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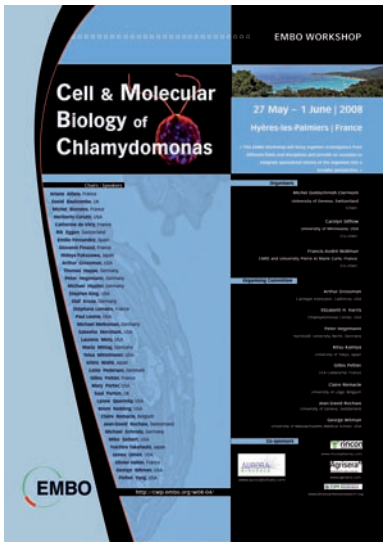
Chlamydomonas: a sexually active, light-harvesting, carbon-reducing, hydrogen-belching 'planimal'

Conference on the Cell & Molecular Biology of *Chlamydomonas*

Kevin E. Redding[1+] *& Douglas G. Cole*[2]

[1]Arizona State University, Tempe, Arizona, USA, and [2]University of Idaho, Moscow, Idaho, USA



The first EMBO workshop and the 13th international conference on the Cell & Molecular Biology of *Chlamydomonas* was held between 27 May and 1 June 2008, in Hyères-les-Palmiers, France, and was organized by M. Goldschmidt-Clermont, C. Silflow & F.-A. Wollman.

Keywords: algae; bioenergy; *Chlamydomonas*; flagella; *Volvox*

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See Glossary for abbreviations used in this article.

Introduction

Imagine walking along a beautiful beach of sand and rock on the Côte d'Azur enjoying the magnificent scenery—and anticipating an excellent meal afterwards—when suddenly, from the waters arises a tiny monstrosity: part plant, part animal and even part bacterium. It is green and gets its energy from the sun; it also swims (doing the breast stroke) in search of quality light, nutrients and mates, and yet it can ferment and emit hydrogen in a manner similar to an anaerobic bacterium. Furthermore, despite its bizarre chimeric nature, this beast seems to be perfectly adapted to its life.

Although this description might seem fantastic, it is none the less accurate and is essentially what 242 *Chlamydomonas* researchers from 19 countries were confronted with during the first European Molecular Biology Organization workshop and the 13th international conference on the Cell & Molecular Biology of *Chlamydomonas* (Fig 1). This conference forced many researchers working on various aspects of the unicellular alga *Chlamydomonas*, or 'Chlamy' as it is affectionately called, to face the fact that all this time they have been working on a 'planimal'—an organism with traits that do not fit easily into nice, neat categories. Although this has long been appreciated by the Chlamy community, the bulk of which has been traditionally divided between those working on photosynthesis and chloroplasts, and those working on flagella and motility, new insights coming primarily from the genomes of *Chlamydomonas reinhardtii* and the related multicellular alga *Volvox carteri* have shown us that the chimeric nature of Chlamy runs deep. After all, how many organisms can, at the same time, serve as models for human disease and biological hydrogen production?

Chlamy sails into the post-genomic era

An important milestone for *Chlamydomonas* research was reached in 2007 with the first publication of the complete nuclear genome of a green alga (Merchant *et al*, 2007). Exhaustively mined by the community over the past few years, the annotated genome (http://www.jgi.doe.gov) has given rise to several powerful tools and approaches. Among the first tools were extensive genomic microarrays that have been used widely in the Chlamy field for transcriptional analysis. Chlamy transcriptomics, however, is changing markedly as high-throughput sequencing approaches are reducing costs and experimental time, while providing greater coverage. Moderate-length reads of a few hundred base pairs have been obtained by the pyrosequencing approach of 454 sequencing (454 Life Sciences, Branford, CT, USA), in which 100 MB of information

[1]Department of Chemistry and Biochemistry, Arizona State University, 1711 South Rural Road, Tempe, Arizona 85287-1604, USA
[2]Department of Microbiology, Molecular Biology and Biochemistry, University of Idaho, Moscow, Idaho 83844-3052, USA
[+]Corresponding author. Tel: +1 480 965 0136; Fax: +1 480 965 2747;
E-mail: kevin.redding@asu.edu

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can be obtained in a single afternoon. To address differential gene expression in Chlamy, the field has also embraced the powerful Solexa sequencing method (Illumina, San Diego, CA, USA), which generates an even greater number of much shorter reads (25–35 base pairs), allowing deeper coverage at a lower cost, as discussed by S. Merchant (Los Angeles, CA, USA) and others.

Proteomic analyses of specific organelles are being used to generate proteome scorecards that provide a more global view of protein expression. Until recently, for example, only a few dozen proteins were known with certainty to be present in the Chlamy mitochondrion. Applying modern mass spectrometry to the purified organelle, A. Atteia (Grenoble, France) was able to identify 513 proteins, increasing our knowledge by approximately tenfold. Interestingly, of 86 enzymes associated with metabolism, 36 had not been identified in similar mitochondrial proteomic analyses of other model organisms; a further eight proteins have no known homologues in eukaryotes.

Proteomics has also been applied to basal bodies, which double as centrioles in this organism (Keller *et al*, 2005). K. Brachhold (Cologne, Germany) has now isolated basal bodies depleted of pericentriolar material, yielding a proteome representing 435 gene products, several of which have been localized to the basal apparatus. The conservation of this organelle was explored in a special lecture by M. Bornens (Paris, France), who pointed out that some centriolar components are well conserved even when the organelle is not. The yeast spindle pole body, for example, contains centrin but lacks centrioles.

G. Kreimer (Erlangen, Germany) and co-workers refined their proteomic analysis of the Chlamy eyespot, which initially identified approximately 200 proteins, including several kinases and phosphatases (Schmidt *et al*, 2006). By using standard techniques to enrich for phosphopeptides, they reported 52 specific phosphorylation sites within 32 different proteins, 27 of which were not previously known to be phosphorylated. The research community is now poised to see how the phosphoproteome changes in response to eyespot-related signalling (Wagner *et al*, 2008).

Metabolic profiling—also known as metabolomics—is being used to understand how Chlamy responds to environmental, genetic and developmental changes. In this approach, mass spectrometry—usually in conjunction with liquid or gas chromatography—is used to quantify the levels of dozens or even hundreds of metabolites in a single sample. C. Bölling (Berlin, Germany) addressed the changes in metabolites that occur when cells adapt to sulphur deprivation. Comparative analyses of wild-type Chlamy and a mutant that was unable to respond to sulphur starvation, *sac1*, revealed a metabolic profile consistent with an inability to enhance the scavenging and uptake of sulphur, along with a substantial inability to sustain many processes that lead to the transient or permanent accumulation of metabolite levels in wild-type cells. E. Hom (Cambridge, MA, USA) and colleagues have generated improved open reading frame models for Chlamy using the highly successful gene-prediction algorithm AUGUSTUS. Hom announced the open availability of these expressed sequence tag-based predictions (which can be found at http://augustus.gobics.de/predictions/chlamydomonas) and the metabolic network-reconstruction efforts of the group—initially focused on central metabolism—both of which should facilitate future high-throughput analyses. The technologies and approaches developed for Chlamy metabolic profiling will undoubtedly be invaluable in similar analyses of other algae; the genomes of several algal species are now being determined by the Joint Genome Institute.

Glossary

CYC6	gene encoding cytochrome c6
Fa2p	NIMA-related kinase required for flagellar excision and cell-cycle progression
LC1	outer dynein arm-associated light chain 1
METE	gene encoding *S*-adenosylmethionine synthetase
NAC2	gene encoding a factor required for maturation/stability of the photosystem II protein D2 messenger RNA
NADH	nicotinamide-adenine dinucleotide
ND5	mitochondrially encoded protein associated with complex I of the electron-transport system
NEK	NIMA-related kinase
NIMA	never-in-mitosis A, a kinase required for mitosis in *Aspergillus nidulans*
ODA	outer dynein arm
PF13	gene required for cytoplasmic assembly of ODAs
PPR	pentatricopeptide repeat
regA	master regulatory gene required for differentiation in *Volvox*, thought to act by repressing reproductive genes in somatic cells
sac1	gene required for acclimation to low-sulphur conditions
THI4	gene encoding enzyme required for thiamine biosynthesis
TPR	tetratricopeptide repeat

Further additions to the molecular and genetic toolbox

One of the most welcome developments has been the increase in the number of tools available for the reliable control of gene expression.

On the repressive side, gene silencing has now become routine. Moreover, work from the laboratory of H. Cerutti (Lincoln, NE, USA) has shown that Chlamy has the core components of the RNA interference (RNAi) machinery discovered elsewhere, including several proteins that are similar to Dicer and Argonaute. Chlamy seems to use many, overlapping systems to keep genomic parasites under conrol. D. Baulcombe (Cambridge, UK)—a major player in the silencing field who recently launched a Chlamy project—gave an encouraging plenary lecture outlining a strategy for the routine design and use of artificial microRNAs to silence Chlamy genes in a specific and permanent manner.

On the activation side, problems with stable, long-term expression of transgenes are a thing of the past. R. Bock (Potsdam-Golm, Germany) has created strains that show high-level, stable expression of foreign proteins, independent of codon usage and genomic context. Not only are we seeing new techniques for constitutive expression and silencing, but also systems for controllable expression are being developed and refined. The *CYC6* promoter can be repressed by the addition of Cu^{2+}, and the use of metal chelators and Ni^{2+} can fine tune this response, resulting in a greater than 200-fold range of expression (Quinn *et al*, 2003). Expression of the *THI4* gene is repressed by the addition of thiamine, which has been shown by M. Croft (Cambridge, UK) and co-workers to be mediated by a riboswitch that modulates splicing (Croft *et al*, 2007), allowing for a transportable repression system. The use of the vitamin B_{12}-responsive *METE* promoter, discovered by the same group, increases the range of repressible systems to three—with more to come.

New tools are also being added to the *Chlamydomonas* genetic toolbox. Perhaps the most powerful of these was described by J.-H. Lee (St Louis, MO, USA), who created diploid cell lines

Fig 1 | A few highlights of the 2008 Chlamy meeting. (**A**) M. Goldschmidt-Clermont opening the meeting. (**B**) Intense discussions during the many poster sessions. (**C**) J.-D. Rochaix explaining the chloroplast. (**D**, **E**) Not the worst place in the world to discuss science. (**F**) J.-H. Lee getting his just desserts. (**G**) Did you say dessert? (**H**) The intrepid band who will give you Chlamy 2010. (**I**) beautiful beaches (but the water was colder than it looks).

capable of undergoing meiosis (Lee *et al*, 2008). The sporulation of diploid cells allows the production, maintenance and study of recessive lethal mutations, which cannot be maintained in the haploid strains that are normally used. Marked improvements were also described in the specific targeting of nuclear-encoded genes. As with many eukaryotic organisms, the replacement of Chlamy genes by homologous recombination has been extremely inefficient. Enhancing this rate by some 5,000-fold, B. Zorin (Berlin, Germany) revealed that part of the solution includes the use of single-stranded DNA. It does not take much imagination to realize that the combination of homologous recombination and diploid sporulation will allow the future study of essential Chlamy genes, which has long been a desire of the community.

Chlamy as a model system for human disease

Chlamy has recently taken a central role in studies that show the molecular basis of specific human ciliary diseases, including primary ciliary dyskinesia and an expanding family of diseases associated with cystic kidneys. One advantage of Chlamy as a disease model is the relative ease with which specific disease genes and their protein products can be studied. Mutations in the conserved ciliary protein hydin, for example, are known to cause mammalian hydrocephalus. After pinpointing its ciliary location to a specific site within the central pair apparatus, K. Lechtreck and G. Witman (Worcester, MA, USA) found that the RNAi-mediated reduction of hydin protein generated Chlamy cells with paralysed flagella that were frozen mid-stroke (Lechtreck & Witman, 2007). In parallel,

hydin-defective mice were shown to display a similar ciliary defect, explaining why the ependymal cilia of the hydrocephalic brain move cerebrospinal fluid so poorly (Lechtreck *et al*, 2008).

Advances in the analysis of the structure and regulation of the flagellar basal body complex were also highlighted at this meeting. To address the function of an outer dynein arm (ODA) subunit known as LC1, the group of S. King (Farmington, CT, USA) transformed Chlamy with a series of *Myc*-tagged mutant proteins, some of which produced a dominant-negative phenotype of reduced swimming velocity. Although these mutant proteins altered the extent of flagellar bending, they did not reduce the beat frequency. The binding of the ODA to the axoneme requires a heterotrimeric docking complex that forms a 55-nm rope-like structure, as described by K. Wakabayashi (Tokyo, Japan), who elegantly reconstituted the complex using heterologously-expressed protein. Before binding to the axoneme, ODA must enter the organelle; D. Mitchell (Syracuse, NY, USA) showed that PF13 aids dynein complex formation and ODA16 assists intraflagellar transport particles in the movement of the dynein complex from the cell body into the flagellum (Ahmed *et al*, 2008). Elegant total internal-reflection fluorescence microscopy was used by B. Engel (San Francisco, CA, USA) to show that the size of these particles is inversely proportional to the length of the organelle, suggesting that it modulates the rate of flagellar assembly.

Chlamy can quickly jettison its flagella in response to certain environmental stresses. G. Wheeler (Plymouth, UK) used bio-ballistic loading of dextran-conjugated Ca^{2+}-responsive dyes to visualize changes in the concentration of cytoplasmic Ca^{2+}; this showed that external increases in concentration trigger rapid (~500 ms) increases in cytoplasmic Ca^{2+}, which coincide with flagellar excision (Wheeler *et al*, 2008). The rapid elevation in Chlamy cytoplasmic Ca^{2+} is faster than any previously visualized in plants or green algae. Flagellar excision also requires the NIMA related Fa2p kinase. As described by L. Quarmby (Burnaby, Canada), related kinases seem to coordinate ciliogenesis with the cell cycle (White & Quarmby, 2008). The importance of this cell-cycle regulation is emphasized by the fact that mutations in the mammalian *Nek1* and *Nek8* genes give rise to polycystic kidneys.

Human disease modelling in Chlamy is not restricted to the flagellum. For example, the study of mitochondrially-linked diseases is especially attractive in Chlamy because it has a NADH dehydrogenase—also known as Complex I—that is not present in *Saccharomyces cerevisiae* and is similar to the mammalian enzyme, which is a hotspot for mutations that cause human disease. This allows the use of Chlamy as a unicellular genetic model system for this complex enzyme, which has subunits encoded by both the mitochondrial and nuclear genomes. By using homologous recombination within the mitochondrial genome, C. Remacle (Liège, Belgium) specifically targeted the ND5 subunit of Complex I to recreate a known human disease mutation, M223L. This mutation resulted in a low abundance of Complex I, suggesting that ND5 might be essential in the assembly or maintenance of human complex I. This approach is particularly powerful because the analysis of mitochondrial disease in human tissues is often complicated by the presence of both healthy and diseased organelles, due to segregation of the mitochondrial genome.

The volvocine lineage: how to become multicellular

Comparisons of the Chlamy genome with the recently released genome of its multicellular evolutionary cousin, *V. carteri*, have become very important. *Volvox* forms spherical colonies that contain approximately 2,000 cells, which are divided into germ-line cells and Chlamy-like somatic cells. Both the *C. reinhardtii* 121-Mb nuclear genome (Merchant *et al*, 2007) and the *V. carteri* 138-Mb nuclear genome contain close to 14,500 genes, as reported by S. Prochnik (Walnut Creek, CA, USA). Direct alignments of the two sequences reveal that most of the gene families present in *Volvox* are also present in Chlamy. This is surprising when one considers the numerous morphological and developmental differences between the two species. One difference is found in the mating locus, which contains both sex-specific and housekeeping genes. The *Volvox* mating locus is much larger and has undergone evolutionary change more rapidly than that of Chlamy, as described by J. Umen (La Jolla, CA, USA). Another difference, described by A. Harryman (Baltimore, MD, USA), is found in the *regA* group of putative transcription factors. *Volvox* has several paralogues of the master cell-fate regulator *regA*, whereas Chlamy has only one *regA*-related gene (Duncan *et al*, 2007). As described by I. Nishii (Wako, Japan), the *Volvox*-specific process of embryonic inversion requires the coordination of *Volvox*-specific genes with additional genes shared by both organisms.

The genomic information available for *C. reinhardtii* and *V. carteri* has made the volvocine lineage one of the hottest systems for studying the evolution of multicellularity. The use of these two organisms as models for cell biology has given us a treasure trove of data, which is now being mined by evolutionary biologists to reconstruct the history of a unicellular eukaryote evolving into a much larger ensemble of differentiated cells with a distinct division between somatic and germ-line cells. It was thought that *Volvox* and *Chlamydomonas* were separated by approximately 50 million years; however, new estimates based on genomic comparisons suggest that the divergence is two to six times greater. The presence of many extant intervening species with intermediate morphologies—from the eight-cell packets of *Gonium* to the 128-cell spheres of *Pleodorina*—makes this system even more powerful. In the future, knowledge about their genomes will allow the testing of many hypotheses. In addition, as highlighted by U. Goodenough (St Louis, MO, USA), the green algal genomes allow one to ask more far-ranging questions about the evolution of crucial differences between algae and plants, such as the switch in dominance from the haploid to diploid state. It is clear that the Chlamy system will have the same pivotal role in the study of green algae as the *Arabidopsis* system has had in the study of plants.

Chloroplasts, photosynthesis and biofuels

Chlamy has long served as a model system for studying the control of chloroplast gene expression. With the increased need for re-engineering of electron-transport and metabolic pathways to increase yields of biofuels, this research has become even more important. Classical genetic analysis has revealed that the regulators of chloroplast genes are often gene specific in Chlamy, typically acting by stabilizing or promoting the translation of a specific messenger RNA. J. Nickelsen (München, Germany) described proteomic approaches that are being taken to understand the function of proteins containing TPR motifs. There are around 80 members of this family in the genome, which are joined by the 11 members of the PPR family; however, it is not clear at present what all of them are doing. By putting one of them, *NAC2*, under the control of a repressible nuclear promoter, the laboratory of J.-D. Rochaix (Genève, Switzerland) has been able to make chloroplast genes (and transgenes) responsive to environmental changes (Surzycki *et al*, 2007) and to bring controllable expression to the chloroplast.

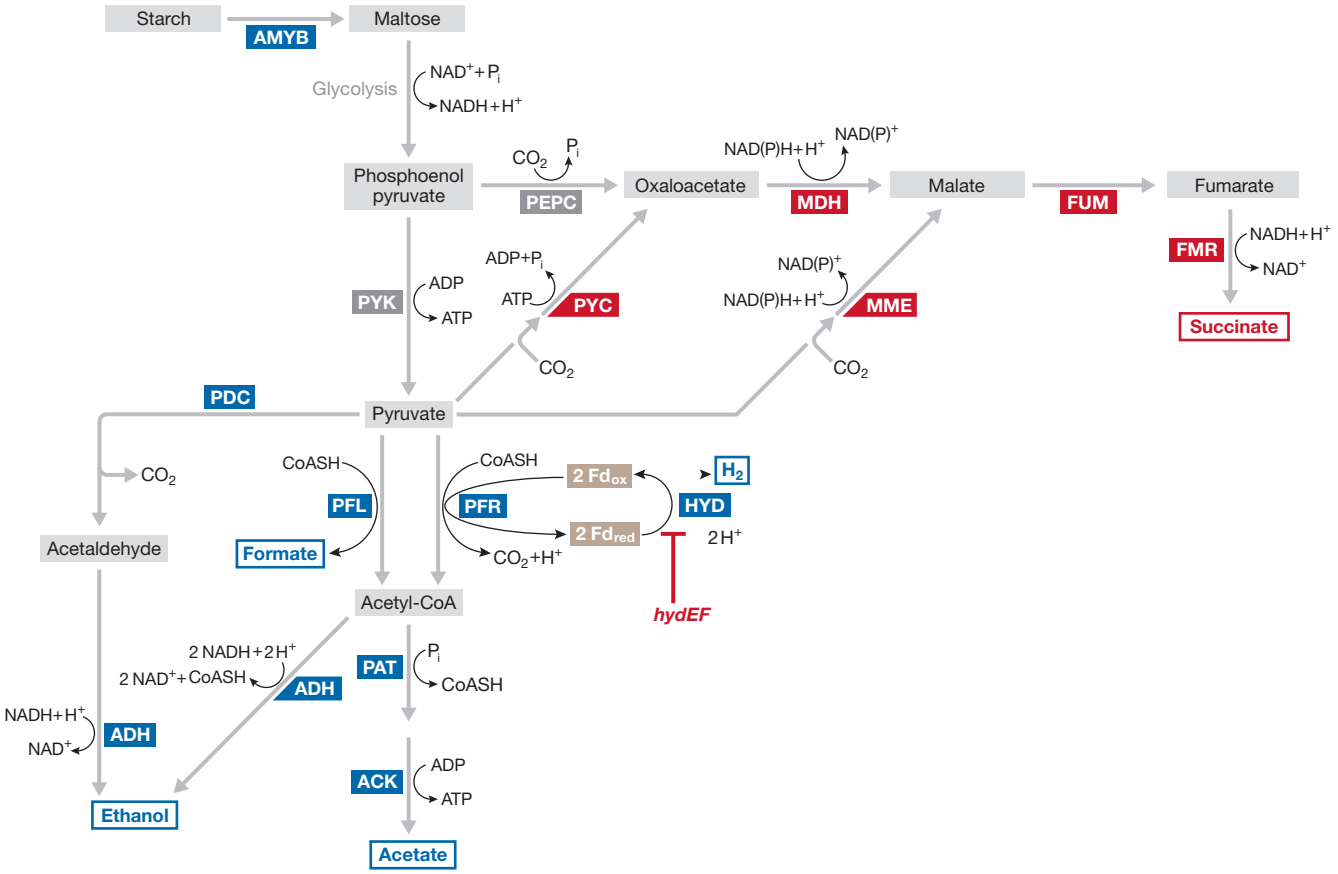

Fig 2 | The effect of dark anaerobiosis on selected metabolic pathways, as revealed by gene expression and metabolite profiling. The enzymes are indicated next to the arrows that depict the specific steps that they catalyse. The enzymes for which expression is induced by growth in dark anaerobic conditions are shown in blue; endpoint metabolites detected by metabolic profiling are boxed. Note that in some cases enzymes are encoded by more than one gene and the effects have been grouped for clarity. The analysis of the *hydEF* mutant, which cannot evolve hydrogen owing to a deficiency in the maturation of the [FeFe]-hydrogenase is also indicated: genes additionally up-regulated in the *hydEF* mutant are shown in red, as well as the new metabolite produced (succinate). The data are based on Mus *et al* (2007) and more recent unpublished results. ('Glycolysis' sums the results of all the enzymes in the sequence from maltase to enolase—note that the amount of NADH produced is expressed per molecule of phosphoenol pyruvate.) ACK, acetate kinase; ADH, acetaldehyde/alcohol dehydrogenase; AMYB, β-amylase; Fd, ferredoxin; FMR, fumarate reductase; FUM, fumarase; HYD, [FeFe]-hydrogenase; MDH, malate dehydrogenase; MME, malic enzyme; PAT, phosphate acetyltransferase; PDC, pyruvate decarboxylase; PEPC, PEP carboxylase; PFL, pyruvate formate lyase; PFR, pyruvate:ferredoxin oxidoreductase; PYC, pyruvate carboxylase; PYK, pyruvate kinase.

Chlamy is now the main experimental eukaryotic system for biological hydrogen production. Several talks focused on the optimization of the photosynthetic electron transport, metabolism and enzymology that cooperate to produce hydrogen. The hydrogenases, which accept electrons from ferredoxin, are thought to act as an 'escape valve' for electrons when photosynthetic electron flow is just starting. Their intolerance to oxygen normally results in a shutdown of hydrogen production soon after photosystem II has started to split water, as explained by M. Ghirardi (Golden, CO, USA). Current efforts to get around this problem include the use of regulatory mechanisms to decrease the activity of photosystem II to a level at which respiration can remove the oxygen produced, selection for more oxygen-resistant hydrogenases and the engineering of metabolic pathways. T. Happe (Bochum, Germany) also reported the identification of the major electron donor to the hydrogenases. Both his group and that of M. Seibert (Golden, CO, USA) have succeeded in heterologously expressing the Chlamy hydrogenases and maturation enzymes. Seibert and A. Dubini (Golden, CO, USA) have also mapped alternative metabolic pathways by using a combination of expression and metabolic profiling (Fig 2). This is where the power of the Chlamy system really shines: being unicellular makes biochemical and metabolic analyses (relatively) simple; and having a genome without huge gene families—as often seems to be the case in higher plants—makes genetic, transcriptomic and proteomic analyses relatively straightforward. Several groups reported the use of both types of large-scale screens to tease apart the intricate relationships between alternate pathways of electron and metabolite flow.

Among the many insights garnered from the various 'omics' studies is the fact that Chlamy has many ways to route electrons and carbon, and it is rarely obvious how this network will respond to changes posed by the environment or the machinations of genetic engineers (Grossman *et al*, 2007). In fact, even when considering individual

steps or short pathways, Chlamy often has alternative mechanisms to achieve the same thing—for example, light-(in)dependent chlorophyll biosynthesis, (non)photochemical reduction of plastoquinone and oxygen-(in)dependent synthesis of several metabolites. This situation is probably due to the retention of genes from the ancestral eukaryote that have since been lost in the lineages leading to plants and animals/fungi, coupled with selection for adaptation to the many ecological niches Chlamy seems to inhabit. Moreover, at least some genes seem to have been imported from prokaryotes, including the photosynthetic endosymbiont that became the chloroplast. Despite the metabolic flexibility of this organism, most of the genes are present as a single copy, which will simplify their functional genetic analysis.

Summary

The Chlamy 2008 EMBO workshop was well attended by scientists from around the globe, and the appearance and active participation of several prominent researchers who have only recently entered these waters were most welcome. Also notable was the fact that the depth and breadth of biological issues being addressed were greater than ever before; unlike past Chlamy meetings, photosynthesis and motility, although still prominent, did not overshadow everything else. The diverse topics discussed—many of which we sadly did not have space to mention here—ranged from biophysics in the picosecond timescale to the morphology of various organelles, from the molecular biology of microRNA-mediated silencing to the evolution of sexual reproduction and multicellularity, and from the production of hydrogen gas and biodiesel oils to understanding the molecular basis of human disease. Part of the credit for how fast the field has moved belongs to the collegial community of Chlamy and *Volvox* researchers, who have freely shared ideas and reagents, and who have welcomed newcomers with open arms and countless mutant strains. Indeed, like the Mediterranean mixing pot in the south of France, the Chlamy community has become a rich Provençal *bouillabaisse* of researchers attacking and solving diverse biological issues. Stay tuned—the best is yet to come.

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Kevin E. Redding

Douglas G. Cole