVA pathway and a plastidic MEP pathway. Green algae only have a plastidic MEP pathway. (Yellow) As a result of the secondary endosymbiotic event, the diatom plastid is surrounded by four membranes, the outer most is the ER (aka chloroplast ER), next is the periplastid, then the outer and inner chloroplast membranes. Resulting from this is the periplastidic compartment and an additional transport requirement for the plastid in diatoms relative to chlorophytes. (Light blue) Carbohydrates are stored as chrysolaminarin in a cytoplasmically localized vacuole in diatoms, and as starch in the chlorophytes. (Dark green) The OPP is cytosolic in diatoms and plastidic in chlorophytes. (Pink) The Entner–Doudoroff pathway in diatom mitochondria. (Orange) The lower half of the EMP pathway in diatom mitochondria. (Lime) The urea cycle in diatoms.

lead to the development of completely novel strains or synthetic organisms that combine evolutionarily-distinct features.

Acknowledgements

Biofuels research in the Hildebrand lab is supported by Air Force Office of Scientific Research (AFOSR) grants FA9550-08-1-0178 and FA9550-08-1-0178, US Department of Energy grants DE-EE0001222 and DE-EE0003373, National Science Foundation grant CBET-0903712, California Energy Commission's 'California Initiative for Large Molecule Sustainable Fuels', agreement number: 500-10-039, and UCMexus grant CN-10-454. RMA was supported by the Department of Energy Office of Science Graduate Fellowship Program (DOE SCGF), made possible in part by the American Recovery and Reinvestment Act of 2009, administered by ORISE-ORAU under contract no. DE-AC05-06OR23100. EMT was supported by an National Institutes of Health Marine Biotechnology Training Grant Fellowship. SRS was supported by the Department of Defense through the National Defense Science & Engineering Graduate Fellowship Program. Biofuels research in the Polle lab was supported by AFOSR grants FA9550-08-1-0170 and FA9550-08-1-0403 as well as by the US Department of Energy grant DE-EE0003046.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Davis R, Aden A, Pienkos PT: **Techno-economic analysis of autotrophic microalgae for fuel production**. *Appl. Energy* 2011, **88**:3524-3531.
This paper establishes baseline economics for microalgal biofuel production in open ponds or enclosed PBRs within a five-year timeframe using mass balances approaches. Economic sensitivity studies were established to identify important cost drivers, and a major set was growth and lipid production. It was recommended that near-term research should focus on maximizing lipid content as it offers more substantial cost reduction potential relative to an improved algae growth rate.

2. •• Keeling PJ: **The endosymbiotic origin, diversification and fate of plastids**. *Philos. Trans. R. Soc. Lond. B* 2010, **365**:729-748.
Describes the current understanding of microalgal evolution related to symbiotic events involving different sources of plastids. The analysis details the diverse events involved in the evolutionary process and offers a glimpse into how genomes evolve and how cells integrate through gene transfers and protein trafficking.

3. Chow WS, Kim EH, Horton P, Anderson JM: **Granal stacking of thylakoid membranes in higher plant chloroplasts: the physicochemical forces at work and the functional consequences that ensue**. *Photochem. Photobiol. Sci.* 2005, **4**:1081-1090.

4. Mullineaux CW: **Function and evolution of grana**. *Trends Plant Sci.* 2005, **10**:521-525.

5. • Lepetit B, Goss R, Jakob T, Wilhelm C: **Molecular dynamics of the diatom thylakoid membrane under different light conditions**. *Photosynth. Res.* 2012, **111**:245-257.
Summarizes current knowledge about structural and functional changes in diatom thylakoid membranes acclimated to different light conditions. Topics include the organization and regulation of light-harvesting proteins, the dissipation of excessively absorbed light energy by the process of non-photochemical quenching, and the lipid composition of diatom thylakoid membranes. A working hypothesis of the domain formation of the diatom thylakoid membrane is presented to highlight the most prominent differences of heterokontic thylakoids in comparison to vascular plants and green algae during the acclimation to low and high light conditions.

6. Buchel C, Wilhelm C: **Evidence for a lateral heterogeneity by patchwork-like areas enriched with photosystem I complexes in the three thylakoid lamellae of *Pleurochloris meiringensis* (Xanthophyceae)**. *J. Crypt. Bot.* 1992, **2**:375-386.

7. • Neilson JAD, Durnford DG: **Structural and functional diversification of the light-harvesting complexes in photosynthetic eukaryotes**. *Photosynth. Res.* 2010, **106**:57-71.
This review discusses the different classes of LHCs within photosynthetic eukaryotes and examines LHC diversification in different groups in a structural and functional context.

8. Mimuro M, Akimoto S: **Carotenoids of light harvesting systems: energy transfer processes from fucoxanthin and peridinin to chlorophyll**. In *Photosynthesis in Algae.* Edited by Larkum AWD, Douglas SE, Raven JA. Dordrecht: Kluwer; 2003:335-349.

9. Fork DC, Satoh K: **The control by state transitions of the distribution of excitation-energy in photosynthesis**. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 1986, **37**:335-361.

10. Wagner H, Jakob T, Wilhelm C: **Balancing the energy flow from captured light to biomass under fluctuating light conditions**. *New Phytol.* 2006, **169**:95-108.

11. • Raven JA: **The cost of photoinhibition**. *Physiol. Plant.* 2011, **142**:87-104.
A detailed analysis of the energetic costs associated with photosystems absorbing excess light. A comparison is also made between the costs of photoinhibition and those of other plant functions impeded by the occurrence of oxygenic photosynthesis, that is, the competitive inhibition of the carboxylase activity of ribulose bisphosphate carboxylase-oxygenase by oxygen via the oxygenase activity, and oxygen damage to nitrogenase in diazotrophic organisms.

12. Casper-Lindley C, Bjorkman O: **Fluorescence quenching in four unicellular algae with different light-harvesting and xanthophyll-cycle pigments**. *Photosynth. Res.* 1998, **56**:277-289.

13. Goss R, Jakob T: **Regulation and function of xanthophyll cycle-dependent photoprotection in algae**. *Photosynth. Res.* 2010, **106**:103-122.

14. Kana R, Kotabova E, Sobotka R, Prasil O: **Non-photochemical quenching in cryptophyte alga *Rhodomonas salina* is located in chlorophyll *a/c* antennae**. *PLoS ONE* 2012, **7**:e29700.

15. Badger MR, Andrews TJ, Whitney SM, Ludwig M, Yellowlees DC, Leggat W, Price DG: **The diversity and coevolution of Rubisco, plastids, pyrenoids, and chloroplast-based CO_2-concentrating mechanisms in algae**. *Can. J. Bot.* 1998, **76**:1052-1071.

16. Tcherkez GGB, Farquhar GD, Andrews TJ: **Despite slow catalysis and confused substrate specificity, all ribulose bisphosphate carboxylases may be nearly perfectly optimized**. *Proc. Natl Acad. Sci. U. S. A.* 2006, **103**:7246-7252.

17. •• Raven JA, Giordano M, Beardall J, Maberly SC: **Algal evolution in relation to atmospheric CO_2: carboxylases, carbon-concentrating mechanisms and carbon oxidation cycles**. *Philos. Trans. R. Soc. B* 2012, **367**:493-507.
An overview of the climatic and evolutionary processes involved in selection of RuBisCO and generation of carbon concentrating mechanisms in microalgae.

18. • Reinfelder JR: **Carbon concentrating mechanisms in eukaryotic marine phytoplankton**. *Annu. Rev. Mar. Sci.* 2011, **3**:291-315.
A review of the biophysics and biochemistry of carbon concentrating mechanimss among the three dominant groups of eukaryotic marine phytoplankton, including evaluation of energetic and nutrient costs.

19. Price JD, Badger MR, Woodger FJ, Long BM: **Advances in understanding the cyanobacterial CO_2-concentrating-mechanism (CCM): functional components, Ci transporters, diversity, genetic regulation and prospects for engineering into plants**. *J. Exp. Bot.* 2008, **59**:1441-1461.

20. Raven JA: **Inorganic carbon acquisition by eukaryotic algae: four current questions**. *Photosynth. Res.* 2010, **106**:123-134.

21. Tachibana M, Allen AE, Kikutani S, Endo Y, Bowler C, Matsuda Y: **Localization of putative carbonic anhydrases in two marine diatoms, *Phaeodactylum tricornutum* and *Thalassiosira pseudonana***. *Photosynth. Res.* 2011, **109**:205-221.

22. Maberly SC, Courcelle C, Groben GR, Gontero B: **Phylogenetically based variation in the regulation of the Calvin cycle enzymes, phosphoribulokinase and glyceraldehyde-3-phosphate dehydrogenase, in algae**. *J. Exp. Bot.* 2010, **61**:735-745.

23. •• Ball S, Colleoni C, Cenci U, Raj JN, Tirtiaux C: **The evolution of glycogen and starch metabolism in eukaryotes gives molecular clues to understand the establishment of plastid endosymbiosis**. *J. Exp. Bot.* 2011, **62**:1775-1801.
Review of cellular and gene transfer events involved in establishing carbon storage flux pathways between plastid endosymbionts and the host and subsequent movement of the pathway to plastids in the Chlorophytes. Reconstruction of starch metabolism in the common ancestor of Archaeplastida suggests that polysaccharide synthesis was ancestrally cytosolic. In addition, the synthesis of cytosolic starch from the ADP-glucose exported from the cyanobacterial symbiont possibly defined the original metabolic flux by which the cyanobiont provided photosynthate to its host.

24. Deschamps P, Haferkamp I, d'Hulst C, Neuhaus HE, Ball SG: **The relocation of starch metabolism to chloroplasts: when, why and how**. *Trends Plant Sci.* 2008, **13**:574-582.

25. Ramazanov Z, Rawat M, Henk MC, Mason CB, Matthews SW, Moroney JV: **The induction of the CO_2-concentrating mechanism is correlated with the formation of the starch sheath around the pyrenoid on *Chlamydomonas reinhardtii***. *Planta* 1994, **195**:210-216.

26. Booy FP, Chanzy H, Boudet A: **An electron-diffraction study of paramylon storage granules from *Euglena gracilis***. *J. Microsc. Oxford.* 1981, **121**:133-140.

27. Hirakawa Y, Howe A, James ER, Keeling PJ: **Morphological diversity between culture strains of a Chlorarachniophyte, *Lotharella globosa***. *PLoS ONE* 2011, **6**:e23193.

28. Chiovitti A, Molino P, Crawford SA, Teng RW, Spurck T, Wetherbee R: **The glucans extracted with warm water from diatoms are mainly derived from intracellular chrysolaminaran and not extracellular polysaccharides**. *Eur. J. Phycol.* 2004, **39**:117-128.

29. Andersen RA: ***Ochromonas moestrupii* sp. nov. (Chrysophyceae), a new golden flagellate from Australia**. *Phycologia* 2011, **50**:600-607.

30. Fettke J, Hejazi M, Smirnova J, Hochel E, Stage M, Steup M: **Eukaryotic starch degradation: integration of plastidial and cytosolic pathways**. *J. Exp. Bot.* 2009, **60**:2907-2922.

31. Keeling PJ, Palmer JD: **Horizontal gene transfer in eukaryotic evolution**. *Nat. Rev. Genet.* 2008, **8**:605-618.

32. • Martin W: **Evolutionary origins of metabolic compartmentalization in eukaryotes**. *Philos. Trans. R. Soc. B* 2010, **365**:847-855.
Explores a hypothesis as to how multiple steps in a metabolic pathway can be re-targeting within a cell. It is suggested that pathways attain novel compartmentation variants via a 'minor mistargeting' mechanism. If protein targeting in eukaryotic cells possesses enough imperfection such that small amounts of entire pathways continuously enter novel compartments, selectable units of biochemical function would exist in new compartments, and the genes could become selected.

33. •• Ginger ML, McFadden GI, Michels PAM: **Rewiring and regulation of cross-compartmentalized metabolism in protists**. *Philos. Trans. R. Soc. B* 2010, **365**:831-845.
Describes the concept that subcompartmentalization of ubiquitous metabolic pathways has been repeatedly remodelled during eukaryotic evolution, and that models of intermediary metabolism established other organisms are not conserved in many protists. Also describes how metabolic remodelling can strongly influence regulatory mechanisms that control carbon flux through the major metabolic pathways.

34. Nils C, May P, Kempa S, Handorf T, Ebenhoh O: **An integrative approach towards completing genome-scale metabolic networks**. *Mol. Biosyst.* 2009, **5**:1889-1903.

35. Krumholz EW, Yang H, Weisenhorn P, Henry CS, Libourel IGL: **Genome-wide metabolic network reconstruction of the picoalga *Ostreococcus***. *J. Exp. Bot.* 2012, **63**:2353-2362.

36. Braeutigam A, Weber APM: **Proteomic analysis of the proplastid envelope membrane provides novel insights into small molecule and protein transport across proplastid membranes**. *Mol. Plant* 2009, **2**:1247-1261.

37. Lunn JE: **Compartmentation in plant metabolism**. *J. Exp. Bot.* 2007, **58**:35-47.

38. •• Smith SR, Abbriano RM, Hildebrand M: **Comparative analysis of diatom genomes reveals substantial differences in the organization of carbon partitioning pathways**. *Algal Res.* 2012, **1**:2-16.
Documentation of differences in predicted intracellular location of glycolysis isozymes in diatoms, and implications for distinct routes for carbon flux. The analysis reveals that fundamental networks of carbon flux are not static, but continue to dynamically evolve.

39. Terashima M, Specht M, Hippler M: **The chloroplast proteome: a survey from the *Chlamydomonas reinhardtii* perspective with a focus on distinctive features**. *Curr. Genet.* 2011, **57**:151-168.

40. Liaud MF, Lichtle C, Apt K, Martin W, Cerff R: **Compartment-specific isoforms of TPI and GAPDH are imported into diatom mitochondria as a fusion protein: evidence in favor of a mitochondrial origin of the eukaryotic glycolytic pathway**. *Mol. Biol. Evol.* 2000, **17**:213-223.

41. Kroth PG, Chiovitti A, Gruber A, Martin-Jezequel V, Mock T, Parker MS, Stanley MS, Kaplan A, Caron L, Weber T *et al.*: **A model for carbohydrate metabolism in the diatom *Phaeodactylum tricornutum* deduced from comparative whole genome analysis**. *PLoS ONE* 2008, **3**:e1426.

42. Fabris M, Matthijs M, Rombatus S, Vyverman W, Goossens A, Baart GJE: **The metabolic blueprint of *Phaeodactylum tricornutum* reveals a eukaryotic Entner–Doudoroff glycolytic pathway**. *Plant J.* 2012:701004-701014.

43. Ruelland E, Miginiac-Maslow M: **Regulation of chloroplast enzyme activities by thioredoxins: activation or relief from inhibition?** *Trends Plant Sci.* 1999, **4**:136-141.

44. Michels AK, Wedel N, Kroth PG: **Diatom plastids possess a phosphoribulokinase with an altered regulation and no oxidative pentose phosphate pathway**. *Plant Physiol.* 2005, **137**:911-920.

45. Gruber A, Weber T, Bartulos CR, Vugrinec S, Kroth PG: **Intracellular distribution of the reductive and oxidative pentose phosphate pathways in two diatoms**. *J. Basic Microbiol.* 2009, **49**:58-72.

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

47. Armbrust EV, Berges JA, Bowler C, Green BR, Martinez D, Putnam NH, Zhou S, Allen AE, Apt KE, Bechner M *et al.*: **The genome of the diatom *Thalassiosira pseudonana*: ecology, evolution, and metabolism**. *Science* 2004, **306**:79-86.

48. • Allen AE, Dupont CL, Obornik M, Horak A, Nunes-Nesi A, McCrow JP, Zheng H, Johnson DA, Hu HH, Fernie AR, Bowler C: **Evolution and metabolic significance of the urea cycle in photosynthetic diatoms**. *Nature* 2011, **473**:203-209.
Experimental testing of the role of the urea cycle in diatoms, demonstrating an involvement in interfacing between carbon and nitrogen metabolism. The results suggest that the urea cycle serves as a distribution and repackaging hub for inorganic carbon and nitrogen and contributes significantly to the metabolic response of diatoms to episodic nitrogen availability. The diatom ornithine-urea cycle represents a key pathway for anaplerotic carbon fixation into nitrogenous compounds that are essential for diatom growth and for the contribution of diatoms to marine productivity.

49. Lee RE, Kugrens P: **Hypothesis: the ecological advantage of chloroplast ER – the ability to outcompete at low dissolved CO_2 concentrations**. *Protist* 1998, **149**:341-345.

50. Moog D, Stork S, Zauner S, Maier UG: ***In silico* and *in vivo* investigations of proteins of a minimized eukaryotic cytoplasm**. *Genome Biol. Evol.* 2011, **3**:375-382.

51. Hildebrand M, Davis AK, Smith SR, Traller JC, Abbriano R: **The place of diatoms in the biofuels industry**. *Biofuels* 2012, **3**:221-240.

52. Yoon K, Han D, Li Y, Sommerfeld M, Hu Q: **Phosolipid:diacylglycerol acyltransferase is a multifunctional enzyme involved in membrane lipid turnover and degradation while synthesize triacylglycerol in the unicellular green**

microalga *Chlamydomonas reinhardtii*. *Plant Cell* 2012 http://dx.doi.org/10.1105/tpc.112.100701.

53. Chen JE, Smith AG: **A look at diacylglycerol acyltransferases (DGATs) in algae**. *J. Biotechnol.* 2012, **162**:28-39.

54. La Russa M, Bogen C, Uhmeyer A, Doebbe A, Filippone E, Kruse O, Mussgnug JH: **Functional analysis of three type-2 DGAT homologue genes for triacylglycerol production in the green microalga *Chlamydomonas reinhardtii***. *J. Biotechnol.* 2012, **162**:13-20.

55. Fan J, Andrew A, Xu C: **A chloroplast pathway for the *de novo* biosynthesis of triacylglycerol in *Chlamydomonas reinhardtii***. *FEBS Lett.* 2011, **585**:1985-1991.

56. Goodson C, Roth R, Wang ZT, Goodenough U: **Structural correlates of cytoplasmic chloroplast lipid body synthesis in *Chlamydomonas reinhardtii* and stimulation of lipid body production with acetate boost**. *Euk. Cell* 2011, **10**:1592-1606.

57. Peralta-Yahya PP, Zhang F, del Cardayre SB, Keasling JD: **Microbial engineering for the production of advanced biofuels**. *Nature* 2012, **488**:320-328.

58. Chu S, Majumdar A: **Opportunities and challenges for a sustainable energy future**. *Nature* 2012, **488**:294-303.

59. Lohr M, Schwender J, Polle JEW: **Isoprenoid biosynthesis in**
• **eukaryotic phototrophs: a spotlight on algae**. *Plant Sci.* 2012, **185**:9-22.
Review on algal isoprenoid metabolism. Current views on the biochemistry and genetics of the core isoprenoid metabolism in land plants and in the major algal phyla are compared and some of the most pressing open questions are highlighted. On the basis of the different evolutionary histories of the various groups of eukaryotic phototrophs, the distribution and regulation of the mevalonate (MVA) and the methylerythritol phosphate (MEP) pathways in land plants and algae and the potential consequences of the loss of the MVA pathway in groups such as the green algae are discussed.