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Review

Anaerobic energy metabolism in unicellular photosynthetic eukaryotes ☆

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ABSTRACT

Anaerobic metabolic pathways allow unicellular organisms to tolerate or colonize anoxic environments. Over the past ten years, genome sequencing projects have brought a new light on the extent of anaerobic metabolism in eukaryotes. A surprising development has been that free-living unicellular algae capable of photoautotrophic lifestyle are, in terms of their enzymatic repertoire, among the best equipped eukaryotes known when it comes to anaerobic energy metabolism. Some of these algae are marine organisms, common in the oceans, others are more typically soil inhabitants. All these species are important from the ecological (O_2/CO_2 budget), biotechnological, and evolutionary perspectives. In the unicellular algae surveyed here, mixed-acid type fermentations are widespread while anaerobic respiration, which is more typical of eukaryotic heterotrophs, appears to be rare. The presence of a core anaerobic metabolism among the algae provides insights into its evolutionary origin, which traces to the eukaryote common ancestor. The predicted fermentative enzymes often exhibit an amino acid extension at the N-terminus, suggesting that these proteins might be compartmentalized in the cell, likely in the chloroplast or the mitochondrion. The green algae *Chlamydomonas reinhardtii* and *Chlorella* NC64 have the most extended set of fermentative enzymes reported so far. Among the eukaryotes with secondary plastids, the diatom *Thalassiosira pseudonana* has the most pronounced anaerobic capabilities as yet. From the standpoints of genomic, transcriptomic, and biochemical studies, anaerobic energy metabolism in *C. reinhardtii* remains the best characterized among photosynthetic protists. This article is part of a Special Issue entitled: The evolutionary aspects of bioenergetic systems.

1. Introduction: life without oxygen

Life without oxygen is common on Earth. Oxygen shortage in biotopes can result from geochemical or physical circumstances but also from bacterial activities. Anoxia can be transient or protracted, extending from diurnal periods to months or years or millennia or more. Examples of diurnal anoxia include tidal zones [1,2], while marine oxygen minimum zones can remain anoxic for many years [3,4], and seafloor brines [5] can remain anoxic for 50,000 years or more [6]; all of these habitats harbor rich eukaryotic floras. Survival in anoxic habitats requires means of anaerobic energy metabolism, which are far more varied in prokaryotes [7] than in eukaryotes [8]. This chapter will focus mainly on photosynthetic eukaryotes which abound in anoxic environments [4,9], anaerobic energy metabolism in heterotrophic eukaryotes having been reviewed in depth recently [8].

Whether in aerobic or anaerobic environments, the challenge of staying alive means staying far from equilibrium, and that means maintaining metabolic flux and redox balance. In anaerobic energy metabolism, just as in aerobic metabolism, the generation of ATP entails the accumulation of reduced cofactors such as NADH and $FADH_2$ that are generated by catabolic pathways, usually glycolysis, and that have to be reoxidized in a process that involves the transfer of electrons to suitable acceptors that are then excreted by the organism to sustain metabolic flux. Among eukaryotes, anoxygenic photosynthesis has not been described, hence for eukaryotes, only two processes are known to maintain redox balance and conserve energy under anoxia: i) fermentations, which usually entail substrate level phosphorylation (SLP), and ii) anaerobic respiration involving chemiosmotic coupling. In eukaryote respiration, electrons, usually stemming from carbohydrates and lipids but sometimes from sulfide [10,11], enter *via* NADH and $FADH_2$ the mitochondrial electron transport chain, which is comprised of membrane proteins, the prosthetic groups of which harbor increasingly positive potentials in the course of electron flux towards the terminal acceptor. Electron flux serves to establish an electrochemical gradient across the inner mitochondrial membrane, resulting in a proton motive force that drives ATP

Abbreviations: ACS, acetate CoA synthetase; ACK, acetate kinase; ADHE, aldehyde/alcohol dehydrogenase; ASCT, acetate:succinate CoA-transferase; ATP, adenosine 5′ triphosphate; PTA, phosphotransacetylase; PFL, pyruvate formate-lyase; PFL-AE, pyruvate formate-lyase activating enzyme; PFO, pyruvate:ferredoxin oxidoreductase; SLP, substrate level phosphorylation

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synthesis. In anaerobic mitochondria, the terminal acceptor is not O_2, but is often fumarate [12]. Fermentations are processes in which the terminal electron acceptor is generated by the cell through metabolism. ATP is generated *via* SLP, where an intermediate contains a high energy phosphate bond (usually a mixed anhydride) which is used to phosphorylate ADP. SLP occurs with the help of soluble, rather than membrane-associated, enzymes. In eukaryotes, some fermentation pathways entail the harnessing of chemiosmotic energy, for example the reduction of endogenously generated fumarate to succinate in the respiratory chain of some anaerobic mitochondria [8,13]. Overall, respiration and fermentation differ with respect to the enzymes employed for cofactor reoxidation and the energy conservation processes involved.

The biochemistry of anaerobes has been far more extensively studied in prokaryotes than in eukaryotes, probably because most of what we know about eukaryotic energy metabolism stems from studies of multicellular organisms, the majority of which are specialized towards oxygen as the terminal acceptor. Most multicellular eukaryotes such as animals and plants can only withstand short periods of anoxia. A few metazoans which withstand longer periods of anoxia [1,8,12] or even total anoxia do exist, as the recently reported marine sediment-dwelling animals *Loriciferans* indicate [6]. Most of the metabolic diversity in eukaryotes is found among unicellular species (protists), among them parasites (many of which live in oxygen deprived environments) and photosynthetic organisms (microalgae), which frequently encounter hypoxic or anoxic conditions in their natural environments, *e.g.* soils, microbial mats, or marine and freshwater sediments.

Much effort has been devoted to the study of parasites with the goal of identifying targets to develop specific treatments, and a comprehensive review on the anaerobic pathways in heterotrophic eukaryotes has been recently published [8]. Compared to medically relevant parasites, our understanding of anaerobic energy metabolism in photosynthetic eukaryotes has always been more limited. This circumstance has changed somewhat over the last ten years, with the growing interest in microalgae for biotechnological applications, for example the production of biofuels such as hydrogen and hydrocarbons [14–18]. Here we provide an update on the knowledge on the anaerobic metabolic routes occurring in unicellular green algae. The significance/relevance of the compartmentalization for some anaerobic pathways is considered as the evolutionary origin of these pathways and the impact of phototrophy origins for anaerobic pathways.

2. Microalgae in anaerobic environments

For photosynthetic algae anoxia is commonplace and often transient. The oxygenation of their habitats, such as soils, fresh and marine waters, is intricately linked to light intensity, the depth of the waters, and to biotic activities. Low light can help to bring the cells to hypoxia when O_2 production in plastids is outpaced by O_2 consumption. Algal blooms also destabilize the ecosystems by attenuation of light and oxygen levels.

The metabolic responses of microalgae to anoxia have been the subject of examination for some time. Green algae, such as *Chlamydomonas reinhardtii*, *Chlamydomonas moewusii*, *Chlorogonoium elongatum*, *Chlorella fusca*, and *Scenedesmus* D3, ferment their plastidic starch to a variety of end products including acetate, ethanol, formate, glycerol, lactate, H_2 and CO_2 [19–23]. The algal heterofermentation patterns observed clearly contrast with the lactate or ethanol homofermentation of yeasts and multicellular organisms (plants, animals), while showing some similarities to the mixed-acid fermentations common to enteric bacteria [24] that lead to the excretion of partially oxidized metabolites [25].

Typical for mixed-acid fermentations, the distribution of excreted end products varies among the green algal species (and sometimes among strains) and furthermore varies with environmental factors, medium composition and carbon source. In *C. reinhardtii*, dark fermentation leads to the production of formate, acetate and ethanol in a 2:1:1 ratio [22,26]. In contrast, *C. moewusii* produced no formate under dark anoxia; the major excreted products in these conditions were acetate, glycerol and ethanol, and their relative amounts appear to diverge between studies [21,27]. During fermentation, some green algae produce hydrogen. H_2 evolution in the dark is low as compared to that measured in the light [19,26]. The amount of H_2 produced appears to be species-specific [22,27,28]. Skjånes et al. [29] have identified three green algal strains from brackish waters and marine habitats (*Chlamydomonas euryale*, *Chlamydomonas vectensis*, *cf Oocystis*) capable of producing H_2 anaerobically, indicating that this ability is not restricted to freshwater species.

Rather than excreting the fermentation end-products of the anaerobic metabolism, some algae accumulate them. Under anaerobiosis, various *Euglena gracilis* species accumulate large amounts of wax esters (C10–C18 fatty acids esterified with C10–C18 aliphatic alcohols), with a simultaneous net production of ATP [30]. The fatty acids are synthesized in the mitochondrion, acetyl-CoA serving as the terminal electron acceptor during carbohydrate oxidation. The wax esters are not excreted but are deposited in the cytosol instead, where they accumulate, constituting up to 65% of the protist's dry weight in some strains [31]. Upon return to oxic conditions, the aliphatic chains can be converted once again to acetyl-CoA, which can be oxidized to CO_2 in mitochondria or used to form paramylon reserves. Wax ester fermentation occurs in the mitochondria as a malonyl-CoA independent synthesis of fatty acids [32]. In acid mine drainage biofilms mainly constituted by *Euglena mutabilis*, large amounts of wax esters have been detected [33].

Diatoms and dinoflagellates can survive in dark anoxic marine sediments after sinking [34]. The sedimented diatoms were found to contain high concentrations of nitrate that they accumulated from sea water [35]. Recently, Kamp et al. [36] showed that the levels of accumulated nitrate and the diatom survival under dark/anoxic conditions are correlated. The consumption of the intracellular nitrate pool was furthermore correlated with excretion of ammonium, in both benthic and pelagic species, suggesting that diatoms might obtain the energy for cell survival by ammonium fermentation, as described for the fungus *Fusarium oxysporum* [37].

Importantly, environmental sequencing has uncovered vast eukaryotic communities in anaerobic environments. Sequence analysis of both nuclear (18S) and plastid (16S) ribosomal RNA clones revealed a great variety of phytoplankton in marine (oxic and anoxic) samples [4,9,38–42] and in freshwaters [43]. Most microalgae newly identified in coastal and open-sea samples belong to known phylogenetic groups, such as prasinophytes, haptophytes, dinoflagellates, cryptophytes, and diatoms [4,42,44–46]. Photosynthetic algae have also been discovered in a variety of chemically extreme environments. In natural rock acid drainages and acid mine drainages, characterized by extreme acidity (pH ~2–3) and high heavy metal concentrations, large eukaryote communities have been discovered in biofilms. Among these eukaryotes, a surprising assortment of phototrophic species was found, including chlorophytes (*Chlamydomonas*, *Chlorella*, *Dunaliella*), rhodophytes (*Galderia*), euglenozoans and diatoms (*Navicula*, *Nitszchia*) [47,48].

3. Enzymes of fermentation and anaerobic respiration in eukaryotes

Especially among heterotrophs, glycolysis is the backbone of eukaryote carbon and energy metabolism, leading to the production of pyruvate, ATP and NADH. The further fate of pyruvate can take place not only in the cytosol and the mitochondria, but also in plastids. In eukaryotes, the most common cytosolic fermentation processes to regenerate NAD^+ emanate from pyruvate, involving either

ethanol fermentations *via* pyruvate decarboxylase (PDC, EC 4.1.1.1) and alcohol dehydrogenase (ADH, EC 1.1.1.1) (typical of plants, certain animals and fungi), or lactate fermentations *via* lactate dehydrogenase (LDH, EC 1.1.1.27) (typical of many eukaryotes, including mammalian muscle). Alcohol and lactate fermentations are typical pathways for dealing with short term anoxia, but some fish, carp for example, can survive weeks in anoxia through ethanol fermentation [49,50]. Among protists, pyruvate metabolism is typically more diversified, including additional anaerobic pathways and enzymes that are not present in higher animals. These pathways often localize to mitochondria or hydrogenosomes, anaerobic forms of mitochondria that lack cytochromes and produce ATP fermentatively [8]. In algae, a plastidic location for fermentative metabolism is also clearly invoked, to which several recent studies bear witness [51,52].

In addition to simple fermentation processes, some facultative anaerobic eukaryotes exhibit fermentation processes linked to the mitochondrial electron transport chain, thereby generating a proton gradient across the membrane [8]. This process is often referred to as anaerobic respiration although the electron acceptor (fumarate) is not obtained from the environment but from the cells' metabolism.

The range of ATP yields from glucose breakdown in eukaryotes is modest [8]. The overall energetic differences between mitochondria using oxygen as a terminal acceptor, or not, boil down to about a factor of six. Taking into account the costs of ADP–ATP exchange and phosphate transport across the mitochondrial inner membrane, it can be calculated that the complete oxidation of glucose *via* mammalian mitochondria yields ~30 molecules ATP [53,54]. Eukaryotes with anaerobic mitochondria gain, in addition to 2 ATP from glycolysis, 2 ATP per glucose through substrate level phosphorylations leading to acetate and propionate production and about 1 additional ATP per glucose from proton pumping at complex I using fumarate as the terminal acceptor, making their overall energy yield about 5 ATP/glucose [8,12,55]. Protists that contain hydrogenosomes, anaerobic forms of mitochondria [56] best studied in the human parasite *Trichomonas vaginalis* [57], obtain about 4 ATP per glucose: 2 from glycolysis and 1 ATP per pyruvate, which is fermented to H_2, CO_2, and acetate [8,58].

Eukaryotes such as *Giardia intestinalis* [59] that possess mitosomes – highly reduced forms of mitochondria with no direct role in ATP synthesis [60,61] – satisfy their energy needs from cytosolic fermentations that deliver 2 ATP per glucose from glycolysis and somewhere between 0 and 1 additional ATP per pyruvate, depending upon environmental conditions. Although *Giardia* does not grow in the presence of atmospheric oxygen levels, low levels of oxygen allows *Giardia* to maintain redox balance with the help of cytosolic NADH oxidases that do not conserve energy but allow it to metabolize pyruvate to acetate, which yields one ATP per pyruvate [8]. If no oxygen is available, they rely on pyruvate metabolism to maintain redox balance, excreting ethanol with no additional ATP gain. Thus, eukaryotes with mitosomes obtain 2–4 ATP/glucose, depending on the environmental conditions [8]. Pure ethanol fermentations that entail no energy conservation from pyruvate metabolism, such as in yeast, which can ferment indefinitely, or carp, which can survive months of complete anoxia [50], deliver 2 ATP/glucose. From this, it is evident that differences among eukaryotes with respect to energy yield, end products and underlying enzymes concern the metabolic fate of pyruvate. It should also be mentioned that some eukaryotes have no *bona fide* energy metabolism at all, namely the energy parasites, such as some microsporidians, that steal ATP from their host cells *via* ADP/ATP carriers in their plasma membrane [62].

3.1. Conversion of pyruvate into acetyl-CoA by PFO and PFL

In typical aerobic mitochondria, the conversion of pyruvate into acetyl-CoA is catalyzed by pyruvate dehydrogenase (PDH), a multi-subunit complex, and the resulting acetyl-CoA is typically oxidized to CO_2 through the citric acid (TCA) cycle, yielding ATP, NADH and $FADH_2$. PDH can also function in some anaerobic mitochondria, for example during malate dismutation in *Fasciola* (liver fluke) mitochondria [12,13] or during wax ester fermentation in *Euglena* [31,63]. More typical of many prokaryotic and eukaryotic anaerobes is pyruvate oxidation *via* pyruvate:ferredoxin oxidoreductase (abbreviated PFO or PFOR, sometimes PFR; E.C. 1.2.7.1) generating CO_2, acetyl-CoA and two electrons. These two electrons are then transferred *via* three [4Fe4S] clusters to the final electron acceptor, either a ferredoxin (FDX) or a flavodoxin [64]. Like PDH, PFO belongs to a large family of enzymes which depend on the cofactor thiamine pyrophosphate (TPP) for the cleavage of the carbon–carbon bond linking the carbonyl and carboxyl groups of pyruvate [65]. In some anaerobic autotrophic prokaryotes though PFO functions in the reverse direction, *i.e.* as pyruvate synthase [66]. Except the PFOs from *Desulfovibrio* species [67], all the other PFOs studied so far are readily inactivated by oxygen. PFO activity in eukaryotes was first discovered in the hydrogenosomes – H_2-producing forms of mitochondria – of trichomonads [68]. Subsequent studies have found PFO in anaerobic parasites from distinct lineages [69,70]. PFO has been extensively studied because it is the target for metronidazole, the current treatment against many anaerobic protistan parasites of humans [71].

An alternative form of PFO has been described in eukaryotes which is a fusion protein consisting of a PFO domain at its N-terminus and an NADPH–cytochrome P450 reductase domain at its C-terminus [72,73]. This pyruvate:$NADP^+$ oxidoreductase (PNO) decarboxylates pyruvate to acetyl-CoA while transferring electrons to the flavoprotein domain to reduce $NADP^+$. PNO was first described in *E. gracilis* [74] and has since been found in apicomplexan parasites [72] and various eukaryotic heterotrophic lineages [70,73,75]. In *Euglena*, the enzyme localizes to mitochondria [74] whereas in the heterotrophic stramenopile *Blastocystis*, PNO is found in the organism's anaerobic mitochondrion [73].

In addition to PFO, there is another route of pyruvate breakdown to acetyl-CoA that operates in various prokaryotes and a few eukaryotic lineages, that is pyruvate formate-lyase (PFL; EC 2.3.1.54) [76–78]. PFL uses a radical-based homolytic mechanism to convert pyruvate into acetyl-CoA and formate [79]. PFL is activated post-translationally by the introduction of a free-radical, a reaction catalyzed by an iron-dependent activating enzyme (PFL-AE; EC 1.97.1.4) which requires the cofactor S-adenosyl methionine (SAM) as well as a reduced ferredoxin (or flavodoxin) [79–81]. PFL is irreversibly inactivated by hypophosphite, a formate analogue [82]. Like PFO, PFL is extremely sensitive to oxygen. The radical-containing enzyme is irreversibly inactivated by oxygen *via* oxygenolytic cleavage at the glycine radical [79]. In some bacteria such as *Escherichia coli* species the iron-dependent aldehyde/alcohol dehydrogenase ADHE plays the role of PFL-deactivase by removing the radical on PFL [83]. Based upon current sampling, it appears that PFL is more widespread than PFO/PNO in photosynthetic protists, in contrast to heterotrophic protist in which PFL is less common than PFO [8].

3.2. Iron-only hydrogenase

Hydrogenases function to dispose of excess electrons accumulated during fermentation or to extract electrons from hydrogen for the reduction of substrates in energy-yielding processes. Different classes of hydrogenases have been identified based on their metal content (FeFe, NiFe, NiFeSe, FeS-free) [84–86]. Hydrogenases are very widespread among prokaryotes while among eukaryotes, they have so far only been found in unicellular species, including parasites and photosynthetic algae [8,28]. All eukaryotic hydrogenases characterized so far belong to the class of Fe-only ([FeFe]) hydrogenases in which the [4Fe4S] cluster is linked through a cysteine residue to a [2Fe2S] cluster [87,88]. In most H_2-producing heterotrophic eukaryotes studied to date, the electrons arising from pyruvate oxidation

by PFO are transferred to a ferredoxin, and subsequently to hydrogenase [8].

Even though it is not known yet to occur among eukaryotes, there is a new and exciting aspect to [FeFe] hydrogenase function in prokaryotes that deserves mention: electron bifurcation. Schut and Adams [89] found that the [FeFe]-hydrogenase of *Thermotoga maritima* generates H_2 with one electron coming from reduced ferredoxin *via* a [2Fe2S] cluster containing subunit and one electron coming from NADH *via* a flavoprotein subunit. This allows *Thermotoga* to generate H_2 in part from NADH, which is a thermodynamically unfavorable reaction; but by coupling it to the oxidation of a low potential reduced ferredoxin, the overall reaction (H_2 production) becomes thermodynamically favorable and goes forward. This is potentially relevant for eukaryotic [FeFe] hydrogenase, because the corresponding [2Fe2S] cluster-containing and flavoprotein subunits are found in eukaryotes and have furthermore been found in association with the [FeFe] hydrogenase catalytic subunit [8]. Electron bifurcation [90] is being found in many prokaryotic anaerobes (reviewed by Buckel and Thauer in this volume), and it is possible that it occurs in eukaryotes as well, with the reaction catalyzed by [FeFe] hydrogenase being a candidate.

3.3. Aldehyde/alcohol dehydrogenase (ADHE)

Acetyl-CoA produced from pyruvate (either by PDH, PFL or PFO) can be used to generate ATP from ADP by conversion to acetate (see below) or to maintain redox balance by conversion to ethanol. In bacterial mixed-acid fermentations, ethanol is typically produced *via* bifunctional aldehyde/alcohol dehydrogenase (ADHE or ADH1) [25]. This enzyme combines a coenzyme A-dependent acetaldehyde dehydrogenase (N-terminal half) and a Fe-dependent alcohol dehydrogenase (C-terminal half). ADHE is a common enzyme among bacteria where it plays a key role under anaerobic conditions by regenerating reducing power and CoASH [83,91,92]. Among eukaryotes, the protein has been found in anaerobic parasites such as *Giardia* [93], *Entamoeba* [94] and in a non-parasitic rumen inhabitant, the chytridiomycete *Piromyces* sp. E2 [95] where it participates in core carbon metabolism. An ADHE has also been identified in two evolutionary related unicellular algae, the green alga *C. reinhardtii* and its colorless relative *Polytomella* sp. [96,97].

3.4. Substrate-level phosphorylation (SLP)

In substrate level phosphorylations, a phosphate group in a high energy bond is harnessed to phosphorylate ADP *via* soluble enzymes without the help of electrochemical gradients. In eukaryotes, acetyl-CoA produced by either PFL or PFO (see above) can be converted into acetate to produce ATP *via* substrate level phosphorylation; at present, three different routes for this SLP are known [98]. The first route is the ASCT cycle, which involves two enzymes: an acetate:succinate CoA-transferase (ASCT; EC 2.8.3.8) which transfers the CoA moiety of acetyl-CoA to succinate, and a succinyl-CoA synthetase (SCS or STK; EC 6.2.1.5) which converts succinyl-CoA back into succinate. The ASCT reaction converts one thioester into another. In the SCS reaction, the energy in the thioester bond of succinyl-CoA is conserved *via* phosphorolysis to generate succinyl phosphate as a reaction intermediate, the mixed anhydride bond of which directly phosphorylates ADP to ATP [99]. The ACST reaction can be catalyzed by three different types of ASCT proteins (ASCT subfamilies 1A, 1B, 1C) which share little sequence homology [98]. The ASCT cycle is present in some hydrogenosome-containing eukaryotes and in anaerobic mitochondria [8]. In the second route, concomitant production of acetate and ATP from anaerobically produced acetyl-CoA is catalyzed by a single enzyme, the ADP-forming acetyl-CoA synthetase ([ADP]-ACS). Among eukaryotes, this enzyme has been characterized in *Entamoeba* [100] and in *Giardia* [101]. A third route of anaerobic production of ATP from acetyl-CoA involves the sequential action of phosphotransacetylase (PTA or PAT; EC 2.3.1.8) and acetate kinase (ACK or AK; EC 2.7.2.15). The ACK-PTA pathway, well-known among prokaryotes [25], has so far only been found in the photosynthetic alga *C. reinhardtii* [97,102]. Of these three SLP pathways, which conserve the energy in the thioester bond of acetyl-CoA, the ASCT cycle involving the Krebs cycle enzyme succinyl-CoA synthetase, appears to be the most common among heterotrophic eukaryotes studied so far.

3.5. Anaerobic respiration

Respiration is the use of a terminal electron acceptor that is obtained from the environment. The respiratory process involves the passage of electrons through an electron transport chain, creating an ion gradient (usually a proton gradient) across the membrane that is further used for ATP synthesis and transport processes. Anaerobic respiration is common place among prokaryotes, which can use a great diversity of environmentally available terminal acceptors for ATP synthesis and which respire across their plasma membrane, eukaryotes across their inner mitochondrial membrane [103]. Anaerobic respiration appears to be comparatively rare in eukaryotes, based on present sampling [12].

Malate dismutation is a process that allows some multicellular eukaryotes such as lower marine animals (mussels, oysters) and parasitic helminths (*Ascaris suum*, *Fasciola hepatica*) to survive in anaerobic environments [12]. In this process, glucose is degraded to phospho*enol*pyruvate which is subsequently reduced to malate by cytosolic malate dehydrogenase, thereby reoxidizing the glycolytic NADH. The produced malate is further metabolized inside mitochondria. To maintain redox balance, some of the malate is oxidized and some is reduced (dismutation). Malate oxidation to acetyl-CoA occurs *via* malic enzyme (ME) and PDH. The CoA group from the acetyl-CoA is then transferred to succinate by an acetate:succinate CoA transferase, yielding acetate and succinyl-CoA. Regeneration of CoASH allows ATP production through SLP *via* the ASCT cycle (see above). Malate reduction to succinate occurs in two steps that reverse part of the Krebs cycle, with fumarate as intermediate. Fumarate reduction which involves a membrane-bound fumarate reductase and a quinone – typically a rhodoquinone – is also linked to the mitochondrial electron transport chain *via* complex I and F_oF_1-ATP synthase [104]. Rhodoquinone is a quinone essential for fumarate reduction as it occurs in some bacteria (such as *Rhodospirillum rubrum*), and it is also essential for fumarate reduction during malate dismutation in eukaryotic species that possess anaerobic mitochondria. The pathway of rhodoquinone synthesis, however, appears to be different in *R. rubrum* compared to eukaryotes as in *R. rubrum* rhodoquinone synthesis proceeds from ubiquinone, whereas this is not the case in eukaryotes [105–108], and A.G.M.T., unpublished observations]. Compared to fermentation, malate dismutation conserves more energy out of the same substrate (phospho*enol*pyruvate).

4. *Chlamydomonas* is the most flexible eukaryotic fermenter so far

C. reinhardtii is a unicellular green alga found in freshwaters and wet soils around the globe. This alga has become a model organism to study photosynthesis and chloroplast biogenesis [109,110], nutrient deprivation [111–115] as well as assembly and function of flagella [116–118]. The ability of *C. reinhardtii* to survive under anoxia has long been known [22,26], with interest renewed following the release of its nuclear genome sequence in 2007 [119]. Metabolic pathways predicted from physiological studies [22] could be confirmed while unexpected routes were also uncovered [97,120,121]. Among the surprises was the extended array of routes for pyruvate stemming from starch breakdown (Fig. 1). In *Chlamydomonas*, pyruvate can be

metabolized to ethanol *via* a PDC–ADH pathway or to lactate *via* LDH. But pyruvate can also be converted into acetyl-CoA by a pyruvate formate-lyase or a pyruvate:ferredoxin oxidoreductase. The existence of a PFL in *C. reinhardtii* had been proposed earlier based on the inhibition of its formate production by sodium hypophosphite [22]. In contrast, the occurrence of a PFO had not been predicted from experimental approaches.

Microarray studies and RT-PCR have been largely used to unravel the metabolic routes used by the alga to adapt to anoxic atmosphere [122–124]. Although these studies have pinpointed various genes, among them genes for fermentative enzymes, it quickly became clear that there was no strict correlation between transcript levels and protein levels or enzyme activity [123,125]. Furthermore, most of these RNA analyses were carried out on cells experiencing anoxia in a medium which contains solely potassium phosphate and magnesium chloride (AIB medium) [126]. The cells resuspended in this medium also face nutrient and carbon source deprivation.

Current approaches to understanding the anaerobic metabolism of *Chlamydomonas* entail a multi-faceted approach: i) the study of cells exposed to different anoxic conditions (light *vs.* darkness, cell resuspension medium), ii) the production of mutant strains deficient in fermentative enzymes, iii) enzymatic characterization of these enzymes, iv) mass spectrometry analyses and v) studies to determine the intracellular localization of the fermentative enzymes, as most of them exhibit an extended N-terminus as compared to their bacterial counterparts, suggesting organellar (chloroplast or mitochondrial) localization.

cDNAs for PFL and its activating enzyme PFL-AE have been sequenced, revealing the relationship to bacterial enzymes, including to the well-studied enzyme of *E. coli* [97,123]. When complemented with the *C. reinhardtii* PFL, *E. coli* cells deficient in *pfl* were shown to produce formate, demonstrating that the algal enzyme was synthesized and post-translationally activated in *E. coli* [123]. Algal strains deficient in PFLs have been recently produced by insertional mutagenesis in two different research laboratories [52,127]. The lack of PFL activity in these mutants led to a rerouting of dark anaerobic metabolism, with an increased production of lactate, ethanol and CO_2 and a lowered production of acetate [52,127]. Immunoblot experiments and mass spectrometry data have provided evidence for the dual localization of the PFL in the chloroplast and mitochondrion [97,102] (Fig. 1). It remains to be ascertained that the enzyme is indeed active in both cell compartments, as the intracellular localization of PFL-AE is currently unknown.

A gene encoding a PFO that is homologous to PFO from other eukaryotes was detected in the *Chlamydomonas* genome [97,121]. *C. reinhardtii* PFO expressed in *E. coli* catalyzes the oxidative decarboxylation of pyruvate, in a CoASH- and TPP-dependent manner; this activity is highly sensitive to oxygen (van Lis et al. in preparation), like most bacterial and parasite PFOs [128]. Early work indicated compartmentation of enzymes involved in pyruvate breakdown [129]. The identification of tryptic peptides specific to the PFO in chloroplast fractions but not in mitochondrial fractions of anaerobic *C. reinhardtii* cells [102], suggests that the enzyme is chloroplast localized. This localization is also supported by immunoblotting experiments (van Lis et al. in preparation) (Fig. 1).

C. reinhardtii is able to produce H_2 after anaerobic adaptation in the light or in the dark [22,23,130]. The metabolic function of the H_2 production in the alga seems to be redox balance in the absence of O_2. *C. reinhardtii* possesses two [FeFe] hydrogenases, HYDA1 and HYDA2, which both exhibit the minimal structure for [FeFe] hydrogenases [131–134]. HYDA1 and HYDA2 localize to the chloroplast, where they can independently catalyze H_2 production in the light and in the dark [135]. In light, HYDA1 is coupled to the reducing site of the photosynthetic electron transport chain (PSII or NADH:Q oxidoreductase) and accepts electrons from FDX1 (petF) [136–138]. From the analysis of the interaction between FDX and HYDA1, Winkler et al. [139] concluded that among the six known chloroplast [2Fe2S] ferredoxins (FDX1–FDX6), FDX1 appears as the most efficient electron mediator to hydrogenase(s). It is thought that in *C. reinhardtii* PFO is the source of electrons to hydrogenases in the dark, as in the case of heterotrophic unicellular eukaryotes [8]. Studies of the dark fermentation products excreted by a *C. reinhardtii* mutant strain deficient in hydrogenase activity, obtained by disruption of the HydEF gene that encodes a hydrogenase maturation protein, revealed an increased production of succinate [124]. In the alga several metabolic routes can potentially lead to the anaerobic production of this organic acid [124], including a pathway common among heterotrophic eukaryotes and that involves a phospho*enol*pyruvate carboxykinase (PCK), a malate dehydrogenase (MDH), a fumarate hydratase (FUM) and a soluble fumarate reductase (FRD) [8] (Fig. 1).

Acetyl-CoA produced by PFL or PFO can be used by either ADHE, allowing the reoxidation of glycolytic NADH, or by the consecutive action of a PTA and an ACK, to generate ATP. Together with formate and acetate, ethanol is one of the major products excreted by *C. reinhardtii* exposed to dark anoxia [22,23,26,122]. The existence of a CoA-acetylating aldehyde dehydrogenase activity in the photosynthetic alga, similar to that found in facultative bacteria or parasites, was suggested by earlier studies [129] and subsequently confirmed by the isolation of its cDNA [97]. When exposed to dark anaerobiosis in AIB medium, an *Adhe* insertional mutant does not excrete ethanol [140], suggesting that in these conditions, ADHE is the main enzyme involved in ethanol production, as in bacterial mixed-acid fermentations. Comparative proteomic studies [102] and immunoblot experiments [R. van Lis, unpublished results] support a chloroplast localization of this enzyme.

Chlamydomonas possesses two sets of genes for phosphotransacetylase and acetate kinase, likely resulting from gene duplication events. These PTA–ACK pathways could participate in anaerobic ATP synthesis when cellular energy demands increase, but they could also be involved in acetate assimilation. From proteomic analysis of organelle-enriched samples, it was inferred that PTA1–ACK2 constitutes a mitochondrial route while PTA2–ACK1 constitutes a chloroplast pathway [97,102] (Fig. 1).

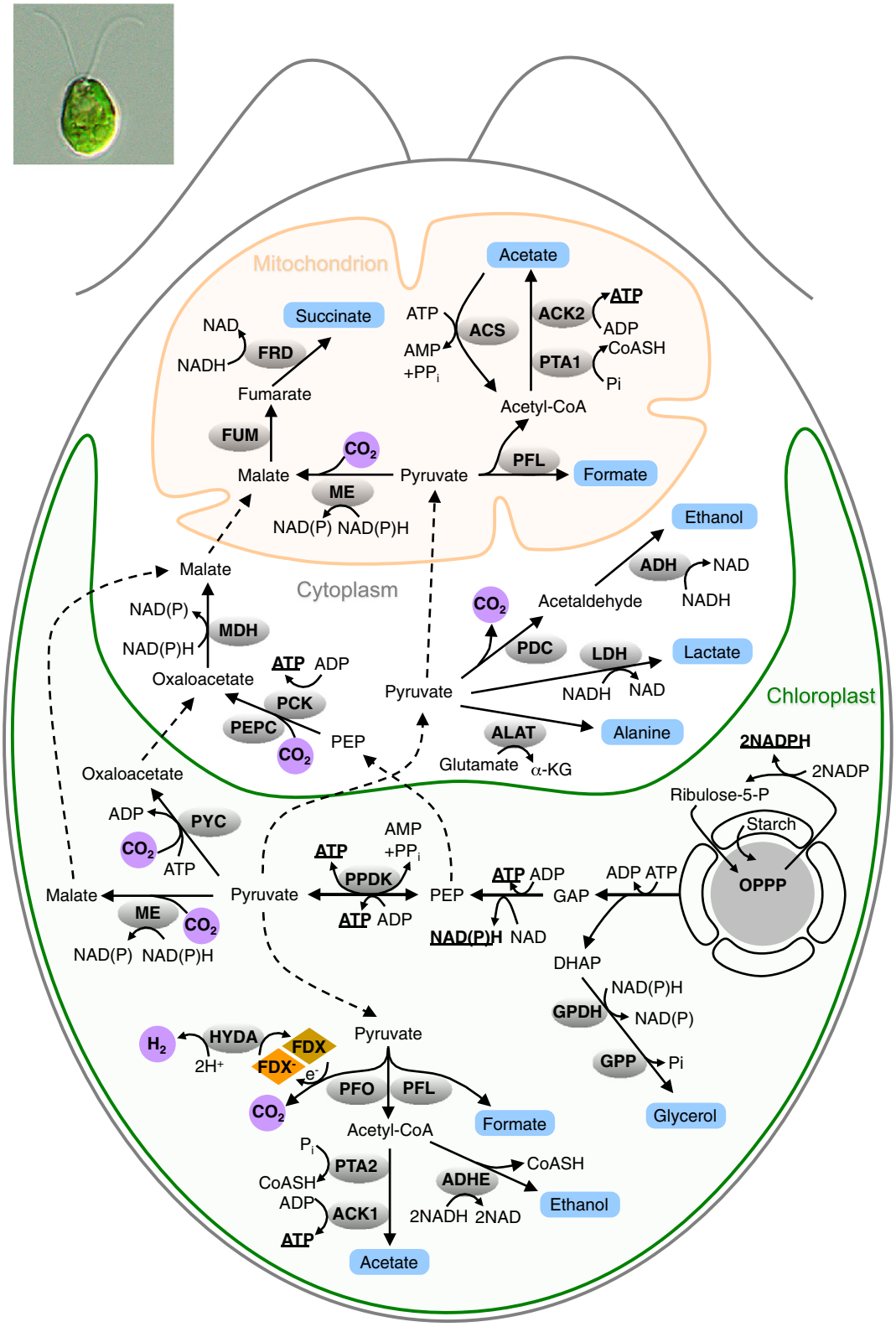
Production of algal mutants has revealed an even more diverse spectrum of fermentation end products, including alanine [52] and

Fig. 1. Schematic representation of the dark anaerobic energy metabolism in *Chlamydomonas reinhardtii*. The multiplicity of the metabolic routes that can be used when exposed to dark anoxia has been revealed by physiological and biochemical studies on wild-type and mutant strains. For most enzymes, subcellular localization was based on previous studies [51,97,102,124,169]. The mitochondrial localization of the fumarate reductase (FRD) is based on targeting prediction by PredAlgo [170]. Although *Pck* transcript levels were previously reported not to increase upon dark anoxia [124], PCK is included in the scheme since different experimental conditions could easily show a different picture. Also, transcript levels do not necessarily translate to enzyme levels or activity. Enzymes that have multiple isoforms and for which no clear location is known or multiple locations of the different isoforms are envisioned, were fit in to serve best schematic continuity (PPDK, ACS, MDH). The different metabolites/redox sinks and/or excreted fermentation products identified so far are highlighted in blue. Acetate is mostly incorporated into growth media for *C. reinhardtii*, and its assimilation by ACS necessitates ATP and produces AMP and pyrophosphate (PP_i). PP_i-dependent PPDK could be engaged instead of pyruvate kinase and yield 4 ATP instead of 2 with the concerted action of adenylate kinase (2 ADP → ATP + AMP). In *Arabidopsis* and in rice, PP_i-dependent enzymes were reported to be likely involved in anoxia tolerance [171]. Enzyme abbreviations are: ACK, acetate kinase; ACS, acetyl-CoA synthase; ADH, NAD-dependent alcohol dehydrogenase; ADHE, aldehyde/alcohol dehydrogenase; ALAT, alanine aminotransferase; FDX, ferredoxin; FRD, soluble fumarate reductase; FUM, fumarate hydratase; GPP, glycerol-3 phosphatase; GPDH, glycerol-3-phosphate dehydrogenase; HYDA, Fe-only hydrogenase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; ME, malic enzyme; OPPP, oxidative pentose phosphate pathway; PCK, PEP carboxykinase; PDC, pyruvate decarboxylase; PEPC, PEP carboxylase; PFL, pyruvate formate-lyase; PFO, pyruvate:ferredoxin oxidoreductase; PPDK, pyruvate phosphate dikinase; PTA, phosphotransacetylase; PYC, pyruvate carboxylase.

glycerol [140]. These alternative routes are depicted in Fig. 1. Alanine remained as a metabolite, which is very common in anaerobic metabolism among animals [12], while the other fermentation products are excreted. Progress on *Chlamydomonas* fermentative pathways and metabolic plasticity continues, but the metabolic switches and the interplay between the multiple metabolic routes are yet obscure.

5. Genome survey/metabolic data of the anaerobic metabolic routes found in photosynthetic algae

Eukaryotic photosynthesis arose more than one billion years ago when a heterotrophic host engulfed a cyanobacterial ancestor [141,142]. Following this primary endosymbiotic event, the



cyanobacterium underwent genome reduction and genetic integration within the host genome *via* gene transfer to the nucleus [143]. Once the endosymbiont was integrated within its host, three major lineages diverged, the green algae (and their land plant relatives), red algae, and glaucophytes [144]. Subsequent rounds of secondary endosymbioses took place, in which red or green algae were engulfed and retained by eukaryote hosts thereby transferring photosynthesis to other eukaryotic lineages. Many algal lineages have acquired their plastids thus through secondary and tertiary endosymbiosis [141,145]. At present seven distinct eukaryotic groups that harbor plastids derived from secondary endosymbiosis have been identified: euglenophytes, chlorarachniophytes, haptophytes, stramenopiles, cryptophytes, dinoflagellates and apicomplexans [145] (Fig. 2).

Experimental studies conducted to reveal the anaerobic potentialities of unicellular eukaryotic phototrophs are so far limited, mainly carried out on green algae (chlorophytes), such as *Chlamydomonas* and *Chlorella*. Another approach to learn about the anaerobic metabolic abilities of these algae is *via* surveys of genome sequences. At the time of writing, 17 algal genome sequences were publicly available (see Table 1). These genomes are of species that can be cultured in the laboratory, but which are not necessarily the most representative, especially with regard to anaerobic metabolism. Nevertheless, as detailed below, this algal genome sample brings a new light on the extent and diversity of the anaerobic energy metabolism among photosynthetic algae.

5.1. PFL/PFL-AE system

Hypophosphite inhibition of anaerobic formate production, indicating PFL pathways, had been reported earlier for various green species such as *C. reinhardtii*, *Chlorella vulgaris*, *Chlorogonium and Scenedesmus* [22,146]. The genome survey indicates that the PFL/PFL-AE system is widespread among the chlorophytes, with the identification of PFL/PFL-AE genes in Chlorophyceae (*Chlamydomonas*), Trebouxiophycea (*Chlorella* NC64) and in Prasinophyceae (*Ostreococcus*, *Micromonas*), marine algae characterized by a compact genome and a minimal cellular organization [147–149] (Table 1; Fig. 2). The PFL/PFL-AE system is also encoded in the glaucophyte *Cyanophora paradoxa*.

Among the algae that possess secondary plastids, genes for a PFL/PFL-AE system are present in the chlorarachniophyte *Bigelowiella natans*, where PFL is 63% identical to *C. reinhardtii* PFL. In the marine centric diatom *Thalassiosira pseudonana* genes for two sets of PFL/PFL-AE are present (Fig. 3). These genes likely result from a duplication event as the PFLs share 94% sequence identity, and the PFL-AE 76% of identity. *Thalassiosira* PFL and PFL-activating enzyme share 54–55% sequence identity with their counterparts in *Chlamydomonas*.

5.2. PFO/PNO

The ability to perform oxidative decarboxylation by PFO, or its alternative form PNO, is encoded in diverse algal lineages (Table 1; Fig. 2). PFO genes are present in the chlorophytes *Chlamydomonas* and *Chlorella*, and in the diatom *Thalassiosira*. The predicted *Chlorella* PFO exhibits 57% sequence identity with *Chlamydomonas* PFO, and is likely to be compartmentalized as it exhibits an N-terminal extension. *Thalassiosira* PFO shares 46% sequence identity with its counterparts in *Chlamydomonas* and *Chlorella*. PNO genes are found in the glaucophyte *Cyanophora paradoxa* [150], in the euglenid *E. gracilis* [72,151,152], in the apicomplexan *Cryptosporidium parvum* [72] and in the cryptophyte *Guillardia theta*. The lack of an extended N-terminus in *G. theta* PNO suggests that the enzyme is cytosolic. Three of the surveyed algae, *Chlamydomonas*, *Chlorella* NC64, and *Thalassiosira*, contain the two enzymatic systems capable of the anaerobic conversion of pyruvate into acetyl-CoA, namely PFO and PFL. The co-occurrence of both routes has not been reported in parasitic eukaryotes so far.

5.3. Hydrogenases

Genes for iron-only hydrogenases have been identified in many algal species. The first sequences of algal HYD obtained were from the green algae *Scenedesmus obliquus* [153] and *Chlamydomonas* [131]. These enzymes were unique as they represented the smallest form of [FeFe]-HYD known, comprising only the cluster H, in contrast to bacterial enzymes which exhibit additional clusters on the N-terminal side of the protein. With the release of the genome sequence of *Chlorella variabilis* NC64A and the identification of two hydrogenases (HYDA1, HYDA2), a novel structure for algal [FeFe]-hydrogenases was uncovered as they exhibited an N-terminal F-cluster domain [28]. *Chlorella* HYDAs are as sensitive to O_2 as the *Chlamydomonas* HYDs [28], therefore ruling out the hypothesis that the F-cluster could protect the enzyme from oxygen inactivation. All the chlorophyte HYD sequences exhibit a transit peptide sequence which likely target the enzyme to the chloroplast, as it is the case for *Scenedesmus* or *Chlamydomonas* [28,131]. The maturation proteins HydEF and HydG required for the assembly and insertion of metal clusters in [FeFe]-hydrogenases were initially discovered in *C. reinhardtii* [154]. In *Cyanophora*, hydrogen metabolism is predicted from the identification of genes for HYDA and its maturation factors [150] (Table 1). The rhodophytes *Porphyridium* and *Porphyra* were found to perform reduction of CO_2 with hydrogen as electron donor under low light (photoreduction) [155]. Among the algae with secondary plastids, *Thalassiosira* is so far the only member that contains a gene encoding a typical [FeFe]-hydrogenase, but as noted before [86], the genes for the maturation factors HydEF and HydG have not been found in its genome (Table 1).

5.4. ADHE

Aldehyde/alcohol dehydrogenase is found in several algal lineages (Table 1; Fig. 2), including two chlorophytes *Chlamydomonas* and *C. variabilis* NC64. While *Chlamydomonas* possesses one gene, *Chlorella* exhibits two distinct *Adhe* genes which encode enzymes sharing 65% sequence identity. The predicted proteins share 64% sequence identity with *C. reinhardtii* ADHE. Genes for ADHE are found in several lineages with secondary plastids. In *G. theta*, two isoforms of ADHE are predicted which differ mostly in their N-terminal sequence. These enzymes share 49% sequence identity with their closest bacterial counterparts *Thermosynechoccocus* and *Clostridium acetobutylicum*, and 45% with their counterpart in *Chlamydomonas*. In *B. natans*, the ADHE exhibits 42% amino acid sequence identity to its counterpart in *Chlamydomonas*, and it is predicted to be organellar as it has an extended N-terminus. The presence of an ADHE in *Chromera veila*, a photosynthetic alveolate closely related to apicomplexan parasites [156], is also inferred from ESTs [157].

5.5. Energy conserving acetate metabolism

The majority of the algae surveyed here exhibit at least one of the three systems for substrate-level phosphorylations involving acetate known in eukaryotes ([98]; see Section 3.4). Therefore it seems that these algae possess a metabolic route for ATP synthesis to contribute to the cell's viability in anaerobic environments. *Chlorella* possesses a gene encoding a putative acetate:succinate CoA-transferase which shares 40% sequence identity with that of *Fasciola hepatica* (ASCT-subfamily 1B). Genes for an ASCT are also present in the genome of the diatoms *Thalassiosira* and *Phaedodactylum*. Predicted diatom ASCTs share up to 47% sequence identity with the mitochondrial ASCT from kinetoplastids (*Trypanosoma* species), which belong to the subfamily 1A [98].

The *Thalassiosira* genome also encodes a gene for a putative acetyl-CoA synthetase (ADP forming) (Table 1; Fig. 3). The predicted protein exhibits 36% amino acid sequence identity with the enzyme from *Giardia lamblia* which has been studied in detail [101]. Unlike

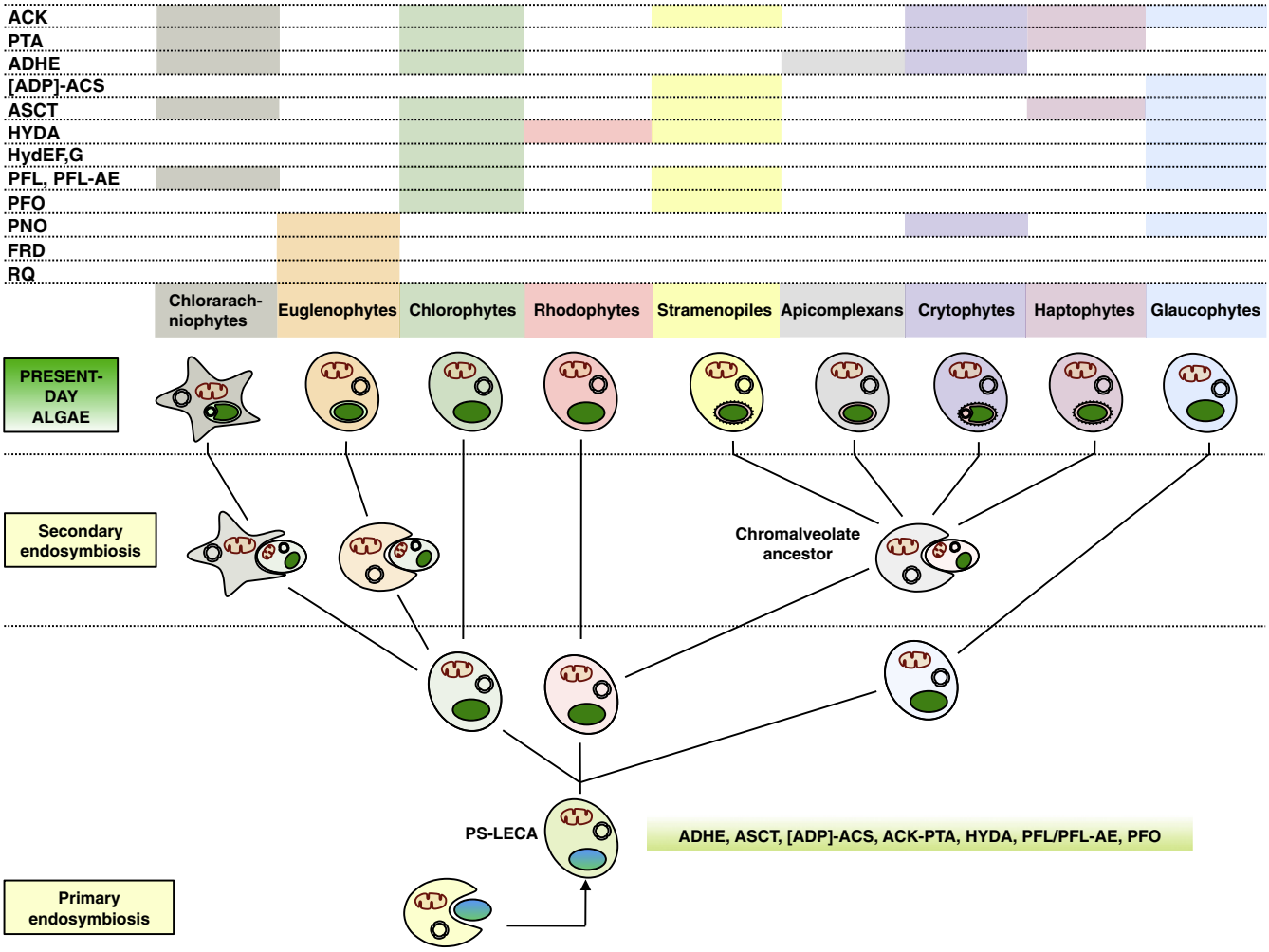


Fig. 2. Schematic representation of the evolution of photosynthetic algae with the distribution of enzymes associated to anaerobic energy metabolism as inferred from a genome-based survey. Only the algal lineages for which genomic data are publicly available are shown. Shading indicates that the corresponding gene is present in the genome (or in the case of RQ, that the compound is present in the mitochondrial membrane). Dinoflagellates and algae resulting from tertiary endosymbiosis are not included as we lack insights into their anaerobic capabilities. Algae are currently seen as resulting from rounds of endosymbiotic events, with the very first one being the engulfment of a cyanobacterium by a heterotrophic eukaryote. From that event, the three contemporary algal lineages emerged: chlorophytes, glaucophytes and rhodophytes. Members of the chlorophytes and rhodophytes were engulfed by independent eukaryotic hosts resulting in lineages with secondary plastids. Chlorophytes and stramenopiles (diatoms) are the most diverse in their anaerobic metabolism, whereas rhodophytes appear to be mostly devoid of the typical anaerobic enzymes found in other algae. Enzyme abbreviations are as in Fig. 1.

its parasite counterpart, the diatom ACS exhibits an N-terminal extension, suggestive of an organellar localization (Fig. 3). In three other diatoms, incomplete DNA sequences suggest that an ACS (ADP-forming) might also be present.

Ack and *Pta* genes have also been identified in several algal lineages with secondary plastids, including the cryptophytes, the haptophytes, and the chlorarachniophytes (see Table 1), suggesting that a PTA–ACK route for anaerobic ATP production (or assimilation of acetate) might occur in these algae as in *Chlamydomonas*.

5.6. Glycerol as an end product

Glycerol is usually a minor product of dark fermentation of green algae [22,26]. However, *Chlamydomonas* can excrete significant amounts of glycerol; this was observed in conditions where the ADHE route is blocked [140]. The typical route to glycerol formation is a two-step reaction involving the reduction of the glycolytic intermediate dihydroxyacetone phosphate to glycerol-3-phosphate, by an NAD^+-dependent glycerol-3-phosphate dehydrogenase (GDPH), followed by the hydrolysis of the glycerol-3-P by a glycerol-3-phosphatase (GPP) (Fig. 1). This pathway which allows the reoxidation of glycolytic NADH is known in some heterotrophic anaerobic eukaryotes such the parasitic protist *T. vaginalis* [8]. The potential for glycerol production is expected to be widespread among algae as genes for GPDH and GPP are found in various algal lineages, such as *Cyanidioschyzon merolae*, *Thalassiosira* and *G. theta*.

5.7. Anaerobic respiration

The anaerobic respiratory pathways that have been found among eukaryotes so far occur in heterotrophic species [12]. An exception is *Euglen*a, whose wax ester fermentation is thought to involve the respiratory chain [158] with the rhodoquinone-dependent reduction of fumarate to succinate being coupled to the mitochondrial electron transport chain [159]. Benthic or pelagic diatoms species can accumulate high concentrations of nitrate. Kamp et al. [36] recently showed that under dark anaerobic conditions, nitrate consumption followed ammonium production. They proposed that nitrate might be respired to ammonium *via* an anaerobic biochemical route known as ammonium fermentation. In *Fusarium*, nitrate reduction to nitrite and the subsequent reduction of nitrite to ammonium are thought to entail ATP synthesis *via* substrate-level phosphorylation only [37] or possibly *via* respiration and electron transport phosphorylation in addition

Table 1
Distribution of genes involved in anaerobic energy metabolisms among unicellular photosynthetic eukaryotes as inferred from genome survey. BLASTP and TblastN searches on NCBI whole genome databases and/or on the website of each given alga were performed to identify the genes encoding fermentative enzymes. Homology to the different enzymes was determined using an E value cutoff of less than 10^{-10} using either the *C. reinhardtii* sequences or other eukaryotic ones and at least 80% coverage.

	F/B/M	Condition	Reference	PFL	PFL-AE	PFO/PNO	HYDA	HydEF	HydG	ADHE	ACK	PTA	ASCT[a]	[ADP]-ACS[b]
Primary plastids														
Chlorophytes														
Chlamydomonas reinhardtii	F	Genome	DOE JGI	✓[c]	✓[c]	PFO[c]	✓[c,d]	✓[c]	✓[c]	✓[c]	✓[c,d]	✓[c,d]	–	–
Chlorella NC64	F	Genome	DOE JGI	✓	✓[c]	PFO[c]	✓[c,d]	✓[c]	✓[c]	✓[c,d]	✓[c,d]	✓[c,d]	1B[c]	–
Coccomyxa sp. C-169	F	Genome	DOE JGI	–	–	–	–	–	–	–	✓	✓[c]	–	–
Ostreococcus tauri	M	Genome	DOE JGI	✓	✓	–	–	–	–	–	–	–	–	–
Ostreococcus lucimarinus	M	Genome	DOE JGI	✓	✓[c]	–	–	–	–	–	–	–	–	–
Micromonas pulsilla CCMP1545	M	Genome	DOE JGI	✓	✓	–	–	–	–	–	–	–	–	–
Micromonas sp. strain RCC299	M	Genome	DOE JGI	–	–	–	–	–	–	–	–	–	–	–
Glaucophytes														
Cyanophora paradoxa	F	Genome fragment	Project[e]	✓[c,d]	✓	PNO	✓[d]	✓	✓	–	✓	?	1B	✓[c]
Rhodophytes														
Cyanidioschyzon merolae	F	Genome Project[f]	–	–	–	–	–	–	–	–	–	–	–	–
Secondary plastids														
Euglenophytes														
Euglena gracilis	F/B	ESTs	NCBI	?	?	PNO[c]	?	?	?	?	?	?	?	?
Chlorarachniophytes														
Bigelowiella natans	M	Genome	DOE JGI	✓	✓	–	–	–	–	✓[c]	✓	✓[g]	1A	–
Cryptophytes														
Guillardia theta	M	Genome	DOE JGI	–	–	PNO	–	–	–	✓[c,d]	✓[c]	✓	–	–
Haptophytes														
Emiliania huxleyi	M	Genome	DOE JGI	–	–	–	–	–	–	–	✓[c]	✓	1A[c]	–
Stramenopiles														
Aureococcus anophagefferens	M	Genome	DOE JGI	–	–	–	–	–	–	–	–	–	–	✓[g]
Fragilariopsis cylindrus	M	Genome	DOE JGI	–	–	–	–	–	–	–	–	–	1B[c,g]	–
Phaedodactylum triconutum	M	Genome	DOE JGI	–	–	–	–	–	–	–	✓	–	1A[c]	✓[g]
Pseudo-nitzschia multiseries CLN-47	M	Genome	DOE JGI	–	–	–	–	–	–	–	–	–	–	✓[g]
Thalassiosira pseudonana	M	Genome	DOE JGI	✓[d]	✓[d]	PFO	✓	–	–	–	–	–	1A	✓[c]
Apicomplexans														
Chromera velia	M	ESTs	NCBI [157]	?	?	?	?	?	?	✓	?	?	?	?

✓, gene was found; –, not found. ?, unknown.
Abbreviations are as follows: DOE JGI, Department of Energy Joint Genome Institute; NCBI, National Center for Biotechnology Information; F/B/M, freshwater, brackish water, marine water.
[a] ASCT, acetate:succinate CoA-transferase; 1A, homolog to members of subfamily ASCT-1A (*Trypanosoma cruzi*, EAN79240); 1B, homolog to members of subfamily ASCT-1B (*Fasciola hepatica*, ACF06126).
[b] Homolog to *Giardia lamblia* acetyl-CoA synthetase [ADP forming] (XM_001705692).
[c] Protein is predicted or known to be compartmentalized, likely in the chloroplast or in the mitochondrion.
[d] At least two distinct genes were detected.
[e] *Cyanophora paradoxa* Genome project http://cyanophora.rutgers.edu/cyanophora/home.php.
[f] *Cyanidioschyzon merolae* Genome Project http://merolae.biol.s.u-tokyo.ac.jp/.
[g] Partial sequence.

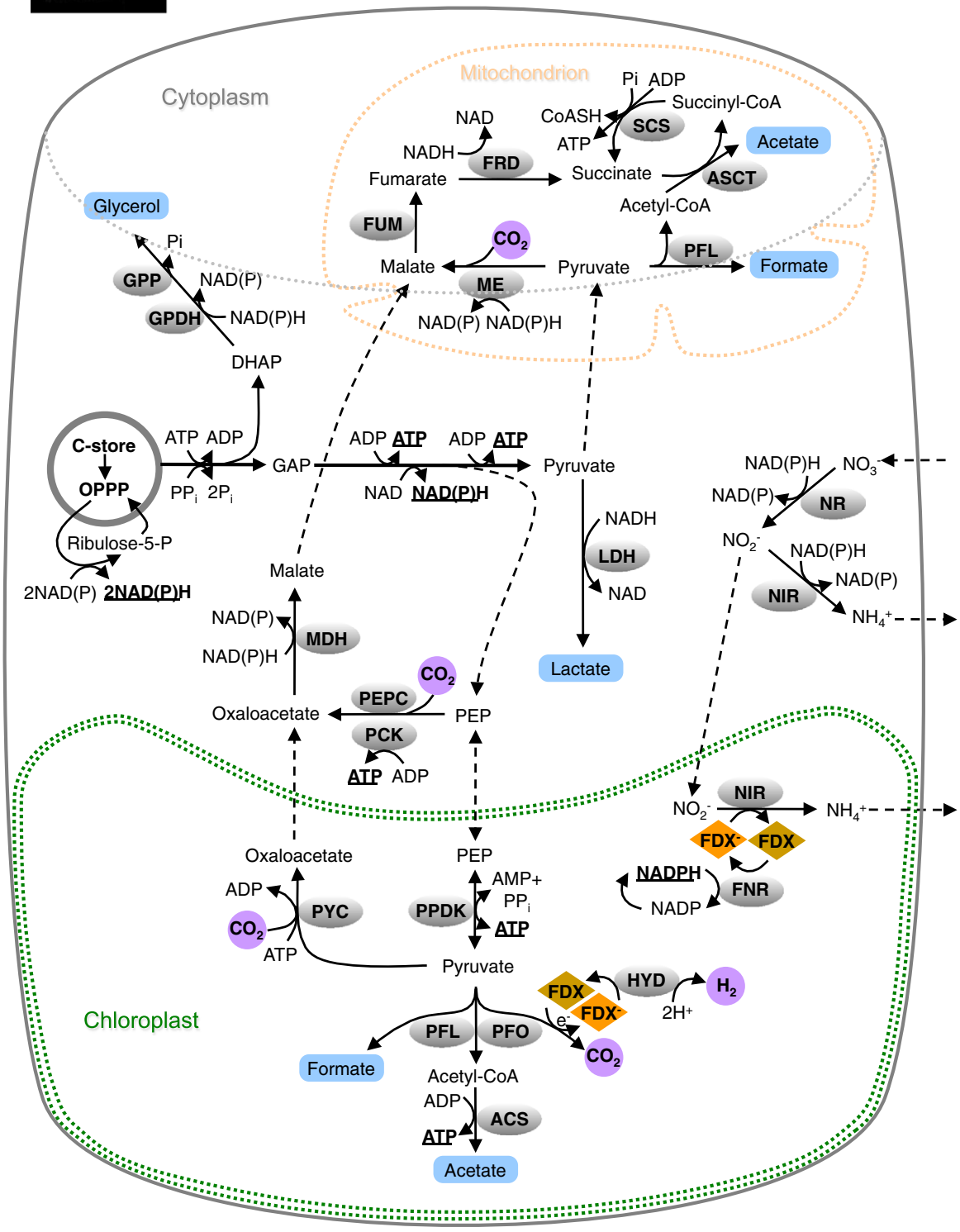
[160]. Though the enzymatic partners of the fungal pathways are not fully identified, nitrite reductase (NirK) from *Fusarium* has been characterized, it is homologous to copper-containing nitrite reductases from proteobacteria, likely has a mitochondrial origin and furthermore has conserved homologues in *Chlamydomonas* [161]. In diatoms, the pathway could provide energy to prepare for the resting stage or long-term survival under dark anoxic conditions, but none of the enzymatic components have been characterized so far.

6. Evolutionary insights

During evolution, enzymes and pathways can readily undergo recompartmentation between mitochondrial, cytosolic, and in the case of algae, plastidic compartments, as the example of ADHE localization shift for *Polytomella* (mitochondrial) *vs. Chlamydomonas* (chloroplast) underscores [51,97]. The movement of whole pathways from one compartment to another became evident through studies of chloroplast-cytosol isoenzymes [162], studies of differential localization of isoprenoid biosynthesis to plastids and the cytosol of algae [163], starch metabolism in algae [164], peroxisomal pathways [165], and the glycosome in trypanosome evolution [166]. The problem of how to transfer whole pathways (or major segments thereof) from one compartment to another has been a longstanding evolutionary puzzle because a single enzyme in a new compartment is of no use and hence hardly selectable in evolution, the whole functional unit has to move. One possible solution to this problem

Fig. 3. Tentative reconstruction of the anaerobic energy metabolism in the diatom *Thalassiosira pseudonana*. Highlighted in blue are the different metabolites, redox sinks and/or fermentation products that are typically identified in other unicellular algae but are currently not known for *T. pseudonana*. Although little is known about intracellular locations of the different enzyme pathways, the cytosolic presence of OPPP and glycolytic enzymes was reported by Kroth et al. [172]. Also, these authors depicted a plastidic location of PPDK and PYC, and probably of [ADP]-ACS. The nitrogen cycle enzymes, soluble nitrate reductase (NR) and nitrite reductase (NIR) are represented as proposed previously [173]. Reducing power in the form of reduced FDX for nitrite reduction in the chloroplast may be obtained *via* ferredoxin:NADP reductase (FNR), using NAPDH derived from the oxidative pentose phosphate pathway, as seen in maize roots [174]. Although the depicted compartmentalization of the different enzymes clearly has to be provisional, as indicated by the dotted lines of the organelles, predictions can be made based upon knowledge on *Chlamydomonas* and other (photosynthetic) eukaryotes. The metabolic routes involving succinate are usually mitochondrial. In *C. reinhardtii*, PFL is dually targeted to both chloroplast and mitochondria; in the diatom two PFL isoforms with N-terminal extensions are found that could be sent to different cellular compartments. Note that no genes for FeFe-hydrogenase maturation factors HydEFG have been found in the diatom genomes so far, and no hydrogen production has so far been reported for *T. pseudonana*, but can be predicted; note in addition that *Giardia intestinalis* also lacks detectable genes for FeFe-hydrogenase maturation factors but does produce H_2 [8]. Enzyme abbreviations are as in Fig. 1.
Image: Courtesy of Nils Kröger (Georgia Tech's School of Chemistry and Biochemistry and the School of Materials Science and Engineering).

involves the concept of minor mistargeting [167]. If protein targeting in eukaryotes is not 100% specific (and many examples of dual targeting of enzymes are known), then minor amounts of whole pathways could conceivably end up in the wrong compartment. Such minor mistargeting is more likely for highly expressed proteins, such as those involved in core carbon and energy metabolism. If a



small amount of a whole pathway is present, it could readily become a unit of function, albeit at a low initial activity, and hence a unit upon which natural selection could efficiently act to generate more or less of a new compartmentation variant. Early in eukaryotic evolution, before the origin of the mitochondrial protein import machinery of the inner and outer mitochondrial membranes, the situation was simpler, because gene transfers from mitochondria to the nucleus would have given rise either to pseudogenes (junk DNA) or to enzymes expressed in the cytosol [168] and similar considerations apply for algal genes acquired from plastids before the origin of the plastid protein import machinery [143]. In that way, pathways ancestrally encoded by organellar genomes can be readily transferred to the eukaryotic cytosol.

With regard to more general evolutionary issues concerning the origin of anaerobic energy metabolism in eukaryotes, it is often assumed that ancestral eukaryotes were aerobes and that in order to inhabit anaerobic habitats, eukaryotes had to acquire, *via* some kind of lateral gene transfer, enzymes that would enable them to generate ATP without the help of oxygen respiration in mitochondria [70]. But that can hardly be true, as the study of anaerobic enzymes and pathways in algae indicates, through three lines of evidence. First, algae are photosynthetic and produce oxygen, hence they are under no evolutionary pressures to inhabit anoxic environments in the first place (they can survive with light); accordingly, no compelling selective pressure for them to specifically acquire genes for that purpose can be construed. Second, algal fermentation pathways consist of the same enzymes of anaerobic energy metabolism as found in eukaryotic heterotrophs; were eukaryotes acquiring genes in order to access anaerobic environments, one would hardly expect different lineages of eukaryotes to acquire the same genes and enzymes (and in the case of [FeFe] hydrogenase and PFL, even the same accessory proteins needed for maturation). In other words, if eukaryotes had to acquire genes in order to access anaerobic environments, eukaryotes would harbor as many different anaerobic energy-producing pathways as prokaryotes do, which is hardly the case, as the present study and other recent surveys [8] indicate. Third, no enzymes of anaerobic energy metabolism have yet been found in eukaryotes that are specific to any particular lineage (with the so far nagging exception of *trans*-2-enoyl-CoA reductase of *Euglena* mitochondria [63] that has so far failed to uncover homologues in any other eukaryote); rather they all trace to the eukaryote common ancestor and as more lineages become sampled, the more pronounced this trend becomes. The first real surprise in that respect came from the *Chlamydomonas* genome, which uncovered all of the major enzymes of anaerobic energy metabolism currently known among eukaryotes [97]. The algae (oxygen producers) turn out to be the most flexible eukaryotic anaerobes known. That insight is quite new and helps to change our views of the phylogenetic distribution and ecological significance of anaerobic energy metabolism in eukaryotes.

7. Conclusions

To date, *C. reinhardtii* is the alga that expresses the most complete repertoire of anaerobic enzymes. It has a flexible mixed-acid fermentation in which aspects of bacterial-, plant- and yeast-type fermentation can be found. Its fermentation capabilities included hydrogen production, which is currently of considerable interest in the context of biofuels. *Chlamydomonas* is so far unique among eukaryotes in that it possesses two enzymatic systems to convert pyruvate into acetyl-CoA under anaerobic conditions: PFL and PFO. The interplay between these two routes, their regulation and their ecological significance warrant further study.

Among the photosynthetic algae, literature and genome surveys show a broad panel of fermentative enzymes and a few cases of anaerobic respiration. On the basis of evidence surveyed here, it appears that anaerobic respiration among eukaryotic algae is comparatively rare and that anaerobic fermentation is widespread. Anaerobic respiration is far more common among prokaryotes, where it is significant at a global scale and influences the global cycling of elements, sulfur, nitrogen and carbon. The diversity of metabolic routes in algal groups shows marked variation among species (*Micromonas*, diatoms) and strains, for example *Chlamydomonas* [27] and *Euglena* [31] in those cases that have been studied. Further investigations are needed to assess the scope and ecological significance, if any, of such variation.

Most of the enzymes for anaerobic pathways that are inferred from genomic studies have not been characterized biochemically. For anaerobic metabolism in eukaryotes, there is still intense reliance upon a handful of well-studied organisms [8], for photosynthetic eukaryotes the anaerobic paradigm currently centers on *Chlamydomonas*. The expression and biochemical properties of these enzymes and pathways need further study and in a broader spectrum of model systems. In addition, the spectrum of end products produced by various algae during anaerobic fermentations is still mostly unknown. This would be important information for understanding metabolic diversity among algae. Finally, fermentation of algae appears to represent a significant ecological component of carbon flux in soil that influences the content in organic acids, alcohols and hydrogen, this poses a challenge for future ecological studies.

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References

[1] M.K. Grieshaber, I. Hardewig, U. Kreutzer, H.O. Pörtner, Physiological and metabolic responses to hypoxia in invertebrates, Rev. Physiol. Biochem. Pharmacol. 125 (1994) 43–147.
[2] L.E. Burnett, The challenges of living in hypoxic and hypercapnic aquatic environments, Am. Zool. 37 (1997) 633–640.
[3] J.M. Bernhard, K.R. Buck, M.A. Farmer, S.S. Bowser, The Santa Barbara Basin is a symbiosis oasis, Nature 403 (2000) 77–80.
[4] T. Stoeck, A. Behnke, R. Christen, L. Amaral-Zettler, M.J. Rodriguez-Mora, A. Chistoserdov, W. Orsi, V.P. Edgcomb, Massively parallel tag sequencing reveals the complexity of anaerobic marine protistan communities, BMC Biol. 7 (2009) 72.
[5] V.P. Edgcomb, W. Orsi, C. Leslin, S.S. Epstein, J. Bunge, S. Jeon, M.M. Yakimov, A. Behnke, T. Stoeck, Protistan community patterns within the brine and halocline of deep hypersaline anoxic basins in the eastern Mediterranean Sea, Extremophiles 13 (2009) 151–167.
[6] R. Danovaro, A. Dell'Anno, A. Pusceddu, C. Gambi, I. Heiner, R.M. Kristensen, The first metazoa living in permanently anoxic conditions, BMC Biol. 8 (2010) 30.
[7] J.P. Amend, E.L. Shock, Energetics of overall metabolic reactions of thermophilic and hyperthermophilic archaea and bacteria, FEMS Microbiol. Rev. 25 (2001) 175–243.
[8] M. Müller, M. Mentel, J. van Hellemond, K. Henze, C. Wöhle, S.B. Gould, R.-Y. Yu, M. van der Giezen, A.G.M. Tielens, W.F. Martin, Biochemistry and evolution of anaerobic energy metabolism in eukaryotes, Microbiol. Mol. Biol. Rev. 76 (2012) 444–495.
[9] T. Fenchel, B.J. Finlay, Ecology and Evolution in Anoxic Worlds, Oxford University Press, 1995.
[10] J.E. Doeller, B.K. Gaschen, V. Parrino, D.W. Kraus, Chemolithoheterotrophy in a metazoan tissue: sulfide supports cellular work in ciliated mussel gills, J. Exp. Biol. 202 (1999) 1953–1961.
[11] U. Theissen, W. Martin, Sulfide:quinone oxidoreductase (SQR) from the lugworm *Arenicola marina* shows cyanide- and thioredoxin-dependent activity, FEBS J. 257 (2008) 1131–1139.
[12] A.G.M. Tielens, C. Rotte, J.J. van Hellemond, W. Martin, Mitochondria as we don't know them, Trends Biochem. Sci. 27 (2002) 564–572.
[13] A.G.M. Tielens, J. van Hellemond, The electron transport chain in anaerobically functioning eukaryotes, Biochim. Biophys. Acta 1365 (1998) 71–78.
[14] A. Melis, T. Happe, Hydrogen production. Green algae as a source of energy, Plant Physiol. 127 (2001) 740–748.
[15] Q. Hu, M. Sommerfeld, E. Jarvis, M. Ghirardi, M. Posewitz, M. Seibert, A. Darzins, Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances, Plant J. 54 (2008) 621–639.

[16] A. Hemschemeier, A. Melis, T. Happe, Analytical approaches to photobiological hydrogen production in unicellular green algae, Photosynth. Res. 102 (2009) 523–540.
[17] R. Radakovits, R.E. Jinkerson, A. Darzins, M.C. Posewitz, Genetic engineering of algae for enhanced biofuel production, Eukaryot. Cell 9 (2010) 486–501.
[18] E. Stephens, I.L. Ross, J.H. Mussgnug, L.D. Wagner, M.A. Borowitzka, C. Posten, O. Kruse, B. Hankamer, Future prospects of microalgal biofuel production systems, Trends Plant Sci. 15 (2010) 554–564.
[19] H. Gaffron, J. Rubin, Fermentative and photochemical production of hydrogen in algae, J. Gen. Physiol. 26 (1942) 219–240.
[20] A. Ben-Amotz, D.I. Erbes, M.A. Riederer-Henderson, D.G. Peavey, M. Gibbs, H_2 metabolism in photosynthetic organisms. 1. Dark H_2 evolution and uptake by algae and mosses, Plant Physiol. 56 (1975) 72–77.
[21] U. Klein, A. Betz, Fermentative metabolism of hydrogen-evolving *Chlamydomonas moewusii*, Plant Physiol. 61 (1978) 953–956.
[22] K. Kreuzberg, Starch fermentation via a formate producing pathway in *Chlamydomonas reinhardtii*, *Chlorogonium elongatum* and *Chlorella fusca*, Physiol. Plant. 61 (1984) 87–94.
[23] S. Ohta, K. Miyamoto, Y. Miura, Hydrogen evolution as a consumption mode of reducing equivalents in green algal fermentation, Plant Physiol. 83 (1987) 1022–1026.
[24] F.C. Neidhardt, J.L. Ingraham, M. Schaechter, Physiology of the Bacterial Cell, Sinauer, Sunderland, Mass, 1990.
[25] A.J. Wolfe, The acetate switch, Microbiol. Mol. Biol. Rev. 69 (2005) 12–50.
[26] R.P. Gfeller, M. Gibbs, Fermentative metabolism of *Chlamydomonas reinhardtii*: I. Analysis of fermentative products from starch in dark and light, Plant Physiol. 75 (1984) 212–218.
[27] J.E. Meuser, G. Ananyev, L.E. Wittig, S. Kosourov, M.L. Ghirardi, M. Seibert, G.C. Dismukes, M.C. Posewitz, Phenotypic diversity of hydrogen production in chlorophycean algae reflects distinct anaerobic metabolisms, J. Biotechnol. 142 (2009) 21–30.
[28] J.E. Meuser, E.S. Boyd, G. Ananyev, D. Karns, R. Radakovits, U.M. Narayana Murthy, M.L. Ghirardi, G.C. Dismukes, J.W. Peters, M.C. Posewitz, Evolutionary significance of an algal gene encoding an [FeFe]-hydrogenase with F-domain homology and hydrogenase activity in *Chlorella variabilis* NC64A, Planta 234 (2011) 829–843.
[29] K. Skjånes, G. Knutsen, T. Källqvist, P. Lindblad, H_2 production from marine and freshwater species of green algae during sulfur deprivation and considerations for bioreactor design, Int. J. Hydrogen Energy 33 (2008) 511–521.
[30] H. Inui, K. Miyatake, Y. Nakano, S. Kitaoka, Wax fermentation in *Euglena gracilis*, FEBS Lett. 150 (1982) 89–93.
[31] S. Tucci, R. Vacula, J. Krajcovic, P. Proksch, W. Martin, Variability of wax ester fermentation in natural and bleached *Euglena gracilis* strains in response to oxygen and the elongase inhibitor flufenacet, J. Eukaryot. Microbiol. 57 (2010) 63–69.
[32] D.E. Buetow, The mitochondrion, in: D.E. Buetow (Ed.), The Biology of Euglena, vol. IV, Academic Press, San Diego, 1989, pp. 247–314.
[33] S. Dasgupta, J. Fang, S.S. Brake, S.T. Hasiotis, L. Zhang, Biosynthesis of sterols and wax esters by *Euglena* of acid mine drainage biofilms: implications for eukaryotic evolution and the early Earth, Chem. Geol. 306–307 (2012) 139–145.
[34] D.H. Jewson, S.F. Lowry, R. Bowen, Co-existence and survival of diatoms on sand grains, Eur. J. Phycol. 41 (2006) 131–146.
[35] E. Lomstein, M.H. Jensen, J. Sørensen, Intracellular NH_4^+ and NO_3^- pools associated with deposited phytoplankton in a marine sediment (Aarhus Bight, Denmark), Mar. Ecol. Prog. Ser. 61 (1990) 97–105.
[36] A. Kamp, D. de Beer, J.L. Nitsch, G. Lavik, P. Stief, Diatoms respire nitrate to survive dark and anoxic conditions, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 5649–5654.
[37] Z. Zhou, N. Takaya, A. Nakamura, M. Yamaguchi, K. Takeo, H. Shoun, Ammonia fermentation, a novel anoxic metabolism of nitrate by fungi, J. Biol. Chem. 277 (2002) 1892–1896.
[38] P. López-García, F. Rodríguez-Valera, C. Pedrós-Alió, D. Moreira, Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton, Nature 409 (2001) 603–607.
[39] S.Y. Moon-van der Staay, R. De Wachter, D. Vaulot, Oceanic 18S rDNA sequences from picoplankton reveal unsuspected eukaryotic diversity, Nature 409 (2001) 607–610.
[40] B. Diez, C. Pedros-Alio, R. Massana, Study of genetic diversity of eukaryotic picoplankton in different oceanic regions by small-subunit rRNA gene cloning and sequencing, Appl. Environ. Microbiol. 67 (2001) 2932–2941.
[41] S.C. Dawson, N.R. Pace, Novel kingdom-level eukaryotic diversity in anoxic environments, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 8324–8329.
[42] M.L. Cuvelier, A.E. Allen, A. Monier, J.P. McCrow, M. Messié, S.G. Tringe, T. Woyke, R.M. Welsh, T. Ishoey, J.H. Lee, B.J. Binder, C.L. DuPont, M. Latasa, C. Guigand, K.R. Buck, J. Hilton, M. Thiagarajan, E. Caler, B. Read, R.S. Lasken, F.P. Chavez, A.Z. Worden, Targeted metagenomics and ecology of globally important uncultured eukaryotic phytoplankton, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 14679–14684.
[43] T.A. Richards, A.A. Vepritskiy, D.E. Gouliamova, S.A. Nierzwicki-Bauer, The molecular diversity of freshwater picoeukaryotes from an oligotrophic lake reveals diverse, distinctive and globally dispersed lineages, Environ. Microbiol. 7 (2005) 1413–1425.
[44] V.P. Edgcomb, D.T. Kysela, A. Teske, A. de Vera Gomez, M.L. Sogin, Benthic eukaryotic diversity in the Guaymas Basin hydrothermal vent environment, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 7658–7662.
[45] T. Stoeck, S. Epstein, Novel eukaryotic lineages inferred from small-subunit rRNA analyses of oxygen-depleted marine environments, Appl. Environ. Microbiol. 69 (2003) 2657–2663.
[46] R. Massana, J. Castresana, V. Balagué, L. Guillou, K. Romari, A. Groisillier, K. Valentin, C. Pedrós-Alió, Phylogenetic and ecological analysis of novel marine stramenopiles, Appl. Environ. Microbiol. 70 (2004) 3528–3534.
[47] S.S. Brake, S.T. Hasiostis, Eukaryote-dominated biofilms and their significance in acidic environments, Geomicrobiology 27 (2010) 534–558.
[48] L.A. Amaral-Zettler, F. Gómez, E. Zettler, B.G. Keenan, R. Amils, M.L. Sogin, Microbiology: eukaryotic diversity in Spain's river of fire, Nature 417 (2002) 137.
[49] G. van den Thillart, M. van Berge-Henegouwen, F. Kesbeke, Anaerobic metabolism of goldfish, *Carassius auratus* (L.): ethanol and CO_2 excretion rates and anoxia tolerance at 20, 10 and 5 °C, Comp. Biochem. Physiol. A Comp. Physiol. 76 (1983) 295–300.
[50] A. van Waarde, G. Van den Thillart, M. Verhagen, Ethanol formation and pH regulation in fish, in: P.W. Hochachka, P.L. Lutz, T. Sick, M. Rosenthal, G. van den Thillart (Eds.), Surviving Hypoxia, CRC Press, Boca Raton, 1993, pp. 157–170.
[51] M. Terashima, M. Specht, M. Hippler, The chloroplast proteome: a survey from the *Chlamydomonas reinhardtii* perspective with a focus on distinctive features, Curr. Genet. 57 (2011) 151–168.
[52] C. Catalanotti, A. Dubini, V. Subramanian, W. Yang, L. Magneschi, F. Mus, M. Seibert, M.C. Posewitz, A.R. Grossman, Altered fermentative metabolism in *Chlamydomonas reinhardtii* mutants lacking pyruvate formate lyase and both pyruvate formate lyase and alcohol dehydrogenase, Plant Cell 24 (2012) 692–707.
[53] P.R. Rich, The molecular machinery of Keilin's respiratory chain, Biochem. Soc. Trans. 31 (2003) 1095–1105.
[54] P.R. Rich, Chemiosmotic theory, in: Encyclopedia of Biological Chemistry, Elsevier, in press.
[55] A.G.M. Tielens, Energy generation in parasitic helminthes, Parasitol. Today 10 (1994) 346–352.
[56] T.M. Embley, M. van der Giezen, D.S. Horner, P.L. Dyal, P. Foster, Hydrogenosomes and mitochondria: phenotypic variants of the same fundamental organelle, Philos. Trans. R. Soc. Lond. B Biol. Sci. 358 (2003) 191–203.
[57] M. Müller, The hydrogenosome, J. Gen. Microbiol. 139 (1993) 2879–2889.
[58] M. Müller, Energy metabolism. Part I: anaerobic protozoa, in: J. Marr (Ed.), Molecular Medical Parasitology, Academic Press, London, 2003, pp. 125–139.
[59] J. Tovar, G. León-Avila, L.B. Sánchez, R. Sutak, J. Tachezy, M. van der Giezen, M. Hernández, M. Müller, J.M. Lucocq, Mitochondrial remnant organelles of *Giardia* function in iron-sulphur protein maturation, Nature 426 (2003) 172–176.
[60] B.A. Williams, R.P. Hirt, J.M. Lucocq, T.M. Embley, A mitochondrial remnant in the microsporidian *Trachipleistophora hominis*, Nature 418 (2002) 865–869.
[61] M. van der Giezen, Hydrogenosomes and mitosomes: conservation and evolution of functions, J. Eukaryot. Microbiol. 56 (2009) 221–231.
[62] A.D. Tsaousis, E.R.S. Kunji, A.V. Goldberg, J.M. Lucocq, R.P. Hirt, T.M. Embley, A novel route for ATP acquisition by the remnant mitochondria of *Encephalitozoon cuniculi*, Nature 453 (2008) 553–556.
[63] M. Hoffmeister, M. Piotrowski, U. Nowitzki, W. Martin, Mitochondrial trans-2-enoyl-CoA reductase of wax ester fermentation from *Euglena gracilis* defines a new family of enzymes involved in lipid synthesis, J. Biol. Chem. 280 (2005) 4329–4338.
[64] M.H. Charon, A. Volbeda, E. Chabrière, L. Pieulle, J.C. Fontecilla-Camps, Structure and electron transfer mechanism of pyruvate:ferredoxin oxidoreductase, Curr. Opin. Struct. Biol. 9 (1999) 663–669.
[65] K. Tittmann, Reaction mechanisms of thiamin diphosphate enzymes: redox reactions, FEBS J. 276 (2009) 2454–2468.
[66] G. Fuchs, Alternative pathways of carbon dioxide fixation: insights into the early evolution of life? Annu. Rev. Microbiol. 65 (2011) 631–658.
[67] L. Pieulle, B. Guigliarelli, M. Asso, F. Dole, A. Bernadac, E.C. Hatchikian, Isolation and characterization of the pyruvate-ferredoxin oxidoreductase from the sulfate-reducing bacterium *Desulfovibrio africanus*, Biochim. Biophys. Acta 1250 (1995) 49–59.
[68] D.G. Lindmark, M. Müller, Hydrogenosome, a cytoplasmic organelle of the anaerobic flagellate, *Tritrichomonas foetus* and its role in pyruvate metabolism, J. Biol. Chem. 248 (1973) 7724–7728.
[69] D.S. Horner, R.P. Hirt, T.M. Embley, A single eubacterial origin of eukaryotic pyruvate: ferredoxin oxidoreductase genes: implications for the evolution of anaerobic eukaryotes, Mol. Biol. Evol. 16 (1999) 1280–1291.
[70] L.A. Hug, A. Stechmann, A.J. Roger, Phylogenetic distributions and histories of proteins involved in anaerobic pyruvate metabolism in eukaryotes, Mol. Biol. Evol. 27 (2010) 311–324.
[71] J. Kulda, Trichomonads, hydrogenosomes and drug resistance, Int. J. Parasitol. 29 (1999) 199–212.
[72] C. Rotte, F. Stejskal, G. Zhu, J.S. Keithly, W. Martin, Pyruvate:$NADP^+$ oxidoreductase from the mitochondrion of *Euglena gracilis* and from the apicomplexan *Cryptosporidium parvum*: a biochemical relic linking pyruvate metabolism in mitochondriate and amitochondriate protists, Mol. Biol. Evol. 18 (2001) 710–720.
[73] Y. Lantsman, K.S.W. Tan, M. Morada, N. Yarlett, Biochemical characterization of a mitochondrial-like organelle from *Blastocystis* sp. subtype 7, Microbiology 154 (2008) 2757–2766.
[74] H. Inui, K. Miyatake, Y. Nakano, S. Kitaoka, Occurrence of oxygen-sensitive, NADP+-dependent pyruvate dehydrogenase in mitochondria of *Euglena gracilis*, J. Biochem. 96 (1984) 931–934.
[75] M.L. Ginger, L.K. Fritz-Laylin, C. Fulton, W.Z. Cande, S.C. Dawson, Intermediary metabolism in protists: a sequence-based view of facultative anaerobic metabolism in evolutionarily diverse eukaryotes, Protist 161 (2010) 642–671.
[76] A. Akhmanova, F.G. Voncken, K.M. Hosea, H. Harhangi, J.T. Keltjens, H.J. op den Camp, G.D. Vogels, J.H.P. Hackstein, A hydrogenosome with pyruvate formate-

lyase: anaerobic chytrid fungi use an alternative route for pyruvate catabolism, Mol. Microbiol. 32 (1999) 1103–1114.
[77] G. Gelius-Dietrich, K. Henze, Pyruvate formate lyase (PFL) and PFL activating enzyme in the chytrid fungus *Neocallimastix frontalis*: a free-radical enzyme system conserved across divergent eukaryotic lineages, J. Eukaryot. Microbiol. 51 (2004) 456–463.
[78] C.W. Stairs, A.J. Roger, V. Hampl, Eukaryotic pyruvate formate lyase and its activating enzyme were acquired laterally from a firmicute, Mol. Biol. Evol. 28 (2011) 2087–2099.
[79] A.F. Wagner, M. Frey, F.A. Neugebauer, W. Schäfer, J. Knappe, The free radical in pyruvate formate-lyase is located on glycine-734, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 996–1000.
[80] J. Knappe, J. Schacht, W. Möckel, T. Höpner, H. Vetter Jr., R. Edenharder, Pyruvate formate-lyase reaction in *Escherichia coli*. The enzymatic system converting an inactive form of the lyase into the catalytically active enzyme, Eur. J. Biochem. 11 (1969) 316–327.
[81] H.P. Blaschkowski, G. Neuer, M. Ludwig-Festl, J. Knappe, Routes of flavodoxin and ferredoxin reduction in *Escherichia coli*. CoA-acylating pyruvate: flavodoxin and NADPH: flavodoxin oxidoreductases participating in the activation of pyruvate formate-lyase, Eur. J. Biochem. 123 (1982) 563–569.
[82] R.K. Thauer, F.H. Kirchniawy, K.A. Jungermann, Properties and function of the pyruvate-formate-lyase reaction in clostridiae, Eur. J. Biochem. 27 (1972) 282–290.
[83] D. Kessler, I. Leibrecht, J. Knappe, Pyruvate-formate lyase-deactivase and acetyl CoA reductase activities of *Escherichia coli* reside on a polymeric protein particle encoded by adhE, FEBS Lett. 281 (1991) 59–63.
[84] P.M. Vignais, B. Billoud, J. Meyer, Classification and phylogeny of hydrogenases, FEMS Microbiol. Rev. 25 (2001) 455–501.
[85] D.S. Horner, B. Heil, T. Happe, T.M. Embley, Iron hydrogenases—ancient enzymes in modern eukaryotes, Trends Biochem. Sci. 27 (2002) 148–153.
[86] J. Meyer, [FeFe] hydrogenases and their evolution: a genomic perspective, Cell. Mol. Life Sci. 64 (2007) 1063–1084.
[87] J.W. Peters, W.N. Lanzilotta, B.J. Lemon, L.C. Seefeldt, X-ray crystal structure of the Fe-only hydrogenase (CpI) from *Clostridium pasteurianum* to 1.8 angstrom resolution, Science 282 (1998) 1853–1858.
[88] D.W. Mulder, E.M. Shepard, J.E. Meuser, N. Joshi, P.W. King, M.C. Posewitz, J.B. Broderick, J.W. Peters, Insights into [FeFe]-hydrogenase structure, mechanism, and maturation, Structure 19 (2011) 1038–1052.
[89] G.J. Schut, M.W.W. Adams, The iron-hydrogenase of *Thermotoga maritima* utilizes ferredoxin and NADH synergistically: a new perspective on anaerobic hydrogen production, J. Bacteriol. 191 (2009) 4451–44657.
[90] G. Herrmann, J. Elamparithi, M. Galina, W. Buckel, Energy conservation via electron-transferring flavoprotein in anaerobic bacteria, J. Bacteriol. 190 (2008) 784–791.
[91] J. Arnau, F. Jørgensen, S.M. Madsen, A. Vrang, H. Israelsen, Cloning of the *Lactococcus lactis* adhE gene, encoding a multifunctional alcohol dehydrogenase, by complementation of a fermentative mutant of *Escherichia coli*, J. Bacteriol. 180 (1998) 3049–3055.
[92] L. Fontaine, I. Meynial-Salles, L. Girbal, X. Yang, C. Croux, P. Soucaille, Molecular characterization and transcriptional analysis of adhE2, the gene encoding the NADH-dependent aldehyde/alcohol dehydrogenase responsible for butanol production in alcohologenic cultures of *Clostridium acetobutylicum* ATCC 824, J. Bacteriol. 184 (2002) 821–830.
[93] L.B. Sánchez, Aldehyde dehydrogenase (CoA-acetylating) and the mechanism of ethanol formation in the amitochondriate protist, *Giardia lamblia*, Arch. Biochem. Biophys. 354 (1998) 57–64.
[94] I. Bruchhaus, E. Tannich, Purification and molecular characterization of the NAD^+-dependent acetaldehyde/alcohol dehydrogenase from *Entamoeba histolytica*, Biochem. J. 303 (1994) 743–748.
[95] B. Boxma, F. Voncken, S. Jannink, T. van Alen, A. Akhmanova, S.W. van Weelden, J.J. van Hellemond, G. Ricard, M. Huynen, A.G. Tielens, J.H. Hackstein, The anaerobic chytridiomycete fungus *Piromyces* sp. E2 produces ethanol via pyruvate: formate lyase and an alcohol dehydrogenase E, Mol. Microbiol. 51 (2004) 1389–1399.
[96] A. Atteia, R. van Lis, G. Mendoza-Hernández, K. Henze, W. Martin, H. Riveros-Rosas, D. González-Halphen, Bifunctional aldehyde/alcohol dehydrogenase (ADHE) in chlorophyte algal mitochondria, Plant Mol. Biol. 53 (2003) 175–188.
[97] A. Atteia, R. van Lis, G. Gelius-Dietrich, A. Adrait, J. Garin, J. Joyard, N. Rolland, W. Martin, Pyruvate:formate lyase and a novel route of eukaryotic ATP-synthesis in anaerobic *Chlamydomonas* mitochondria, J. Biol. Chem. 281 (2006) 9909–9918.
[98] A.G.M. Tielens, K.W. van Grinsven, K. Henze, J.J. van Hellemond, W. Martin, Acetate formation in the energy metabolism of parasitic helminths and protists, Int. J. Parasitol. 40 (2010) 387–397.
[99] K. Hamblin, D.M. Standley, M.B. Rogers, A. Stechmann, A.J. Roger, R. Maytum, M. van der Giezen, Localisation and nucleotide specificity of *Blastocystis* succinyl-CoA synthetase, Mol. Microbiol. 68 (2008) 1395–1405.
[100] R.E. Reeves, L.G. Warren, B. Susskind, H.S. Lo, An energy-conserving pyruvate-to-acetate pathway in *Entamoeba histolytica*. Pyruvate synthase and a new acetate thiokinase, J. Biol. Chem. 252 (1977) 726–731.
[101] L.B. Sánchez, M. Müller, Purification and characterization of the acetate forming enzyme, acetyl-CoA synthetase (ADP-forming) from the amitochondriate protist *Giardia lamblia*, FEBS Lett. 378 (1996) 240–244.
[102] M. Terashima, M. Specht, B. Naumann, M. Hippler, Characterizing the anaerobic response of *Chlamydomonas reinhardtii* by quantitative proteomics, Mol. Cell. Proteomics 9 (2010) 1514–1532.
[103] N. Lane, W. Martin, The energetics of genome complexity, Nature 467 (2010) 929–934.
[104] J.J. van Hellemond, A.G.M. Tielens, Expression and functional properties of fumarate reductase, Biochem. J. 304 (1994) 321–333.
[105] T. Jonassen, P.L. Larsen, C.F. Clarke, A dietary source of coenzyme Q is essential for growth of long-lived *Caenorhabditis elegans* clk-1 mutants, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 421–426.
[106] E.B. Kayser, M.M. Sedensky, P.G. Morgan, C.L. Hoppel, Mitochondrial oxidative phosphorylation is defective in the long-lived mutant clk-1, J. Biol. Chem. 279 (2004) 54479–54486.
[107] B.C. Brajcich, A.L. Iarocci, L.A. Johnstone, R.K. Morgan, Z.T. Lonjers, M.J. Hotchko, J.D. Muhs, A. Kieffer, B.J. Reynolds, S.M. Mandel, B.N. Marbois, C.F. Clarke, J.N. Shepherd, Evidence that ubiquinone is a required intermediate for rhodoquinone biosynthesis in *Rhodospirillum rubrum*, J. Bacteriol. 192 (2010) 436–445.
[108] Z.T. Lonjers, E.L. Dickson, T.P. Chu, J.E. Kreutz, F.A. Neacsu, K.R. Anders, J.N. Shepherd, Identification of a new gene required for the biosynthesis of rhodoquinone in *Rhodospirillum rubrum*, J. Bacteriol. 194 (2012) 965–971.
[109] J.-D. Rochaix, The three genomes of *Chlamydomonas*, Photosynth. Res. 73 (2002) 285–293.
[110] S. Eberhard, G. Finazzi, F.A. Wollman, The dynamics of photosynthesis, Annu. Rev. Genet. 42 (2008) 463–515.
[111] A.R. Grossman, Acclimation of *Chlamydomonas reinhardtii* to its nutrient environment, Protist 151 (2000) 201–224.
[112] S.S. Merchant, M.D. Allen, J. Kropat, J.L. Moseley, J.C. Long, S. Tottey, A.M. Terauchi, Between a rock and a hard place: trace element nutrition in *Chlamydomonas*, Biochim. Biophys. Acta 1763 (2006) 578–594.
[113] B. Naumann, A. Busch, J. Allmer, E. Ostendorf, M. Zeller, H. Kirchhoff, M. Hippler, Comparative quantitative proteomics to investigate the remodeling of bioenergetic pathways under iron deficiency in *Chlamydomonas reinhardtii*, Proteomics 7 (21) (2007) 3964–3979.
[114] S. Yehudai-Resheff, S.L. Zimmer, Y. Komine, D.B. Stern, Integration of chloroplast nucleic acid metabolism into the phosphate deprivation response in *Chlamydomonas reinhardtii*, Plant Cell 19 (2007) 1023–3810.
[115] A.M. Terauchi, G. Peers, M.C. Kobayashi, K.K. Niyogi, S.S. Merchant, Trophic status of *Chlamydomonas reinhardtii* influences the impact of iron deficiency on photosynthesis, Photosynth. Res. 105 (2010) 39–49.
[116] C.D. Silflow, P.A. Lefebvre, Assembly and motility of eukaryotic cilia and flagella. Lessons from *Chlamydomonas reinhardtii*, Plant Physiol. 127 (2001) 1500–1507.
[117] D.G. Cole, The intraflagellar transport machinery of *Chlamydomonas reinhardtii*, Traffic 4 (2003) 435–442.
[118] W.J. Snell, J. Pan, Q. Wang, Cilia and flagella revealed: from flagellar assembly in *Chlamydomonas* to human obesity disorders, Cell 117 (2004) 693–697.
[119] S.S. Merchant, S.E. Prochnik, O. Vallon, E.H. Harris, S.J. Karpowicz, G.B. Witman, A. Terry, A. Salamov, L.K. Fritz-Laylin, L. Maréchal-Drouard, W.F. Marshall, L.H. Qu, D.R. Nelson, A.A. Sanderfoot, M.H. Spalding, V.V. Kapitonov, Q. Ren, P. Ferris, E. Lindquist, H. Shapiro, S.M. Lucas, J. Grimwood, J. Schmutz, P. Cardol, H. Cerutti, G. Chanfreau, C.L. Chen, V. Cognat, M.T. Croft, R. Dent, S. Dutcher, E. Fernández, H. Fukuzawa, D. González-Ballester, D. González-Halphen, A. Hallmann, M. Hanikenne, M. Hippler, W. Inwood, K. Jabbari, M. Kalanon, R. Kuras, P.A. Lefebvre, S.D. Lemaire, A.V. Lobanov, M. Lohr, A. Manuell, I. Meier, L. Mets, M. Mittag, T. Mittelmeier, J.V. Moroney, J. Moseley, C. Napoli, A.M. Nedelcu, K. Niyogi, S.V. Novoselov, I.T. Paulsen, G. Pazour, S. Purton, J.P. Ral, D.M. Riaño-Pachón, W. Riekhof, L. Rymarquis, M. Schroda, D. Stern, J. Umen, R. Willows, N. Wilson, S.L. Zimmer, J. Allmer, J. Balk, K. Bisova, C.J. Chen, M. Elias, K. Gendler, C. Hauser, M.R. Lamb, H. Ledford, J.C. Long, J. Minagawa, M.D. Page, J. Pan, W. Pootakham, S. Roje, A. Rose, E. Stahlberg, A.M. Terauchi, P. Yang, S. Ball, C. Bowler, C.L. Dieckmann, V.N. Gladyshev, P. Green, R. Jorgensen, S. Mayfield, B. Mueller-Roeber, S. Rajamani, R.T. Sayre, P. Brokstein, I. Dubchak, D. Goodstein, L. Hornick, Y.W. Huang, J. Jhaveri, Y. Luo, D. Martínez, W.C. Ngau, B. Otillar, A. Poliakov, A. Porter, L. Szajkowski, G. Werner, K. Zhou, I.V. Grigoriev, D.S. Rokhsar, A.R. Grossman, The *Chlamydomonas* genome reveals the evolution of key animal and plant functions, Science 318 (2007) 245–250.
[120] A. Atteia, A. Adrait, S. Brugière, R. van Lis, M. Tardif, O. Deusch, T. Dagan, L. Kuhn, B. Gontero, W. Martin, G. Garin, J. Joyard, N. Rolland, A proteomic survey of *Chlamydomonas reinhardtii* mitochondria sheds new light on the metabolic plasticity of the organelle and on the nature of the α-proteobacterial mitochondrial ancestor, Mol. Biol. Evol. 29 (2009) 1533–1548.
[121] A.R. Grossman, M. Croft, V.N. Gladyshev, S.S. Merchant, M.C. Posewitz, S. Prochnik, M.H. Spalding, Novel metabolism in *Chlamydomonas* through the lens of genomics, Curr. Opin. Plant Biol. 10 (2007) 190–198.
[122] F. Mus, A. Dubini, M. Seibert, M.C. Posewitz, A.R. Grossman, Anaerobic acclimation in *Chlamydomonas reinhardtii*: anoxic gene expression, hydrogenase induction, and metabolic pathways, J. Biol. Chem. 282 (2007) 25475–25486.
[123] A. Hemschemeier, J. Jacobs, T. Happe, Biochemical and physiological characterization of the pyruvate formate-lyase Pfl1 of *Chlamydomonas reinhardtii*, a typically bacterial enzyme in a eukaryotic alga, Eukaryot. Cell 7 (2008) 518–526.
[124] A. Dubini, F. Mus, M. Seibert, A.R. Grossman, M.C. Posewitz, Flexibility in anaerobic metabolism as revealed in a mutant of *Chlamydomonas reinhardtii* lacking hydrogenase activity, J. Biol. Chem. 284 (2009) 7201–7213.
[125] J. Jacobs, S. Pudollek, A. Hemschemeier, T. Happe, A novel, anaerobically induced ferredoxin in *Chlamydomonas reinhardtii*, FEBS Lett. 583 (2009) 325–329.
[126] M.L. Ghirardi, R.K. Togasaki, M. Seibert, Oxygen sensitivity of algal H_2-production, Appl. Biochem. Biotechnol. 63–65 (1997) 141–151.
[127] G. Philipps, D. Krawietz, A. Hemschemeier, T.A. Happe, A pyruvate formate lyase-deficient *Chlamydomonas reinhardtii* strain provides evidence for a link

between fermentation and hydrogen production in green algae, Plant J. 66 (2011) 330–340.
[128] E. Chabrière, M.H. Charon, A. Volbeda, L. Pieulle, E.C. Hatchikian, J.C. Fontecilla-Camps, Crystal structures of the key anaerobic enzyme pyruvate: ferredoxin oxidoreductase, free and in complex with pyruvate, Nat. Struct. Biol. 6 (1999) 182–190.
[129] K. Kreuzberg, G. Klöch, D. Grobheiser, Subcellular distribution of pyruvate-degrading enzymes in *Chlamydomonas reinhardtii* studied by an improved protoplast fractionation procedure, Physiol. Plant. 69 (1987) 481–488.
[130] A.R. Grossman, C. Catalanotti, W. Yang, A. Dubini, L. Magneschi, V. Subramanian, M.C. Posewitz, M. Seibert, Multiple facets of anoxic metabolism and hydrogen production in the unicellular green alga *Chlamydomonas reinhardtii*, New Phytol. 190 (2011) 279–288.
[131] T. Happe, A. Kaminski, Differential regulation of the Fe-hydrogenase during anaerobic adaptation in the green alga *Chlamydomonas reinhardtii*, Eur. J. Biochem. 269 (2002) 1022–1032.
[132] T. Happe, J.D. Naber, Isolation, characterization and N-terminal amino acid sequence of hydrogenase from the green alga *Chlamydomonas reinhardtii*, Eur. J. Biochem. 214 (1993) 475–481.
[133] T. Happe, B. Mosler, J.D. Naber, Induction, localization and metal content of hydrogenase in the green alga *Chlamydomonas reinhardtii*, Eur. J. Biochem. 222 (1994) 769–774.
[134] M. Forestier, P. King, L.P. Zhang, M. Posewitz, S. Schwarzer, T. Happe, M.L. Ghirardi, M. Seibert, Expression of two [Fe]-hydrogenases in *Chlamydomonas reinhardtii* under anaerobic conditions, Eur. J. Biochem. 270 (2003) 2750–2758.
[135] J.E. Meuser, S. D'Adamo, R.E. Jinkerson, F. Mus, W. Yang, M.L. Ghirardi, M. Seibert, A.R. Grossman, M.C. Posewitz, Genetic disruption of both *Chlamydomonas reinhardtii* [FeFe]-hydrogenases: Insight into the role of HYDA2 in H_2 production, Biochem. Biophys. Res. Commun. 417 (2012) 704–709.
[136] F. Mus, L. Cournac, V. Cardettini, A. Caruana, G. Peltier, Inhibitor studies on non-photochemical plastoquinone reduction and H_2 photoproduction in *Chlamydomonas reinhardtii*, Biochim. Biophys. Acta 1708 (2005) 322–332.
[137] A. Hemschemeier, S. Fouchard, L. Cournac, G. Peltier, T. Happe, Hydrogen production by *Chlamydomonas reinhardtii*: an elaborate interplay of electron sources and sinks, Planta 227 (2008) 397–407.
[138] A. Hemschemeier, T. Happe, Alternative photosynthetic electron transport pathways during anaerobiosis in the green alga *Chlamydomonas reinhardtii*, Biochim. Biophys. Acta 1807 (2011) 919–926.
[139] M. Winkler, S. Kuhlgert, M. Hippler, T. Happe, Characterization of the key step for light-driven hydrogen evolution in green algae, J. Biol. Chem. 284 (2009) 36620–36627.
[140] L. Magneschi, C. Catalanotti, V. Subramanian, A. Dubini, W. Yang, F. Mus, M.C. Posewitz, M. Seibert, P. Perata, A.R. Grossman, A mutant in the ADH1 gene of *Chlamydomonas reinhardtii* elicits metabolic restructuring during anaerobiosis, Plant Physiol. 158 (2012) 1293–1305.
[141] S.B. Gould, R.R. Waller, G.I. McFadden, Plastid evolution, Annu. Rev. Plant Biol. 59 (2008) 491–517.
[142] L.W. Parfrey, D.J.G. Lahr, A.H. Knoll, L.A. Katz, Estimating the timing of early eukaryotic diversification with multigene molecular clocks, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 13624–13629.
[143] W. Martin, T. Rujan, E. Richly, A. Hansen, S. Cornelsen, T. Lins, D. Leister, B. Stoebe, M. Hasegawa, D. Penny, Evolutionary analysis of *Arabidopsis*, cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 12246–12251.
[144] S.M. Adl, A.G.B. Simpson, M.A. Farmer, R.A. Andersen, O.R. Anderson, J.R. Barta, S.S. Bowser, G. Brugerolle, R.A. Fensome, S. Fredericq, T.Y. James, S. Karpov, P. Kugrens, J. Krug, C.E. Lane, L.A. Lewis, J. Lodge, D.H. Lynn, D.G. Mann, R.M. McCourt, L. Mendoza, O. Moestrup, S.E. Mozley-Standridge, T.A. Nerad, C.A. Shearer, A.V. Smirnov, F.W. Spiegel, M.F. Taylor, The new higher level classification of eukaryotes with emphasis on the taxonomy of protists, J. Eukaryot. Microbiol. 52 (2005) 399–451.
[145] G.I. McFadden, Primary and secondary endosymbiosis and the origin of plastids, J. Phycol. 37 (2001) 951–959.
[146] K. Kreuzberg, Pyruvate degradation via pyruvate formate-lyase (EC 2.3.1.54) and the enzymes of formate fermentation in the green alga *Chlorogonium elongatum*, Planta 163 (1985) 60–67.
[147] E. Derelle, C. Ferraz, S. Rombauts, P. Rouzé, A.Z. Worden, S. Robbens, F. Partensky, S. Degroeve, S. Echeynié, R. Cooke, Y. Saeys, J. Wuyts, K. Jabbari, C. Bowler, O. Panaud, B. Piégu, S.G. Ball, J.P. Ral, F.Y. Bouget, G. Piganeau, B. De Baets, A. Picard, M. Delseny, J. Demaille, Y. Van de Peer, H. Moreau, Genome analysis of the smallest free-living eukaryote *Ostreococcus tauri* unveils many unique features, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 11647–11652.
[148] B. Palenik, J. Grimwood, A. Aerts, P. Rouzé, A. Salamov, N. Putnam, C. Dupont, R. Jorgensen, E. Derelle, S. Rombauts, K. Zhou, R. Otillar, S.S. Merchant, S. Podell, T. Gaasterland, C. Napoli, K. Gendler, A. Manuell, V. Tai, O. Vallon, G. Piganeau, S. Jancek, M. Heijde, K. Jabbari, C. Bowler, M. Lohr, S. Robbens, G. Werner, I. Dubchak, G.J. Pazour, Q. Ren, I. Paulsen, C. Delwiche, J. Schmutz, D. Rokhsar, Y. Van de Peer, H. Moreau, I.V. Grigoriev, The tiny eukaryote *Ostreococcus* provides genomic insights into the paradox of plankton speciation, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 7705–7710.
[149] A.Z. Worden, J.H. Lee, T. Mock, P. Rouzé, M.P. Simmons, A.L. Aerts, A.E. Allen, M.L. Cuvelier, E. Derelle, M.V. Everett, E. Foulon, J. Grimwood, H. Gundlach, B. Henrissat, C. Napoli, S.M. McDonald, M.S. Parker, S. Rombauts, A. Salamov, P. Von Dassow, J.H. Badger, P.M. Coutinho, E. Demir, I. Dubchak, C. Gentemann, W. Eikrem, J.E. Gready, U. John, W. Lanier, E.A. Lindquist, S. Lucas, K.F. Mayer, H. Moreau, F. Not, R. Otillar, O. Panaud, J. Pangilinan, I. Paulsen, B. Piegu, A. Poliakov, S. Robbens, J. Schmutz, E. Toulza, T. Wyss, A. Zelensky, K. Zhou, E.V. Armbrust, D. Bhattacharya, U.W. Goodenough, Y. Van de Peer, I.V. Grigoriev, Green evolution and dynamic adaptations revealed by genomes of the marine picoeukaryotes *Micromonas*, Science 324 (2009) 268–272.
[150] D.C. Price, C.X. Chan, H.S. Yoon, E.C. Yang, H. Qiu, A.P. Weber, R. Schwacke, J. Gross, N.A. Blouin, C. Lane, A. Reyes-Prieto, D.G. Durnford, J.A. Neilson, B.F. Lang, G. Burger, J.M. Steiner, W. Löffelhardt, J.E. Meuser, M.C. Posewitz, S. Ball, M.C. Arias, B. Henrissat, P.M. Coutinho, S.A. Rensing, A. Symeonidi, H. Doddapaneni, B.R. Green, V.D. Rajah, J. Boore, D. Bhattacharya, *Cyanophora paradoxa* genome elucidates origin of photosynthesis in algae and plants, Science 335 (2012) 843–847.
[151] H. Inui, K. Miyatake, Y. Nakano, S. Kitaoka, The physiological role of oxygen-sensitive pyruvate dehydrogenase in mitochondrial fatty acid synthesis in *Euglena gracilis*, Arch. Biochem. Biophys. 237 (1985) 423–429.
[152] H. Inui, K. Ono, K. Miyatake, Y. Nakano, S. Kitaoka, Purification and characterization of pyruvate:$NADP^+$ oxidoreductase in *Euglena gracilis*, J. Biol. Chem. 262 (1987) 9130–9135.
[153] L. Florin, A. Tsokoglou, T. Happe, A novel type of iron hydrogenase in the green alga *Scenedesmus obliquus* is linked to the photosynthetic electron transport chain, J. Biol. Chem. 276 (2001) 6125–6132.
[154] M.C. Posewitz, P.W. King, S.L. Smolinski, L. Zhang, M. Seibert, M.L. Ghirardi, Discovery of two novel radical S-adenosylmethionine proteins required for the assembly of an active [Fe] hydrogenase, J. Biol. Chem. 279 (2004) 25711–25720.
[155] E. Kessler, Hydrogenase, photoreduction and anaerobic growth. Algal physiology and biochemistry, in: W.D.P. Stewart (Ed.), Botanical monograph, vol. 10, University of California Press, Berkeley and Los Angeles, California, 1974.
[156] R.B. Moore, M. Oborník, J. Janouskovec, T. Chrudimsky, M. Vancová, D.H. Green, S.W. Wright, N.W. Davies, C.J. Bolch, K. Heimann, J. Slapeta, O. Hoegh-Guldberg, J.M. Logsdon, D.A. Carter, A photosynthetic alveolate closely related to apicomplexan parasites, Nature 451 (2008) 959–963.
[157] C. Woehle, T. Dagan, W.F. Martin, S.B. Gould, Red and problematic green phylogenetic signals among thousands of nuclear genes from the photosynthetic and apicomplexa-related *Chromera velia*, Genome Biol. Evol. 3 (2011) 1220–1230.
[158] T. Schneider, A. Betz, Wax ester fermentation in *Euglena gracilis* T. Factors favouring the synthesis of odd-numbered fatty acids and alcohols, Planta 166 (1985) 67–73.
[159] M. Hoffmeister, A. van der Klei, C. Rotte, K.W.A. van Grinsven, J.J. van Hellemond, K. Henze, A.G.M. Tielens, W. Martin, *Euglena gracilis* rhodoquinone:ubiquinone ratio and mitochondrial proteome differ under aerobic and anaerobic conditions, J. Biol. Chem. 279 (2004) 22422–22429.
[160] Z. Zhou, N. Takaya, H. Shoun, Multi-energy metabolic mechanisms of the fungus *Fusarium oxysporum* in low oxygen environments, Biosci. Biotechnol. Biochem. 74 (2010) 2431–2437.
[161] S.W. Kim, S. Fushinobu, S.M. Zhou, T. Wakagi, H. Shoun, Eukaryotic nirK genes encoding copper-containing nitrite reductase: originating from the protomitochondrion? Appl. Environ. Microbiol. 75 (2009) 2652–2658.
[162] W. Martin, C. Schnarrenberger, The evolution of the Calvin cycle from prokaryotic to eukaryotic chromosomes: a case study of functional redundancy in ancient pathways through endosymbiosis, Curr. Genet. 32 (1997) 1–18 W.
[163] H.K. Lichtenthaler, The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants, Annu. Rev. Plant Physiol. Plant Mol. Biol. 50 (1999) 47–65.
[164] P. Deschamps, C. Colleoni, Y. Nakamura, E. Suzuki, J.L. Putaux, A. Buléon, S. Haebel, G. Ritte, M. Steup, L.I. Falcón, D. Moreira, W. Löffelhardt, J. Nirmal Raj, C. Plancke, C. d'Hulst, D. Dauvillée, S. Ball, Metabolic symbiosis and the birth of the plant kingdom, Mol. Biol. Evol. 25 (2008) 536–548.
[165] T. Gabaldon, Peroxisome diversity and evolution, Philos. Trans. R. Soc. Lond. B Biol. Sci. 365 (2010) 765–773.
[166] M. Gualdrón-López, A. Brennand, V. Hannaert, W. Quiñones, A.J. Cáceres, F. Bringaud, J.L. Concepción, P.A. Michels, When, how and why became glycolysis compartmentalized in the Kinetoplastea? A new look at an ancient organelle, Int. J. Parasitol. 41 (2011) 1–20.
[167] W. Martin, Evolutionary origins of metabolic compartmentalization in eukaryotes, Philos. Trans. R. Soc. Lond. B Biol. Sci. 365 (2010) 847–855.
[168] W. Martin, M. Müller, The hydrogen hypothesis for the first eukaryote, Nature 392 (1998) 37–41.
[169] A.M. Terauchi, S.F. Lu, M. Zaffagnini, S. Tappa, M. Hirasawa, J.N. Tripathy, D.B. Knaff, P.J. Farmer, S.D. Lemaire, T. Hase, S.S. Merchant, Pattern of expression and substrate specificity of chloroplast ferredoxins from *Chlamydomonas reinhardtii*, J. Biol. Chem. 284 (2009) 25867–25878.
[170] M. Tardif, A. Atteia, M. Specht, G. Cogne, N. Rolland, S. Brugière, M. Hippler, M. Ferro, C. Bruley; G. Peltier, O. Vallon and L. Cournac, PredAlgo, a new subcellular localization prediction tool dedicated to green algae, Mol. Biol. Evol. (in press) http://dx.doi.org/10.1093/molbev/mss178.
[171] S. Huang, T.D. Colmer, A.H. Millar, Does anoxia tolerance involve altering the energy currency towards PP_i? Trends Plant Sci. 13 (2008) 221–227.
[172] P.G. Kroth, A. Chiovitti, A. Gruber, V. Martin-Jezequel, T. Mock, M.S. Parker, M.S. Stanley, A. Kaplan, L. Caron, T. Weber, U. Maheswari, E.V. Armbrust, C. Bowler, A model for carbohydrate metabolism in the diatom *Phaeodactylum tricornutum* deduced from comparative whole genome analysis, PLoS One 3 (2008) e1426.
[173] N.L. Hockin, T. Mock, F. Mulholland, S. Kopriva, G. Malin, The response of diatom central carbon metabolism to nitrogen starvation is different from that of green algae and higher plants, Plant Physiol. 158 (2012) 299–312.
[174] C.G. Bowsher, D.M. Long, A. Oaks, S.J. Rothstein, Effect of light/dark cycles on expression of nitrate assimilatory genes in maize shoots and roots, Plant Physiol. 95 (1991) 281–285.