Meeting report

A bright future for *Chlamydomonas*

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A report on the 12th International Conference on the Cell and Molecular Biology of *Chlamydomonas*, Portland, USA, 9-14 May 2006.

The biennial meeting on the cell and molecular biology of *Chlamydomonas* brings together those who work on this photosynthetic unicellular eukaryote and use it as a model system. This year's meeting provided an overview of the advances this organism has helped to make in areas ranging from studies of flagella to photosynthesis. One underlying theme was the status of *Chlamydomonas reinhardtii* as a model organism - what is it a model for, and how appropriate is that model? In some aspects *C. reinhardtii* most closely models plant systems, in others mammalian cellular processes; regardless, *Chlamydomonas* is a powerful system for the study of a variety of molecular and cellular processes.

This was the first meeting of the *Chlamydomonas* community since the completion of the nuclear genome sequence of *C. reinhardtii*. Dozens of groups have contributed annotation and curation to the genome database and browser developed at the US Department of Energy's Joint Genome Institute (DOE JGI), fine tuning more than 15,000 candidate genes that currently appear in the database. Simon Prochnik (DOE JGI, Walnut Creek, USA) reported on the current status of the genome project and the plans for the release and publication of this sequence later this year. A comparative phylogenomic analysis of *C. reinhardtii* with other sequenced genomes has examined the evolutionary origin of *Chlamydomonas* genes, and identified *Chlamydomonas*-specific genome expansions. The benefits of the availability of the *C. reinhardtii* nuclear genomic sequence were clear in many of the talks.

Chlamydomonas as a model plant

Chlamydomonas has long been billed as a model plant - it requires very little space for growth, has a short generation time compared with higher plants, the nuclear and chloroplast genomes have been sequenced and annotated, and new genes can be introduced into both these genomes by transformation. Photosynthetic function can be replaced by carbon sources in the medium, allowing the study of non-photosynthetic mutations or growth in complete darkness. Studies of the chloroplast have been a trademark of *C. reinhardtii*, and include work on photosynthesis, carbon-concentrating mechanisms and gene expression. In his keynote address, Francis-André Wollman (Institut de Biologie Physico-Chimique, Paris, France) reviewed studies of gene expression in the *Chlamydomonas* chloroplast, highlighting the autoregulatory processes that control the expression of genes encoding subunits of multiprotein complexes. Subunits in each of the four photosynthetic membrane protein complexes require the presence of at least one of their partner subunits (a dominant subunit, DS) in order to be actively expressed. This mode of regulation is referred to as 'control by epistasy of synthesis'. The question remains as to how membrane-bound DSs are able to affect the translation of chloroplast mRNAs that are presumably not associated with membranes. Wollman outlined one possible mechanism in which the DS and the factors that limit the translation of a regulated subunit have an affinity for the same binding site. When the DS is not present, the limiting factors are bound and sequestered away from the mRNA of the regulating subunit, so it cannot be translated; when the DS is present, it binds instead, releasing the limiting factors and allowing expression of the regulated subunit. Mitochondria have a similar mechanism for regulating gene expression, so generalities can be drawn between chloroplasts, mitochondria and bacteria.

Studies of gene expression in the chloroplast have taken an interesting turn into applications for *Chlamydomonas* in biotechnology. The chloroplast genome is easily altered via homologous recombination, and this has been used to study basic aspects of chloroplast gene expression. This technology is now being used to express recombinant proteins in the

chloroplast. One of us (S.M.) described a transformation strategy for the chloroplast that allows recombinant proteins to accumulate to more than 5% total protein. An endogenous coding region (*psbA* in this case) is replaced with the transgene of interest, eliminating competition with or autoattenuation from the endogenous gene, allowing high levels of recombinant protein synthesis. This replacement renders the strain nonphotosynthetic, but reintroduction of a *psbA* coding region driven by a *psbD* promoter into a different site on the genome restores photosynthetic activity without losing the ability to accumulate high levels of recombinant protein. The high levels of expression might allow *C. reinhardtii* to compete with commonly used expression systems such as bacteria and mammalian CHO cells. Scott Franklin (Rincon Pharmaceuticals, La Jolla, USA) presented a comprehensive analysis of the feasibility and cost benefits of using the *Chlamydomonas* chloroplast as a platform for the production of human therapeutic proteins. He showed that such transgenic proteins purified from *C. reinhardtii* chloroplasts assemble into the correct complexes and have the appropriate biological activity.

Another biotechnological application to come out of studies of the *C. reinhardtii* chloroplast is concerned with hydrogen production. *Chlamydomonas* can adopt an anaerobic metabolism, producing hydrogen gas and metabolites such as formate and ethanol (Figure 1). Anja Hemschemeier (Ruhr-Universität Bochum, Bochum, Germany) presented details of the different fermentation pathways active in the chloroplast and showed that hydrogenase activity may function as an electron 'valve' when photosynthetic electron sinks are impaired. Photofermentation is also being pursued for biotechnological applications in this era of alternative fuel options. Matthew Posewitz (Colorado School of Mines and National Renewable Energy Laboratory, Golden, USA) presented work that his group has done to identify genes required for hydrogen production. Some of these genes are involved in the pathway itself, whereas others affect the accumulation of starch, an important input for fermentation under nonphotosynthetic conditions (see Figure 1). While hydrogen production from *Chlamydomonas* tanks, instead of gas tanks, is not on the horizon just yet, this alga may prove a useful bioreactor for energy production.

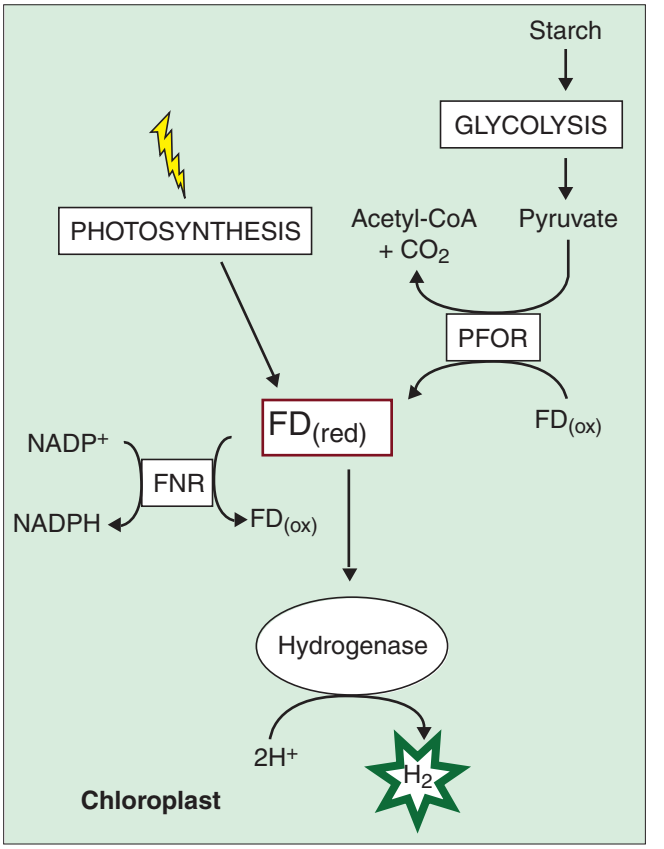

Figure 1
Hydrogen production in the *C. reinhardtii* chloroplast. Normally, the protein ferredoxin (FD) transfers electrons to an enzyme that reduces $NADP^+$ to NADPH, which is required for chloroplast metabolic processes. Reduced ferredoxin ($FD_{(red)}$) can instead transfer electrons to a chloroplast hydrogenase, which produces molecular hydrogen (H_2) from protons (H^+). Hydrogen production thus acts as an alternate electron sink. Reduced ferredoxin can also be produced via glycolysis from the breakdown of starch, which enables hydrogen production in the absence of photosynthesis. FNR, ferredoxin $NADP^+$ oxidoreductase; PFOR, pyruvate ferredoxin oxidoreductase. Figure courtesy of and adapted from M. Posewitz.

Silencing gene silencing

Although the nuclear genome is easily transformed, transgenes introduced into the nuclear genome are often silenced, a major difficulty in *C. reinhardtii* as it is in many other organisms. Markus Heitzer (University of Regensburg, Germany) presented a strategy for creating expression constructs that can minimize silencing effects by enabling efficient and robust selection of only highly expressed constructs. Addition of an internal ribosome-entry (IRES) site element allows the linkage of the gene of interest and a selectable marker into a single transcript from which both can be translated. Heitzer showed that using this strategy, increasingly stringent antibiotic selection yielded very highly expressed genes of interest. Mukesh Lodha (University of Freiburg, Germany) reported a strategy to counteract silencing effects that are normally induced through the use of a strong promoter like that of *RBCS2* in transgene constructs. Certain domains of the promoter of the heat-shock gene *HSP70A*, when added upstream of the *RBCS2* promoter in transgene constructs, were able to abrogate transcriptional silencing effects due to the *RBCS2* promoter.

Groups working with RNA interference (RNAi) also need to make sure that the introduced DNA encoding the interfering RNA is not itself silenced in the nucleus. Kempton Horken (University of Nebraksa-Lincoln, Lincoln, USA) presented the use of an opposing promoter system for RNAi, coupled

with acetamidase selection. This strategy, like that outlined by Heitzer, couples a robust selection system directly to the expression of a desired insert, in this case the template DNA for the interfering RNA.

Determining the possible functions of naturally occurring small RNAs in the regulation of gene expression in a single-celled organism is of considerable interest, as in multicellular organisms much of the RNA silencing by these small RNAs is involved in embryonic development, and specifically in setting up developmental gradients of gene expression. Attila Molnar (John Innes Centre, Norwich, UK) presented an analysis of the small RNAs found in both vegetative *C. reinhardtii* cells and gametes. Differences in the small cytoplasmic RNAs were identified between gametes and vegetative cells, and environmental effects were also shown to affect the identity of the small RNAs that accumulate. Fadia Ibrahim (University of Nebraska-Lincoln) presented results on the involvement of a polymerase beta nucleotidyltransferase in RNA-mediated gene silencing. Cells mutant for this enzyme were deficient in RNAi of an introduced transgene, and the intermediate RNA cleavage products resulting from RNAi were stabilized. In wild-type cells, the cleavage products receive nontemplated oligo(A)$^+$ tails that seem to target them for degradation via the exosome, an exoribonuclease complex similar in architecture to the proteasome.

Chlamydomonas as a model for microtubule-based processes

Another 'old faithful' for studies in *C. reinhardtii* is the flagellum. A large number of groups presented work on everything from microtubule organization and sliding, to basal bodies (centrioles) and intraflagellar transport. In this field, *Chlamydomonas* serves in many ways as a model for microtubule-based mammalian cell processes. Lotte Pedersen (University of Copenhagen, Denmark) presented a well worked out model for the mechanism of trafficking of axonemal precursors (complexes comprised of tubulin, dynein and radial spokes, for example) from the base of the flagellum to the tip and back again. This model outlines the mechanisms by which intraflagellar transport complexes A and B are shuttled via a bidirectional microtubule-based transport system during assembly and maintenance of the flagella. In this model, complex A binds to the active motor proteins and complex B binds to complex A for trafficking. Turnaround of the complexes at the flagellar tip involves unloading of all cargos from the active motor, followed by reassembly on the retrograde motor for recycling to the flagellar base. Ben Lucker (University of Idaho, Moscow, USA) presented data on the composition of complex B, and outlined both a salt-stable core for this complex and specific interactions between various subunits. There was also a report from Qian Wang (University of Texas Southwestern Medical Center at Dallas, USA) on the involvement of intraflagellar transport in signal transduction, in the form of gamete activation in response to flagellar adhesion. A specific flagellar protein kinase was found to be activated by flagellar adhesion, and was also shown to be a cargo for intraflagellar transport. Both the biophysical properties and location of the protein kinase were altered in flagellar-adhering gametes with mutations affecting intraflagellar transport.

An interesting mutant that may help in dissecting the formation of the 9+2 microtubule arrangement in cilia and flagella was described by Yuuki Nakazawa (University of Tokyo, Japan). This mutant, *variable doublet number 1* (*vdn1*), has basal-body defects and assembles axonemes with varying numbers of outer doublet microtubules. In some cases the defect in the outer doublet microtubules affected the presence of the central microtubule pair, and double mutants that also lack radial spokes support the hypothesis that the presence of a central pair of microtubules depends on the space defined by the outer doublets and the radial spokes.

Jessica Feldman (University of California, San Francisco, USA) described an interesting study on the positioning of centrioles, the structures from which flagella arise, in the cell. Mutants with abnormal phototaxis were isolated, and one mutant, *askew2*, was found to have variable numbers of flagella as well as centriole-positioning defects. In an *askew2* double mutant that was only able to produce flagella from the original 'mother' centriole but not daughter centrioles, Feldman's group showed that mother centrioles were positioned correctly, but that daughter centrioles were randomly positioned, and proposed that the mother centriole needs to communicate to the daughter centriole to ensure its proper positioning within the progeny cell.

The meeting showed clearly that, with the nuclear genome sequence completed, and continually improving methods for nuclear and chloroplast transformation, *C. reinhardtii* remains an attractive model organism. Whether comparisons are required between *Chlamydomonas* and higher plants, mammalian cells or bacterial systems, biochemical and genetic studies are easy to carry out in this single-celled alga. *Chlamydomonas* also sits on the horizon of biotechnology, with a future as both a bioreactor and as a protein-expression platform. We look forward to seeing the progress that will undoubtedly be made before the next meeting in two years' time.