April 2007

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Published in *Current Opinion in Plant Biology* 10:2 (April 2007), pp. 190–198. In an issue on the theme of "Genome Studies and Molecular Genetics," edited by Stefan Jansson and Edward S Buckler. doi:10.1016/j.pbi.2007.01.012

Published online February 8, 2007.

Novel metabolism in Chlamydomonas through the lens of genomics

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Abstract: *Chlamydomonas* has traditionally been exploited as an organism that is associated with sophisticated physiological, genetic and molecular analyses, all of which have been used to elucidate several biological processes, especially photosynthesis and flagella function and assembly. Recently, the genomics of *Chlamydomonas* has been combined with other technologies to unveil new aspects of metabolism, including inorganic carbon utilization, anaerobic fermentation, the suite and functions of selenoproteins, and the regulation of vitamin biosynthesis. These initial findings represent the first glimpse through a genomic window onto the highly complex metabolisms that characterize a unicellular, photosynthetic eukaryote that has maintained both plant-like and animal-like characteristics over evolutionary time.

Introduction

Chlamydomonas reinhardtii is a member of the green algal lineage that diverged from the streptophytes approximately one billion years ago. It has served as an outstanding model organism, especially for analyzing eukaryotic chloroplast biology and the biogenesis and action of flagella and basal bodies [1, 2, 3]. Genetic analyses with this organism began in the mid 20th century and developed into sophisticated molecular and genomic technologies for dissecting biological processes. Unique attributes that make *Chlamydomonas* ideal for dissecting photosynthesis are its ability to grow heterotrophically in the dark by metabolizing exogenous acetate, and its maintenance of a normal green chloroplast that retains the capacity to perform oxygenic photosynthesis when illuminated following growth in the dark. These characteristics have allowed the isolation of a range of mutants in which the function and biogenesis of the photosynthetic apparatus is adversely affected [1, 4]. Most other photosynthetic organisms and all vascular plants either do not survive or exhibit growth retardation and pigment loss in the absence of photosynthesis. Recent work on photosynthesis in *Chlamydomonas* has focused on the discovery of molecules that catalyze the assembly of the photosynthetic apparatus and determine the abundance and rate of synthesis of individual complexes, and regulatory molecules that control the distribution of excitation energy (state transitions) or dissipation of excess absorbed light energy (non-photochemical quenching) [2, 5, 6].

Many molecular technologies have also been applied to studies of *Chlamydomonas*. The chloroplast and nuclear genomes of this alga are readily transformed [7]. Plasmid, cosmid, and bacterial artificial chromosome (BAC) libraries are available. Methods have been developed to generate and identify tagged mutant alleles. Alleles that are not tagged can be identified by map-based cloning [8, 9]. Gene function can be evaluated by suppression of specific gene activities using antisense or RNA interference (RNAi) constructs [10], and reporter genes have been developed to identify regulatory factors and sequences that are involved in regulating gene expression [11].

In this review, we discuss how the genomics of *Chlamydomonas* are being combined with these other technologies to unveil new aspects of metabolism, including inorganic carbon utilization, anaerobic fermentation, the suite and functions of selenoproteins, and the regulation of vitamin biosynthesis. The facts and concepts discussed below represent initial insights into the highly complex metabolisms that characterize a unicellular, photosynthetic eukaryote.

Current status of the genome and genome resources

The value of the technologies described above is augmented by the nearly 300,000 expressed sequence tags (ESTs) [12, 13, 14] and a draft *Chlamydomonas* genome sequence (http://genome.jgi-psf.org/Chlre3/Chlre3.home.html). Several different *Chlamydomonas* cDNA libraries were constructed using RNA from cells grown under different environmental conditions (see Table 1; [12]). The EST sequences were assembled on the basis of sequence similarity, paired-end sequence information, and genomic information to generate a set of Unigenes designated ACEGs (for 'Assembly of Contiguous ESTs verified by Genome sequences') (M Jain *et al*., unpublished). Unigene sets were used to develop both cDNA- and oligonucleotide (70mer)-based microarrays [13, 15].

The current draft release (version 3.0) of the *C. reinhardtii* genome (a detailed description to be published elsewhere), generated at the Joint Genome Institute (JGI), used genomic DNA from strain CC-503 *cw92 mt+*. A whole-genome shotgun sequencing strategy was used to generate a draft genome sequence assembly (http://genome.jgi-psf.org/Chlre3/Chlre3.info.html). The assembly was constructed from ~3 million reads (13-fold redundant coverage) that yielded scaffolds containing 121 Mb and gaps of ~15.0 Mb within scaffolds. The assembled sequence was used for *ab initio* gene prediction, which was assisted by transcriptome data. Over 15 000 gene models were predicted and ~4000 have been manually curated. Genome-associated resources for *Chlamydomonas* are available at several websites; some presently active sites are given in Box 1.

Novel metabolisms

Although many aspects of basic metabolism have been explored using *Chlamydomonas*, the genome has also revealed or augmented our understanding of novel metabolic processes ranging from the acquisition of inorganic carbon to the exploitation of selenocysteine-containing enzymes.

The carbon concentrating mechanism

The carbon concentrating mechanism (CCM) uses energy to increase intracellular CO_2 concentrations, allowing high rates of photosynthetic CO_2 fixation when the concentration of external Ci (where Ci is CO_2, HCO_3^- and CO_3^{-2}) is low. A CCM is crucial for *Chlamydomonas* and most aquatic photosynthetic organisms because of highly variable concentrations of dissolved Ci in aqueous environments, the impact of pH on the relative distribution between dissolved CO_2 and bicarbonate, and a 10^4 slower rate of diffusion of CO_2 in water relative to air. CCM function depends on active Ci transport and intracellular bicarbonate accumulation, combined with carbonic anhydrase (CA) activity. CA activity facilitates the dehydration of bicarbonate at the site of localization of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), providing near-saturating CO_2 concentrations [CO_2] for carboxylation. Components that are involved in CCM are discussed below and depicted in Figure 1.

The genome of *Chlamydomonas* has revealed an unexpectedly large number of encoded CAs. Nine putative CAs that are encoded by the *Chlamydomonas* genome might contribute to CCM, of which only five (CAH1–CAH5) were known previously. CAH1 (synthesized in ambient [CO_2]) and CAH2 (synthesized when [CO_2] >1%) are in the periplasm. Their roles in

Box 1. Genome-associated resources for *Chlamydomonas*.

JGI *Chlamydomonas* Genome Portal: http://genome.jgi-psf.org/Chlre3/Chlre3.home.html
cDNA libraries: http://www.chlamy.org/libraries.html (and the associated sites)
Kazusa EST database: http://www.kazusa.or.jp/en/plant/chlamy/EST/
BAC libraries: http://www.genome.clemson.edu/
BAC end sequences: http://genome.jgi-psf.org/cgi-bin/getPlateSeq?db=chlre2andprefix=PTQanddatalib=chlre_bacends
Flagellar proteome: http://laboratories.umassmed.edu/chlamyfp/index.php
Genome annotation: http://www.chlamydomonas.info/

Table 1. *Chlamydomonas* cDNA libraries constructed using RNA from cells.

Library	Growth conditions	Project IDs
Core	High and low [CO_2] conditions in minimal salt and acetate-supplemented media	**874, 894, 1024**
Stress I	Media devoid of either nitrogen, sulfur or phosphorus	**963**
Stress II	Grown following a shift to different nitrogen sources, under different osmotic conditions, conditions that facilitate H_2 production, and following exposure to cadmium	**1031**
Stress III	Copper-free or low-iron medium, high light, anoxic	**1115**
Deflagellation	Recovering from deflagellation	**1030**
Gamete–Zygote	Developing into gametes and zygotes	**1112**
S1D2	S1D2, a polymorphic strain interfertile with the laboratory strain	**925**

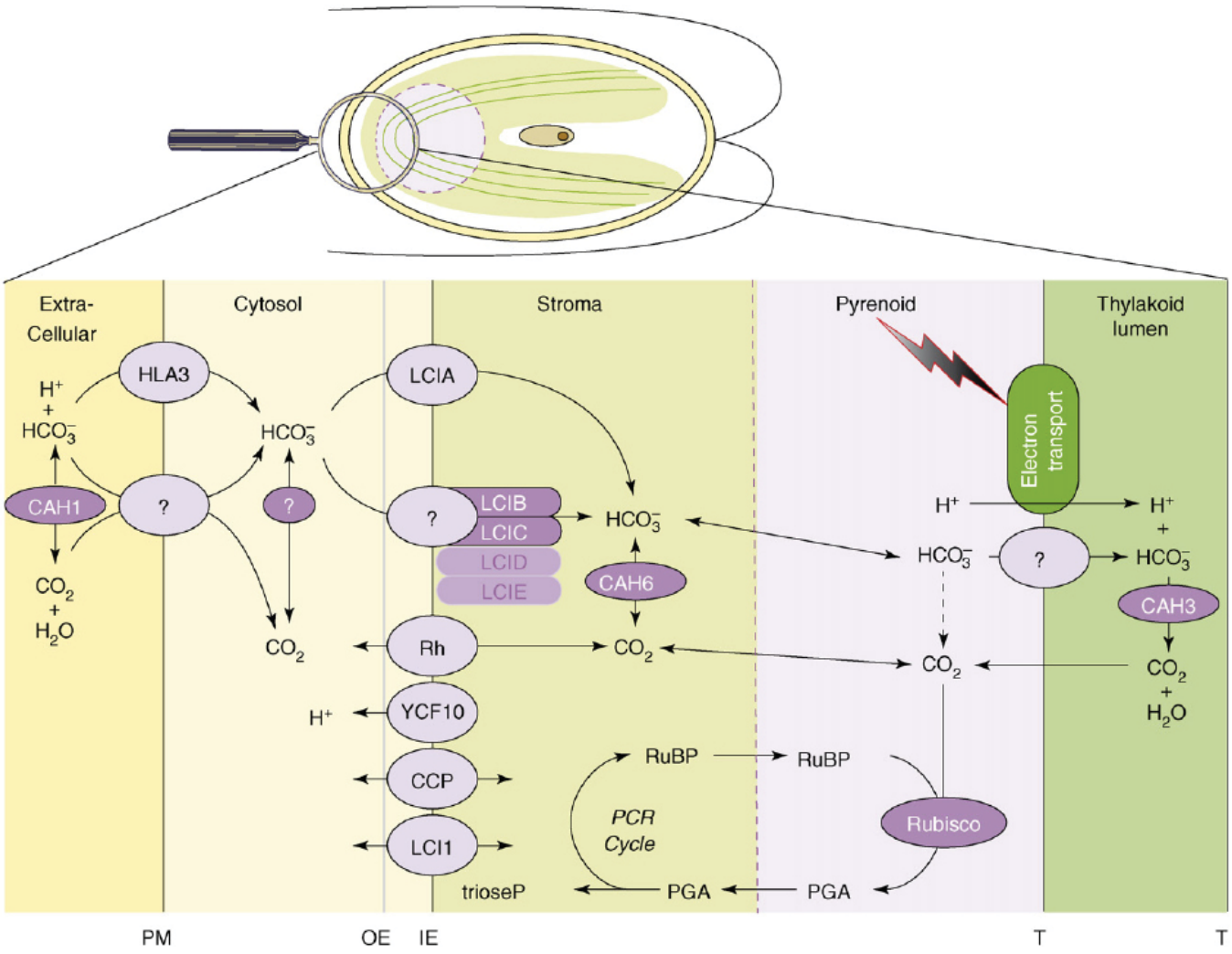

Figure 1. The cell biology of the carbon concentrating mechanism. The movement of inorganic carbon (Ci) from the extracellular space (yellow) to the thylakoid lumen (green) via the cytosol (uncolored) and stroma (light green) is shown. The pyrenoid is a non-membrane-bound compartment (boundary indicated as dashed line) within the stroma, and is the site of Rubisco localization. Membranes that serve as barriers for transport are shown as solid black lines. The lilac and purple ovals indicate transporters and enzymes, respectively, that catalyze steps in Ci uptake. Question marks indicate unidentified, postulated carriers. Putative Ci transporters without supporting functional data are indicated without substrates. IE, inner envelope; OE, outer envelope; PM, plasma membrane; T, thylakoid membrane.

the CCM have not been established, but they are probably important when the surrounding milieu is more alkaline [16, 17]. The *CAH1* and *CAH2* genes are clustered with other genes that are possibly important for the CCM. CAH3 is located in the thylakoid lumen and its importance was established genetically [18]. The roles of the mitochondrial isoforms CAH4 and CAH5, which are induced coordinately with CAH1, are not clear, but these CAs have been implicated in anapleurotic reactions [19]. The newly discovered CA isoforms are CAH6, CAH7, CAH8 and CAH9. The chloroplast stromal location of CAH6 suggests that it might be involved in trapping Ci as bicarbonate in the alkaline stroma [20], but the cellular locations and functions of the other novel CAs are unknown.

Mutant characterizations have been essential to our understanding of the CCM, revealing both functional and regulatory elements of the system. The *cia5* (*ccm1*) mutant of *Chlamydomonas* cannot acclimate to limiting [CO_2] and the CIA5 protein is probably a transcription factor that is crucial for acclimation [21, 22]. The *pmp1* mutant was reported to be a high-[CO_2]-requiring mutant that has a deficiency in Ci transport. This strain grew poorly in low or ambient [CO_2] (~0.04%) but like the wildtype strain at high (5%) [CO_2] when the CCM is not necessary. This strain also exhibits normal growth at very low (≤0.01%) [CO_2], however, indicating that a multi-tiered regulatory program, controlled by CO_2 levels, is crucial for the acclimation of *Chlamydomonas* to limiting [CO_2] [23]. Further

characterizations established three acclimation states: high [CO_2] (≥0.5% CO_2), low [CO_2] (0.4%–0.03% CO_2), and very low [CO_2] (≤0.01% CO_2). The biology of *Chlamydomonas* has several defining features in each of these three states: in high [CO_2], genes that are induced by limiting CO_2 concentrations are not expressed and the photosynthetic $K_{1/2}(CO_2)$ is similar to that of the $K_m(CO_2)$ of Rubisco. In the low [CO_2] state, limiting-[CO_2]-regulated genes are induced and photosynthetic $K_{1/2}(CO_2)$ is markedly decreased. In the very low [CO_2] state, the photosynthetic V_{max} is decreased and the $K_{1/2}(CO_2)$ is further decreased. The abundance of specific transcripts that are associated with CO_2 limitation was elevated in both low [CO_2] and very low [CO_2] states, suggesting that differences between these two states might arise from a quantitative difference in transcript levels [23], although there might be other, as yet unidentified, genes with expression characteristics that differentiate the two states.

Identification of the defective gene in *pmp1* was facilitated by both recognition of its novel 'air-dier' phenotype and the *Chlamydomonas* draft genome sequence. A tagged mutant gene *ad1* (air dier) was found to be allelic to *pmp1*. The defect in the *ad1* mutant was shown to be in *LCIB*, a gene activated following exposure of *Chlamydomonas* to limiting [CO_2] [24]. This gene is a member of a unique, small gene family consisting of *LCIB*, *LCIC*, *LCID* and *LCIE*; homologs to these genes were detected only in the genomes of the green microalgae *Ostreococcus taurii*, *Ostreococcus lucimarinus* (http://genome.jgi-psf.org/Ost9901_3/Ost9901_3.home.html ; http://genome.jgi-psf.org/Ostta4/Ostta4.home.html) and the colonial alga *Volvox carterii*. Although presented as being associated with the membrane transport components in Figure 1, LCIB, LCIC and LCID appear to be localized in the plastid, and are soluble polypeptides that function in some unknown way to enable Ci transport.

The draft genome sequence has also allowed the identification of several candidate genes that encode Ci transporters. Some of these genes, *CCP1*, *CCP2* and *LCI1*, were previously shown to be responsive to low [CO_2] and encode putative plastid envelope proteins [25, 26], although their role in CCM is still uncertain [27]. Another candidate transporter YCF10 encodes a plastid envelope protein with similarity to CotA in cyanobacteria. Although YCF10 might not be directly involved in Ci transport, it might influence transport through its proton translocation activity [28]. Additional candidate transporter genes include *HLA3*/*MRP1* and *LCIA*. *LCIA* (also called *NAR1.2*) is induced under limiting-[CO_2] conditions and encodes a formate/nitrite transporter [29]. *LCIA* expressed in *Xenopus* oocytes displays low-affinity bicarbonate transport activity [30] and high-affinity nitrite transport activity, making its role in Ci uptake uncertain. *HLA3*, originally identified as a high-light-induced gene and further characterized as being activated during [CO_2]-limited growth [31], is an ABC transporter of the multidrug-resistance associated protein (MRP) group.

Another interesting development in recent years is the report that Rh1 proteins in *Chlamydomonas* function as CO_2 gas channels [32]. Transcripts from the *RH1* gene increase in high [CO_2], suggesting that the encoded proteins might help to provide adequate [CO_2] for photosynthesis in the absence of a CCM, although the relationship between these proteins and CO_2 assimilation in *Chlamydomonas* has yet to be established. Because of the close relationship between Rh in *Chlamydomonas* and Rh blood factors, these findings have generated considerable interest with regard to potential roles of blood Rh in CO_2 transfer [33].

Recently, several genes that are induced by low [CO_2] were localized to a 75 kbp region [24] of scaffold 15 of version 3.0 of the *Chlamydomonas* draft genome. This cluster includes the limiting-[CO_2]-induced genes *CCP2* and *CCP1*, which encode plastid envelope proteins of the mitochondrial carrier family of proteins [27], LCID, LCIE, CAH1 and CAH2. The arrangement of the gene models suggests a duplication of an ancestral *LCI–CCP* gene pair flanked by an ancestral periplasmic *CAH* gene, but the functional importance of this arrangement, if any, is not yet understood.

Fermentation and hydrogen metabolism

Anoxic metabolism in *Chlamydomonas* (Figure 2) utilizes fermentative pathways that are typically present in strict anaerobes to metabolize starch into a variety of fuels, including H_2 and ethanol [34, 35, 36]. The use of fermentative pathways that are associated with anaerobic chemotrophs by an oxygenic phototroph was initially considered to be paradoxical; however, it is now apparent that photosynthetic microbes may experience extended periods of anoxia. When *Chlamydomonas* is grown with limited aeration under low light intensities, O_2 consumption by respiration can exceed the rate of photosynthetic O_2 production, leading to hypoxia [37], a common condition in the natural environment. Previous biochemical analyses have demonstrated that formate, acetate, and ethanol are major fermentative products formed in parallel with H_2 and CO_2 ([38, 39]; F Mus *et al*., unpublished). Trace amounts of lactate and glycerol have also been reported in *Chlamydomonas*.

The current draft genome for *Chlamydomonas* has revealed a unique repertoire of proteins that facilitate metabolic acclimation according to energetic fluxes arising from diurnal light/dark cycles [40, 41]. Several of these proteins function in fermentative production of ATP during anaerobiosis. Intriguingly, *Chlamydomonas* encodes key enzymes that are required for each of the five predominant pathways used to catabolize pyruvate (shown in Figure 2). This metabolic

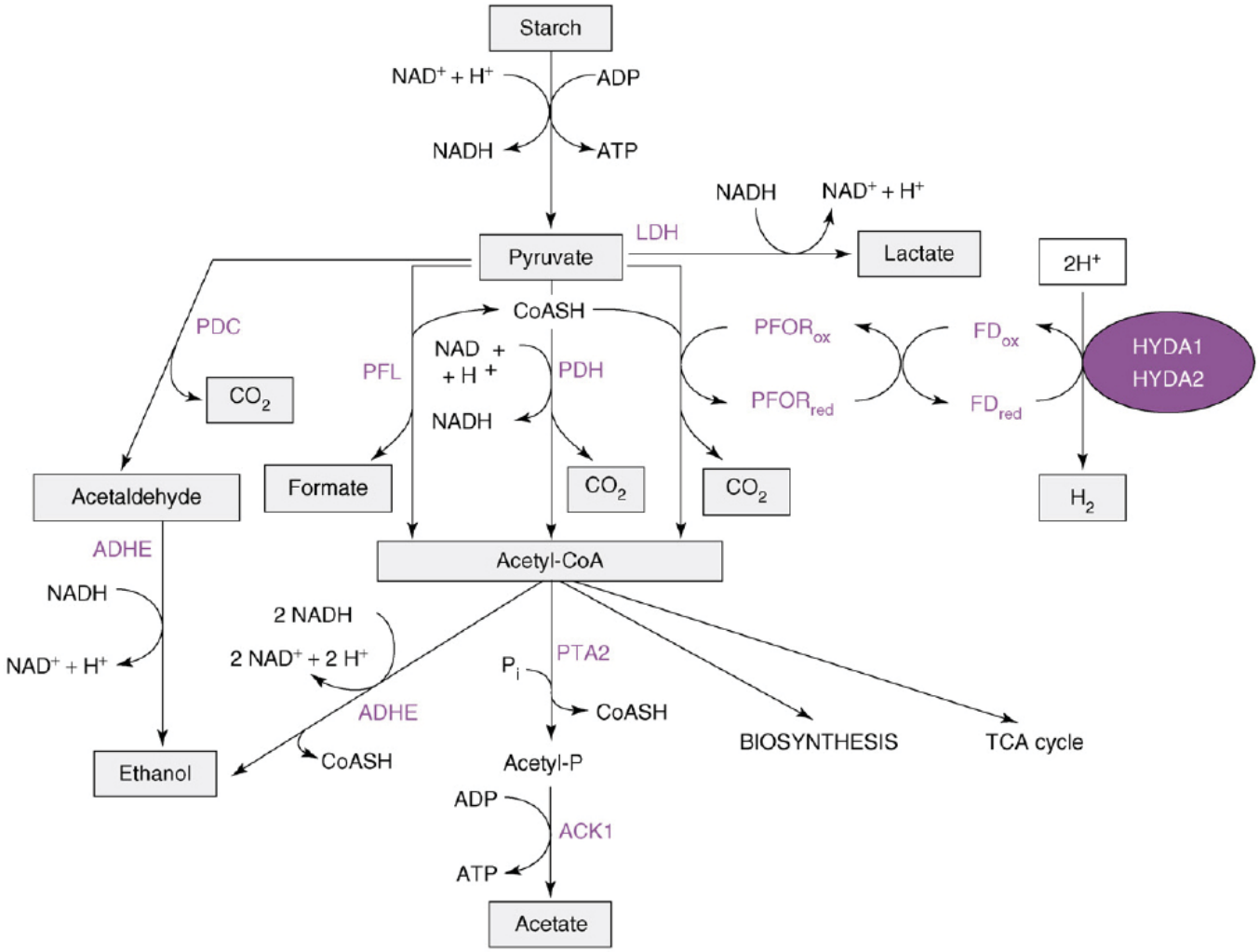

Figure 2. Pyruvate metabolism in *Chlamydomonas*. The four predominant pathways available in *Chlamydomonas* for the catabolism of pyruvate are illustrated. Oxygen availability, NAD(H) and ATP levels influence the partitioning of pyruvate into the appropriate pathways. Parallel processes in mitochondria and the chloroplast are expected for some aspects of pyruvate metabolism. In aerobic conditions, pyruvate is oxidized by the pyruvate dehydrogenase complex (PDH). The NAD^+ that is required for pyruvate production from starch can be regenerated by the respiratory chain (not shown). In anaerobic conditions, pyruvate can be oxidized via pyruvate ferredoxin oxidoreductase (PFOR) yielding acetyl-CoA, reduced ferredoxin and CO_2. Alternatively, pyruvate can be cleaved by pyruvate formate lyase (PFL), yielding acetyl-CoA and formate. HYDA1 and HYDA2 are two isoforms of (FeFe)-hydrogenase that oxidize the ferredoxin reduced by the action of PFOR. Pyruvate might also be decarboxylated by the pyruvate decarboxylase (PDC) homolog, resulting in the production of acetaldehyde and CO_2. Under hypoxic conditions, NADH is reoxidized by alcohol/aldehyde dehydrogenase (ADHE) activity. NADH might also be reoxidized by a putative lactate dehydrogenase (LDH). The conversion of acetyl-CoA to acetate and CoA via acetate kinase (ACK1) and phosphotransacetylase (PTA2) results in the fermentative production of ATP.

diversity is extraordinary in a single organism, suggesting marked metabolic flexibility suited for survival in a dynamic environment.

In addition to the pyruvate dehydrogenase complex, which catalyses the oxidation of pyruvate to CO_2 and acetyl-CoA (the latter is further oxidized by the TCA cycle under aerobic conditions), the *Chlamydomonas* genome encodes homologs of pyruvate formate lyase (PFL), pyruvate decarboxylase (PDC) and pyruvate ferredoxin oxidoreductase (PFOR). These enzymes direct organic carbon into distinct anaerobic pathways that are required to balance levels of NADH oxidation and ATP production during fermentation. PFOR couples the oxidation of pyruvate to acetyl-CoA and CO_2 with the reduction of ferredoxin, which can be used by the [FeFe]-hydrogenase to reduce protons to H_2, as shown in Figure 2. The two *Chlamydomonas* [FeFe]-hydrogenases, encoded by *HYDA1* and *HYDA2*, together with PFOR and the [FeFe]-hydrogenase maturation proteins HYDEF and HYDG [42] are examples of proteins usually only associated with strict anaerobes.

Interestingly, *Chlamydomonas* contains both the PFL and PFOR pathways for generating acetyl-CoA, yielding formate and CO_2 as co-products, respectively. Conversion of acetyl-CoA into acetate via the phosphotransacetylase (PTA)/acetate kinase (ACK) pathway results in ATP production. NADH is not reoxidized by the acetate production pathway, however, and consequently the synthesis of ethanol from either acetaldehyde (PDC pathway) or acetyl-CoA (PFL or PFOR pathways) is used to reoxidize NADH to sustain glycolysis. Alcohol and aldehyde dehydrogenase activities are both likely to be catalyzed by the aldehyde/alcohol dehydrogenase (ADHE) homolog in *Chlamydomonas* [40, 41]. The presence of both the acetaldehyde and acetyl-CoA pathways for ethanol production and the PFOR and PFL pathways for acetyl-CoA generation is remarkable for a eukaryote. Oxidation of NADH could also be coupled to H_2 production, as observed for *Clostridia* [43].

Interestingly, the *Chlamydomonas* genome has two copies of *PTA* and *ACK*. PTA1 and ACK2 are localized to mitochondria, whereas PTA2 and ACK1 are probably in the chloroplast [40]. The cellular location of PFOR is unknown, but it is expected, at least to some extent, to be in the chloroplast with the hydrogenases. PFL, which is encoded by a single *Chlamydomonas* gene, appears to be in mitochondria and chloroplasts [40], indicating dual-organelle targeting. No homolog of formate dehydrogenase is found on the *Chlamydomonas* genome, suggesting the absence of formate-hydrogen-lyase activity in *Chlamydomonas*, in accord with previous biochemical observations [38, 39].

In addition to anaerobiosis in the dark, deprivation of sulfate leads to reduced levels of oxygenic photosynthesis [44], resulting in anaerobiosis in the light [45]. Hence, *Chlamydomonas* can perform hypoxic fermentative metabolism in the light, creating a unique situation during which oxygenic photosynthesis, respiration and fermentation occur simultaneously. Recent microarray data examining gene expression as *Chlamydomonas* acclimates to hypoxic conditions has demonstrated that there are marked changes in the expression of genes that are associated with parallel metabolic processes that occur both in mitochondria and chloroplasts (F Mus *et al*., unpublished). Further examination of these pathways in the different organelles, and of crosstalk among them, will reveal insights that could be leveraged into strategies for engineering metabolism to optimize the production of energy-rich fermentative products. Furthermore, more than 60% of the differentially expressed genes observed on the microarrays encode putative proteins of unknown function; some are likely to participate in the function and/or regulation of the extraordinary metabolic networks of *Chlamydomonas*.

Selenoproteins

Selenoproteins, which contain selenocysteine (Sec), are present in bacteria, Archaea and many Eukarya [46], but not in vascular plants. In bacteria, these proteins are often involved in catabolic processes, whereas in eukaryotes they can have both biosynthetic and antioxidant functions. *Chlamydomonas* was previously shown to make selenoproteins [47], and a new analysis of the complete genome (VN Gladyshev, unpublished) led to the identification of 12 selenoproteins representing ten families, as well as Sec biosynthesis and insertion machinery, including a Sec-specific elongation factor, selenophosphate synthetase, SECIS-binding protein 2 (SBP2) and a single, previously identified Sec tRNA [48]. It is surprising that so many selenoprotein genes have been found in *Chlamydomonas* considering that these genes have been lost during the evolution of the streptophytes.

The selenoproteome of *Chlamydomonas* consists of selenoprotein K (SELK), selenoprotein M (SELM), thioredoxin reductase (TR), methionine-*S*-sulfoxide reductase (MSRA), glutathione peroxidase 1 (GPX1), glutathione peroxidase 2 (GPX2), selenoprotein U (SELU), selenoprotein T (SELT), selenoprotein W1 (SELW1), selenoprotein W2 (SELW2), selenoprotein H (SELH) and membrane selenoprotein (MSP). Five of the selenoproteins (TR, MSRA, GPX1, GPX2 and SELM) have homologs that have redox functions. The functions of the remaining seven are not known. Of these seven, five (SELK, SELW1, SELW2, SELH and SELT) have homologs in the human selenoproteome, one (SELU) is found in some animals (fish and birds) and one (MSP) appears to be present only in other unicellular organisms including other green algae (i.e. *Volvox carteri* and *Ostreococcus tauri*). *Chlamydomonas* MSP is predicted to have two Sec and is only the second eukaryotic selenoprotein, after SELP, to utilize more than one Sec. Furthermore, *Chlamydomonas* has recently been found to contain the most efficient MSRA enzyme known [49]. These data suggest that *Chlamydomonas* is dependent on the supply of selenium and that this dependence is due to the presence of this trace element in twelve selenoproteins with diverse functions. The selenium demand is probably low, however, because selenoproteins are present at low abundances in *Chlamydomonas* (and in other organisms). In fact, the growth of *Chlamydomonas* does not require the specific addition of selenium because enough is included as a contaminant of the other reagents. Given the role of many of the selenoproteins in dealing with oxidative stress, it is possible that the impact of selenium nutrition might be evident only under situations of exacerbated stress, such as excess excitation energy or poor nutrition.

Vitamins

Eukaryotic algae, like humans, need several vitamins for their growth. Vitamin B_{12} (cobalamin), vitamin B_1 (thiamine) and vitamin H (biotin) are required by a wide variety of algae, although not all algae require all three vitamins [50]. *Chlamydomonas* does not, itself, require an external supply of any of these micronutrients, but the recent genome sequence gives us

some fascinating insights into both vitamin metabolism in algae and the evolution of biosynthetic pathways.

Cobalamin is a tetrapyrrole-derived molecule that is synthesized exclusively by prokaryotic organisms. In humans, the vitamin is required as a cofactor for vitamin B_{12}-dependent methionine synthase. Vascular plants do not require this vitamin because they contain an alternative vitamin B_{12}-independent isoform of methionine synthase. The *Chlamydomonas* genome sequence has revealed two isoforms of methionine synthase: one is human-like and requires vitamin B_{12} as a cofactor (METH), whereas the other is plant-like and functions without this vitamin (METE) [51]. This arrangement, in which an individual organism contains two types of methionine synthases, is analogous to the situation in many bacteria, but it has not been observed in vascular plants or animals. The observation that *Chlamydomonas* has retained both isoforms of the enzyme, and has the ability to differentially control their expression [51], suggests that vitamin B_{12} is only transiently available in the alga's natural environment. The results also suggest specialization and concomitant gene loss in the animal and plant lineages.

Another vitamin, thiamine, plays a key role in intermediary metabolism. The active compound, thiamine pyrophosphate (TPP) is essential for all organisms. Although some algae cannot synthesize TPP *de novo*, others, such as *Chlamydomonas*, can [4 and 50]. In all organisms studied to date, TPP is synthesized from a thiazole and a pyrimidine moiety that are joined to form thiamine phosphate, which is then phosphorylated to form TPP. In bacteria, the thiazole moiety is derived from 1-deoxy-d-xylulose 5-phosphate, while the pyrimidine moiety is made from 5-aminoimidazole ribonucleotide [52]. Much less is known about thiamine biosynthesis in eukaryotes, but in *Saccharomyces cerevisiae* it appears that the thiazole and pyrimidine moieties are synthesized through different routes [52]. Interestingly, the genome sequence of *Chlamydomonas* indicates that the pyrimidine moiety is synthesized by a bacterial route (THIC) and the thiazole moiety made in the same way as in fungi (THI4).

Several of the thiamine biosynthetic genes in *Chlamydomonas* appear to contain regions that are highly similar to TPP riboswitches [53, 54, 55]. These regulatory elements are structures within the mRNA of individual genes that specifically bind TPP, and in so doing regulate the expression of the cognate gene. Several TPP riboswitches have been studied in bacteria, but little work has been carried out in eukaryotes. Furthermore, EST evidence from the *Chlamydomonas* genome project suggests that the mRNAs of some thiamine biosynthetic genes are alternatively spliced in riboswitch-containing regions, suggesting that TPP binding to the pre-mRNA might influence splicing. One region of particular interest is in the *THIC* gene, where EST evidence suggests that the 7th exon can be alternatively spliced. If this exon is included in the mature mRNA, a premature stop codon is incorporated, and a truncated (presumably non-functional) THIC protein is synthesized. If this exon is removed from the mature message, however, a full-length THIC protein is translated. It is therefore intriguing to speculate that TPP can bind to the pre-mRNA and thereby influence the way in which it is spliced.

Conclusions

The presentation above describes the union of physiological, biochemical and genetic data with near full genome information to reveal new metabolic routes and their control, and to expand our view of known metabolic networks. The examples provided represent only first explorations into topical areas of energy conversion and nutrient utilization. As we learn more about the nutrient requirements of *Chlamydomonas* (e.g. [56]) and apply developing technologies to examine both metabolite profiles (e.g. [57]) and how nutrient levels alter genome-wide expression patterns [13], we will not only learn more about individual pathways but also gain insights into the ways in which these pathway are integrated to optimize energy production and utilization.

Acknowledgements

We would like to acknowledge that funds supporting part of this work were from National Science Foundation (NSF) grants in support of the *Chlamydomonas* Genome Project (MCB 0235878 awarded to ARG). The work was also made possible by the efforts of Dan Rokhsar and others at the Joint Genome Institute in generating a draft sequence of the *Chlamydomonas* genome. All of the authors that have contributed to this article acknowledge support from US Department of Agriculture (USDA), National Institutes of Health (NIH), Department of Energy (DOE), Air Force Office of Scientific Research (AFOSR) and NSF.

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- • of special interest;
- •• of outstanding interest

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