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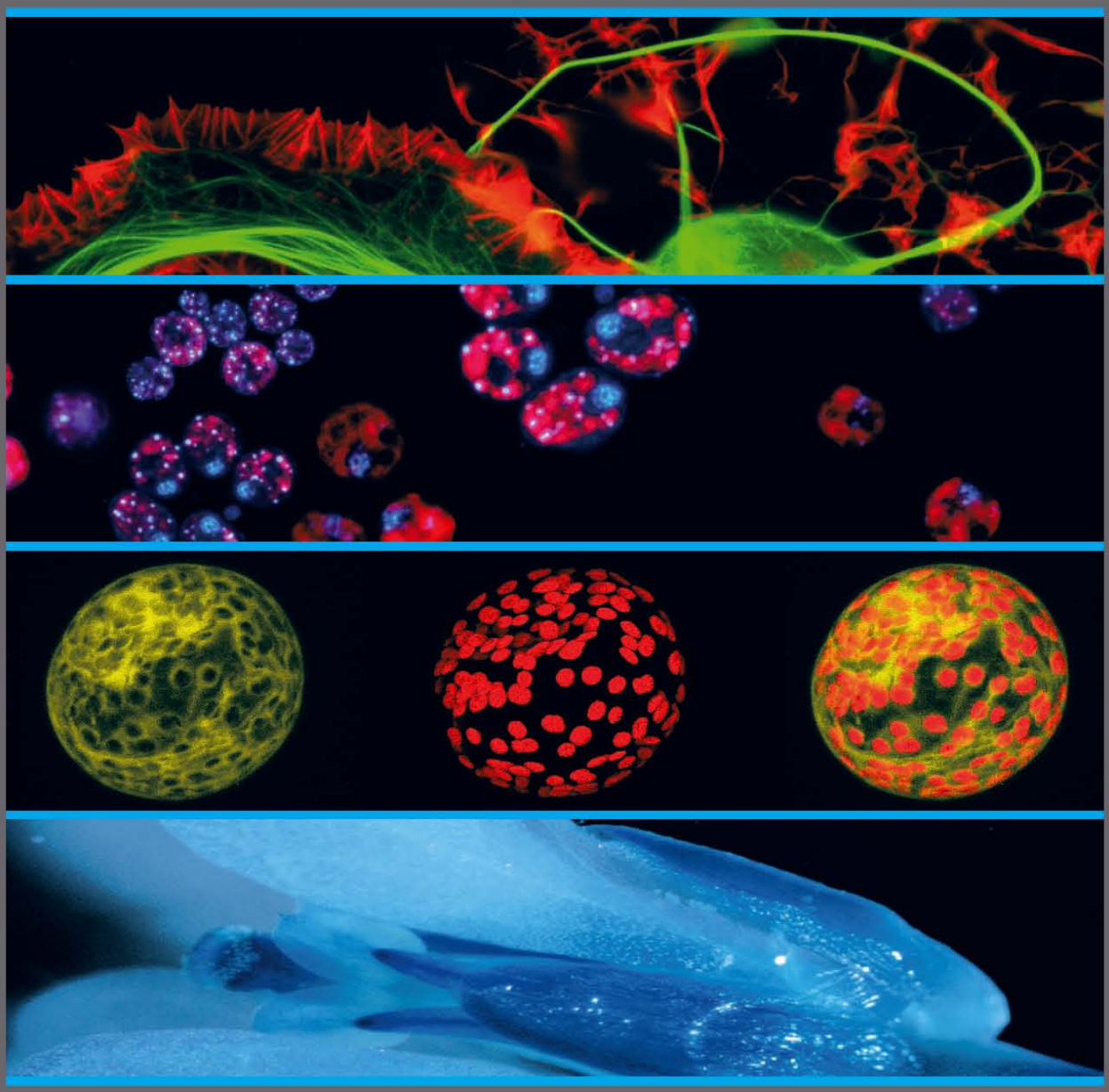
Potsdam-Golm BMBF- FORschungseinrichtung zur SYStembiologie

FORSYS
Research Units for Systems Biology

Scientific Report 2007-2009

Addendum



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GoFORSYS

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Photosynthesis and Growth:

A Systems Biology-based Approach

Scientific Report

- Addendum -

Reporting Period

01/2007 – 07/2009

Universität Potsdam
Max Planck Institute of Molecular Plant Physiology
Max Planck Institute of Colloids and Interfaces
FORSYS Research Units for Systems Biology

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Research Reports

(in alphabetical order)

1. Research Group

PI	Prof. Thomas Altmann
Group Member	Berit Ebert (postdoc)
	Date of employment: 03/09

2. Tasks worked on

WP 4 Testing of applicability of models developed in Chlamydomonas by comparison with the response of model higher plants

WP 4.3 Identification of genotypes differing in photosynthesis and growth

3. Achievements made

Collections of Arabidopsis recombinant inbred lines (RILs) and near isogenic lines (NILs) derived from crosses of naturally occurring accessions ("ecotypes") have been characterised in close collaboration with *Go*FORSYS partner 16 (Group Willmitzer) for their growth/biomass accumulation characteristics and their metabolic composition. Thus, 429 C24/Col-0 RILs and 369 RIL test crosses (RIL-TCs) to both parents, as well as 97 C24/Col-0 NILs and 41 NIL test crosses (NIL-TCs) cultivated in soil under controlled environmental conditions were analysed for shoot dry mass and metabolite composition (via GC-MS). Using RIL genotype and phenotype data, 6 biomass QTL and 157 metabolite QTL were detected. In addition to verification of most QTL by the NIL data, additional biomass and metabolite QTL were identified. In line with previous observations of a close relation between metabolite composition and plant growth, two biomass QTL coincided with significantly more metabolite QTL than expected for a random distribution. Furthermore, 7 QTL for biomass absolute mid-parent heterosis and 147 QTL for metabolite absolute mid-parent heterosis were detected by analysis of the RILs and RIL-TCs. Again, in addition to verification of most of the heterosis QTL in the RIL / RIL-TC population, additional heterotic QTL were detected using the NIL / NIL-TC data. The RIL / RIL-TC genotype and phenotype data were furthermore used in collaboration by GoFORSYS partner 10 to develop a procedure for improved heterosis prediction.

A major biomass QTL has been fine mapped to a genomic segment containing 14 protein coding genes that are under analysis for their contribution to growth control by investigation of mutants and transgenic plants. Similarly, 30 candidate enzyme-encoding genes located in QTL regions of 15 metabolites are being studied for their involvement in metabolite content determination. A set of 100 deeply genotyped Arabidopsis natural accession has been provided to GoFORSYS partner 12 for in-depth analysis of growth and metabolic traits such as enzyme activities, protein, starch, sugars, amino acids, chlorophylls, and metabolic intermediates. Analysis of growth / biomass accumulation of the complete set of 136 NILs plus their NIL-TCs under elevated light intensity has been initiated.

The assembled information lays a solid foundation for a comparative analysis between Chlamydomonas and higher plant growth-related molecular and metabolic features and for testing the applicability of models derived for the unicellular system in the other WPs.

4. Deviations from Work Program and Corrective Action

None

5. Future Perspectives

The information assembled for the Arabidopsis accessions, RILs, and NILs lays a solid foundation for a comparative analysis between Chlamydomonas and higher plant growth-related molecular and metabolic features and for testing the applicability of models derived for the unicellular system in the other WPs. Thus, Arabidopsis homologs to Chlamydomonas genes with prominent positions in expression networks and with connections to growth characteristics can be investigated for sequence variation associated with biomass and metabolism, information on Chlamydomonas gene-metabolism interactions can be used to test similar relations in Arabidopsis and growth-related metabolites and metabolite signatures associated with strong or weak growth can be compared between the two systems. In formation gained on the growth characteristics of NILs under standard and elevated light intensity will foster the selection of contrasting lines for in-depth analyses of the composition of the photosynthetic apparatus, gas exchange, chlorophyll fluorescence parameters, and direct measurements of photosynthetic light and dark reactions.

6. Cooperation

6.1 Internal Cooperation (within GoFORSYS)

GoFORSYS partner 16 (Prof. Dr. Lothar Willmitzer): Metabolic analysis of RILs / RIL-TCs, NILs / NIL-TCs and QTL detection

GoFORSYS partner 10 (Prof. Dr. Joachim Selbig): Identification of growth-related metabolic signatures and complex predictors of biomass and biomass heterosis

GoFORSYS partner 12 (Prof. Dr. Mark Stitt): Identification of integrators of the metabolic status of plants using natural accession collections

GoFORSYS partner 13 (Prof. Dr. Ralph Tiedemann): Analysis of sequence variation in photosynthetic Arabidopsis genes among natural accession collections

GoFORSYS partner 2 (Prof. Dr. Ralph Bock): Analysis of light reaction in diverse Arabidopsis genotypes

6.2 External Cooperation

Prof. Dr. Ulrich Schurr (Research Center Jülich): Quantification of plant growth rates using automated image acquisition and analysis

Prof. Dr. Albrecht Melchinger (Univ. Hohenheim): Growth and heterosis QTL analyses

Prof. Dr. Maarten Koornneef, Dr. Mathieu Reymond (Max Planck Institute for Plant Breeding Research, Cologne): Meta-QTL analysis

Prof. Dr. D. Weigel (Max Planck Institute for Developmental Biology, Tübingen): Analysis of Arabidopsis genome-wide sequence variation

7. Publications

[1] R. Sulpice, H. Tschoep, M. von Korff, D. Büssis, B. Usadel, M. Hoehne, H. Witucka-Wall, T. Altmann, M. Stitt and Y. Gibon (2007) Description and application of a rapid and sensitive non-radioactive microplate-based assay for maximum and initial activity of D-ribulose-1,5-bisphosphate carboxylase/oxygenase, Plant Cell Env. 30:1163-75

[2] J. Lisec, R. C. Meyer, M. Steinfath, H. Redestig, M. Becher, H. Witucka-Wall, O. Fiehn, O. Törjek, J. Selbig, T. Altmann and L. Willmitzer (2008) Identification of metabolic and biomass QTL in Arabidopsis thaliana in a parallel analysis of RIL and IL populations, Plant J. 53:960-72

[3] T. Gärtner, M. Steinfath, S. Andorf, J. Lisec, R. C. Meyer, T. Altmann, L. Willmitzer and J. Selbig (2009) Improved heterosis prediction by combining information on DNA- and metabolic markers, PLoS ONE 4(4):e5220

[4] R. Sulpice, E.-T. Pyl, H. Ishihara, S. Trenkamp, M. Steinfath, H. Witucka-Wall, Y. Gibon, B. Usadel, F. Poree, M. Conceicao Piques, M. von Korff, M. C. Steinhauser, J .J. B. Keurentjes, M. Guenther, M. Hoehne, J. Selbig, A. R. Fernie, T. Altmann and M. Stitt (2009) Starch as a major integrator in the regulation of plant growth, Proc. Natl. Acad. Sci. USA 106:10348-53

[5] J. Lisec, M. Steinfath, R. C. Meyer, J. Selbig, A. E. Melchinger, L. Willmitzer and T. Altmann (2009) Identification of heterotic metabolite QTL in *Arabidopsis thaliana* RIL and IL populations, Plant J. doi: 10.1111/j.1365-313X.2009.03910.x

[6] R. C. Meyer, B. Kusterer, J. Lisec, M. Steinfath, M. Becher, H. Scharr, A. E. Melchinger, J. Selbig, U. Schurr, L. Willmitzer and T. Altmann (2009) QTL analysis of early stage heterosis for biomass in Arabidopsis, Theor. Appl. Genet. doi: 10.1007/s00122-009-1074-6

8. Poster presentations

None

9. Teaching and Training activities

None

10. Organisation of Scientific Events

None

11. Invited talks

Trinational Arabidopsis Meeting; Vienna (Austria); Arabidopsis biomass natural variation and heterosis; 09/2007

Genetics and Plant Improvement; Versailles (France); Arabidopsis heterosis; 03/2008

Plant Breeding: from High Throughput Genotyping to Whole Genome Selection; Göttingen/Einbeck; Genome-wide genotyping and association testing; 11/2008

23. FSP-Kolloqium; Stuttgart-Hohenheim; Biomarkers; 11/2008

Heterosis and epigenetic Variation; Clermont-Ferrand (France); Arabidopsis heterosis; 12/2008

Int. Symp. Bioenergy: Harnessing Plant Metabolism; Tel Aviv (Israel); Arabidopsis biomass natural variation and heterosis; 02/2009

12. Commercialisation (Patents, Cooperation with Industrial Partners etc.)

Patent application: PCT/EP2007/011392 “Determination and prediction of the expression of traits…”

1. Research Group

PI	Prof. Ralph Bock, Mark A. Schöttler (PhD)
Group Members	Christin Albus (PhD student 03/07)
	Markus Rott (PhD student 02/07)
	Adam Idoine (PhD student 10/08)

2. Tasks worked on

WP3.1 and 3.2:
- *Chlamydomonas* growth
- *Chlamydomonas* transformation

WP3.3 and 4:
- Analysis of the light reactions in *Chlamydomonas* (also WP2.1: quantitation of all components of the electron transport chain *in vivo:* difference absorption spectroscopy, chlorophyll fluorescence)
- Analysis of gene expression: determination of transcriptional and translational responses in the three major classes of non-polyadenylated RNAs (chloroplast and mitochondrial transcripts, microRNAs): (i) steady-state transcript levels of chloroplast and mitochondrial transcripts, (ii) translational activities (analyzing polysomal RNAs on microarrays) and (iii) identification of *Chlamydomonas* non-coding RNAs by an experimental RNomics approach.

3. Achievements made

WP3.1, WP3.2, WP3.3: The group was heavily involved in the design of the GoFORSYS bioreactor for controlled growth of *Chlamydomonas reinhardtii*. An optimized bioreactor that allows single parameter variation experiments is now available. In cooperation with Heinz Walz GmbH (Effeltrich, Germany), an optimized fluorometer was designed which communicates with the LED-system of the bioreactor and triggers saturation pulse sequences. This enables us to directly determine chlorophyll-a fluorescence parameters in the bioreactor.

To facilitate the genetic analysis of photosynthesis and the in vivo monitoring of gene expression, new tools for *Chlamydomonas* research were developed, including new reporter genes (publications [1] and [2]) and new strains that efficiently express foreign genes and display higher transformation efficiencies (publication [2]). Also, the possible involvement of phytohormones in the growth and regulation of physiological processes in *Chlamydomonas* was critically assessed (publication [3]).

WP3.3 and 4: All methods required for the physiological analysis of photosynthesis have been established. All components of the photosynthetic electron transport chain can now be reliably quantified (PSI, PSII, plastocyanin and Cytb6f by difference absorption spectroscopy, plastoquinone by fluorescence induction measurements, ATP synthase by electrochromic shift measurements).

Methods for organellar transcriptomics and translatomics were successfully developed (publication [4]). An oligonucleotide microarray containing all genes and open reading frames in the plastid and

mitochondrial genomes of *Chlamydomonas reinhardtii* was designed and has been tested in transcriptomics and translatomics (polysome profiling) experiments. It allows the reliable detection and highly reproducible quantitation of all organellar transcripts in *Chlamydomonas*.

Libraries of small non-coding RNAs were constructed to assess the regulatory contribution of microRNAs and similar ncRNAs to the control of photosynthesis and related physiological processes. The libraries cover more than 25 growth conditions and developmental stages and thus should provide a nearly complete representation of the non-coding transcriptome of *Chlamydomonas*. Mass sequencing using Solexa technology has been completed (>88 million sequences) and has resulted in the identification of hundreds of new microRNA candidates. The potential microRNAs and their precursors have been analyzed bioinformatically. Target gene prediction is underway. A microRNA microarray has been designed and will be used for microRNA profiling in all GoFORSYS experiments.

4. Deviations from Work Program and Corrective Action

None.

5. Future Perspectives

Work will continue according to plan (organellar transcriptomics and translatomics, microRNA profiling as well as physiological measurements in all GoFORSYS experiments). In addition to the routine profiling of microRNAs in all GoFORSYS experiments, the detailed functional analysis of photosynthesis-relevant miRNAs and their targets will be pursued.

6. Cooperation

6.1 Internal Cooperation (within GoFORSYS)

Stitt: polysome analysis (method optimization, sharing of preparations)
Walther: microRNAs (bioinformatic identification, precursor folding, target prediction)
Schroda: photosynthesis physiology (bioreactor optimization)
Lokstein: photosynthetic electron transport

6.2 External Cooperation

FORSYS Partner project: O. Kruse (Univ. Bielefeld), M. Hippler (Univ. Münster), C. Posten (Univ. Karlsruhe) – hydrogen evolution and photosynthesis (transcriptomics, translatomics, proteomics and metabolomics under hydrogen-producing conditions; modelling; design of a full-genome microarray)

7. Publications

[1] N. Shao, R. Bock (2008) A codon-optimized luciferase from *Gaussia princeps* facilitates the *in vivo* monitoring of gene expression in the model alga *Chlamydomonas reinhardtii*, Curr. Genet. 54:381-388

[2] J. Neupert, D. Karcher, R. Bock (2009) Generation of *Chlamydomonas* strains that efficiently express nuclear transgenes, Plant J., 57:1140-1150

[3] S. Lau, N. Shao, R. Bock, G. Jürgens, I. De Smet (2009) Auxin signaling in algal lineages: fact or myth? Trends Plant Sci., 14:182-188

[4] S. Kahlau, R. Bock (2008) Plastid transcriptomics and translatomics of tomato fruit development and chloroplast-to-chromoplast differentiation: Chromoplast gene expression largely serves the production of a single protein. Plant Cell, 20:856-874

8. Poster presentations

[1] N. Shao, R. Bock: A codon-optimized luciferase from *Gaussia princeps* facilitates the *in vivo* monitoring of gene expression in *Chlamydomonas reinhardtii*, DFG meeting "Gene Expression and Proteome Dynamics in *Chlamydomonas reinhardtii*", Freiburg, Oct. 12-14, 2007

[2] J. Neupert, D. Karcher, R. Bock: Generation of *Chlamydomonas* strains that efficiently express nuclear transgenes, DFG meeting "Gene Expression and Proteome Dynamics in *Chlamydomonas reinhardtii*", Freiburg, Oct. 12-14, 2007

[3] N. Shao, R. Bock (2008) A codon-optimized luciferase from *Gaussia princeps* facilitates the *in vivo* monitoring of gene expression in *Chlamydomonas reinhardtii*, EMBO Workshop "Cell and Molecular Biology of Chlamydomonas", Hyeres-les-Palmiers (France), May 27 – June 1, 2008

9. Teaching and Training activities

„Molecular plant science: Photosynthesis, primary metabolism and growth", lecture series (MPI- MP, 4 h, Feb. 2008)

"Photosynthesis Research Methods", seminar series (MPI-MP, 20h, started Feb. 2009)

10. Organisation of Scientific Events

Co-Organizer of the International Chlamydomonas Conference, Berlin, Germany (2012)

8. Invited talks

[1] DFG meeting "Gene Expression and Proteome Dynamics in *Chlamydomonas reinhardtii*", Freiburg, "Generation of Chlamydomonas expression strains", Oct. 12-14, 2007

[2] EMBO Workshop "Cell and Molecular Biology of Chlamydomonas", Hyeres-les-Palmiers (France), "Generation of *Chlamydomonas* strains that efficiently express nuclear transgenes", May 27 – June 1, 2008

[3] EMBO Workshop "Cell and Molecular Biology of Chlamydomonas", Hyeres-les-Palmiers (France), "Towards systems biology of photosynthesis", May 27 – June 1, 2008

[4] Gordon Research Conference "Photosynthesis", Mount Holyoke (USA), "Metabolic control of photosynthetic electron transport" June 22-27, 2008

[5] Joint Annual Meeting of the Association for General and Applied Microbiology (VAAM); Bochum; Organelle transcriptomics and translatomics of *Chlamydomonas* reinhardtii; March 2009

12. Commercialisation (Patents, Cooperation with Industrial Partners etc.)

(Patent application, company name, subject, type and scope of activities etc.)

1. Junior Research Group

PI	Oliver Ebenhöh
Group Members	Erim Solmaz (PhD, 06/07)
(*Go*FORSYS)	Önder Kartal (PhD, 07/07)
	Georg Basler (programmer, 12/07)
	Katrin Tirok (postdoc, 09/08)
	Heike Aßmus (postdoc, 09/08)
	Kai Kruse (student, 10/08)
	Carolin Heeger (student, 01/09)
(non-*Go*FORSYS)	Nils Christian (PhD, 11/06 – IRTG 1360)
	Xiaoqing Li (PhD, 10/07 – IMPRS)
	Moritz Schütte (PhD, 11/07 – IRTG 1360)
	Heike Hameister (PhD, 12/07 – SFB 740)
	Alexander Skupin (postdoc, 12/08 – FORSYS-Partners)

2. Tasks worked on

The research group "Systems biology and mathematical modelling" focuses around the development of mathematical models and theoretical concepts of central processes in plant metabolism. We contribute mainly to *Go*FORSYS work package 1.

WP1.3 Integrated data analysis: We integrate metabolite profile data, proteomics and genomics into modelling approaches to predict missing reactions in the network of *Chlamydomonas reinhardtii*. Based on our predictions which genes may code for the missing enzymes we are currently designing new experiments together with experimental partners in GoFORSYS (esp. AG Schroda). On a theoretical basis, we compare our heuristic approaches with other theoretical descriptions developed by AG Schaub.

Based on the framework of metabolic control analysis (MCA), novel theoretical approaches are developed to gain insight into the origin of observed correlations, e.g. between metabolite levels or metabolites and enzyme activities. This understanding will lead to a better interpretation of these data and to more targeted experimental designs.

On a more fundamental level, we are investigating the structure of genome-scale metabolic networks to obtain hints of their evolutionary past. To test for pronounced properties that distinguish biological networks from comparable chemical networks as may occur in inanimate matter, we are presently, in cooperation with AG Selbig, developing concepts how metabolic networks can be randomized while still describing theoretically possible interaction networks.

WP1.4. Modelling and simulation methods: We analyze several aspects of plant carbon metabolism. Detailed models of the enzymatic activity of RuBisCO, in particular to study the fallover effect, have been developed (diploma thesis F. Witzel). Kinetic models of the Calvin cycle are established to investigate regulatory principles and adaptive behaviour of the system in response to external changes. Predictions will be experimentally tested in the bioreactor system.

The long known phenomenon that the fixed carbon dioxide is asymmetrically distributed in the produced starch is still not completely understood. Various hypotheses aiming to explain this so-called Gibb's effect are simulated with kinetic models and the predictions compared to experiments presently carried out in chloroplast extracts by Dr. Kempa.

Models are being developed simulating the breakdown of transitory starch. One focus is the initial attack on the granule's surface which is theoretically challenging since surface physics and biochemistry have to be integrated into a common mathematical framework. The functionality of the disproportionating enzyme is addressed by applying concepts from statistical physics. Predictions are presently tested experimentally by AG Steup.

On a faster time scale, kinetic models are formulated to simulate the dynamics of the light reactions. In particular, the adaptive properties are studied and predictions compared to fluorescence experiments carried out by AG Lokstein.

To describe cell population growth in the bioreactor, we develop population dynamical models which are fitted to the observed growth and medium exchange rates. Prediction of the models will help optimize the experimental setup of the bioreactor.

In the context of a FORSYS-partners program we are developing in cooperation with Prof. Mittag and Prof. Schuster, FSU Jena, models describing the autonomous circadian rhythm in Chlamydomonas. In cooperation with AG Kurths, we improve existing models for the clock in *Arabidopsis thaliana* with the specific aim to understand the temperature compensating mechanisms and unravel the coupling of the single cellular oscillators.

Further activities in cooperation with other theoretical groups aim at improving methods to fit models to experimental data as well as to discriminate the quality of different models.

3. Achievements made

Most kinetic models are implemented and are currently being studied on structural and dynamic levels. Close interaction with experimental groups have been established and our predictions enter in the planning and design of new experiments. Simultaneously, new experimental findings enter our models. The work has been presented on several conferences. Some articles have been published and three undergraduate theses have been supervised.

4. Deviations from Work Program and Corrective Action

5. Future Perspectives

All developed modelling and theoretical approaches bear great potential to be expanded. Our method to predict missing links in metabolic networks can be further expanded to include more data from other mass-spec techniques. A long term goal is to extend the concept such that not only reactions recorded in databases can be predicted, but completely novel synthesis routes can be suggested.

The systems investigated in the kinetic models are closely interlinked. We aim at developing an integrated model incorporating photosynthesis, carbon fixation, starch synthesis and starch degradation. A combination of such heterogeneous models is very challenging.

We aim at achieving a suitable degree of simplification based on the understanding of the single systems, which is gained by present modelling activities. The integrative model aims at characterizing the overall performance and efficiency of an organism under different and permanently changing environmental conditions. I expect that such a model is extremely useful to support targeted genetic modifications aiming at improving, for example, the carbon fixing capabilities.
Our basic research regarding the structure and evolution of metabolic networks will in the long term help to refine current theories on the evolution of metabolism and to elucidate under which constraints the present interaction networks have been shaped.

6. Cooperation

6.1 Internal Cooperation (within GoFORSYS)

AG Holschneider. Parameter estimation and model discrimination. Alternative parameter estimation techniques are developed with the goal to assess qualities of model fits and to discriminate between models.

AG Kurths. Circadian oscillations in *A. thaliana*. We aim at estimating the required strength of the coupling of the single cellular oscillators in order to explain the observed stable rhythms under circadian conditions.

AG Lokstein. Light adaptation in photosynthesis. The fluorescence measurements support model development and allow for hypothesis testing.

AG Saalfrank. Enzymatic mechanism of RuBisCO. Calculated activation energies of single enzymatic steps in the carbon fixating process enter our models to describe the temperature dependency of the fallover effect.

AG Schaub. Network optimization. Our algorithm to fill gaps is compared to methods developed by the computer scientists allowing to find global optima. We aim at understanding optimization principles in the organization of metabolism.

AG Selbig. Algorithmic complexity. The knowledge of the complexity of an algorithm has practical consequences for the planned development of widely applicable software tools and web services.

AG Steup. Degradation of transitory starch. Experiments support model development and allow to test new predictions.

AG Stitt. Calvin cycle. Model development and hypothesis testing.

AG Walther. Network completion. The knowledge of this bioinformatics research group allowed to effectively include sequence information in our predictive approach.

AG Schroda. Main resource for experimental data on Chlamydomonas: Metabolites, fluxes, proteins, growth rates etc. Model development and validation.

6.2 External Cooperation

Prof. Mittag, Prof. Schuster, FSU Jena, Circadian Clock in Chlamydomonas.

Prof. Kanehisa, Prof. Goto, Kyoto University. We plan to provide our developed algorithms as added services accessible via the KEGG database.

Prof. Segrè, Boston University. Projects to elucidate the evolutionary history of metabolism are being developed.

Dr. Rohwer, University Stellenbosch. Theoretical concepts to understand metabolite/flux/activity correlation data.

7. Publications

[1] Ö. Kartal and O. Ebenhöh (2009) Ground State Robustness as an Evolutionary Design Principle in Signaling Networks, ***submitted***

[2] G. Basler, O. Ebenhöh, J. Selbig and Z. Nikoloski, Uniform randomization of mass balanced metabolic networks, ***submitted***

[3] N. Christian, P. May, T. Handorf, S. Kempa and O. Ebenhöh (2009) An integrative approach towards completing genome-scale metabolic networks, Molecular BioSystems, in press, DOI:10.1039/B915913B

[4] M. Mutwil, B. Usadel, M. Schütte, A. Loraine, O. Ebenhöh and S. Persson (2009) Assembly of an interactive correlation network fort he Arabidopsis genome employing a novel heuristic clustering algorithm, ***submitted***

[5] O. Ebenhöh and T. Handorf (2009) Functional classification of genome-scale metabolic networks, EURASIP Journal on Bioinformatics and Systems Biology, ***in press***

[6] K. Kruse and O. Ebenhöh (2008) Comparing flux balance analysis to network expansion: Producibility, sustainability and the scope of compounds, Genome Inform, ***in press***

[7] G. Basler, Z. Nikoloski, O. Ebenhöh and T. Handorf (2008) Biosynthetic potentials from species-specific metabolic networks, Genome Inform, ***in press***

[8] J. Numata, O. Ebenhöh and E. W. Knapp (2008) Measuring correlations in metabolomic networks with mutual information, Genome Inform, ***in press***

[9] Z. Nikoloski, S. Grimbs, J. Selbig and O. Ebenhöh (2008) Hardness and approximability of the inverse scope problem, Lecture Notes in Bioinformatics, ***in press***

[10] Ö. Kartal and O. Ebenhöh (2008) The glucan water dikinase – a kinetic model to understand the initial step in starch mobilization in plant leaves. In: From Computational Biophysics to Systems Biology, NIC Series, Vol. 40:245-248.

[11] P. May, S. Wienkoop, S. Kempa, B. Usadel, N. Christian, J. Rupprecht, J. Weiß, L. Recueno-Munoz, O. Ebenhöh, W. Weckwerth and D. Walther. (2008) Metabolomics and proteomics assisted genome annotation and analysis of the draft metabolic network of *Chlamydomonas reinhartii*, *Genetics* 179:157-166.

[12] F. Matthäus, C. Salazar and O. Ebenhöh. (2008) Biosynthetic potentials of metabolites and their hierarchical organization (2008). PLoS Comput Biol 4(4).

[13] T. Handorf, N. Christian, O. Ebenhöh and D. Kahn (2008) An environmental perspective on metabolism, J Theor Biol 252:530-537.
[14] N. Christian, T. Handorf and O. Ebenhöh (2007) Metabolic Synergy: Increasing biosynthetic capabilities by network cooperation, Genome Inform 18:320-329.

8. Poster presentations

1) N. Christian and O. Ebenhöh; Temperature Dependence of Metabolic Networks of Archaea and Bacteria; German Conference on Bioinformatics; Potsdam; 09/2007
2) E. Solmaz and O. Ebenhöh; Kinetic modeling of the Calvin cycle; German Conference on Bioinformatics; Potsdam; 09/2007
3) Ö. Kartal and O. Ebenhöh; Mathematical modelling of starch breakdown in leaves: the role of Disproportionating Enzyme; German Conference on Bioinformatics; Potsdam; 09/2007
4) H. Hameister and O. Ebenhöh; A mathematical model of the proliferating cell nuclear antigen (PCNA); International Conference on Systems Biology of Mammalian Cells; Dresden; 05/2008
5) Ö. Kartal and O. Ebenhöh; The Glucan, Water Dikinase – A kinetic model to understand the initial step in starch mobilization in plant leaves; From Computational Biophysics to Systems Biology; Jülich; 05/2008;
6) H. Hameister and O. Ebenhöh; A mathematical model of the proliferating cell nuclear antigen (PCNA); European Conference on Mathematical and Theoretical Biology; Edinburgh; 06/2008
7) N. Christian, P. May, T. Handorf, D. Walther and O. Ebenhöh; Classification of Missing Enzymes in Large Scale Metabolic Networks; 5th International Conference on Plant Metabolomics; Yokohama; 07/2008
8) E. Solmaz and O. Ebenhöh; Kinetic modeling and regulation mechnisms of the Calvin cycle; International Study Group for System Biology; Helsingör; 08/2008
9) E. Solmaz and O. Ebenhöh; Kinetic modeling of the Calvin cycle; 9th International Conference on System Biology; Göteborg; 08/2008
10) G. Basler, Z. Nikoloski and O. Ebenhöh; Randomization of metabolic networks with biochemical constraints; European Conference on Computational Biology; Cagliari; 09/2008
11) M. Schütte, E. Kramer, J. Rohwer and O. Ebenhöh; The effects of parameter deviations on reactant concentrations in metabolic networks; RECOMB 2008; Boston; 10/2008

9. Teaching

Theoretical Evolution (1SWS) WS 2008/2009
Mathematical Modelling of Biological Systems (2SWS) SS 2008
Ausgewählte Kapitel der Systembiologie (2SWS) WS 2007/2008
Molecular Plant Science: Photosynthesis and Primary Metabolism (1 lecture in series) WS 2007/2008

10. Organisation of Scientific Events

Workshop *Metabolic Networks, Dynamics, Evolution, and Topology*, MPIMP, 11/2008
IRTG and *Go*FORSYS lecture & seminar, MPIMP, 02/2008

11. Invited talks

Abdus Salam School and Conference 'From Biological Networks to Cellular Functions'; Trieste; *Modeling large-scale metabolic networks: Structure, Function and Evolution;* 06/2009

SIAM Conference on Dynamical Systems; Snowbird; *An integrative approach towards completing genome-scale metabolic networks;* 05/2009

Trinational Arabidopsis Meeting; Zurich; *Filling the gaps in genome scale metabolic networks;* 09/2008

Workshop on Integrative Network Analysis; Potsdam; *Predicting function from structure: Inferring minimal nutrient requirements from the structure of organism-specific metabolic networks;* 04/2008

German Conference on Bioinformatics; Potsdam; *GoFORSYS;* 09/2007

Molecular Interactions Workshop; Berlin; *Genomes, Organisms and their Environments: A new level of complexity;* 09/2007

The Third UniNet Workshop 'Robustness of Network Models'; Girona; *Robustness and Flexibility in the Evolution of Metabolic Networks;* 06/2007

Bioinformatics & Biomathematics Symposium; Amsterdam; *Structural and functional analysis of large-scale metabolic networks;* 04/2007

1. Research Group

PI	Prof. Matthias Holschneider
Group Members	Bruno Schwenk, Postdoc (08/08)

2. Tasks worked on (break down by WPs and tasks)

Development of Bayesian approach for the identification of functional relations in metabolomic datasets. Visualisation of the space of all possible linear relations through Hesse normal form. Extension to higher dimensional data sets and non linear relations. Interactive extraction of local correlations through integrated software tool. Pre-treatment of data for biologic networks. The major contribution of this partner will be to WP1.

3. Achievements made

We contributed to WP1.3 by developing a data analytical method for the robust and rotationally n-variant detection, representation and visualisation of 2-dimensional linear functional relationships in 3-dimensional profile data.

Our method ExPlanes (Exploring Planes in profile data) consists of two steps: In a first step we compute from a rotational and translational invariant flat prior, the Bayesian posterior in the space of all possible 2-planes in 3-space. This is done by exploring a suitable natural parametrization of this space. In a second step we define a family of test statistics as functionals of this posterior distribution that enable the detection of specific relationships. In addition we use the plane posterior to perform a classification task.

In additionally contributing to WP1.3 and WP1.4 we created a method to analyse the relationship between kinetic models and time course data that allows us to fit the parameters of a given model, explore parameter correlations resulting from incomplete data and to compare the likelihood of structurally different models.

On the basis of the so called Ensemble-Kalman-Filter (EnKF) already described in literature we developed an stochastic algorithm for the fully Bayesian inversion of non linear differential equations and non Gaussian Markov models that gives an estimator for the likelihood of the data given the model, it's parameter values and the co-variances of a Gaussian error model.

We augmented this core part of our method by a two hierarchical Bayesian error models, that allow the estimation of the measurement error co-variances from the time series data themselves without relying on biological or technical replicates of the measurements. This enables treatment of dynamical processes which are highly sensitive with respect to parameters and initial conditions, that therefore cannot be reproduced experimentally to reliably obtain replications.

We devised an homogeneous Gaussian error model that assumes an unknown error co-variance that is identical for all time points of measurement and an inhomogeneous model which assumes different and unknown co-variance matrices for each of the time points that interact with a common hyperprior distribution.

The Bayesian nature of the model allows us to gradually incorporate prior information of the dynamical parameters as well as on the measurement errors. In addition we expect from the heterogeneous error model an increased robustness against single, outlying data points, that is deemed to be essential for the application on in vivo data.

In our practical work with Bayesian methods we found that a great amount of manpower is used to represent, visualize and explore posterior densities. The technical work of programming and debugging the computer code can be very time consuming, especially for stochastical methods of representation. We therefore designed an object-oriented computer code we call “PostEx” that facilitates representation, visualization and exploration of Bayesian posteriors for a wide class of models. The code will be distributed as an R-package and contains both stochastical and grid-representations and is provided with a minimalistic command-line interface. Because of the object oriented structure it can be easily maintained and extended, which we think will become important as spectral methods of posterior-representation will become popular. We set a high value in a professional design of the software and extensive testing.

4. Deviations from Work Program and Corrective Action

Our experience with the Ensemble-Kalman-Filter suggested us to follow the line of investigating the underlying dynamical and causal processes that generate biological profile data. In this we see three different aspects that we will try to elucidate: We will apply the EnKF to the analysis of models and data generated in the GoFORSYS project, to see its practical limitations. In addition we will investigate methods of numerical representations of Bayesian posteriors in complex structured models. Especially we will focus on sparse matrix representations of Generalized Linear Mixture Models (GLMM).

Finally we will study the structure of chemical reaction systems themselves trying to obtain insight in their functionality and properties as data generating processes.

5. Future Perspectives

At a theoretical level we think that the bottleneck for the successful and reliable application of Bayesian methods lies in the numerical representation of posteriors. This encompasses the aspects of appropriate sets of basis functions and the development of algorithms using them. At present we see three approaches in literature, namely sample-representations, point-representations (either grid or weighted sample) and semi-analytical approaches using density functions from the exponential family. We believe that spectral methods, using Fourier-type polynomials will come under general investigation in the near future. We will therefore concentrate on mathematical techniques founded in functional analysis. On the practical level we see that object oriented programming will go along with the development of such more abstract views on approximation in the space of densities.

6. Cooperation

6.2 Internal Cooperation (within GoFORSYS)

- Group of Prof. Dr. Martin Steup; Kinetic measurements and models of biochemical processes. Obtain better understanding of the techniques of measurement used in experimental biology.
- Dr. Oliver Ebenhöh; Kinetic modelling of metabolic and genetic processes.
- Prof. Dr. Joachim Selbig; Analysis of functional relationships in profile data.

6.2 External Cooperation

Group of Prof. Dr. Reinhold Kliegl; Generalized Linear Mixture Models.

7. Publications

Bruno Schwenk, Joachim Selbig, Yehuda Ben-Zion, Matthias Holschneider (2009)
ExPlanes: Exploring Planes in Profile Data.
IEEE Transactions on Computational Biology and Bioinformatics; Submitted.

8. Poster presentations

Bruno Schwenk, Joachim Selbig, Matthias Holschneider:
"Planes in 3-Dimensional Metabolite Triplet Data: A Robust, Bayesian Approach"
RECOMB 2009, Singapore, (March 2008)

Bruno Schwenk, Matthias Holschneider:
"The Ensemble-Kalman-Filter (EnKF) for Parameter Estimation"
German Symposium on Systems Biology 2009
Heiderlberg (May 2009)

9. Teaching and Training activities

"Introduction into Bayesian Data Analysis for Biologists and Bioinformaticians"
Golm (July 2009)

10. Commercialisation

Patent Application:
Matthias Holschneider, Bruno Schwenk:
"Method for investigating ternary relationships"
European Patent Office;
Application Number: EP09158291.6
Submission Number: 563613

1. Research Group

PI	Prof. Dr. Dr.h.c Jürgen Kurths
Group Members	C. Zhou, postdoc 01/2007-08/2007
	Aneta Koseska, postdoc, 09/2007-12/2009

2. Tasks worked on

WP1:	Developing mathematical models in order to understand underlying regulatory mechanisms for cellular decision making;
WP1.1, WP1.3:	Developing algorithms for reconstruction of regulatory networks from experimental data (transcriptomics);
WP1.3, WP4.2:	Understanding fundamental design principles of genetic and metabolic networks;
WP1.4, WP4:	Understanding dynamical properties of circadian clock.

3. Achievements made

WP1: We have shown, via theoretical modeling of a population of noise-driven bistable genetic switches, that reliable timing of decision-making processes can be accomplished for large enough population sizes, as long as cells are globally coupled by chemical means. Moreover, we have investigated the mechanisms responsible for cellular differentiation of cells and shown that a specific type of dynamical behavior, the oscillation death phenomena, similar to Turing structures, only without space localization is the background for morphogenesis.

WP1.1, WP1.3: We have developed a novel algorithm for reconstruction of TF networks from experimental data, and are currently testing it on ANAC042 regulatory network (publication in preparation). The algorithm will be used to reconstruct TF regulatory networks in C.reinhardtii, using experimental data from the core experiments (work planned for November/December 2009).

WP1.3, WP4.2: We have shown that symmetry breaking instabilities are very important regulators of the genetic networks dynamics. Moreover, we have shown that this phenomenon has direct implications to the dynamics of metabolic processes, by investigating in particular a modified Zhu model of the Calvin cycle (publication in preparation).

WP1.4, WP4: We investigate the co-regulation and synchronization between single circadian clocks in *A.thaliana* leafs. We account for the specificity of the leaf structure by developing a specific type of "fractal" coupling, which we are currently implementing in an *A.thaliana* circadiian clock model.

4. Deviations from Work Program and Corrective Action

- none

5. Future Perspectives

We plan to develop mathematical methods and models for dynamical reconstruction of gene regulatory networks from experimental data of *C.reinhardtii* (proteomics, transcriptomics, metabolomics) using random Boolean networks and principles from selforganization of complex systems. Moreover, we will expand our previous investigations on cellular differentiation, by investigating the influence of network motifs on the dynamic properties of this mechanism. Next year, we will also develop a dynamical model characterizing the electron transport chain in *C.reinhardtii*, performing additional stability analysis of the proton fluxes etc. Furthermore, we will work on stress response analysis, especially connected to the regulatory networks reconstructed with the methods and models developed from our group, and look in details in the processing of the information, using for e.g. transfer entropies and/or similar measures.

6. Cooperation

Internal Cooperation (within GoFORSYS)

1*. AG Müller-Röber:* (a) Reconstruction of TF regulatory networks in *C. reinhardtii* (cooperation also with *AG Schroda)*, (b) Development of synthetic oscillators for regulation of photosynthetic processes in *A. thaliana:* The theoretical and experimental investigation of synthetic genetic regulatory networks we have accomplished in the previous years are the basis for construction and experimental realization of a synthetic oscillator which coupled to the circadian oscillator should provide regulation and improvement of photosynthetic processes;

2. *AG Holschneider and AG Ebenhöh:* Investigation of circadian clock properties in *A. thaliana* - we have developed a C++ toolbox for integration and analysis of gene regulatory networks.

3. *AG Selbig:* Investigations of symmetry breaking instabilities in metabolic processes and analysis of its implications and importance on the functionality of biological systems.

6.2 External Cooperation

1. *Prof. Dr. E.Volkov*, Department of Theoretical Physics, Lebedev Physical Inst., Moscow, Russia and *Prof. Dr. A.Zaikin*, Department of Mathematics, University College London, UK: Investigation of dynamical regimes in gene regulatory networks;

2. *Prof. Dr. J.Garcia-Ojalvo*, Departament de Fisica i Enginyeria Nuclear, Universitat Politecnica de Catalunya, Terrassa, Spain and *Dr. E. Ullner*, School of Natural and Computing Sciences, University of Aberdeen, UK: Investigations of cellular differentiation mechanisms, clustering, attractor properties;

3. *Prof. Dr. C. Arevalo Ferro*, Universidad Nacional De Colombia – Bogotá, Colombia: Engineering synthetic circuits;

4. *Prof. R. Ramaswamy*, J. Nehru University, Delhi, India: nonlinear methods to analyze genetic networks.

7. Publications

[1] A. Koseka, A. Zaikin, J. Garcia-Ojalvo, J. Kurths (2007), Stochastic suppression of gene expression oscillators under intercell coupling, Phys. Rev. E 75: 031917;

[2] A. Koseska, E. Volkov, A. Zaikin, J. Kurths (2007), Inherent multistability in arrays of autoinducer coupled genetic oscillators, Phys. Rev. E 75: 031916;

[3] A. Koseska, E. Volkov, A. Zaikin, J. Kurths (2007), Quantized cycling time in artificial genetic network induced by noise and intercell communication, Phys. Rev. E 76: 020901(R);

[4] G.Z.Lopez, V.Zlatic, C.Zhou, H.Stefancic, J.Kurths (2008), Reciprocity of networks with degree correlations and arbitrary degree sequences, Phys. Rev. E. 72: 016106;

[5] E.Ullner, A.Koseska, E.Volkov, J.Garcia-Ojalvo, H.Kantz, J.Kurths (2008) , Multistability of synthetic genetic network with repressive cell to cell communication, Phys. Rev. E 78: 031904

[6] A. Arenas, A. Diaz-Guilera, J. Kurths, Y. Moreno, C. Zhou (2008): Synchronization in Complex Networks, Physics Reports 469: 93;

[7] A.Koseska, A. Zaikin, J.Kurths and J. Garcia-Ojalvo (2009), Timing cellular decision making under noise via cell-cell communication, PloS ONE 4: e4872

[8] A.Koseska, E.Volkov and J.Kurths (2009), Detuning-dependent dominance of oscillation death in globally coupled synthetic genetic oscillators, Europhys. Lett. **85:** 28002

[9] A. Koseska, E. Ullner, E. Volkov, J. Kurths and J. Garcia-Ojalvo (2009), Cooperative differentiation through clustering in multicellular populations, submitted, Journal of theoretical biology

[10] A. Koseska, E. Volkov and J. Kurths (2010), Oscillation quenching mechanims: amplitude vs. oscillation death, Invited review for Physics Reports, to appear in spring, 2010

Book chapters: [11] E. Ullner, A. Koseska, A. Zaikin, E. Volkov, J, Kurths and J. Garcia-Ojalvo, Dynamics of multicellular synthetic gene networks, in "*Handbook on biological networks*", World Scientific Press, to appear in fall, 2009

8. Poster Presentations

1. A. Koseska, E. Ullner, E. Volkov, J. Kurths and J. Garcia-Ojalvo, *Cooperative behavior of multicellular populations,* German Symposium on Systems Biology, Heidelberg, Germany, 05/2009;
2. A. Koseska, E. Volkov and J. Kurths, *Synthetic gene networks for cellular regulation,* 1st FORSYS Symposium, Berlin, Germany, 06/2008;
3. A. Koseska, A. Zaikin, E. Volkov and J. Kurths, *Detuning-dependent effects in synthetic genetic circuits: quantized cycling and oscillation quenching dominance,* BioSysBio - Synthetic Biology, Systems Biology and Bioinformatics, Imperial College London, UK, 03/2008.

9. Teaching and Training activities

1. Workshop (lecture plus exercise): Modeling small gene networks, University of Potsdam, 05/2009-06/2009;
2. 4 SWS course: Introduction to theoretical systems biology, University of Potsdam, SS2009

10. Organisation of Scientific Events

1. 3rd International IEEE Scientific Conference on Physics and Control, Potsdam, 09/07
2. 3rd Conference: BioSim – Network of excellence, Potsdam, 10/07

11. Invited talks

1. J. Kurths: SynCoNet, Leuven, Belgium, Dynamics on complex networks, 07/2007

2. A. Koseska: 3rd PhysCon conference, Potsdam, *Dynamical properties of genetic networks,* 09/2007;

3. J. Kurths: Int. Conf. Life Systems Modeling and Simulations, Shanghai, *Synchronization in complex networks and its application in life sciences*, plenary talk, 09/2007;

4. A. Koseska: 2nd BioSim network of excellence conference, Potsdam, *Modeling of synthetic regulatory networks,* 10/2007;

5. J. Kurths: ERA – Network workshop, Budapest, *Complex networks in systems biology* , 11/2007;

6. J. Kurths:Dutch Phys Soc Meeting, Nijmegen, *Emerging properties from interacting units*, 04/2008;

7. J. Kurths: Workshop Complexity, Academ. Europaea, Heidelberg, *Complex systems approach in systems biology*, 04/2008;

8. J. Kurths: 3rd KIAS Conf Stat. Phys. Seoul, *Complex networks - fashionable or useful?*, 07/2008;

9. J. Kurths: 3rd Conf. BIOSIM-EU network, Budapest, *Network of networks*, 09/2008;

10. J. Kurths: Conf. Science of Complexity, Elat, *Inferring connectivity in genetic networks*,03/2009;

11. A. Koseska: German Symposium on Systems Biology, Heidelberg, *Symmetry breaking instabilities in metabolic processes*, 05/2009;

12. J. Kurths: Era-Network opening Conf., Brussels, *Complex networks from brain to climate*,05/2009, keynote lecture;

13. J. Kurths: EMBO Conf., Cambridge, *Bifurcation analysis of synthetic genetic networks*, 06/2009;

14. A. Koseska: Dynamics in System Biology, Aberdeen, UK, *On the reconstruction of gene regulatory networks*, 09/2009.

12. Commercialisation (Patents, Cooperation with Industrial Partners etc.)

None

1. Research Group

PI	Prof. Reinhard Lipowsky
Group Members	Angelo Valleriani (group leader)
	Apoorva Nagar (postdoc, since June 1st 2008)
	Michael Rading (PhD student, since February 1st 2008)

2. Tasks worked on

WP1:

Project 1: Description of cell cycle and cell size distribution of Chlamy.
(Michael Rading, PhD student); Cooperation with: Martin Steup and Heiko Lokstein.
Project 2: Polysome size and ribosome traffic on mRNA
(Apoorva Nagar, Postdoc); Cooperation with Mark Stitt.

3. Achievements made

Description of cell cycle and cell size distribution of Chlamy. (Michael Rading, PhD student)

We have first concentrated on the mathematical description of the cell size distribution for Chlamy cells under constant conditions. We have derived the general continuity equations that govern the stationary size distribution under constant environmental conditions. Using some simplifying assumptions about the growth rate and cell division rate, we can obtain analytical solutions. Furthermore, we have developed a numerical algorithm, by which we can numerically calculate the stationary distributions for realistic growth rates and cell division rates. The second subproject addresses the scenario related to synchronized cells. Since experiments on such cells have already been performed by the Steup group, the synchronization problem offers an attractive example to test some aspects of our model. In fact, we find that it is possible to compute the distribution of the cells at the beginning of each light period using relatively simple but general assumptions. Indeed, experiments performed so far suggest that (i) the cell size increases linearly with time and that (ii) the number of divisions is determined by the size of the cell at the start of the dark phase. With our model, we can show that, under these assumptions, the size distribution of the daughter cells at the beginning of the light phase should become narrower and narrower with increasing number of generations. Since such an evolution of the size distribution does not seem to be observed, experiments are being set up in order to find deviations from the two basic assumptions.

Modelling polysome size and ribosome traffic on mRNA (Apoorva Nagar, Postdoc)

The aim of this project is to model mRNA/ribosome interactions and to calculate the number of ribosomes attached to mRNAs. Experiments conducted on plant leaves (Arabidopsis Thaliana) and on Chlamy cells show that there is a bimodal distribution concerning the number of ribosomes on mRNA. In fact, many mRNA turn out to have no ribosomes attached to them, they are "unloaded", whereas the remaining fraction carry a variable number of ribosomes.

One important feature of this observation is that the fraction of unloaded mRNA changes after changing light conditions from dark to light and vice versa. Since ribosomes attach to a given mRNA and then move along this mRNA in a directed manner in order to perform translation, the loading of mRNA by ribosomes can be modelled as an asymmetric simple exclusion process. Using such a model, we can calculate the distribution of the number of ribosomes attached to the mRNAs and the dependence of this distribution on the length of the mRNA. Such a calculation has been performed for those mRNAs, for which the Stitt group has experimentally measured the ribosome number distribution. Currently, such data are available for about a hundred mRNAs but this number could be increased to several thousands. The asymmetric simple exclusion process leads to nonequilibrium phase transitions that depend on the effective loading rate of the mRNA with ribosomes. It should be possible to deduce this effective loading rate from the observed ribosome number distributions. This effective loading rate could be different for different mRNAs because of the various processes that are involved in the initiation and elongation phases of translation. One nontrivial question that we currently address is whether this effective rate attains a typical value independent of the coding segment of the mRNA or if it differs strongly between different mRNAs. Our current translation model is relatively simple and is not intended to describe all aspects of the initiation, elongation, and termination phases involved in the translation process. Our general modelling philosophy is to look for discrepancies between the model predictions and the data. If we can identify such discrepancies, they provide important hints for the development of more detailed models. A third part of the project deals with the effect of mRNA turn-over on polysomal statistics and on the translation efficiency. We have developed a simple stochastic model to address this question. More detailed modeling that also takes the sequence of specific targeted mRNA into account will be performed. A comparison between polysomes in chloroplasts and cells will reveal if polysomal activity and turn-over of mRNA have different effects.

4. Deviations from Work Program and Corrective Action

The objective of Project 1 is to understand and model the experimental observations about the size and age distribution of cells in the culture. This should give us insights into the metabolic states of the cells under different environmental conditions. This project arose during the initial setup of the research network as an important link between the experimental and theoretical activities.

Project 2 arose more recently as a need to predict theoretically the conditions under which polysomes are observed.

5. Future Perspectives

Project 1 aims at first comparing the cell size distributions with those that arise in cell cultivations under synchronous conditions and under continuous light. Further goals are to understand the dependence of the model parameters on environmental conditions.

Project 2 aims at constructing a model for the prediction of the distribution of polysomes and its dependence on environmental conditions. Taking into account also the turn-over of the mRNA, this study will also give us a precise tool to compute the effective translation rate.

6. Cooperation

6.1 **Internal Cooperation (within GoFORSYS)**

Project 1: Martin Steup and Heiko Lockstein

Project 2: Mark Stitt

6.2 External Cooperation

None

7. Publications

None

8. Poster presentations

None

9. Teaching

- Reinhard Lipowsky and Angelo Valleriani, Course, “Theory of biosystems”, IMPRS on Biomimetic Systems, WS0607
- Reinhard Lipowsky, Course, “Biosystems: From Molecules to Networks”, HU-Berlin and IMPRS on Biomimetic Systems, WS0708 and WS0809
- Reinhard Lipowsky and Angelo Valleriani, Seminar, “Recent advances in Biophysics”, IMPRS on Biomimetic Systems, SS07
- Angelo Valleriani and Oliver Ebenhöh, Course, “Theoretical Evolution”, University of Potsdam, WS0809
- Angelo Valleriani, Course and Practicum, “Stochastic Modelling in Evolution”, University of Potsdam, WS0809

10. Organisation of Scientific Events

- “Summer School on Biomimetic Systems”, Max Planck Institute of Colloids and Interfaces,
- Golm, June 2007. Organizers: Reinhard Lipowsky and Angelo Valleriani.
- Workshop “Active Biomimetic Systems”, Max Planck Institute of Colloids and Interfaces, Golm, July 2007. Organizers: Reinhard Lipowsky and Angelo Valleriani.

11. Invited talks

04.05.2007 Evry07 “Modellling of complex biological systems”, Paris

21.05.2007 CNLS conference on “Complexity of biological and soft materials”, Santa Fe

11.06.2007 Colloquium, Hahn Meitner Institute, Berlin

15.01.2008 Colloquium, University of Stuttgart

07.05.2008 Workshop on “Bio-inspired complex networks”, MPI-PKS, Dresden

15.05.2008 Symposium on “Complexity in materials far from equilibrium”, Blacksburg

21.04.2009 HFSP workshop on “Synthetic molecular motors”, Bristol

20.05.2009 Colloquium, Humboldt University, Berlin

03.06.2009 Colloquium, University of Duisburg

1. Research Group

PI	Heiko Lokstein
Group Members	Stephanie Schlede, PhD student, 07/07
	Andreas Garz, PhD student, 06/08
	Katrin Scholtyssek, student worker, 03/09

2. Tasks worked on

WP2 and WP3:

Elucidation of the regulation of photosynthesis and photoprotection in *Chlamydomonas,* in particular the network of influences on, and the mechanisms of photoprotective non-photochemical quenching of chlorophyll fluorescence (NPQ). Bulk fluorescence and pigment measurements with *Chlamydomonas* cultures, also in the framework of the *core-experiments*, are complemented with fluorescence and growth measurements with single *Chlamydomonas* cells to facilitate qualified modelling of photosynthesis, growth and their regulation by external and internal stimuli in *Chlamydomonas.*

3. Achievements made

We have established pulse-modulated chlorophyll (PAM) fluorescence measurements with *Chlamydomonas* cultures to elucidate regulation of photosynthesis and photoprotection. To achieve this aim an adapter to reproducibly measure PAM fluorescence parameters with *Chlamydomonas* cultures with a commercial fluorometer has been devised. We were able to show that different culturing conditions (e.g., autotrophic, heterotrophic, CO_2 limited or -replete) will result in a different capacity for photoprotective non-photochemical quenching of chlorophyll fluorescence (NPQ). Moreover, it was shown that the major fraction of NPQ in autotrophic *Chlamydomonas* cultures is of qE type (i.e., membrane- ɘnergetization dependent). Since NPQ (qE) capacity in *Chlamydomonas* apparently depends on the accumulation of the novel LhcSR ("stress related") protein we have started to isolate and functionally characterize the LhcSR protein in collaboration with the FORSYS-Partner group of M. Hippler, Münster (*vide infra*). Measurements with different *npq*-mutants of *Chlamydomonas* and *Arabidopsis* were done. These data have also been used in modelling photosynthesis and its regulation (*vide infra*). The room-temperature fluorescence measurements were complemented by 77 K-fluorescence measurements to elucidate underlying mechanistic aspects (in collaboration with the group of M.A. Schöttler). The first results have been presented as a poster at the 13th International Chlamydomonas Confererence (*vide infra*).

We have adapted a commercial confocal microscope (in combination with home-made *optical tweezers* and *microfluidic devices*) for manipulation and fluorescence (including chlorophyll fluorescence using a modified PAM technique) and growth measurements with single *Chlamydomonas* cells.

Collaboration was established with the groups of O. Ebenhöh and P. Saalfrank to enable modelling of photosynthesis and its regulation, in particular also the role of xanthophylls in NPQ in *Chlamydomonas* and *Arabidopsis*. First results were presented as a poster (*vide infra*), a respective paper is in preparation.
In the framework of the *Go*FORSYS *core-experiments* chlorophyll fluorescence, photosynthetic pigment (in particular, xanthophyll cycle activity) and LhcSR accumulation were measured.

4. Deviations from Work Program and Corrective Action

Very recently it has become apparent that NPQ (qE) in *Chlamydomonas* depends on the accumulation of the LhcSR protein. Thus we have started a collaboration with the FORSYS-Partner group of M. Hippler (Münster) to isolate and functionally characterize the LhcSR protein.
It was originally planned to conduct single cells measurements with *Chlamydomonas* in collaboration with the TU Berlin. This, however, proved to be not very practicable. The problem was overcome by adaptation of a commercial confocal microscope (starting in 06/2008) at the Institut für Physik und Astronomie, Universität Potsdam, to the specific requirements of *Chlamydomonas* single cell analyses (in collaboration with Prof. Dr. R. Menzel)

5. Future Perspectives

Our ultimate goal is to understand and to model *Chlamydomonas* photosynthesis, in particular, chlorophyll fluorescence signals and NPQ (under varying growth conditions) and its impact on growth and productivity as a systemic approach. We strive to isolate and functionally characterize the novel LhcSR protein and the regulation of its expression at all systemic levels. We aim at measuring chlorophyll fluorescence parameters and crucial metabolites/proteins as well as cell growth with single *Chlamydomonas* cells.

6. Cooperation

6.1 Internal Cooperation (within GoFORSYS)

Dr. Oliver Ebenhöh, Modelling of algal and plant photosynthesis, in particular chlorophyll fluorescence signals and stress responses
Prof. Dr. Peter Saalfrank, Modelling of the photophysical properties of carotenoids/xanthophylls in their native protein environments to understand their roles in regulation of photosynthesis and photoprotection
Dr. Mark Aurel Schöttler, Spectroscopic measurements of algal and plant photosynthesis and its regulation

6.2 External Cooperation

Prof. Dr. Michael Hippler, Institut für Biochemie und Biotechnologie der Pflanzen, Westfälische Wilhelms-Universität Münster; Isolation, purification and functional characterization of the LhcSR protein and its role in NPQ in *Chlamydomonas*

7. Publications

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8. Poster presentations

S. Schlede, S. Rheinhardt, H. Lokstein; Investigations on the regulation of photosynthesis and photoprotection in *Chlamydomonas rheinhardtii* with special regard to systems biology; 13th International Chlamydomonas Conference, Hyeres-les-Palmiers, France, Mai 27-June 1, 2008

K. Tirok, T. Houwaart, H. Lokstein, S. Schlede, O. Ebenhöh; Modeling photosynthetic electron transport and its adaptability; German Symposium on Systems Biology, Heidelberg, May 12-15, 2009

A. Garz, S. Schlede, K. Tirok, O. Ebenhöh, R. Menzel, H. Lokstein; Chlorophyll fluorescence measurements with single cells of the photosynthetic model organism *Chlamydomonas rheinhardtii* (in the frame-work of a systems biology approach); Gordon Research Conference-Photosynthesis, Bryant University, USA, June 28-July 03, 2009

9. Teaching

H. Lokstein: Lecture series: „**Biophysik der Photosynthese**“, Universität Potsdam, (winter semester 2006/07 and summer semester 2008)

2 Ring lectures „**Molecular plant science: Photosynthesis, primary metabolism and growth**“ (winter semester 2007/08)

Lecture series: „**Photosynthese**“, Universität Potsdam, (winter semester 2008/09)

Practical course „**Pflanzenphysiologie**“, Universität Potsdam, (winter semester 2006/07 and summer semester 2007)

S. Schlede: Practical course „**Pflanzenphysiologie**“, Universität Potsdam, (winter semester 2007/08 and summer semesters 2008 and 2009)

A. Garz: Practical course: “**Grundpraktikum Physik**”, Universität Potsdam, (summer semesters 2008, 2009 and winter semester 2008/09)

10. Organisation of Scientific Events

None

11. Invited talks

1. Pflanzenwissenschaftliches Kolloquium; Universität Münster; “The role(s) of xanthophylls in photoprotection: mechanistic aspects”; November 25, 2008

2. Institutscolloquium MPIBPC Göttingen; “Pigment-pigment interactions in the peridinin-chlorophyll *a*-protein“; May 06, 2009

3. Seminar für Optik und Photonik, TU Berlin, “Chlorophyll-carotenoid interactions in photosynthetic pigment-protein complexes”; July 17, 2009

1. Research Group

PI	Prof. Bernd Müller-Röber
Group Members	PhD students Flavia Vischi Winck (since 08/2007) and Raúl Trejos Espinosa (07/2007 – 05/2009)

2. Tasks worked on (break down by WPs and tasks)

Contribution mainly to WP1.2 and WP3.1

- Establishment of RNA isolation procedure;
- Establishment of multi-parallel qRT-PCR based expression profiling platform for transcription factor genes, photosynthetic genes, cell cycle genes, and starch synthesis and degradation genes; identification of suitable reference genes;
- Establishment of a protocol for the isolation of nuclei from Chlamydomonas based on principles and methods of differential centrifugation;
- Comparative analysis of nuclear protein sample preparation for shotgun proteomics. This step was performed by testing the efficiency of protocols for nuclear protein extraction/precipitation. Different methods using urea buffer, acetone and trizol as extraction chemical agents were compared;
- Development of the website Chlamydomonas Transcriptional Regulation Initiative "ChlamyTRI" (http://plntfdb.bio.uni-potsdam.de/ChlamyTRI/) to enhance the interaction of researchers interested in Chlamydomonas transcription factors/regulators. The site was developed using a pre-established blog platform, were information related to transcription factor analysis was included, e.g. weblinks to TF databases, weblinks to nuclear localization prediction tools and open discussion blog sessions;
- Calculation of the codon bias distribution for Chlamydomonas coding DNA sequences (CDSs). The CDSs were extracted from the JGI website/database. The Chlamydomonas codon frequency table was generated using around 9000 CDSs (a previous table was generated from only 846 CDSs; published in http://www.kazusa.or.jp/codon/cgi-bin/showcodon.cgi?species=3055). The CAI (codon adaptation index), CB (codon bias) and amino acid frequencies of Chlamydomonas and Arabidopsis were compared;
- Prediction of nuclear / nuclear subcellular localization signals of the whole Chlamydomonas proteome using the three tools BaCelLo, NucPred and PredictNLS (http://gpcr.biocomp.unibo.it/bacello/), http://www.sbc.su.se/~maccallr/nucpred/, http://cubic.bioc.columbia.edu/predictNLS/);
- Nuclear proteome analysis of Chlamydomonas CC503 grown in standard phototrophic condition. Proteins were pre-fractionated by 1D-PAGE and identified by shotgun proteomics. A target-decoy search strategy was used for protein identification and determination of the FDR (False Discovery Rate);
- Expression profiling of 736 genes including 234 genes encoding for transcription factors (TFs) of Chlamydomonas under light shift experimental condition (200µE to 700µE);

3. Achievements made

- In a genome study of *Chlamydomonas reinhardtii* transcription factors (TFs) and transcription regulators (TRs) we identified 234 genes encoding 147 TFs and 87 TRs of more than 30 families. A comparison between this set of genes and the respective orthologues of *Arabidopisis thaliana*, *Oryza sativa*, *Populus trichocarpa*, *Vitis vinifera*, *Cyanidioschyzon merolae* and *Ostreococcus tauri* was performed with the aim to understand the evolutionary relationships of these genes. Twelve out of 34 plant-specific TF families were found in at least one algal species, indicating their early evolutionary origin. Twenty-two plant-specific TF families and one plant-specific TR family were not observed in algae, indicating their specific association with developmental and/or physiological processes characteristic to multicellular plants. A search for orthologues of genes of the light-regulated transcriptional network of Arabidopsis in Chlamydomonas was done.
- Putative binding sites for E2F-DP transcription factors (c/gGCGCg/c) were identified in promoters of Chlamydomonas genes (genes coding for transcription factors, cell cycle genes, carbon metabolism/photosynthetic genes, and starch related genes). A comparative study was done in *Ostreococcus tauri*, *Arabidopsis thaliana* and *Oryza sativa* promoter regions for orthologous genes.
- A multi-parallel qRT-PCR platform for expression profiling in *Chlamydomonas reinhardtii* was established. The platform covers 736 genes: carbon metabolism/ photosynthetic genes (410); transcription factors (245), cell cycle genes (30), starch synthesis and regulation genes (48), and reference genes (3). Reference genes encode actin (PI:24392), ubiquitin (PI:190824), elongation factor (PI:24524).
- Expression profiles of the 736 genes included in the qRT-PCR platform were obtained for Core Experiment I (light shift from 200 µE to 700 µE). The experiment was conducted with four biological replicates and respective controls. The experiment had ten time points and the data of the expression levels were normalized by the Quantile method. The clustering approach (Euclidean distance) indicated the appropriate separation of the data into two main groups: control and treatment samples.
- An improved protocol for the isolation of nuclei of Chlamydomonas was established and a manuscript describing it submitted (Winck *et al*.).
- More than 141 proteins were identified from the nuclear preparations (cells grown under standard phototrophic conditions) by shotgun proteomics. Some of them could be functionally annotated, including 15 transcription factor/regulatory proteins. Most of the proteins identified have never been described in standard plant nuclear proteomic analyses before. Validation of the protein identification was done by verifying the FDR (False Discovery Rate) and FPR (False Positive Rate) with a target-decoy search strategy.

- A list of proteins predicted to contain nuclear localization signals (NLS) was generated. A comparative analysis of the Chlamydomonas proteome using the three different prediction tools BaCelLo, NucPred, and PredictNLS revealed 47.9%, 1.0% and 6.0%, respectively, NLS-containing proteins. These data indicate the need for an improvement of nuclear localisation prediction tools.
- A Chlamydomonas codon frequency table using the genomic data set was created. A comparison of the CAI and CB distribution for the collected CDSs of Chlamydomonas and Arabidopsis datasets using the software CodonW revealed differences in bias between the two plants.
- Release of the first version of the ChlamyTRI website (http://plntfdb.bio.uni-potsdam.de/ChlamyTRI/).
- For quantitative growth analysis in Arabidopsis we set up a LemnaTec Scanalyzer system in spring 2009. A number of control experiments was performed to establish growth and analysis protocols for Arabidopsis plants grown in 12-well microtitre plates and in soil, which allows analysing growth for up to about 2 weeks (before leaves start to overlap). A further protocol was established, using 96-well plates, to quantify germination and early seedling growth at high throughput. The Lemnatec Scanalyzer will be used to define growth parameters with high resolution in Arabidopsis wild-type and mutant plants. A publication describing analysis protocols and limitations of the system is in preparation (S. Arvidsson *et al*.).

4. Deviations from Work Program and Corrective Action

No deviations in the field of transcriptome analysis. The proteome analysis was extended to include nuclear proteomics to advance the knowledge about nuclear proteins and assist in their annotation. For the Arabidopsis work we additionally included quantitative growth analysis using the LemnaTec Scanalyzer system. We also searched for genes (focusing on transcription factors) that are prominently expressed during the leaf expansion phase in Arabidopsis. Functional analysis of such genes has been included.

5. Future Perspectives

- Expression profiling of Chlamydomonas genes (photosynthetic genes, transcription factor genes etc.) under various experimental conditions (using qRT-PCR and micro-arrays).
- Modelling of gene expression behaviour (together with biochemical studies performed in other groups in GoFORSYS).
- Establishment of the conditions and methods to perform comparative and quantitative nuclear proteomic analysis of Chlamydomonas using shotgun proteomics and N15 labelling method.
- Comparative nuclear proteomic analysis of Chlamydomonas grown in different conditions to assist in the identification of nuclear proteins and genome annotation.
- Application of methods for the recognition of protein sequence motifs (e.g. MEME) for nuclear proteins of so far unknown function.

- Development of a new strategy to identify protein-DNA interactions.
- Integration of the codon bias and the NLS prediction data into the ChlamyCyc webportal.
- Further elaboration on the use of the LemnaTec Scananlyzer system to integrate molecular data with growth processes in Arabidopsis.
- Further characterization of transcription factors in Arabidopsis that control leaf expansion under normal and stressful conditions.

6. Cooperation

6.1 Internal Cooperation (within GoFORSYS)

- Jens Rupprecht. Preparation of Chlamydomonas cultures for growth at different conditions with the aim to identify genes regulated by developmental stages or environmental stimuli (light, nutrients, stresses).
- Patrick May, Diego Riaño-Pachón, and Sabrina Kleessen. Analysis of transcription factor binding sites in promoters of Chlamydomonas genes using bioinformatic tools.
- Stefanie Wienkoop. Establishment of a method for protein extraction/precipitation; training in proteomics.
- Michael Schroda, Frederik Sommer. Nuclear proteome analysis by shotgun proteomics.
- Diego Riaño-Pachón. Analysis of the stability of secondary structures of RNA molecules of Chlamydomonas.

6.2 External Cooperation

- Systems approach to study the role of tRNAs for translational control in Chlamydomonas and Arabidopsis; with Prof. Dr. Zoya Ignatova, University of Potsdam
- Identification of proteins using mass spectrometry: collaboration with a research group for protein sequencing (Laboratory of Protein Chemistry, UNICAMP, Campinas, São Paulo, Brazil).
- Analysis and data interpretation of the codon bias distribution in Chlamydomonas: collaboration with the research group of Dr. Stefan Rensing (FRISYS, University of Freiburg, Germany).
- Analysis of growth regulatory genes in Arabidopsis, in collaboration with Prof. Dr. Lazlo Bögre, Royal Holloway, University of London
- Analysis of chloroplast development control genes in Arabidopsis, in collaboration with Dr. Enrique Lopez, Royal Holloway, University of London
- Analysis of signalling pathways induced by cytokinins in Chlamydomonas cells, in collaboration with Dr. Alexander Heyl, Free University, Berlin.

- Study of the stability of secondary structures of RNA molecules of Chlamydomonas, in collaboration with Dr. Paulino Pérez-Rodriguez, Colégio de Pós-Graduados, Texcoco, Mexico.

7. Publications

Winck, F.V., Kwasniewski, M., Wienkoop, S., Mueller-Roeber, B. An optimized method for the isolation of nuclei from *Chlamydomonas reinhardtii* (*Chlorophyceae*). J. Phycology, submitted.

Pérez-Rodríguez, P., Riaño-Pachón, D., Guedes Corrêa, L., Rensing, S., Kersten, B., Mueller-Roeber, B.: PlnTFDB: Updated content and new features of the Plant Transcription Factor Database. Nucl. Acids Res., submitted.

D. M. Riano-Pachon, L. G. Correa, R. Trejos-Espinosa, B. Mueller-Roeber (2008) Green transcription factors: a chlamydomonas overview , Genetics 179, 31-39.

Merchant, S.S., Prochnik, S.E., Vallon, O. *et al*. (2007) The Chlamydomonas genome reveals the evolution of key animal and plant functions. Science, 318, 245-251.

Winck F.V., Riano-Pachon D.M., Mueller-Roeber B. Transcriptional regulation initiative, http://plntfdb.bio.uni-potsdam.de/ChlamyTRI/ (2008).

9. Poster presentations

- Raúl Trejos Espinosa, Diego Mauricio Riaño-Pachón, Luiz Gustavo Guedes Corrêa and Bernd Mueller-Roeber; Identification and functional analysis of *Chlamydomonas* transcriptional regulators for systems biology; 1st FORSYS Symposium on Systems Biology; Berlin; 19-20 (June/2008)
- Winck FV, Kwasniewski M, Mueller-Roeber B; Towards Chlamydomonas nuclear proteomics: Optimizing the isolation of nuclei; "Cell and Molecular Biology of Chlamydomonas "*(13th International Chlamydomonas Conference*); Hyères-les-Palmieres -France; (May 27-June 01/2008).
- Winck FV, Kwasniewski M, Mueller-Roeber B; Towards Chlamydomonas nuclear proteomics: Optimizing the isolation of nuclei; Forsys meeting; Berlin-Germany; (June 19, 2008).
- Winck FV, Kwasniewski M, Riaño-Pachón DM, Mueller-Roeber B; Towards Chlamydomonas nuclear proteomics: Optimizing the isolation of nuclei and aggregating information; Bridging Public and Private Research on Bioinformatics and Proteomics; Geneva, Switzerland (Dec. 03-04, 2008).
- Winck FV, Kwasniewski M, Riaño-Pachón DM, Wienkoop S, Mueller-Roeber B. Towards the nuclear proteome of Chlamydomonas reinhardtii; THE DNA-PROTEOME: Recent advances towards establishing the protein-DNA interaction space; Barcelona, Spain (April 20-22, 2009) .
- Winck FV, Kwasniewski M, Riaño-Pachón DM, Wienkoop S, Sommer F, Mueller-Roeber B. Towards the nuclear proteome of *Chlamydomonas reinhardtii*. German Symposium on Systems Biology; Heidelberg, Germany (May 12-15, 2009).

10. Teaching

The PI is full professor at the University of Potsdam and teaches general molecular biology, genomics, molecular plant biology, and synthetic biology in each semester.

The PhD students taught some basic concepts in molecular biology (practical courses for Bachelor students) in one semester.

11. Organisation of Scientific Events

- Co-organiser of conference “Synthetic Bio(techno)logy" (together with acatech and DECHEMA); 9.-10. November 2009; Frankfurt/Main, Germany
- Co-organiser of workshop "Synthetic Biology" (acatech/DFG/Leopoldina), 27. February 2009, Berlin, Germany

12. Invited talks

- Systems Biology meeting, MPI for Mathematics, Leipzig; Presentation of the GoFORSYS research concept; January 12, 2007;
- Tri-National Genomics Meeting, Vienna: ´Global and local views on *green* transcription factors´. Sept. 12-15, 2007;
- 10 Years BioBilanz, Potsdam; ´GoFORSYS – Photosynthesis and Growth: A Systems Biology based Approach´; December 4, 2007;
- Palacky University Olomouc, Czech Republic, ´Genomics of plant transcription factors´; January 29, 2008;
- University of Silesia, Katowice, Poland, ´Genomics of plant transcription factors´; January 31, 2008;
- Adam Mickiewicz University, Poznan, Poland, ´Genomics of plant transcription factors´; February 28, 2008;
- ´Cell and Molecular Biology of Chlamydomonas´, *13th International Chlamydomonas Conference*); Hyères-les-Palmieres, France; ´Nuclear proteomics´ (segment of a discussion session in the Workshop ´Genomes´); May 31, 2008.
- 17. March 2009: Centro de Citricultura Sylvio Moreira; “Plant systems biology”, Cordeirópolis, Brazil.
- 18. March 2009: University of Campinas; “Plant systems biology”, Campinas, Brazil.
- 27. March 2009: University of São Paulo; “Plant systems biology”, Piracicaba, Brazil.
- 18. June 2009: University of Göttingen; „Transcription factors - from genes to genomes to regulatory networks in plant systems”
- 6. July 2009: University of Kiel; „Transcription factors - from genes to genomes to regulatory networks in plant systems”
- 17./18. July 2009: “Synthetische Biologie – auf dem Weg zu einer neuen Zukunftstechnologie”; Symposium “Neue Technologien im Spannungsfeld von Wissenschaft, Politik, Öffentlichkeit und Wirtschaft”, München

- 14. September 2009: „Systems biology of plant growth control "; Treffpunkt Bioinformatik, BioTOP Veranstaltung, Berlin
- 16. September 2009: “Gene regulatory networks and transcription factor transcriptomics”;14th European Congress on Biotechnology, Barcelona

12. Commercialisation (Patents, Cooperation with Industrial Partners etc.)

None at present.

1. Management Education

PI Carsten Müssig

2. Tasks worked on (break down by WPs and tasks)

WP E, Implementation of an educational concept

3. Achievements made

The educational concept includes new curricula for MSc students and doctoral students. The following achievements were made:

- **I.** Establishment of a new Master of Science programme at the University of Potsdam (MSc Bioinformatics, start October 2008)
- **II.** Establishment of an interdisciplinary doctoral programme in systems biology
- **III.** Installation of a learning management system based on the Open Source software Moodle and development of an eLecture facility (start February 2008 and January 2009, respectively).

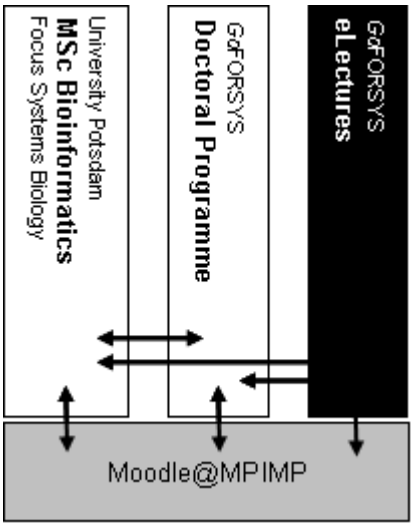

Fig. 1. The *Go*FORSYS teaching activities.

The different teaching activities are complementary (Fig. 1). Several courses of the MSc Bioinformatics programme are open for PhD students, and several courses of the doctoral programme (e.g., the lectures series) are open for MSc students. eLearning activities were initiated during the past months. The technical and didactical basics for the creation of eLectures were established and templates were defined. The LMS Moodle is used to organize courses.

I. Master of Science in Bioinformatics with focus on systems biology

A Master of Science programme in Bioinformatics with a focus on systems biology was established. It became an integral element of the curriculum of the University of Potsdam, and represents one of four MSc programmes offered by the Institute of Biochemistry and Biology. The design of the new curriculum corresponds to the plans initially described in the *Go*FORSYS proposal. Our curriculum offers a unique training for students with different backgrounds, and the students learn to communicate and work in all disciplines that are relevant for bioinformatics and systems biology. Detailed information is available via the web page http://www.bio.uni-potsdam.de/professuren/bioinformatik/lehre/informationen-zum-masterstudiengang (in German). The programme spans four semesters (120 credit points). The first semesters include courses at the undergraduate level (‘bridging courses’) that allow students with different scientific backgrounds to achieve a common knowledge base (in total 18 CP). Students with a background in biology attend courses in computer science (modules “Informatics for science students”), students with a background in computer science visit courses in biology (modules “Biology for informatics students”).

Semesters 2 and 3 offer courses in bioinformatics (required courses “Algorithmic bioinformatics”, “Statistical bioinformatics”, “Sequence bioinformatics”, “Analysis of networks and profiles”, and “Structural bioinformatics”) and courses in systems biology (required course „Theoretical systems biology“, elective courses „Methods and techniques: systems biology, informatics“, „Experimental systems biology“). Further elective courses focus on nutrigenomics (module „Nutrigenomics“) and model organisms and their analysis (module „Model systems and methods of genome science“). Semester 4 is reserved to work on the Master’s thesis (30 CP).

II. A PhD programme in Systems Biology

A structured doctoral programme in systems biology was implemented. A mission statement and guidelines for doctoral students and supervisors were developed. The *Go*FORSYS doctoral programme provides intellectual and technical training through original research, interdisciplinary dialogue, and training of transferable skills. The programme also offers support regarding personal concerns.

Supervision: The thesis research is carried out under the direct supervision of a member of the *Go*FORSYS faculty. Each doctoral project within *Go*FORSYS is discussed amongst the group leaders, ideally within the first three months after the start of the project. The discussion takes place within the frame of the monthly *Go*FORSYS seminar. The respective supervisor introduces the new project. In addition to the direct supervisor, each doctoral student within the *Go*FORSYS programme is supported by an independent thesis committee. The thesis committee is designed to give advice and guidance to the student and evaluate the progress of the research project during yearly meetings. In these meetings, the progress of the research project and possible future experiments and research directions are discussed. Written and oral progress reports allow the doctoral students, their supervisors and thesis committees to evaluate the progress of the thesis project.

Curriculum: The doctoral candidates benefit from several teaching activities. These activities have two major aims:

- Support of doctoral students and their scientific projects
- Development of transferable skills (in particular communication skills)

The curriculum within the *Go*FORSYS doctoral programme consists of different elements: lectures, seminars, practical courses, complementary skills courses, teaching experience, and conference visits. Doctoral students should collect at least 20 credit points (1 CP = 25-30 h) within the curricular activities. 13 CP are awarded for compulsory training elements, the remaining 7 (or more) CP can be distributed over selectable elements. It is also possible to collect CP in another institute, e.g. via lab visits and external courses.

III. eLearning@GoFORSYS

Moodle: Moodle is a learning management system (LMS). I am operating a Moodle server for the *Go*FORSYS programme (http://moodle2008.mpimp-golm.mpg.de/). It is the central communication

and organisation platform for teaching activities. About 280 active Moodle@MPIMP users are registered. The platform also serves to link different PhD programmes. For example, two events (the seminar “Applying for Grants” [August 29 2008] and the workshop “Protein Expression and Analysis” [October 13.-15. 2008]) were jointly organised with the VaTEP doctoral programme via this platform.

Another example is the use as communication platform for coordinators. Moodle@MPIMP currently houses the web space for a network of about 40 PhD programme coordinators, founded by the PoGS (Potsdam Graduate School; http://www.uni-potsdam.de/pogs).

eLectures: An e-learning facility that is based on eLectures is in development. The aim of this facility is to provide digital courses that impart basic knowledge in the disciplines relevant for Systems Biology. First eLecture examples can be found via a preliminary web page:
http://www-de.mpimp-golm.mpg.de/veranstaltungen/veranstLehre/eLectures/index.html

4. Deviations from Work Program and Corrective Action

The educational concept was implemented. Additional activities were initiated.

5. Future Perspectives

The following aspects will be addressed in the future:

- Establishment of a network of doctoral programmes (both local and with external institutes). This will happen in close cooperation with the PoGS.
- Further development of the *Go*FORSYS curriculum for doctoral students (e.g., establish a set of core courses that runs regularly and represents courses that are in high demand).
- Establishment of an eLearning facility based on eLectures and Moodle@MPIMP.

6. Cooperations

There is a close interaction with the PoGS (Potsdam Graduate School) which is led by Heike Küchmeister. The scheduled eLearning activities are discussed with the workgroup eLearning at the University of Potsdam (in particular with Jörg Hafer) and the eLearning advisory committee of the University of Potsdam. The *Go*FORSYS doctoral programme interacts with the IMPRS-PMPG doctoral programme that is led by Ina Talke. Ina Talke establishes a monitoring procedure that will be used in an adapted form by the *Go*FORSYS doctoral programme.

1. Research Group

PI	Jens Rupprecht	
Group Members	Adam Idoine	PhD (October 2008)
	Liliya Yaneva-Roder	Technician (January 2009)

2. Tasks worked on

The research group focuses mainly on the development, establishment and improvement of a photo-bioreactor system suitable for a systems biology approach. The demands on such a system are: (1) Allocation of large volume, sufficient to provide experimental groups samples from the same time point; (2) constancy of parameters; (3) optimal light supply; (4) rapid sampling; (5) non-invasive technologies via on-line monitoring systems; (6) data (on-line & off-line) acquisition. Beside the technical challenges of reactor design we are involved in the development of downstream methods of sample processing.

With the biological system changing under different environmental conditions it is of utmost importance to be able to define, control and maintain the environment of an organism in order to achieve reliable data. The photo-bioreactor is the central device which must guarantee a traceable controlled environment where certain relevant parameters must be measurable and data can be acquired. Standard on-line parameters are pH, temperature, dissolved oxygen, cell density and exhaust gas analysis (via gas chromatography).

An important parameter for photoautotrophic culturing is the carbon dioxide (CO_2) flux. Beside its effect for the growth performance of a culture, CO_2 is a valuable tool to gain more information about the metabolism. On-line data of gas composition and flux can be measured via a multi-valve gas-chromatography system.

Realizing evenly distributed light inside the bioreactor often fails. Conventional fluorescence tubes are not appropriate as the tubes (i) show edge effects where light intensity is altered, (ii) season in short time which makes continuing control of intensities necessary, (iii) cause variation in light entry already at the surface. Therefore we decided to employ a unique LED system. Two half shells were equipped with LEDs of white, blue, red and far red colour to be able to perform light dependent experimental variations (fig. 1).

A useful parameter for the status of a photosynthetic culture is the fluorescence of protein-pigment complexes, elements of the photosynthetic electron chain. Instruments able to measure this light emission are Pulse Amplitude Modulation (PAM) fluorometer. Primary biophysical events in photosynthesis including the efficiency of PSII photochemistry can be measured on-line. In the large volume of a photo-bioreactor it is necessary to support the PAM system with excitation light provided by the light system.

Many biochemical reactions are fast and therefore difficult to resolve. The separation of cells and medium is a difficult task as the procedure may take too long or the cells are harmed or stressed. A fast sampling device allows, together with an appropriate quenching procedure, to tackle even reactions in millisecond resolution and freeze the metabolic processes for further analysis.

The 2nd focus of the group is the investigation of transcriptomics/translatomics of *Chlamydomonas* chloroplast and mitochondrial encoded genes. The complete set of genes from the two organelles is not covered on the *Chlamydomonas* chip from the Carnegie Institution of Washington. Therefore important information is missing. We designed and spotted a macro-array containing all those genes and use this for the GoFORSYS core experiments but also the FORSYS partner project.

3. Achievements made

We have established a newly designed photo-bioreactor system which meets the requirements of a systems biology approach. The bioreactor system can control critical environmental conditions and the changing of single actuators. That allows targeted investigations on the influence of one selectable culturing parameter on the organism. Mixotrophic and photoautotrophic batch and chemostat cultivation has been established. A quenching procedure to stop metabolic processes immediately was developed with the AGs Stitt and Weckwerth/Schroda.

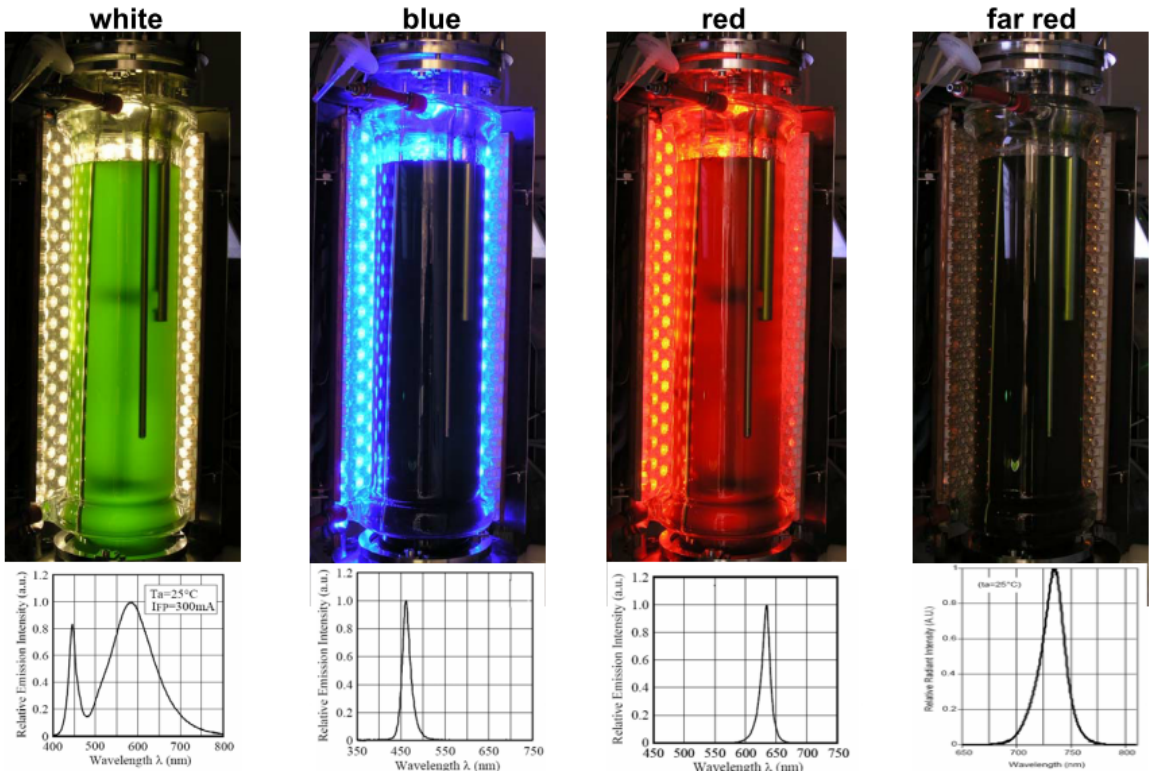

Fig. 1: LED light system. Independent control of 4 different light qualities is possible allowing a range of light dependent experiments on the physiology. The lower panel shows the emitting spectra of the single LED bulbs.

The macro-array chip with all genes from chloroplast and mitochondrium genome was designed and established. The procedure for polysome isolation in *Chlamydomonas* was established and new software for the analysis of isolated polysome fractions has been developed with AG Walther. The regulation of mitochondrium and chloroplast genes seems to take place mostly on the level of translation.

The macro-array data does not show significant changes of transcription due to the light shift. However, we see significant changes in the loading mRNA with ribosomes in the early time points already. That points to an amplification of the translation machinery.

We got precise data from the fermenter runs, providing us with a nice data framework for the further physiological studies. For example, we can see that with increase/decrease of light intensity the cells response immediately with changing in O_2 production rate. With the increase of light intensity by factor 3.5 we see an elevation of cell doubling time by almost the same factor (3.6).

We are currently working on the establishment of an *in situ* microscope. This is intended to be used as an additional instance of the cell density controlling system and allows monitoring the cell cycle to obtain valuable information on the cells shape and division.
The function of the light system has been evaluated by measuring the light penetration at different culture densities for various light intensities. Culture densities up to 4E+06 cells/ml are appropriate. Continuous culturing can be performed independent of a controlling device via a constant medium in-flow, or by the use of a turbidometer controlling the optical culture density. Here however, some adaptations are still necessary in order to improve the result.
The medium composition was investigated during the fermenter run with ICP-MS. Due to the continuous addition of medium none of the components is limiting.

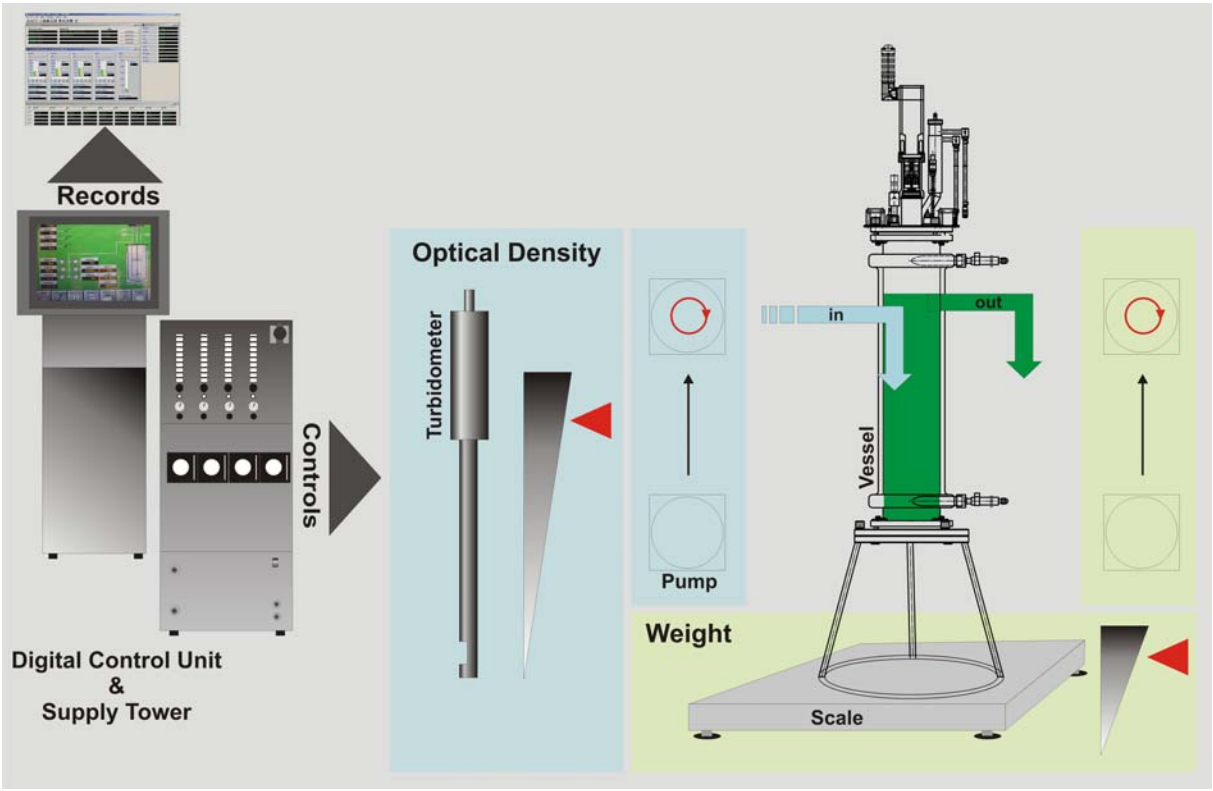

Fig. 2: Controlling principle of the photo-bioreactor system. The Digital Control Unit is the central interface responsible for the data acquisition and regulation of actuators. In the turbidometer driven cultivation process the device controls the culture density by triggering pumps. In a continuous flow process the turbidometer delivers data only without triggering a pump. The culture density is maintained by a continuous medium inflow, the volume has been determined in preliminary experiments.

4. Deviations from Work Program and Corrective Action

The turbidometer driven cultivation was disturbed by a malfunction of the instrument, which caused fluctuations in culture density. We have identified the cause and are working on different aspects of the problem.

The choice of the most suitable strain turned out to be a problem. The sequenced strain was too sensitive to be cultivated in a fermenter. Other suitable strains had to be identified and tested.

5. Future Perspectives

Further joint experiments (core experiments) of the *Go*FORSYS biological group are going to be performed. In future the photo-bioreactor system is used for detailed physiological investigations. Further optimization and adaptation of the system regarding new findings and technical developments (e.g. culture density measurements; nutrient probes) are planned.

According to plan work will continue on organellar transcriptomics/translatomics at different physiological conditions as well as under anaerobic/H_2-inducing conditions. In the near future the fermenter will be established for H_2-producing conditions. First preliminary experiments are done and we were able to detect H_2 gas. However, the levels were low compared to achievements we made in other simpler systems.

6. Cooperation

6.1 Internal Cooperation

6.1.1 Internal Cooperation (within GoFORSYS)

AG Bock. Transcriptomics/translatomics of *Chlamydomonas* chloroplast and mitochondrial encoded genes (with A. Idoine). Transcriptomics/translatomics at H_2-producing conditions. Aim: Identification of markers important for adaptation to a changing environment.

AG Valleriani. *Chlamydomonas* cell cycle. Aim: Modelling of the cell cycle of *C reinhardtii*.

AG Walther. Development of software tools for the analysis of polysomal fractionation. Aim: The relative amounts of RNA, ribosomes and polysomes give interesting insights into the regulation of translation. This tool helps to analyze the chromatograms.

AG Stitt. Polysome isolation and analysis in *Chlamydomonas*. Aim: The relative amounts of RNA, ribosomes and polysomes give interesting insights into the regulation of translation.

AG Ebenhöh. Modeling the growth behaviour of *Chlamydomonas*. Aim: The growth is an important marker for the situation of a culture. Modeling the data will allow to get further insights.

6.1.2 Internal Cooperation (outside GoFORSYS)

AG van Dongen. Regulation of respiration in *Chlamydomonas*. Aim: Respiration is a key player in the H_2-production process, but so far is its regulation neglected.

AG Willmitzer. On the function of TOR kinase: Influence of rapamycin on *Chlamydomonas* growth and metabolism. Aim: How is TOR influencing growth and what is the physiological basis.

6.2 External Cooperation

Prof. Kruse (Univ. Bielefeld), Prof. Hippler (Univ. Muenster), Prof. Posten (Univ. Karlsruhe). Hydrogen evolution and photosynthesis (transcriptomics, translatomics, proteomics and metabolomics under hydrogen-producing conditions; modelling; design of a full-genome microarray). Aim: Improvement of H_2-production.

Prof. Löhmannsröben (Univ. Potsdam), Prof. Posten (Univ. Karlsruhe), Prof. Pulz (Institute for Cereal Processing, Potsdam), Dr. van Dongen (MPI-MP). Development of internal O_2-sensor. Aim: Development of photo-bioreactor system for improved H_2 production by sensitive regulation of O_2 concentration and respiration.

Prof. Buchanan (Univ. of California, Berkeley), Dr. Kempa (Max-Delbrueck Center, Berlin). Metabolic studies on the regulation of the Calvin-Benson Cycle. Aim: New insights in the Calvin-Benson Cycle.

7. Publications

[1] Anh Vu Nguyen, Skye R. Thomas-Hall, Alizée Malnoë, Matthew Timmins, Jan H. Mussgnug, Jens Rupprecht, Olaf Kruse, Ben Hankamer and Peer M. Schenk (2008): The transcriptome of photo-biological hydrogen production induced by sulphur deprivation in the green alga *Chlamydomonas reinhardtii.* Eukaryotic Cell*,* 7**,** 1965-1979

[2] May P, Wienkoop S, Kempa S, Usadel B, Christian N, Rupprecht, J, Weiß J, Recueno-Munoz L, Ebenhöh O, Weckwerth W, Walther D. (2008): Metabolomics and proteomics assisted genome annotation and analysis of the draft metabolic network of *Chlamydomonas reinhardtii*. Genetics, 179 (1): 157-66

[3] Rupprecht, J (2009): From Systems Biology to Fuel – *Chlamydomonas reinhardtii* as a model for a systems biology approach to improve biohydrogen production. Journal of Biotechnology 142: 10-20

[4] Matthew Timmins, Wenxu Zhou, Jens Rupprecht, Lysha Lim, Skye R. Thomas-Hall, Anja Doebbe, Olaf Kruse, Ben Hankamer, Ute C. Marx, Steven M. Smith, and Peer M. Schenk (2009). *The metabolome of Chlamydomonas reinhardtii following induction of anaerobic H2 production by sulphur deprivation*. Journal of Biological Chemistry. *In press*

8. Poster presentations

1) J. Rupprecht; Photosynthesis and Growth: *GoFORSYS - A Systems Biology-based Approach; 14th Photosynthesis Congress*; Glasgow 07/2007
2) J. Rupprecht: *GoFORSYS – Photo-Bioreactor Requirements*; German Conference on Bioinformatics; Potsdam; 09/2007
3) J. Rupprecht: *GoFORSYS – Photo-Bioreactor Requirements*; 1st GoFORSYS Meeting; Berlin 06/08
4) J. Rupprecht: *Systems Biology & Bioengineering - Photo-Bioreactor-Requirements for Biofuel Production*; Solar Biofuels Conference; Bielefeld 08/2008

9. Teaching and Training activities

Ringvorlesung: “Molecular Plant Science: Photosynthesis, Primary Metabolism and Growth”:

Chlamydomonas and systems biology – an overview; 11/2007

Several students were supervised in the laboratory.

10. Organisation of Scientific Events

None

11. Invited talks

European society of Microalgal Biotechnology Workshop: *The Solar Bio-H_2 Project*; Potsdam, 05/2007

12. Commercialisation (Patents, Cooperation with Industrial Partners etc.)

None

1. Research Group

PI	Prof. Peter Saalfrank
Group Members	Jan Götze, PhD student, funded since 03/07
	Dominik Kröner, Postdoc, funded since 12/08

2. Tasks worked on

Contribution mainly to WP1.4:

- Photoresponse of LOV (Light-Oxygen-Voltage) domains of *C. reinhardtii* studied by quantum chemical *ab initio* methods
- Quantum chemical analysis of the general mechanism of the BLUF (blue light sensing using flavine) photoreceptor family on the example of the AppA protein (*R. sphaeroides*) giving hints to both the dark and signalling state on the basis of structural data
- Identification of possibly functional amino acids within tryptophane containing BLUF domains, which have not been experimentally investigated before
- Establishing a QM/MM model of the *C. reinhardtii* RuBisCO for reaction pathway analysis
- Support of Ebenhöh group in creating a valid kinetic model for the RuBisCO reaction pathway, including fallover and inhibitor effects
- Calculation of the equilibrium constants along the RuBisCO reaction pathway using force field methods
- Medium-sized model of the LHCII photoreceptor (spectra calculation, comparison to experiment and further improvement of the model)

General contributions:

- Development of a high throughput application for isotope labelling correction of GC-MS data, for AG Kopka (MPI-MP, Jan Hüge)

3. Achievements made

- Spin-orbit coupling of the electronic processes following the chromophore excitation was modelled on different levels of model complexity.
- A possible model for the dark and signalling state of the tryptophan containing BLUF domains was proposed on the basis of our calculations.
- The transition from the experimental dark state to signalling state BLUF UV/Vis spectra was successfully modelled on the basis of the proposed mechanism.
- Within the explicit AppA BLUF model, Ser41 was identified to be possibly a key player for spectral properties and thus for functional properties as well.
- The RuBisCO QM/MM model predicts qualitatively the binding of the substrate to the enzyme. Further results are to be expected soon.

- Successfully connected the structural knowledge on RuBisCO with the predicted reaction pathway with the Ebenhöh group.

- The LHCII photoreceptor was found to be treatable with quantum chemical methods. However, more data need to be produced to establish a reliable prediction of the capabilities of our model.

4. Deviations from Work Program and Corrective Action

No general deviations or corrective actions so far. The focus of the first funding period was on the photochemical and photophysical response of photoreceptor proteins.

5. Future Perspectives

- The BLUF model is still used for hydrogen bonding analysis for the study of the structural changes between the dark and the signalling state. More results are to be expected soon.
- Direct interaction with external experimental groups allow for mutational studies on the photoreceptor, if required.
- Excited state properties and vibrational spectra of the BLUF domain are analyzed to support or falsify our proposed model, and to investigate to processes connecting the dark and signalling states.
- The RuBisCO model will deliver more data in the following months, which will elucidate the first steps of the pathway. Further steps can be included if time permits.
- Using quantum chemical approaches for selected intermediates or transition states in the RuBisCO pathway may increase the accuracy of the predicted constants.
- The LHCII model will be refined with coupled configuration interaction and time-dependent DFT methods, and finally embedded in environmental systems to analyze protein influence on the carotenoids and chlorophylls.

6. Cooperation

6.1 Internal Cooperation (within GoFORSYS)

- JRG Ebenhöh, Franziska Witzel (now Charitè Berlin), Heike Aßmus and Oliver Ebenhöh; we deliver structural knowledge to support the RuBisCO kinetics model.
- AG Lokstein, Heiko Lokstein. The Lokstein group is the experimental counterpart in our modeling attempts concerning the LHCII.
- Dr. Stefan Kempa (MPI-MP), Susann Irgang; the experimental support for RuBisCO kinetics, aiming at labeling the RuBisCO substrate to gain explicit information on the fate of each carbon atom.

6.2 External Cooperation

- AG Bonačić-Koutecký, Dr. Claudio Greco (HU Berlin); studying the dark and signaling state of the BLUF domains was split between our groups to connect our efforts. We aim at describing the functional differences between the two states. Each group is tasked to describe a single state.
- AG Hegemann, Dr. Tilo Mathes (HU Berlin); the experimental support for the BLUF domains, the Hegemann group is reflecting and working partially on the basis of our results and vice versa. Primary interests are mutational and spectroscopical studies, nut also low temperature data.

7. Publications

[1] K. Zenichowski, M. Gothe, and P. Saalfrank (2007) Exciting Flavins: Absorption Spectra and Spin-orbit Coupling in Light-Oxygen-Voltage (LOV) Domains, J. Photchem. Photobiol. A: Chemistry, 190:290-300.

[2] J. Götze and P. Saalfrank (2009) Serine in BLUF Domains Display Spectral Importance in Computational Models , J Photochem Photobiol B: Biology, 94:87-95

8. Poster presentations

- Karl Zenichowski, Marcel Gothe, and Peter Saalfrank: Vertical Singlet Excitation Energies for Protein Emnedded Isoalloxazines: Simulation of Solvent Effects. Symposium für Theoretische Chemie, Erkner (Oct. 2006.)
- J. Götze and Peter Saalfrank: Modelling the Carbon Isotope Effect of RuBisCO, Otto Warburg Summer School, Berlin (Aug. 2007).
- Bastian Klaumünzer, Jan Götze and Peter Saalfrank: Computational Analysis of BLUF domains: Spectroscopy, photophysics and reaction dynamics, UniCat SAB meeting Berlin (Mar. 2009)

9. Teaching

- Peter Saalfrank, Ringvorlesung ``Molecular Plant Science: Photosynthesis, Primary Metabolism and Growth'': “Quantum chemistry and biological applications in a nutshell” (Nov. 2007).
- Jan Götze: VaTEP and GoFORSYS PhD training program workshop “Protein expression, analysis and structure”, lecture “Approaches to *In silico* Protein Analysis”, MPI-MP Golm (Oct. 2008).
- Götze, Peter Saalfrank, Bastian Klaumünzer: “Computational Analysis of Enzymes and Photoreceptor Proteins”, presentation to members of graduate school “BIG-NSE” (Nov. 2008).

10. Organisation of Scientific Events

None.

11. Invited talks

At conferences:

1. P. Saalfrank: “Vibrational Energy Transfer from Adsorbates to Surfaces”. CECAM-Workshop on ``Energy Flow Dynamics in Biomaterial Systems'', Paris, (Oct 2007).

Others:

1. J. Götze: “Computational Modelling of BLUF Domains”. Talk within seminar of Hegemann group, HU Berlin (June 2008).
2. P. Saalfrank: “Computational Analysis of Enzymes and Photoreceptor Proteins.” Talk within GoFORSYS monthly colloquium, MPI-MP (April 2008).
3. J. Götze: “RuBisCO - a Mechanistic Introduction”, seminar in the Ebenhöh group, MPI-MP (Nov.2007).
4. P. Saalfrank: “Quantum Chemical Investigation of Enzymatic Reactions”. Talk within the ``Retreat Meeting'' of GoFORSYS (April 2007).
5. P. Saalfrank: “Molecular Structure and Dynamics in Complex Environments”. Talk within the colloquium of “Cluster of Excellence UniCat”, Technical University Berlin, Berlin (Feb. 2007).

1. Research Group

PI	Prof. Torsten Schaub
Group Members	Steve Dworschak, Dipl Inform. (03/07) - (09/08)
	Sven Thiele, Dipl. Inform. (05/07)
	Max Ostrowski, Dipl. Inform. (04/09)
	Philippe Gilles Veber, Postdoc (12/07) - (02/08
	Sylvain Blachon, Postdoc, (08/09)

2. Tasks worked on

WP1: Development of constraint based methods to analyze biological networks

- Representation of genetic-regulative and metabolic networks as BC-problems
- Development of methods for consistency checking, diagnosis and prediction of profil data
- Development of a web service for the online analysis
- Development of a graphical back-end for the presentation of diagnosis results in large biological networks
- Development of an answer set solver for constraint logic programs
- Application: micro array data A. Thaliana, Yeast, Ecoli

Development and Implementation of formal languages for modeling biological networks:

- Encoding of the language (Compiler 'al2asp', interface to boolean constraint solving systems (BCS))
- Development of a graphical front-end (Firefox Plugin 'cedit')
- Development of a back-end for statistical analysis
- Extension of ASP language for multivalued resource constraints
- Development of a web service for the server based application of the whole system (front-end, compiler, BCS-tools, back-end)
- Application: Sulfur Starvation Pathway; Potatoe Tuberization

Further development of the existing BCS-Systems:

- Development of parsing- and preprocessing tools (gringo)
- Integration into existing BCS-tools (clasp)
- Development of new techniques for efficient computation of BC-problems
- Development of compilation techniques to transform multivalued constraint problems into BC-problems

3. Achievements made

- Provision of a web service for modelling, planning and statistical analysis of biological models: http://www.cs.uni-potsdam.de/wv/bioasp/adl.html

- CTAID Firefox 2 front-end for the graphical modelling:
  http://www.cs.uni-potsdam.de/~akoenig/cedit/cedit.xpi
- Provision of a web service for the analysis of biological models and profile data:
  http://www.cs.uni-potsdam.de/wv/bioasp/sign_consistency.html
- Implementation and provision of several tools for parsing, compiling and solving of BC-problems:
  http://potassco.sourceforge.net/
- The clasp ASP solver wins 1st in all categories of the ASP Competition 2009
- 1st in two categories (Crafted SAT+UNSAT and Crafted SAT) and
  2nd in one category (Crafted UNSAT) at the SAT Competition 2009

4. Deviations from Work Program and Corrective Action

The withdrawal of Steve Dworschak from the research group shifts the focus of our research. The utilization of action description languages for modelling has taken a back seat and the development of methods for solving multivalued high-resolution problems by means of modern constraint solving mechanisms gains importance.

5. Future Perspectives

The research goals of the group stay unchanged. We plan to continue the modelling and solving of further biological questions as BC-problems. These are amongst others the prediction of reactions in metabolic networks, assistance for the identification of the metabolites as well as protein classification as BC-problems.

In future we hope, by advancing our assisting solving technologies and embedding new technologies from the field of constraint solving, to scale better and solve larger problems and high-resolution problems.

6. Cooperation

6.1 Internal Cooperation (within GoFORSYS)

- Group Ebenhöh: Metabolic Network Expansion; BCS based Methods for Predicting Metabolic Reactions and propose Repairs of Metabolic Networks
- Group Dr. Dirk Walther: Analysis of Metabolic Networks;
  Consistency Checking, Diagnosis and Predication of the Metabolic Network of Chlamydomonas

6.2 External Cooperation

- Dr. Jacques Nicolas (Rennes, France): Reactomics
  Advanced Querying and Reasoning on Biological Databases
- Dr. Andrea Formisano (Perugai, Italy): Resource ASP
  Modelling Biological Resources in BSC
- Prof. Dr. Alexander Bockmayr (Berlin): Thomas Networks;
  discrete modeling approach that capture the qualitative behaviour of gene regulatory networks

- Dr. Philippe Veber (Lyon, France): Analysis of Biological Networks;
  Enhanced methods for Diagnosis of Biological networks
- Dr. Björn Usadel: Analysis of Metabolic Networks;
  Consistency Checking, Diagnosis and Predication on the Metabolic Network of Arabidobsis Thaliana
- Ina Koch (Berlin): Structural Analysis of Petri Nets with ASP
- Andrzej Kierzek (Surrey, UK): Planning and Prediction with Petri Nets with ASP
- Carito Guziolowski (Rennes, France): Repair and Prediction under Inconsistency

7. Publications

[1] Martin Gebser, Torsten Schaub, Sven Thiele, Björn Usadel and Philippe Veber, Detecting Inconsistencies in Large Biological Networks with Answer Set Programming. In M. Garcia de la Banda and E. Pontelli. Proceedings of the Twenty-fourth international Conference on Logic Programming (ICLP'08), LNCS, volume 5366, S. 130-144, Springer Verlag, 2008

[2] Martin Gebser, Roland Kaminski, Benjamin Kaufmann, Max Ostrowski, Torsten Ostrowski, Torsten Schaub and Sven Thiele. Engineering and Incremental ASP Solver. In M. Garcia de la Banda and E. Pontelli. Proceedings of the Twenty-fourth international Conference on Logic Programming (ICLP'08), LNCS, volume 5366, Springer Verlag, 2008

[3] Steve Dworschak, Torsten Grote, Arne König, Torsten Schaub, Philippe Veber: The System BioC for Reasoning about Biological Models in Action Language C. ICTAI Vol. 1, S. 11-18, 2008

[4] Steve Dworschak, Torsten Grote, Arne König, Torsten Schaub and Philippe Veber: Tools for Representing and Reasoning about Biological Models in Action Language C. In M. Pagnucco and M. Thielscher. Proceedings of the Twelfth International Workshop on Nonmonotonic reasoning (NMR'08), School of Computer Science and Engineerin, The University of South Wales, Technical Report Series, Nr. UNSW-CSE-TR-0819, S. 94-102, 2008

[5] Steve Dworschak, Susanne Grell, Victoria Nikiforova, Torsten Schaub and Joachim Selbig. Modelling biological networks by action languages via answer set programming. Constraints Journal, Vol. 13, Nr. 1-2, S. 21-65, 2008

[6] Torsten Schaub and Sven Thiele. Metabolic Network Expansion with ASP. In P. Hill and D. Warren. Proceedings of the Twenty-fifth International Conference on Logic Programming (ICLP'09), LNCS, volume 5649, S. 312-326, Springer Verlag, 2009

[7] Martin Gebser, Max Ostrowski and Torsten Schaub. Constraint Answer Set Solving. In P. Hill and D. Warren. Proceedings of the Twenty-fifth International Conference on Logic Programming (ICLP'09), LNCS, volume 5649, S. 235-249, Springer Verlag, 2009

[8] T. Henin and Torsten Schaub. A Transport Reaction Language: Preliminary Report. In A. Herzig and B. Johnston. Proceedings of the Eighth Workshop on Nonmonotonic Reasoning, Action and Change, S. 41-46, University of Technology, Sydney, Australia, 2009.

[9] Martin Gebser, Carito Guziolowski, Mihail Ivanchev, Torsten Schaub, Anne Siegel, Sven Thiele and Philippe Veber. Prediction and Repair in Large Biological Networks with Answer Set Programming. Submitted

8. Poster presentations

None

9. Teaching and Training activities

- Angewandte Bioinformatik I, University of Potsdam, (WS06/07)
- Angewandte Bioinformatik II, University of Potsdam, (SS07)
  Knowledge in Action Seminar, University of Potsdam, (SS07)
- Lectures as part of the Course Wissensverarbeitung/Wissensrepräsentation (SS07 and SS08) and Antwortmengenprogrammierung, (WS07/08)
- Modellierung biologischer Systeme, University of Potsdam, (SS08)
- Talks as part of the Seminar Wissensverarbeitung SS/WS 07, 08 and 09

10. Organisation of Scientific Events

None

11. Invited talks

- Colloquium Talk, Institute of Sports Medicine and Prevention, University of Potsdam
- Invited Talk, Computer Science Dept., University of Western Sydney

12. Commercialisation (Patents, Cooperation with Industrial Partners etc.)

None

1. Junior Research Group

PI PD Michael Schroda (PI, employed since 10/08)

Group Members

financed by GoFORSYS

Frederik Sommer (postdoc, employed since 10/08)
Julia Weiss (PhD student, employed since 11/07)
Dorothea Hemme (PhD student, employed since 12/08)

financed by other resources, but strongly involved in GoFORSYS

Timo Mühlhaus (PhD student, employed since 10/08; financed by MPI-MP)
Stefan Schmollinger (PhD student, employed since 07/08,financed by MPI-MP)
Daniela Strenkert (PhD student, employed since 07/08, financed by DFG)
Daniel Veyel (PhD student, employed since 07/09, financed by IMPRS)

2. Tasks worked on

WP 1 General bioinformatics and computational methods

- *WP 1.1 Data Warehousing*

WP 2. Development of new analytic tools for systems analysis

- *WP 2.1 Quantification of Proteins*
- *WP 2.3 Metabolite profiling*

WP 3. Multilevel systems analysis of responses in *Chlamydomonas*

- *WP 3.1 Different steady state conditions*
- *WP 3.2 Genetic manipulations*

3. Achievements made

WP 2.1 and 1.1: Our goal was to develop new, mass spectrometry-based analytical tools for *Chlamydomonas* to **(i) determine components of protein complexes, and (ii) monitor proteome dynamics in response to changing environmental conditions**. To achieve the first goal, we have successfully applied (and improved) the QUICK approach to *Chlamydomonas*, which was originally developed by Selbach and Mann in 2006 for human cell cultures. This approach is based on immunoprecipitation, stable isotope labelling, RNAi, and quantitative mass spectrometry. Our QUICK protocol allows for the unequivocal identification of the components of protein complexes in soluble and membrane fractions from *Chlamydomonas*.

Our second goal, to monitor proteome dynamics in *Chlamydomonas*, was essential for the Core Experiments and turned out to be particularly challenging, as the following criteria had to be met: as many proteins as possible needed to be identified in an unbiased fashion in soluble and membrane

fractions and in all samples of a kinetic time course experiment, and furthermore be quantified with sufficient accuracy to detect small changes.

We have indeed managed to establish a method that meets these criteria. Briefly, we mix reference cells having their proteome completely labelled with ^{15}N at a constant ratio to the ^{14}N-labelled samples taken at different time points from the bioreactor culture.
Mixed cells are disrupted by freeze-thawing, and membranes are separated from soluble proteins by centrifugation. Both fractions are precipitated, digested tryptically and subjected to nano LC-MS/MS analysis. Peptide sequences are identified by Mascot and ion intensities of heavy and light peptides are quantified by XPRESS and stored in our newly developed database, primaq. Finally, from primaq the heavy-to-light ratios for all peptides belonging to the same protein are calculated for each time point. About ~900.000 peptide spectra in 66 raw files are generated for each Core Experiment replica, resulting in ~53 GB raw data. The processing of these data is realized by an automatized pipeline constructed by my group and requires ~4 days of processing time. Currently, we are able to monitor the dynamics of ~650 *Chlamydomonas* proteins, including many integral membrane proteins.
WP 2.3: Metabolite analyses for the Core Experiments were supposed to be done by Dr. Kempa from the predecessor group, who has left GoFORSYS in April 2009. As nobody else in my group had yet expertise on metabolite analyses, we outsourced them and determined metabolite profiles by GC-MS in collaboration with the group of Dr. Kopka at the MPI-MP. Here, the dynamics of ~90 metabolites were determined.
WP 3.1: Losses in crop yield during heat waves were reported to dramatically increase as a consequence of the climate change. To devise counteractive measures it is **essential to understand the fundamental mechanisms of the heat shock response in plants**. In higher plants this is difficult because they contain at least 21 heat shock transcription factors (HSFs). In contrast, *Chlamydomonas reinhardtii* contains only a single canonical HSF, which is highly homologous to that of higher plants and serves as key regulator of the stress response. Hence, **one goal of GoFORSYS was to study cellular responses to increased temperatures**. We did this by monitoring changes in transcript and protein levels of heat-stressed cultures that were exposed to various inhibitors or in which HSF1 and a chaperone were downregulated by RNAi and antisense approaches. The data obtained allowed to draw a sketch of the heat shock response in *Chlamydomonas*, which Dr. Skupin from the AG Ebenhöh could work into a mathematical model. Dr. Skupin's model is able to simulate the basic stress response in *Chlamydomonas* and the perturbations induced by the inhibitors used.
To identify the genes regulated by HSF1 we have in collaboration with Prof. Hess and Dr. Kurz at FRISYS, University of Freiburg, performed microarray hybridizations with *Chlamydomonas* wild-type and HSF1-RNAi lines.
In order to perform the Core Experiments, **controlled growth of *Chlamydomonas* in a bioreactor was crucial**. My group was strongly involved in the final steps of the setting up of bioreactor cultures by choosing suitable *Chlamydomonas* strains and by controlling optimal and contamination-free growth conditions.

WP 3.2: Models derived from the top-down and bottom-up approaches taken in GoFORSYS need to be tested by focussed experiments. For these, the **specific and ideally inducible downregulation of key genes is essential**.

We have developed a vector for *Chlamydomonas* that allows for the inducible silencing of target genes. Our vector uses the nitrate-inducible *Chlamydomonas NIT1* promoter driving the expression of an artificial microRNA specifically affecting any target gene of choice, including genes for (phospho)glucan water dikinase (for AG Steup) and TOR (for AG Willmitzer).

4. Deviations from Work Program and Corrective Action

WP 2.1: The approaches intended for the "absolute" quantification of proteins (QCAT; Mass Western) do not take into account the potential loss of proteins during the isolation procedure that is due to the physico-chemical properties of individual proteins and variations in sample handling. Hence, these methods probably only allow for "absolute" quantification at the peptide level and also preclude the unbiased quantification of as many proteins as possible. Hence, we decided to use relative quantification by the method outlined above.

5. Future Perspectives

- Improving our pipeline for the automatized identification and quantification of peptides by (i) including identification programs OMSSA, X!Tandem, and Sequest; (ii) including a second quantification program, ASAPRatio; (iii) including an algorithm for outlier detection, also based on the additional information obtained by the sequence-specific peptide mass shift by ^{15}N-labelling. These improvements are likely to extend the number of quantifiable proteins to close to 1000.
- Parametrizing the heat shock response model with quantitative data on cellular chaperone and HSF1 protein levels, HSF1 phosphorylation state, etc.
- Genome-wide identification of transcription factor targets in *Chlamydomonas* and *Volvox* using ChIP-Seq
- Establishment of a protocol aiming at the analysis of protein synthesis rates using our shotgun proteomics approach combined with ^{13}C-arginine feeding in collaboration with the AG Stitt

6. Cooperation

6.1 Internal Cooperation (within GoFORSYS)

Prof. Steup, underexpression of a *Chlamydomonas* dikinase involved in starch metabolism

Prof. Willmitzer, underexpression of the *Chlamydomonas* TOR (target of rapamycin)

Prof. Bock, localization of proteins in *Chlamydomonas* by fluorescence microscopy

Prof. Stitt, determination of translation rates of *Chlamydomonas* proteins visible by shotgun proteomics

Drs. Ebenhöh and Skupin, modelling of the *Chlamydomonas* stress response

Drs. Walther and May, provide strong support for data warehousing

Dr. Nikoloski, development of algorithm for outlier detection for peptide identification/quantification

All groups: provide help to adapt techniques to the special needs of *Chlamydomonas*

6.2 External Cooperation

Prof. Hess and Dr Kurz (ZBSA) within FRISYS at the University of Freiburg, identification of *Chlamydomonas* genes regulated by heat shock factor 1 by microarray analysis

Prof. Miller, University of Maryland, Baltimore County, USA, on a 1-year sabbatical in my group since 07/2009, Identification of transcription factor targets in *Volvox* by ChIP-Seq

Dr. Kopka, MPI-MP, analysis of *Chlamydomonas* metabolites by GC-MS

7. Publications

[1] M. Schulz-Raffelt, S. Schmollinger, D. Strenkert, D. Veyel, O. Vallon, A. Skupin, O. Ebenhöh, and M. Schroda (2009) Dissection of the stress response in *Chlamydomonas* reveals a role for chloroplast HSP70B in stress signalling. Submitted.

[2] H. Heide, A. Nordhues, F. Drepper, S. Nick, M. Schulz-Raffelt, W. Haehnel, and M. Schroda (2009) Application of quantitative mass spectrometry combined with immunoprecipitation, knock-down and crosslinking (QUICK-X) to *Chlamydomonas* reveals the interaction of VIPP1 with chloroplast HSP90C. Proteomics, 9, 3079-3089.

[3] S. S. Merchant, S. E. Prochnik, O. Vallon et al. (2007). The *Chlamydomonas* genome reveals the evolution of key animal and plant functions, Science 318:245-51.

8. Poster presentations

[1] Schroda, M., Schulz-Raffelt, M., Schmollinger, S., Strenkert, D., Veyel, D., Voss, B., Hess, W.R., Skupin, A., Ebenhöh, O., Hemme, D., Mühlhaus, T., Sommer, F. Modeling of the HSF1-dependent stress response in *Chlamydomonas reinhardtii*. German Symposium on Systems Biology, Heidelberg, May 2009.

[2] Hemme, D., Mühlhaus, T., Weiss, J., Schmollinger, S., Sommer, F., Mettler, T., Schöttler, M.A., Schroda, M. Using quantitative proteomics for the unbiased monitoring of proteome dynamics in *Chlamydomonas reinhardtii.* German Symposium on Systems Biology, Heidelberg, May 2009.

[3] Strenkert, D., Schmollinger, S., Schulz-Raffelt, M., Lodha, M., Schroda, M. Evidence for a role of heat shock factors in *HSP70A* promoter mediated activation of transgene expression. German Symposium on Systems Biology, Heidelberg, May 2009.

9. Teaching and Training activities

Teaching: Methods in System Biology (lecture, literature seminar and class experiment) starting in Winter Semester 2009.

Training: 3 Diploma students (Vittoria Offeddu, Franz Mußotter, Mark Rütgers)

10. Organisation of Scientific Events

None yet within GoFORSYS.

11. Invited talks

[1] University of Mainz. Functions and regulation of the chloroplast HSP70-HSP90 chaperone machineries". December 2008.

[2] Humboldt University, Berlin. Functions and regulation of the chloroplast HSP70-HSP90 chaperone machineries". April 2009.

[3] German Symposium on Systems Biology. Application of QUICK-X to *Chlamydomonas* reveals the presence of VIPP1 in a common complex with chloroplast HSP90C. May 2009.

[4] Wallenfelser Rundgespräche. Dissection of the stress response in *Chlamydomonas reinhardtii*. May 2009.

[5] The 5th Germany-Japan Binational Seminar. Quantitative proteomics for Systems Biology with *Chlamydomonas reinhardtii*. June 2009.

1. Research Group

PI	Prof. Joachim Selbig
Group Members	Zoran Nikoloski, postdoc since June 15, 2007

2. Tasks worked on (break down by WPs and tasks)

The major contributions of the group consider WP1, in particular to WP1.2, WP1.3, and WP1.4. According to the *Go*FORSYS proposal, the group is mainly concerned with the application of graph-theoretical approaches to the comparative network analysis and the characterization of dynamic properties of metabolic and regulatory networks. In addition, the group's focus includes development and application of computational methods to the integrative analysis of the omics data from the *Go*FORSYS core experiment.

3. Achievements made

A phylogenomics approach to improving *Chlamydomonas reinhardtii* gene function annotation was evaluated. Specifically, protein sequences of *C. reinhardtii* and selected eukaryotic and prokaryotic taxa were clustered into protein families. Families with *C. reinhardtii* representatives that also contained members from other taxa known to be involved in carbon concentrating mechanisms (CCM) were further analyzed.

In the context of the integrated data analysis, we proposed an approach for investigating ternary relationships between cellular components relying on the Bayesian formalism, which extends previous approaches and gives a method to investigate otherwise inaccessible types of interrelations.

In order to answer the question whether the Calvin cycle can operate in multiple steady states under fixed environmental conditions, we analyzed the existing Calvin cycle models in the framework of Feinberg's Chemical Reaction Network Theory. For instance, simplification of one model by collapsing the reactions associated to Glyceraldehyde 3-phosphate results in a model version that exhibits multiple steady states. We investigated the complexity of the so-called inverse scope problem, which consists of determining, for a given metabolic network, the minimum cardinality set of substrate compounds necessary for synthesizing a set of target metabolites and showed that it is NP-hard. We also developed the so-called active subnetwork approach that combines high-throughput time-series transcriptome data with graph-theoretic methods and aims at discovering and quantifying the emergence of dynamic global properties in a molecular network challenged by different environmental conditions. This approach can be used to detect metabolites and genes responsible for adaptation to changing environments, which otherwise cannot be detected by classical approaches relying on large changes in expression levels and concentration.

4. Deviations from Work Program and Corrective Action

Due to the delay in the *Go*FORSYS core experiment on *C. reinhardtii* the first results from the integrated data analysis were obtained at the beginning of June 2009. The group is presently involved in applying sophisticated network-based approaches to further refine the results from the classical bioinformatics approaches (*e.g.*, clustering, PCA, ICA). The integrated analysis of data from all omics technologies included in the experiment will be immediately performed once the differential analysis from all separate pieces of omics data is fully resolved.

5. Future Perspectives

The group will continue to work on the issues formulated in the *Go*FORSYS proposal and, in particular, will strengthen the exchange with the experimental working groups of the project.

The plylogenomics approach described provided insights into the evolution of selected genes, it also allowed an improved annotation of selected CCM genes in *C. reinhardtii*. The goal is to extend these analyses and to provide large-scale protein family information, including multiple sequence alignments and phylogenies, for the ChlamyCyc database, cf. http://chlamycyc.mpimp-golm.mpg.de.

The methods developed for analysis of biological systems reconstructed from time-series data will be applied to the data sets from *C. reinhradtii*. As gene annotations are already made possible with a novel clustering method which takes into consideration integrated ontologically structured biological knowledge, the transcriptomics data from the first core experiment are currently analyzed with the purpose of determining function of genes associated with light shift.

6. Cooperation

6.1 Internal Cooperation (within *Go*FORSYS)

Thomas Altmann	integrative data analysis	biomass prediction
Oliver Ebenhöh	network analysis	pathways dynamics
Matthias Holschneider	profile data analysis	bio-marker identification
Michael Schroda	proteome database	protein profile analysis
Mark Stitt	Calvin cycle	stability analysis
Dirk Walther	phylogenetic analysis	annotation improvement
Lothar Willmitzer	metabolite profile analysis	bio-marker identification

6.2 External Cooperation

Aaron Faith, Ben Gurion University of the Negev, Israel	network analysis and modelling	stress response
Kristina Gruden, National Institute of Biology Ljubljana, Slovenia	profile database development	bio-marker identification

Hermann-Georg Holzhütter, Charité Berlin	structural kinetic modelling	pathway dynamics
Björn Junker, IPK Gatersleben, FORSYS partner group	gene lethality prediction	annotation improvement
Michal Or-Guil, Humboldt University Berlin	combining metabolite and peptide profiles	bio-marker identification
Yves van de Peer, Flanders Institute of Biotechnology, Gent, Belgium	network analysis	gene duplication
Christian Schichor, Ludwig Maximilians University Munich	integrated profile data analysis	bio-marker identification
Lee Sweetlove, University of Oxford, UK	profile data analysis	understanding stress response

7. Publications

[1] **Z. Nikoloski**, S. Grimbs, P. May, **J. Selbig** (2008) Metabolic networks are NP-hard to reconstruct, J Theor Biol. 254:807-16.

[2] G.Basler, **Z. Nikoloski**, O.Ebenhöh, T. Handorf (2008) Biosythetic potentials from species-specific metabolic networks, Genome Informatics 20.

[3] **Z. Nikoloski**, S. Grimbs, **J. Selbig**, O. Ebenhöh (2008) Hardness and approximability of the inverse scope problem. In K.A. Crandall, J. Lagergren (Eds.) Algorithms in Bioinformatics, Lecture Notes in Computer Science 5251, Springer-Verlag Berlin Heidelberg, 99-112.

[4] L. Childs, **Z. Nikoloski**, P. May, D. Walther (2009) Identification and classification of ncRNA molecules using graph properfties, Nucleic Acid Research 37 (9) e66.

[5] G. Basler, **J. Selbig**, O. Ebenhöh, **Z. Nikoloski** (2009) Uniform randomization of metabolic networks, Submitted to IEEE/ACM Transactions on Bioinformatics and Computational Biology.

[6] S. Klie, **Z. Nikoloski**, **J. Selbig** (2009) Cluster validation for gene function prediction, Submitted to Journal of Computational Biology.

[7] J. Szymansky, S. Jozefcuk, **Z. Nikoloski**, **J. Selbig**, V. Nikiforova, L. Willmitzer (2009) Stability of metabolic correlations under changing environmental conditions in Escherichia coli – a systems approach, Submitted to PLoS ONE.

[8] J. Lisec, **Z. Nikoloski**, L. Roemisch-Margl, M. Steinfath, H.-P. Piepho, **J. Selbig**, A. Gierl, L. Willmitzer (2009) Heterosis in metabolic networks of maize root, Submitted to PNAS

[9] **Z. Nikoloski**, **J. Selbig**, A. Fait (2009) Getting to grips with plant metabolism: Reducing complexity via network analysis, Submitted to Plant Science.

[10] **Z. Nikoloski**, P. May, **J. Selbig** (2009) Algebraic connectivity explains the evolution of gene regulatory architectures, Submitted to RECOMB 2009.

[11] B. Schwenk, **J. Selbig**, Y. Ben-Zion, M. Holschneider (2009) ExPlanes: Exploring planes in profile data, Submitted to IEEE/ACM Transactions on Bioinformatics and Computational Biology.

6. Poster presentations

[1] G. Basler, **Z. Nikoloski**, O. Ebenhöh; Random generation of metabolic networks with biochemical contraints; European Conference on Computational Biology (ECCB); Cagliary, Italy; Sept 22-26, 2008.

[2] **Z. Nikoloski**, S. Grimbs, J**. Selbig**; Dynamic features of Calvin cycle models; International Conference on Systems Biology (ICSB); Göteborg, Sweden; Aug 22-28, 2008.

[3] **Z. Nikoloski**, S. Grimbs, **J. Selbig**, Refinement of Calvin cycle models with allosteric regulations inferred from metabolomic data, European Conference on Computational Biology (ECCB); Stockholm, Sweden; June 27 – July 2, 2009.

[4] F. M Giorgi, T. Bolger, M. Mutwil, **Z. Nikoloski**, S. Persson, B. Usadel, A novel centrality framework for causal gene regulatory network reverse engineering, 20th International Conference on Arabidopsis Research, Edinburgh, Scotland, UK; June 30 – July 4, 2009.

7. Teaching

Z. Nikoloski

[1] Bioinformatics Working Seminar; University of Potsdam / Max Planck Institute of Molecular Plant Physiology; Sept 2007.

[2] Bioinformatics Affinity Seminar; University of Potsdam / Max Planck Institute of Molecular Plant Physiology; Nov 2007.

[3] Profile data and network analysis, 2009 summer semester course of the Master in Bioinformatics Program at University of Potsdam.

J. Selbig

Professor Dr. Selbig is responsible for teaching Bioinformatics at the University of Potsdam within the Bachelor program Life Sciences and the Master program Bioinformatics with the focus on Systems Biology. He and his co-workers provide courses such as Bioinformatics of biological sequences, Structural bioinformatics, Algorithmic and mathematical bioinformatics, Profile data and network analysis, Theoretical systems biology, and Experimental systems biology.

8. Organisation of Scientific Events

[1] German Conference on Bioinformatics (GCB); Potsdam; Sept 2007.

[2] Workshop on Integrative Network Analysis; Potsdam; Apr 2008.

[3] Workshop on Metabolic Networks: Dynamics, Evolution, and Topology; Potsdam; Nov 2008.

9. Invited talks

Z. Nikoloski

[1] Workshop Evolution across Scales; Potsdam; Does evolution lead to different properties of metabolic networks? Apr 2008.

[2] 8th Workshop on Algorithms in Bioinformatics (WABI); Karlsruhe; Hardness and approximability of the inverse scope problem; Sept 2008.

[3] 14th International IEEE Conference on Engineering of Complex Computing Systems, From intra- to inter-organism transfer of functional knowledge, Potsdam, June 2-5, 2009

J. Selbig

[1] DECHEMA Workshop on Systems Biology; Frankfurt Main; Photosyntheis and growth: A systems biology-based approach; Jan 2007.

[2] International spring school on omic data analysis; Jena; Integrated analysis of transcriptome, proteome, and metabolome data; Mar 2007.

[3] International Workshop on gene regulatory network inference; Jena; Comparative network analysis; Sept 2008.

[4] Max Planck Institute of Psychiatry Workschop; Schloß Ringberg; Application of mutual information in HIV drug resistance and cancer research; May 2009.

1. Research Group

PI	Prof. Martin Steup
Group Members	Henrike Brust (PhD) 03/07
	José Chan Navarrrete (PhD) 04/07-10/08
	Michael Sandmann (PhD) 01/09
	Dr. Mahdi Hejazi (Post-Doc) 06/08-08/09

2. Tasks worked on (break down by WPs and tasks)

WP 3. Multilevel systems analysis of responses in *Chlamydomonas reinhardtii*

3. Achievements made

[1] Synchronization of several photoautotrophic lines from *Chlamydomonas reinhardtii*, including a cell wall deficient line, has been achieved. Currently, the synchronized cells are being characterized using various biochemical and immunological techniques. Of special relevance is the transition from light to dark which results in a massive reorganization of the intracellular carbon fluxes. During this transition the content of starch of glucosyl 6-phosphate residues increases indicating an increased activity of the starch phosphorylating enzymes. Biochemical characterization of the cells throughout the cell cycle is being performed. For the various stages of the cell cycle, secretion of metabolites into the medium is being followed.

[2] The biochemistry of the initial steps of the A-type allomorph of starch has been studied by using crystalline maltodextrins, representing a defined allomorph, and recombinant proteins, such as the glucan, water dikinase, the phosphoglucan, water dikinase and dephosphorylating enzymes. The implications of the phosphorylation and dephosphorylation of glucans on the physical order of the helices and the phase transition from the insoluble to the soluble state have been determined.

[3] In order to alter the strength of the intracellular sinks of the carbon fixed, synchronized *Chlamydomonas* cells were transferred to a sulphate-deficient medium at the end of the dark period. In the three subsequent light-dark cycles cells were diluted in a sulphate-deficient medium. Both biochemical parameters and the cell cycle were followed during the three days of sulphate deficiency. During sulphate deficiency the *Chlamydomonas* cells continue to synthesize the A-type starch. The advantage offered by sulphate deficiency is that intracellular carbon fluxes are massively altered without the complications of induction of gametogenesis (as it is the case with nitrogen deficiency).

[4] The interaction of the various starch synthase and branching enzyme isoforms are being studied by using both plant-derived and recombinant proteins. Functionality of the individual isozymes and their interacting complexes are being analysed by various techniques including immunological studies. The preferred carbohydrate substrates and products are being analyzed by ion exchange chromatography, mass spectrometry and capillary electrophoresis following coupling to a fluorochrome. A functional analysis includes the use of various single and double knock-out mutants.

4. Deviations from Work Program and Corrective Action

None

5. Future Perspectives

[1] The biochemical characterization of the synchronized *Chlamydomonas* cells enables us to estimate the cell-cycle dependent variation of biochemical parameters. This information is relevant for modelling of cellular processes in the unicellular alga. During the late light period, the population of the synchronized cells is heterogeneous as (at least) two size population can be distinguished. This phenomenon will be further analyzed by monitoring replication throughout the light period.

[2] Sulphate deficiency has been established as a suitable tool to alter intracellular sinks of the carbon fixed. Cell division and the release of zoospores are retarded in sulphate deficiency. A detailed biochemical analysis of the sulphate-deprived cells will focus on the interaction between primary metabolism and cell division. Of special interest will be the transition of sulphate-deprived cells to normal growth conditions.

[3] From mutants of higher plants is it known that starch is a major intracellular sink of the carbon fixed whose metabolism strongly determines plant growth and development. Both, starch synthesis and degradation will be analyzed focussing on two aspects: the interaction of various starch synthesizing and branching isozymes and the regulation of starch-phosphorylating enzymes. Of special relevance will be the biochemical characterization of Chlamydomonas cells in which the expression of the predominant starch-related dikinase is inhibited at defined periods of the cellular development.

These experiments will enable us to determine the function of starch phosphorylation both during biosynthesis and degradation.

6. Cooperation

6.1 Internal Cooperation (within GoFORSYS)

[1] A close collaboration exists with the group of Prof. Ebenhöh on the modelling of the plastidial maltodextrin metabolism and the empirical verification of the prediction.

[2] A collaboration exists with the group of Dr. Schroda in order to obtain Chlamydomonas mutants with an inducible RNAi inhibition of the expression of a starch-related dikinase.

[3] A close collaboration exists with the group of Prof. Willmitzer focussing on the lipidomics in synchronized *Chlamydomonas* cells.

[4] A close collaboration exists with the group of Prof. Tiedemann focussing on a functional characterization of the genetic variability of various starch-related enzymes.

[5] A collaboration exists with the group of Prof. Holschneider focussing on a mathematical analysis of the action of glucan hydrolysing enzymes from plants.

6.2 External Cooperation

[1] A close collaboration exists with the group of Prof. Ball (University of Lille) on the primary metabolism in eukaryotic algae, especially in *Chlamydomonas*.

[2] A collaboration exists with the group of Prof. Altmann (IPK Gatersleben) focussing on biochemical and genetic variability of *Arabidopsis* accessions.

[3] A close collaboration exists with the MPI of Colloid and Surfaces (Potsdam-Golm) focussing on the characterization of physical properties of native starch granules.

7. Publications

[1] J. Fettke, M. Hejazi, J. Smirnova, E. Höchel, M. Stage and M. Steup (2009) Eukaryotic starch degradation: integration of plastidial and cytosolic pathways. J. Exp. Bot. 60:2907-2922.

[2] M. Hejazi, J. Fettke, O. Paris and M. Steup (2009) The two plastidial starch-related dikinases sequentially phosphorylate glucosyl residues at the surface of both the A- and the B-type allomorphs of crystallized maltodextrins but the mode of action differs. Plant Physiol. 150:962-976.

8. Poster presentations

n.a.

9. Teaching and Training activities

[1] A two weeks lab course on Expression and Topogenesis of Plant Proteins/Protein Complexes was held in April 2009 at the University of Potsdam (Diploma and Master Students).

[2] A lecture on Plant Primary Metabolism was given on November 2008 at the University of Potsdam.

[3] A lecture within the series Molecular Plant Science: Photosynthesis, Primary Metabolism and Growth was held in January 2008 (Topic: Starch: Structure, Biosynthesis and Degradation; IMPRS Max-Planck Institute of Molecular Plant Physiology)

10. Organisation of Scientific Events

n.a.

11. Invited talks

[1] Starch Round Table San Antonio, Texas October 2007: Plastidial and cytosolic control of starch metabolism in higher plants.

[2] International Workshop on Plant Biomass for Food and Energy: Future and Reality, Baeza, Spain October 2008: Starch metabolism in eukaryots: Contributions of the plastidial and the cytosolic compartment.

[3] University of Lund, November 2009.

12. Commercialisation (Patents, Cooperation with Industrial Partners etc.)

[1] Cooperation with Bayer BioScience GmbH until 2008 focussing on covalent modifications of starch.

[2] Cooperation with Emsland-Stärke GmbH since 2008 focussing on carbohydrate analytics.

1. Research Group

PI	Prof. Mark Stitt
GoFORSYS-supported PhDs	Daniel Vosloh, Sunil Pal

Other relevant contributors:

Björn Usadel, Group Leader; Waltraud Schulze (Group Leader), Ronan Sulpice, John Lunn, Maria Piques, Stephanie Arrivault (PostDocs, AG Stitt); Alexander Ivakov, Tabea Mettler (PhDs AG Stitt)

2. Tasks worked on (break down by WPs and tasks)

WP2, Development of analytic methods: - Develop/optimise technologies to measure enzyme activities and activation, phosphorylated intermediates, subcellular compartmentation; and for quantitative analysis of ribosomes, and transcripts in extracts and polysome gradients.

WP3, Chlamydomonas: - Use these methods in 'core' experiments (in collaboration with other GoFORSYS partners) to provide data sets for modelling of photosynthesis and growt. Also planned are short-term perturbations of photosynthesis (in combination with Mark Schöttler) to study adjustments of metabolism, electron transport and energy dissipation

WP4. Higher plants Use natural diversity in Arabidopsis for systems analysis of C fixation and growth.

3. Achievements made

- Collection and storage of metadata describing experimental conditions is an important part of a systems approach. WP1: We have developed a XML-based tool that captures information about genotypes, growth conditions and experimental information in experiments with complex dynamic temporal changes via a graphical user interface. Hannemann et al. (in press).

- Development and validation of methods to measure levels of all phosphorylated metabolites in the Calvin cycle and end product synthesis. Quantitative information on all intermediates in photosynthesis is needed for metabolic modelling. *WP2:* We have developed and validated a reverse phase ion pair chromatography(ICP)-MS/MS platform that allows quantitative measurement of ~30 metabolites. Recoveries of small representative amounts of metabolite added before extraction is 80-121%. Together with an existing anion exchange chromatography platform and conventional enzymatic tests, we can now measure almost all Calvin cycle and end product synthesis intermediates (Arrivault et al., 2009). The method was developed for Arabidopsis (Stephanie Arrivault), and has been optimised for Chlamydomonas (PhD, Tabea Mettler). *WP3:* It has already been applied in Chlamydomonas core experiments, providing data that was is being used for modelling by Zoran Nikolski (see Part C of the General report) *WP4:* In a pilot application, we showed that end-product synthesis is inhibited at compensation point [CO_2] but the levels of Calvin cycle intermediate remain almost unaltered compared to ambient conditions.

- *Non-aqueous fractionation to determine subcellular metabolite levels*. *WP2:* This is optimised for robotised analyses for determining marker enzyme activity for the plastid, cytosol and vacuole. Attempts to apply it to Chlamydomonas were unsuccessful, probably due to the organisation of the cells. *WP4:* It has been combined with the newly developed IPC-MS/MS to determine subcellular levels of most of the Calvin cycle intermediates in Arabidopis leaves (PhD project, Daniel Vosloh).

- *Development of methods to measure the activation status of Calvin cycle enzymes.* Many Calvin cycle enzymes are subject to redox regulation by thioredoxin. It is a challenge to monitor these post-translational changes, because they are highly unstable in extracts. This information is needed to model the regulation of the Calvin cycle and its interaction with electron transport. *WP2:* We have used highly sensitive enzyme assays established in our group to develop very short-duration stopped-assays of phosphoribulokinase and plastidic fructose-1,6-bisphosphatase, and demonstrate light-dark differences in Arabidopsis and Chlamydomonas (PhD Thesis, Daniel Vosloh).

- *Development and application of methods for the quantitative analysis of ribosomes and polysome loading*. This topic has been expanded since the GoFORSYS application. The methods are of importance for two reasons (i) While expression profiling provides large amounts of information about transcript levels it remains an open question if, how quickly and to what extent, changes of transcripts lead to changes of the levels of the encoded proteins. This information is essential to move beyond modelling transcriptional networks, in order to understand how transcription regulates metabolism and growth. (ii) The first large scale analyses of proteins levels are now emerging (e.g., at the ETHZ, also in house from Waltraud Schulze). But comparison of protein abundances with transcript levels will remain phenomenological unless information is available that mechanistically links these two levels. Quantitative information about translation could provide an important link between molecular events and protein synthesis rates and allow modelling of the energy-balance and growth. *WP2:* We have developed methods to quantify ribosomes and thus measure ribosome numbers per unit FW using quantitative RT-PCR of rRNA combined with spiking of external RNA standards (Maria Piques), validated by measurements of ribosomal protein abundance with LC-MS/MS (Waltraud Schulze). Absolute transcript concentrations are determined by quantitative RT-PCR with external standards. These measurements are made in extracts and polysome gradients. Using literature values for ribosomal progression and some assumptions, we use these data to estimate overall protein synthesis rates, and the rates of synthesis of individual proteins. The latter are compared with protein abundance in leaves, measured with LC-MS/MS analyses or estimated from maximum enzyme activity normed on literature values for specific activity. These methods were initially developed in Arabidopsis. *WP3:* They are being applied to the Chlamydomonas core project. A key finding is that polysome loading increase within minutes of increasing the light intensity. (PhD project, Sunil Pal). *WP4*: In a pilot application in Arabidopsis, we have shown the global rate of protein synthesis, as estimated form ribosome number and polysome loading, is similar to that required for growth and represents a major part of the total carbon and energy balance. Further, we have also shown that most enzymes are synthesised at rates resembling those required for growth (Piques et al., in press).

- *Large scale analyses of enzyme activities, metabolites and growth in >100 Arabidopsis accessions.* Natural genetic diversity provides a useful resource for systems analysis - it reveals the consequences of thousands of small changes at many different points in a network. We have analysed >35 enzyme activities, 80 metabolites and biomass in >100 Arabidopsis accessions.

(i) *Network analysis* revealed that enzyme activities show highly correlated changes. Many metabolites also correlate with each other. However, there are few correlations between enzymes and metabolites. This has many implications for the (f)utility of trying to use correlation networks link protein levels (let alone transcripts) with metabolites in complex metabolic networks, and imply that models will be essential to establish connectivity between these functional levels. They triggered a modelling project between Ebenhoh and Rohwer.

(ii) *Multivariate analysis* revealed that one major metabolic/physiological determinant of growth is linked to carbon allocation. Starch is the major C store laid down in the light to be used to support metabolism and growth at night. It correlates strongly and negatively with biomass (R^2 ~0.23). PLS regression revealed that the same set of metabolites, with the same VIP, are predictive of biomass and starch (Sulpice et al., 2009). A second major determinant relates to the fraction of total protein invested in enzymes of central metabolism (R^2 ~0.12). This parametercorrelates positively with growth. It is almost additive with starch. Thus, about a third of the genetic variance in growth is explained by two integrative metabolic parameters, one related to the efficiency of investment of protein for photosynthesis, and the other for the efficiency of C utilisation (Sulpice, in revision). These findings (and polysome analyses, see above) have prompted us to start to develop models that link C and energy costs of protein synthesis with C allocation strategies and growth. We are currently quantifying ribosomes in <20 Arabidopsis accessions, to integrate this information into growth models.

4. Deviations from Work Program and Corrective Action

See above

5. Future Perspectives

- Activities connected to the 'core' experiments in Chlamydomonas will be pursued with high priority.
- Monitor enzyme activities in parallel with exhaustive measurements of Calvin cycle intermediates and (together with Schöttler) electron transport and energy dissipation in Chlamydomonas. This unique combination of technologies should provide a rich multilayered data set for modelling. In parallel, we are exploring the use of MS-based proteomics linked with chemical derivitisation of cys to quantify rthe edox state of cys residues to achieve wider coverage of enzymes (PhD, Tabea Mettler).
- Studies of the relation between metabolism and growth in Arabidopsis accessions will be integrated with the quantitative analyses of translation, and with models of whole plant growth. More specifically, we are currently extending the analyses of accessions to investigate ribosome numbers, loading and carbon balance, to test the hypothesis that there is a trade-off between a high ribosome concentration (with a potential for generating a high protein content and allowing more rapid protein turnover) and maximising the rate of growth. The trade-off is likely to depend on the irradiance regime, photoperiod,

and the stability of the environmental conditions. These efforts will be integrated with the development of ^{15}N-Arg to measure protein synthesis rates.

6. Co operations

6.1 Internal Co operations (within GoFORSYS)

- Bock, Müller-Röber, Ruprecht, Schroda, Willmitzer et al. –Chlamydomonas 'core experiments
- Schöttler – analysis of substrates and enzymes in parallel with determinations of electron fluxes in rapid transients in Chlamydomonas;
- Ebenhöh – basic formal analysis of regulatory features of the Calvin cycle; derivation of theoretical relations to explain the lack of connectivity between enzyme activities and metabolite levels; modelling of whole plant growth in relation to carbon and energy balance, including ribsome biogenesis and protein synthesis and the impact thereon of photoperiod;
- Selbig, Steinfath – multivariate analysis of large data sets;
- Lipowsky – provisionof data for modelling of polyosme loading;
- Schaub – use of logical reasoning to identify trait sets that show mututally consistent changes.

6.2 External Co operations

- Rohwer (Stellenbosch, SA, Humboldt Fellow): derivation of theoretical relations to explain the lack of connectivity between enzymes and metabolites; modelling protein synthesis rates and levels.
- Millar, Husmeier (CSBE, Edinburgh), Gruissem (ETH Zürich), Smith (JIC Norwich), Rodriguez (CRAG, Barcelona): FP7 collaborative project, Systems analysis of clock-metabolism interactions

7. Publications

[1] **B. Usadel, O.E. Bläsing, Y. Gibon, M. Höhne, M. Günter and M. Stitt**. ( ) Temporal Responses of Metabolites and Global Transcript Levels to Progressive Exhaustion of Carbohydrates in Arabidopsis Rosettes ,Plant Physiol. 146, 1834-1861

[2] P. May, S Weinkoop, S Kempa, **B Usadel,** J. Rupprecht, J. Weiß, L. Recunco-Munoz, O. Ebenhöh, W. Weckwerth, D. Walther , (2008) Metabolomics and proteomics assisted genome annotation and analysis of the draft metabolic network of *Chlamydomonas reinhardtii.* Genetics 179, 157-166

[3] **S. Arrivault, M .Günter, I. Ivakov, R. Feil, D. Vosloh**, J.T.von Doongen, **R. Sulpice, and M. Stitt**. (2009) Measurement of Calvin cycle and other metabolic intermediates in Arabidopsis leaves at different carbon dioxide concentrations using reverse-phase liquid chromatography linked to tandem mass spectrometry. Plant J 10.1111/j.1365-313X.2009.03902.x

[4] **R. Sulpice, E.-T. Pyl,** S. Trenkamp, M. Steinfath**, H. Tschoep**, H. Witucka-Wall, Y. **Gibon, B. Usadel, F. Poree, M. Piques,** M. Von Korff, M.C. Steinhauser, **M. Guenther, M. Hoehne, J.** Selbig, A.R. Fernie, A.T. Altmann, and **M. Stitt**, (2009) Starch as a major integrator in the regulation of growth in Arabidopsis thaliana. Proc. Natl. Acad. Sci .USA 104, 4759-4764

[5] M**. Piques, W.X. Schulze, Y. Gibon, J. Rohwer and M. Stitt** (2009) Quantitative Analysis of Ribosome Recruitment and Transcript Occupancy by Polysomes: The Dynamics of Protein Turnover in Central Metabolism in Arabidopsis Rosettes. Mol. Syst. Boil. In press

[6] **J. Hanneman,** H. Poorter, **B. Usadel,** F. Tardieu, O. Atkin, T Pons, **M. Stitt and Y. Gibon** (2009) XEML Lab: a tool for a standardised description of the growth environment of plants. ,Plant Cell Environ., in press

8. Poster presentations

Numerous. The poster 'Use of reverse phase liquid chromatography linked to tandem mass spectrometry to profile Calvin cycle and other metabolic intermediates in Arabidopsis rosette leaves' presented by S. Arrivault, which won the poster prize at the Metabolomics Conference, Norwich 2009.

9. Teaching and Training activities

GoFORSYS Ring-Vorlesung, Part 'Dark reactions' December 2007

10. Organisation of Scientific Events

Convenor for (and speaker in) the Session 'Plant Systems Biology' at the International Systems Biology Meeting 2008, Göteborg, Sweden August 2008

Organising committee, International Conference on Systems Biology, 2010

Organiser of the section 'Benson-Calvin cycle', Photosynthesis conference, Peking 2010

11. Invited talks

Of numerous talks at major conferences or workshops, directly relevant for Systems Biology are

'From Functional Genomics to Systems Biology', MPI-CNRS workshop on Systems Biology, Berlin September 2007

'Systems Biology of C Sensing and Growth in Arabidopsis', EPSO Meeting Teneriffe, October 2007

'Systems Biology of C Sensing and Growth in Arabidopsis', International Systems Biology Meeting, Göteborg, Sweden, August 2008

'Systems Analysis of Diurnal Regulation', Plenary lecture, International Conference on Arabidopsis Research, Edinburgh June 2009.

'Gauging starch turnover, protein content and growth to the carbon supply, International Congress on Plant Molecular Biology, St Louis, October 2009

'How plants avoid a credit crunch in a fluctuating envornment' at the Symposium "3rd Frontiers in The New Biology: Models of Life", Karolinska Institutet, Sweden, October 2009

12. Commercialisation (Patents, Cooperation with Industrial Partners etc.)

System and method for producing weighed portions of powder from at least one biological material at cryotemperatures (Cryogenic Grinder System). 2007. European patent application (EP 07118640) 17.10.2007; US patent application (US 11/975129) 17.10.2007

1. Research Group

PI	Prof. Ralph Tiedemann
Group Members	Maren Dieckvoß (PhD) 03/07
	Sandra Schwarte (PhD) 04/07
	Anne Kuschel (diploma student) 12/08
	Fanny Wegner (student worker) 09/07
	Madlen Stange (student worker) 09/07

2. Tasks worked on

Functional genomic variation among ecotypes/strains of *Arabidopsis thaliana* and *Chlamydomonas reinhardtii*

3. Achievements made

In *Arabidopsis thaliana* we completed the sequence analysis of all RubisCO genes (1 large subunit, 4 small subunits) in 26 accessions. All genes are relatively conserved and show only a few substitutions at the amino acid level. Interestingly, we found a gene duplication/loss event, where rbcS-1b was lost and substituted by a duplicated rbcS-2b (hereafter called rbcS-2b*) in 3 accessions (Bur-0, Cvi-0 and Sha(kdara)). We developed a PCR assay by which we analyzed this gene duplication/lost event in 74 additional accessions. We detected the same gene duplication/loss in 5 additional accessions such that it occured altogether in 8 out of 100 analyzed accessions. We also sequenced an about 1 kb promotor region for all RubisCO genes. This analysis revealed that the gene duplication/loss event was linked to a promotor insertion (~ 1.3 kb) in rbcS-2b and a promotor deletion (~2.3 kb) in rbcS-2b* in all 8 affected accessions.

Furthermore we have sequenced 16 CALVIN cycle genes including an about 1 kb promotor region (RCA, RMT, 2 x PGK, 3 x GAP-DH, TPI, 2 x SFBA, FBPase, TKL, SBPase, RPI, RPE, PRK) in 6 diverse accessions (Bur-0, Cvi-0, Nok-1, Sap-0, Sha(kdara), Tsu-1). In that data set RubisCO methyltransferase and SBPase exhibit the highest number of amino acid substitutions.
Additionally, we have sequenced genes (incl. ~ 1 kb promotor region) involved in starch synthesis: starch synthases 1-4 (SS 1-4), granule bound starch synthase (GBSS), branching enzyme 1-3 (BE 1-3), debranching enzymes (ISA1,2), and glucan phosphorylases (PHS1,2). Comparing the nucleotide and amino acid sequences among the accessions is under way, as well as correlation of genomic variability to gene expression as well as functional and biochemical properties of the resulting enzymes.

In *Chlamydomonas reinhardtii* the large RubisCO subunit gene was sequenced in 28 strains, whereof 21 are *Chlamydomonas reinhardtii* and 7 are other *Chlamydomonas* species. Sequencing of 2 genes encoding the small subunit is almost complete in 21 *Chlamydomonas reinhardtii* strains. Like in *Arabidopsis*, these genes are very conserved and exhibit only a few amino acid substitutions.

4. Deviations from Work Program and Corrective Action

None

5. Future Perspectives

The next logical step is to evaluate, whether the expressed variability (at the amino acid level) in *Arabidopsis* genes has functional implications. To this end, we envision to characterize phenotypic traits/physiological properties (e.g., photosynthesis and growth rates or RubisCO activity) in insertion mutants for RubisCO small subunit. Additionally, we will measure growth rates and expression patterns in some selected accessions to evaluate functional consequences of the gene duplication/lost event described above.

We will plot our functional classification of accessions/ecotypes onto a robust phylogeny of Arabidopsis, which we will establish by analysis/data retrieval of phylogenetic house keeping genes. Regarding starch synthesis genes, we will compare genomic variation to functional and biochemical properties of the resulting enzymes.

In *Chlamydomonas* we will complete the sequence analysis of CALVIN cycle genes as well as we will analyze starch synthesis genes. As in Arabidopsis, we will evaluate whether expressed variability (at the amino acid level) in *Chlamydomonas* genes has functional implications.

6. Cooperation

6.1 Internal Cooperation (within GoFORSYS)

We collaborate with the group of Prof. Müller-Röber regarding the characterization of insertion mutants for RubisCO small subunit genes (e.g., measure photosynthesis, growth rates, and expression patterns in selected accessions)

We collaborate with the group of Prof. Steup where we combine genomic data set and biochemical data sets to look for evidence of functional variability in starch synthesis genes (SS1-4; GBSS; BE1-3; ISA1,2; PHS1,2).

We collaborate with the group of Prof. Stitt which made available to us measures of RubisCO activity for our insertion mutants for RubisCO small subunit genes.

6.2 External Cooperation

We collaborate with Prof. Altmann (IPK Gatersleben) regarding functional genomic variation in Arabidopsis.

7. Publications

The group has many recent publications on evolutionary biology (http://www.bio.uni-potsdam.de/professuren/evolutionsbiologie/publikationen/publikationen). Publications from this project are under way.

8. Poster presentations

Sandra Schwarte, Madlen Stange, Fanny Wegner, Ralph Tiedemann: A gene duplication/loss event in the otherwise highly conserved Ribulose-1,5-Bisphosphate-Carboxylase/Oxygenase (RubisCO) small subunit gene family among ecotypes of *Arabidopsis thaliana*; ESEB 2009; Turin; 08/2009

9. Teaching and Training activities

A 2 weeks lab course on Molecular Evolutionary Biology focussed on Rubisco genes of *Arabidopsis* was held in February 2009 at the University of Potsdam.

10. Organisation of Scientific Events

15th – 16th of June 2009: DFG-Rundgespräch "Evolutionary Genomics" in Potsdam.

11. Invited talks

None

12. Commercialisation (Patents, Cooperation with Industrial Partners etc.)

None

1. Research Group

PI	Dirk Walther, PhD
Group Members	Patrick May, PhD (06/2007)

2. Tasks worked on

The group has been a major contributor to WP1, General Bioinformatics and Computational Methods; in particular, WP 1.1 Data Warehousing, WP 1.2 Genome Analysis and Comparison, and WP 1.3 Integrated Data Analysis.

3. Achievements

Among other achievements listed below, the work of this group contributed significantly to a major joint *Go*FORSYS publication on the improved Chlamydomonas genome annotation using integrated metabolomics, proteomics, and bioinformatics approaches (May et al., 2008) and developed the ChlamyCyc metabolic network database and web-portal (May et al., 2009) as a global resource for the Chlamydomonas research community, which was recently included into the curated BioCyc family of Pathway/Genome databases (http://biocyc.org/otherpgdbs.shtml).

- Established the ChlamyCyc database and web-portal as a central repository for visualization and analysis of integrative data from various high-throughput technologies before the background of molecular pathways in Chlamydomonas and genome as well as gene centric views. ChlamyCyc also serves as an integration platform for datasets generated by our *Go*FORSYS partner groups, especially for metabolomics and proteomics data sets. Results from this work yielded an improved Chlamydomonas genome annotation.
- Reconstructed draft metabolic network of Chlamydomonas reinhardtii.
- Generated and provided MapMan functional annotation for all Chlamydomonas genes.
- Generated orthology relationships of Chlamydomonas genes against more than 23 other species.
- Identified of candidate miRNAs in Chlamydomonas from deep sequencing data sets.
- Microarray design for a new Chlamydomonas chip.
- Contributed towards establishment of global Chlamydomonas resource (ChlamyBase) as part of an international effort.

4. Deviations from Work Program and Corrective Action

None

5. Future Perspectives

Future efforts will focus on integrating and presenting the results obtained from the Core Experiment. Especially the establishment of an online resource that makes the results available and queryable to the global Chlamydomonas research community, which integrates with the resources already developed as part of the GoFORSYS project (ChlamyCyc), will be at the center of activities.

It is also planned to integrate the datasets with the infrastructure developed under the GABI-PD framework (collaboration with Dr. Birgit Kersten and colleagues) resulting in a seamless integration of Chlamydomonas data with data from other model and crop plants.

- Integrating different database systems such as Promex, Golm Metabolome Database, and a new repository for transcript analysis into the ChlamyCyc portal.
- Upgrade to v4.0 genome annotation once released;
- microRNA analysis and research on gene regulatory networks in Chlamydomonas;
- Systematic data warehousing for *Go*FORSYS data using InterMine technology (established contact with InterMine developers, participated in workshop);
- Support of experimental processes via dataflow software (integration of LIMS) and other bioinformatics service activities.
- Establishment of an online interface to access and query the results obtained from the core experiment; Integration with GABI-PD.

6. Co-operations

6.1 Internal Cooperation (within *Go*FORSYS)

AG Selbig – Gene annotation, phylogeny, Metabolic network reconstruction

AG Ebenhöh - Metabolic network reconstruction for Chlamydomonas, *E.coli* and Arabidopsis.

AG Weckwerth – Metabolomics and proteomics assisted genome annotation, proteomics data analysis workflow, Promex database, primer design.

AG Schroda – Data management, Proteomics data analysis pipeline

AG Schaub – Metabolic network analysis based on ChlamyCyc.

AG Stitt – Gene annotation and primer design, processing ribosomal load data.

AG Müller-Röber - Transcription factor analysis, primer design, gene annotation.

AG Bock – miRNAs discovery from deep sequencing data in Chlamydomonas, small RNA annotation, chloroplast microarray data analysis.

AG Willmitzer - Chlamydomonas orthology relationships to rice, human, Arabidopsis, annotation of lipid related pathways.

6.2 *Go*FORSYS partner groups

Maria Mittag (U Jena) - Development of central Chlamydomonas proteomics experiment, annotation of nitrogen assimilation pathway

Olaf Kruse – Development of a new Chlamydomonas microarray.

6.3 External Cooperation

Lukas Müller (Cornell U) - Development of international Chlamydomonas resource Chlamybase.

Mario Stanke (U Göttingen) – Chlamydomonas genome annotation.

Marc Friedländer, Nikolaus Rajewski (MDC Berlin) – Plant specific miRNA prediction from deep sequencing data.

Wolf Scheible (MPIMP) – Analysis of deep sequencing data from Arabidopsis and Brassica napus.

AG Kehr (MPIMP) – Analysis of deep sequencing data and miRNA predictions from Brassica napus.

Wolfram Weckwerth (U Vienna) – Proteomics assisted genome-annotation in Chlamydomonas.

Stefan Kempa (MDC Berlin) – Metabolomics assisted network reconstruction in Chlamydomonas.

Robert Preissner (Charité) – Protein Docking.

Knut Reinert (FU Berlin) – Sequence analysis, RNA alignments (www.plane-lisa.net).

Prof. **Sebastian Doniach (Stanford U)** Discovery of riboswitches.and regulatory RNA molecules.

7. Publications

GoFORSYS Publications:

- **May P**, Christian JO, Kempa S, and **Walther D**. (2009) ChlamyCyc: an integrative systems biology database and web-portal for Chlamydomonas reinhardtii. BMC Genomics. 10: 209
- Childs L, Nikoloski Z, **May P**, and **Walther D**. (2009) Identification and classification of ncRNA based on graph properties. Nucleic Acid Research. 37(9):e66.
- Pant BD, Musialak-Lange M, Nuc P, **May P**, Buhtz A, Kehr J, **Walther D**, and Scheible WR (2009) Identification of nutrient-responsive *Arabidopsis* and rapeseed microRNAs by comprehensive real-time PCR and small RNA sequencing. Plant Physiol. 150:1541-1555
- **May P**, Wienkoop S, Kempa S, Usadel B, Christian N, Rupprecht J, Weiss J, Recuenco-Munoz L, Ebenhoeh O, Weckwerth W, and **Walther D**. (2008) Metabolomics and proteomics assisted genome annotation and analysis of the draft metabolic network of *Chlamydomonas reinhardtii*. Genetics, 179: 157-166
- Nikoloski Z, Grimbs S, **May P**, and Selbig J.(2008) Metabolic Networks are NP-hard to reconstruct. J Theor Biol 254: 807-816
- Günther S, Eichborn J, **May P**, and Preissner R. (2008) JAIL: a structure-based interface library for macromolecules. Nucleic Acids Res., doi:10.1093/nar/gkn599
- Kempa S, **Walther D**, Ebenhoeh O, and Weckwerth W. (2008) Metabolic Engineering. In: Molecular Biology and Biotechnology. Eds Walker and Rapley.
- Christian N, **May P**, Kempa K, Handorf T, and Ebenhöh E (2009). An integrative approach towards completing genome-scale metabolic networks. *Molecular BioSystems,* DOI:10.1039/B915913B, in press.

- Nikoloski N, Strassburg K, Selbig J, **Walther D**, and Kopka J. Properties of active subnetworks reflect the temperature stress response of Saccharomyces cerevisiae. Submitted to OMICS – Journal of Integrative Biology

8. Poster presentations

- **May P**, Usadel B, Ebenhöh O, Weckwerth W, **Walther D**. Reconstruction and extension of the draft metabolic network of Chlamydomonas reinhardtii. In Abstract book, 9th International Conference on Systems Biology, 89, Gothenburg, 2008;
- Ebenhöh O, Christian N, **May P, Walther D**, Handorf T. Filling the gaps in genome scale metabolic networks. In Abstract book, 5th International Conference on Plant Metabolomics, 34, Yokohama, Japan, 2008;
- **May et al.** GCB 2007, GoFORSYS - Potsdam-Golm BMBF-Research Project on Systems Biology, Potsdam Sep, 2007;
- **May P,** Poster; MPG Postdoc – Meeting, MPI für Züchtungsforschung, Köln, Nov 2008

9. Teaching

- Dirk Walther: Introduction to Systems Biology, Postdam University, (Lecture series jointly given by Prof. Selbig, Prof. Weckwerth, Dr. Dirk Walther);
  - Spring Term 2007
  - Fall Term 2007
- Dirk Walther, Patrick May: Structural Bioinformatics, Lecture, Potsdam University, Spring Term 2009, as part of the Master in Bioinformatics Programme
- Dirk Walther: Bioinformatics Working Seminar, U Potsdam/ MPIMP, ongoing;
- Dirk Walther: Basic statistics, PhD Course, MPIMP, (organized by K. Köhl (MPIMP)) Nov 2008
- Dirk Walther: Network analysis, Lecture as part of PhD IMPRS/GoFORSYS PhD Seminar Series, Jun 2008.

10. Organisation of Scientific Events

- Dirk Walther: Data Management Workshop, BMBF-FORSYS Meeting, Berlin, Jun 2008
- Dirk Walther: German Conference on Bioinformatics (GCB), Local Organizer, Sep 2007
- Dirk Walther: Tri-national workshop on “Omics Technologies and Bioinformatics”, Japan-Sweden-Germany, Golm, Apr 2007

11. Invited talks

- Patrick May: Biocuration 2009 conference, Berlin, April 2009, Filling the gaps in genome scale metabolic networks – an application to *Chlamydomonas reinhardtii*.
- Patrick May: ChlamyBase annotation and database meeting, JGI user meeting, March 2009, Walnut Creek, CA, USA, ChlamyCyc – an integrative systems biology database and web-portal for *Chlamydomonas reinhardtii*.

- Patrick May: Goforsys-Partner Kickoff Meeting, Jena, Nov 2008, ChlamyCyc and genome annotation within *Go*FORSYS.
- Dirk Walther: Invited Talk, Stanford University, Stanford, USA, Sep 2008
- Patrick May: AG Schaub, Universität Potsdam, Institut für Informatik, ChlamyCyc, Sep 2008
- Patrick May: Data Management Workshop, Forsys Meeting, Berlin, Data management within *Go*FORSYS, Jun 2008
- 28-30. April 2008. Conference: Non-coding RNA, Antalya, Turkey
  - Capturing RNA functionality in graph properties**.** Liam Childs, Zoran Nikoloski, **Patrick May** and **Dirk Walther**
  - Exploring RNA-Ligand docking: Riboswitch-Metabolite Interactions. **Patrick May**, Christian Schudoma, **Dirk Walther**
  - Exploring Motif-based RNA 3D Modeling, Christian Schudoma, **Patrick May** and Frank Cordes
- Dirk Walther: Keynote Speaker, PhD Student Conference, Halle, Germany, Jun 2007
- Dirk Walther: Invited Speaker, “New Frontiers in Biosciences”, Nara, Japan, Jan 2007

12. Commercialization

None.

1. Research Group

PI	Prof. Lothar Willmitzer
Group Members	Bettina Seiwert (postdoc, paid from GoFORSYS)
	date of employment: 09/07 – 07/09
	J. Lisec, T. Degenkolbe, P. Giavalisco, A. Eckardt

2. Tasks worked on

WP2: Development of new analytical tools for systems analysis here: WP2.3.
WP3: Multilevel systems analysis of responses in Chlamydomonas here: WP3.1 and WP 3.3.
WP4: Testing of the applicability of models developed in Chlamydomonas by comparison with the response of a model higher plant here: WP4.1. and WP4.3.

3. Achievements made

WP2.3: A metabolome analysis platform targeting secondary metabolism was established which based on reverse phase chromatography (UPLC) and coupled FT-ICR MS analysis combined with the use of a fully 13C isotope labelled metabolome allows the unambiguous annotation of the chemical composition based on the exact mass. In case of *A. thaliana* leaf extracts more than 1000 compounds could be annotated.
As a second technology development a lipidomics platform was established allowing the analysis of more than 120 complex lipids from 12 lipid classes in case of Chlamydomonas. The approach combines a 2-dimensional LC with high resolution MS and isotope labelled metabolomes.

WP3.1: Synchronized batch-type cultures of C reinhardtii have been analyzed on the level of transcripts (nRNA) and metabolites (GC-TOF MS; secondary metabolites; Lipidomics) as a function of division. Parallel cultures have been treated with rapamycin (inhibitor of TOR (**t**arget **o**f **r**apamycin) to evaluate the influence of TOR in a photosynthetically active organism.

WP3.3: In the framework of the core experiments lipid pattern was analyzed using the newly established lipidomics platform. Data have been analyzed in cooperation with J. Selbig/ Z. Nikoloski.

WP4.1: The response of *Arabidopsis thaliana* to eight different environmental conditions covering four different light intensities and three different temperatures was followed on the metabolite and the transcript level over a period of 12 hours after transfer to a novel environment with samples taken every 20 minutes. Using a variety of statistical tools it becomes clear that there is a broad agreement between the responses of transcripts and metabolites, and that the degree of co-regulation depends on the environmental condition. We also observed that interaction of light and temperature tends to modulate the same responses, rather than leading to complete condition-specific reprogramming.

Further, there is very little if any conserved response over all seven novel conditions. At low temperature (4°C) the cold response dominates over the dark response whereas at high temperatures (32°C) the dark response dominates over the temperature response.

Application of graph-based methods allowed the construction of metabolic networks for each of the eight conditions analyzed and the subsequent determination of network parameters. Comparison of the eight networks revealed the presence of both common and conditional features. Transformation of condition specific networks into one another followed both temperature and light gradients. More detailed analysis of the different networks identified novel and conditional connections between a range of amino acids including 4-aminobutyric acid and the branched chain amino acids with intermediates of the TCA cycle suggestive of protein degradation products fueling energy metabolism in the absence of photosynthesis.

WP 4.3. Collections of Arabidopsis recombinant inbred lines (RILs) and near isogenic lines (NILs) derived from crosses of naturally occurring accessions ("ecotypes") have been characterised in close collaboration with the group of Thomas Altmann (PI 1) 16 for their growth/biomass accumulation characteristics and their metabolic composition. 6 biomass and 157 metabolite QTL were detected. Furthermore, 7 QTL for biomass absolute mid-parent heterosis and 147 QTL for metabolite absolute mid-parent heterosis were detected by analysis of the RILs and RIL-TCs. The RIL / RIL-TC genotype and phenotype data were furthermore used in collaboration by GoFORSYS partner 10 to develop a procedure for improved heterosis prediction.

4. Deviations from Work Program and Corrective Action

None

5. Future Perspectives

Development of the analytical platforms has been finished. Thus the major focus will lie on the joint analysis of additional core-experiments performed in the framework of GoFORSYS, the role of TOR in photosynthetic organisms (*C. reinhardtii* and *A. thaliana*) and the comparative analysis of *A. thaliana* and *C. reinhardtii* with respect to their response towards changing environmental conditions.

6. Cooperation

6.1 Internal Cooperation (within GoFORSYS)

GoFORSYS partner 1 (Prof. Dr. Thomas Altmann): Metabolic analysis of RILs / RIL-TCs, NILs / NIL-TCs and QTL detection

GoFORSYS partner 10 (Prof. Dr. Joachim Selbig/ Dr. Zoran Nikoloski): Development of metabolic predictors of biomass and biomass heterosis; network analysis of transcriptome and metabolome data

GoFORSYS partner 17 (PD Dr. M. Schroda): Metabolic profiling; Construction of C. reinhardtii strains inhibited for TOR via inducible RNAi

GoFORSYS partner 11 (Prof. Dr. Martin Steup): Comprehensive analysis of synchronized cell cultures of C. reinhardtii

6.2 External Cooperation

Prof. Dr. Albrecht Melchinger (Univ. Hohenheim): Growth and heterosis QTL analyses

7. Publications

[1] J. Lisec, R. C. Meyer, M. Steinfath, H. Redestig, M. Becher, H. Witucka-Wall, O. Fiehn, O. Törjek, J. Selbig, T. Altmann and L. Willmitzer (2008) Identification of metabolic and biomass QTL in Arabidopsis thaliana in a parallel analysis of RIL and IL populations, Plant J. 53:960-72

[2] T. Gärtner, M. Steinfath, S. Andorf, J. Lisec, R. C. Meyer, T. Altmann, L. Willmitzer and J. Selbig (2009) Improved heterosis prediction by combining information on DNA- and metabolic markers, PLoS ONE 4(4):e5220

[3] J. Lisec, M. Steinfath, R. C. Meyer, J. Selbig, A. E. Melchinger, L. Willmitzer and T. Altmann (2009) Identification of heterotic metabolite QTL in *Arabidopsis thaliana* RIL and IL populations, Plant J. doi: 10.1111/j.1365-313X.2009.03910.x

[4] R. C. Meyer, B. Kusterer, J. Lisec, M. Steinfath, M. Becher, H. Scharr, A. E. Melchinger, J. Selbig, U. Schurr, L. Willmitzer and T. Altmann (2009) QTL analysis of early stage heterosis for biomass in Arabidopsis, Theor. Appl. Genet. doi: 10.1007/s00122-009-1074-6

[5] P. Giavalisco, K. Köhl, J. Hummel, B. Seiwert, L. Willmitzer (2009) ^{13}C Isotope-labeled metabolomes allowing for improved compound annotation and relative quantification in liquid chromatography-mass spectrometry-based metabolomic research. Anal. Chem. 81, 6546 – 6551

[6] B. Seiwert, P. Giavalisco, L. Willmitzer (2009) Advanced mass spectrometry methods for analysis of lipids from photosynthetic organisms in: Lipids in Photosynthesis (Murata and Wada ed.) Springer New York Berlin, in press

*Go*FORSYS Publications

(in alphabetical order)

1. Arenas A, Diaz-Guilera A, Kurths J, Moreno Y, Zhou CS. 2008. Synchronization in complex networks. *Physics Reports - Review Section of Physics Letters* **469**: 93-153
2. Arrivault S, Guenther M, Ivakov A, Feil R, Vosloh D, van Dongen JT, Sulpice R *et al.* 2009. Use of reverse-phase liquid chromatography, linked to tandem mass spectrometry, to profile the Calvin cycle and other metabolic intermediates in Arabidopsis rosettes at different carbon dioxide concentrations. *Plant J*: Epub ahead of print
3. Basler G, Nikoloski Z, Ebenhoh O, Handorf T. 2008. Biosynthetic potentials from species-specific metabolic networks. *Genome Inform* **20**: 135-48
4. Bassler OY, Weiss J, Wienkoop S, Lehmann K, Scheler C, Dolle S, Schwarz D *et al.* 2009. Evidence for novel tomato seed allergens: IgE-reactive legumin and vicilin proteins identified by multidimensional protein fractionation-mass spectrometry and in silico epitope modeling. *J Proteome Res* **8**: 1111-22
5. Childs L, Nikoloski Z, May P, Walther D. 2009. Identification and classification of ncRNA molecules using graph properties. *Nucleic Acids Res* **37**: e66
6. Christian N, Handorf T, Ebenhoh O in *7th Annual International Workshop on Bioinformatics and Systems Biology*, edited by Miyano, DeLisi, Holzhutter, Kanehisa (Imperial College Press, Tokyo, JAPAN, 2007), p. 320-9.
7. Christian N, Handorf T, Ebenhoh O. 2007. Metabolic synergy: increasing biosynthetic capabilities by network cooperation. *Genome Informatics* **18**: 320-9
8. Christian N, May P, Handorf T, Kempa S, Ebenhoeh O. 2009. An integrative approach towards completing genome-scale metabolic networks. *Molecular BioSystems*: in press (DOI:10.1039/B915913B)
9. Dworschak S, Grell S, Nikiforova VJ, Schaub T, Selbig J. 2008. Modeling biological networks by action languages via answer set programming. *Constraints* **13**: 21-65
10. Dworschak S, Grote T, Konig A, Schaub T, Veber P in *2008 20th IEEE International Conference on Tools with Artificial Intelligence* (IEEE, Dayton, OH, USA, 2008), p. 11-8.
11. Dworschak S, Grote T, König A, Schaub T, Veber P in *Twelfth International Workshop on Nonmonotonic Reasoning*, edited by Pagnucco, Thielscher, School of Computer Science and Engineering, The University of New South Wales, 2008), p. 94-102.
12. Ebenhoh O, Handorf T. 2009. Functional classification of genome-scale metabolic networks. *EURASIP Journal on Bioinformatics & Systems Biology*: 570456
13. Fettke J, Hejazi M, Smirnova J, Hochel E, Stage M, Steup M. 2009. Eukaryotic starch degradation: integration of plastidial and cytosolic pathways. *J Exp Bot* **60**: 2907-22
14. Gartner T, Steinfath M, Andorf S, Lisec J, Meyer RC, Altmann T, Willmitzer L *et al.* 2009. Improved heterosis prediction by combining information on DNA- and metabolic markers. *PLoS One* **4**: e5220
15. Gebser M, Kaminski R, Kaufmann B, Ostrowski M, Schaub T, Thiele S in *24th International Conference on Logic Programming (ICLP)*, edited by DelaBanda, Pontelli (Springer-Verlag Berlin, Udine, ITALY, 2008), p. 190-205.
16. Gebser M, Schaub T, Thiele S in *Logic Programming and Nonmonotonic Reasoning. 9th International Conference, LPNMR 2007. Proceedings*, edited by Baral, Brewka, Schlipf (Springer, Tempe, AZ, USA, 2007), p. 266-71.
17. Gebser M, Schaub T, Thiele S, Usadel B, Veber P in *24th International Conference on Logic Programming (ICLP)*, edited by DelaBanda, Pontelli (Springer-Verlag Berlin, Udine, ITALY, 2008), p. 130-44.
18. Giavalisco P, Hummel J, Lisec J, Inostroza AC, Catchpole G, Willmitzer L. 2008. High-resolution direct infusion-based mass spectrometry in combination with whole (13)c metabolome isotope labeling allows unambiguous assignment of chemical sum formulas. *Analytical Chemistry* **80**: 9417-25

1. Research Group

PI	Prof. Thomas Altmann
Group Member	Berit Ebert (postdoc)
	Date of employment: 03/09

2. Tasks worked on

WP 4 Testing of applicability of models developed in Chlamydomonas by comparison with the response of model higher plants

WP 4.3 Identification of genotypes differing in photosynthesis and growth

3. Achievements made

Collections of Arabidopsis recombinant inbred lines (RILs) and near isogenic lines (NILs) derived from crosses of naturally occurring accessions ("ecotypes") have been characterised in close collaboration with *Go*FORSYS partner 16 (Group Willmitzer) for their growth/biomass accumulation characteristics and their metabolic composition. Thus, 429 C24/Col-0 RILs and 369 RIL test crosses (RIL-TCs) to both parents, as well as 97 C24/Col-0 NILs and 41 NIL test crosses (NIL-TCs) cultivated in soil under controlled environmental conditions were analysed for shoot dry mass and metabolite composition (via GC-MS). Using RIL genotype and phenotype data, 6 biomass QTL and 157 metabolite QTL were detected. In addition to verification of most QTL by the NIL data, additional biomass and metabolite QTL were identified. In line with previous observations of a close relation between metabolite composition and plant growth, two biomass QTL coincided with significantly more metabolite QTL than expected for a random distribution. Furthermore, 7 QTL for biomass absolute mid-parent heterosis and 147 QTL for metabolite absolute mid-parent heterosis were detected by analysis of the RILs and RIL-TCs. Again, in addition to verification of most of the heterosis QTL in the RIL / RIL-TC population, additional heterotic QTL were detected using the NIL / NIL-TC data. The RIL / RIL-TC genotype and phenotype data were furthermore used in collaboration by GoFORSYS partner 10 to develop a procedure for improved heterosis prediction.

A major biomass QTL has been fine mapped to a genomic segment containing 14 protein coding genes that are under analysis for their contribution to growth control by investigation of mutants and transgenic plants. Similarly, 30 candidate enzyme-encoding genes located in QTL regions of 15 metabolites are being studied for their involvement in metabolite content determination. A set of 100 deeply genotyped Arabidopsis natural accession has been provided to GoFORSYS partner 12 for in-depth analysis of growth and metabolic traits such as enzyme activities, protein, starch, sugars, amino acids, chlorophylls, and metabolic intermediates. Analysis of growth / biomass accumulation of the complete set of 136 NILs plus their NIL-TCs under elevated light intensity has been initiated.

36. Koseska A, Zaikin A, Garcia-Ojalvo J, Kurths J. 2007. Stochastic suppression of gene expression oscillators under intercell coupling. *Phys Rev E Stat Nonlin Soft Matter Phys* **75**: 031917

37. Koseska A, Zaikin A, Kurths J, Garcia-Ojalvo J. 2009. Timing cellular decision making under noise via cell-cell communication. *PLoS ONE* **4**: e4872

38. Kruse K, Ebenhoh O. 2008. Comparing flux balance analysis to network expansion: producibility, sustainability and the scope of compounds. *Genome Inform* **20**: 91-101

39. Lau S, Shao N, Bock R, Jurgens G, De Smet I. 2009. Auxin signaling in algal lineages: fact or myth? *Trends in Plant Science* **14**: 182-8

40. Lehmann U, Wienkoop S, Tschoep H, Weckwerth W. 2008. If the antibody fails - a mass Western approach. *Plant Journal* **55**: 1039-46

41. Lisec J, Meyer RC, Steinfath M, Redestig H, Becher M, Witucka-Wall H, Fiehn O *et al.* 2008. Identification of metabolic and biomass QTL in *Arabidopsis thaliana* in a parallel analysis of RIL and IL populations. *Plant J* **53**: 960-72

42. Lisec J, Steinfath M, Meyer RC, Selbig J, Melchinger AE, Willmitzer L, Altmann T. 2009. Identification of heterotic metabolite QTL in *Arabidopsis thaliana* RIL and IL populations. *Plant J*: Epub ahead of print

43. Matthaus F, Salazar C, Ebenhoh O. 2008. Biosynthetic potentials of metabolites and their hierarchical organization. *PLoS Computational Biology* **4**: e1000049

44. May P, Christian JO, Kempa S, Walther D. 2009. ChlamyCyc: an integrative systems biology database and web-portal for *Chlamydomonas reinhardtii*. *BMC Genomics* **10**: 209

45. May P, Wienkoop S, Kempa S, Usadel B, Christian N, Rupprecht J, Weiss J *et al.* 2008. Metabolomics- and proteomics-assisted genome annotation and analysis of the draft metabolic network of *Chlamydomonas reinhardtii*. *Genetics* **179**: 157-66

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49. Nguyen AV, Thomas-Hall SR, Malnoe A, Timmins M, Mussgnug JH, Rupprecht J, Kruse O *et al.* 2008. Transcriptome for photobiological hydrogen production induced by sulfur deprivation in the green alga *Chlamydomonas reinhardtii*. *Eukaryotic Cell* **7**: 1965-79

50. Nikoloski Z, Grimbs S, May P, Selbig J. 2008. Metabolic networks are NP-hard to reconstruct. *Journal of Theoretical Biology* **254**: 807-16

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52. Numata J, Ebenhoeh O, Knapp EW. 2008. Measuring correlations in metabolomic networks with mutual information. *Genome Informatics* **20**: 112-22

53. Piques MC, Schulze WX, Gibon Y, Rohwer J, Stitt M. 2009. Quantitative Analysis of Ribosome Recruitment and Transcript Occupancy by Polysomes: The Dynamics of Protein Turnover in Central Metabolism in Arabidopsis Rosettes. In *Mol Syst Biol*, pp. in press

54. Reumann S, Babujee L, Ma CL, Wienkoop S, Siemsen T, Antonicelli GE, Rasche N *et al.* 2007. Proteome analysis of Arabidopsis leaf peroxisomes reveals novel targeting peptides, metabolic pathways, and defense mechanisms. *Plant Cell* **19**: 3170-93

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56. Riano-Pachon DM, Ruzicic S, Dreyer I, Mueller-Roeber B. 2007. PlnTFDB: an integrative plant transcription factor database. *BMC Bioinformatics* **8**: 42

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Figures

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Figure 2: Assignment of PI to WPs

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Figure 4: Oxygen production and response to light shifts (2 consecutive experiments). Yellow: Light intensity; red: O_2 evolution control; blue: O_2 evolution treatment.

Figure 5: HCA of qRT-PCR data derived from samples of high-light exposed (induced) and control cultures (control) at different time points (1, 2, 5, 7, 8, 9, 10: correspond to -60, 0, 20, 60, 120, 240, 480 minutes after the light shift).

Figure 6: MapMan Metabolic Overview diagram (TP1, TP2, TP7, TP8, TP9, TP10). Overview of the dynamic transcriptional response observed in the core experiment illustrated by control-normalised time profiles associated with transcripts grouped by their annotation into MapMan functional bins. To generate this informative and integrative view, systematic annotation of all Chlamydomonas genes into the respective MapMan functional bins was necessary.

Figure 7: Polysome loadings of Chlamydomonas exposed to high light (right panel) or low light (left panel) conditions. Blue: polysomal fraction; red: non-polysomal rRNA fraction. The y-axis shows A_{254}, the x-axis the time in minutes, with 0 equalling the onset of the light shift.

Figure 8: Representative protein data for the light shift experiment

Figure 9: Levels of intermediates from the Calvin cycle, sucrose and starch synthesis, organic acids and amino acids in cells grown continuously at 200 µmol/m^2.s (dark bars) and cells shifted from 200 to 700 µmol/m^2.s at time point 0 x-axes show the time after the light shift [min], y-axes correspond to [pmol/10^6 cells].

Figure 10: Kinetics of exemplary lipids in light shifted (T) and control (C) samples. X-axis: relative units; y-axis: time scale (minutes before resp. after light shift).

Figure 11: Lipid networks of high light shifted samples resulting from correlation analysis distinguishing four different time intervals derived from PCA analysis (left) and distribution of correlation values at different time intervals (right).

Figure 12: Zero-order (A) and first-order (B) partial correlations of the Calvin cycle intermediates from the high light treatment, and zero-order correlations for the control data set (C). p-value < 0.01 are coloured from yellow to white, red squares indicate no significant correlation (represented as 0)

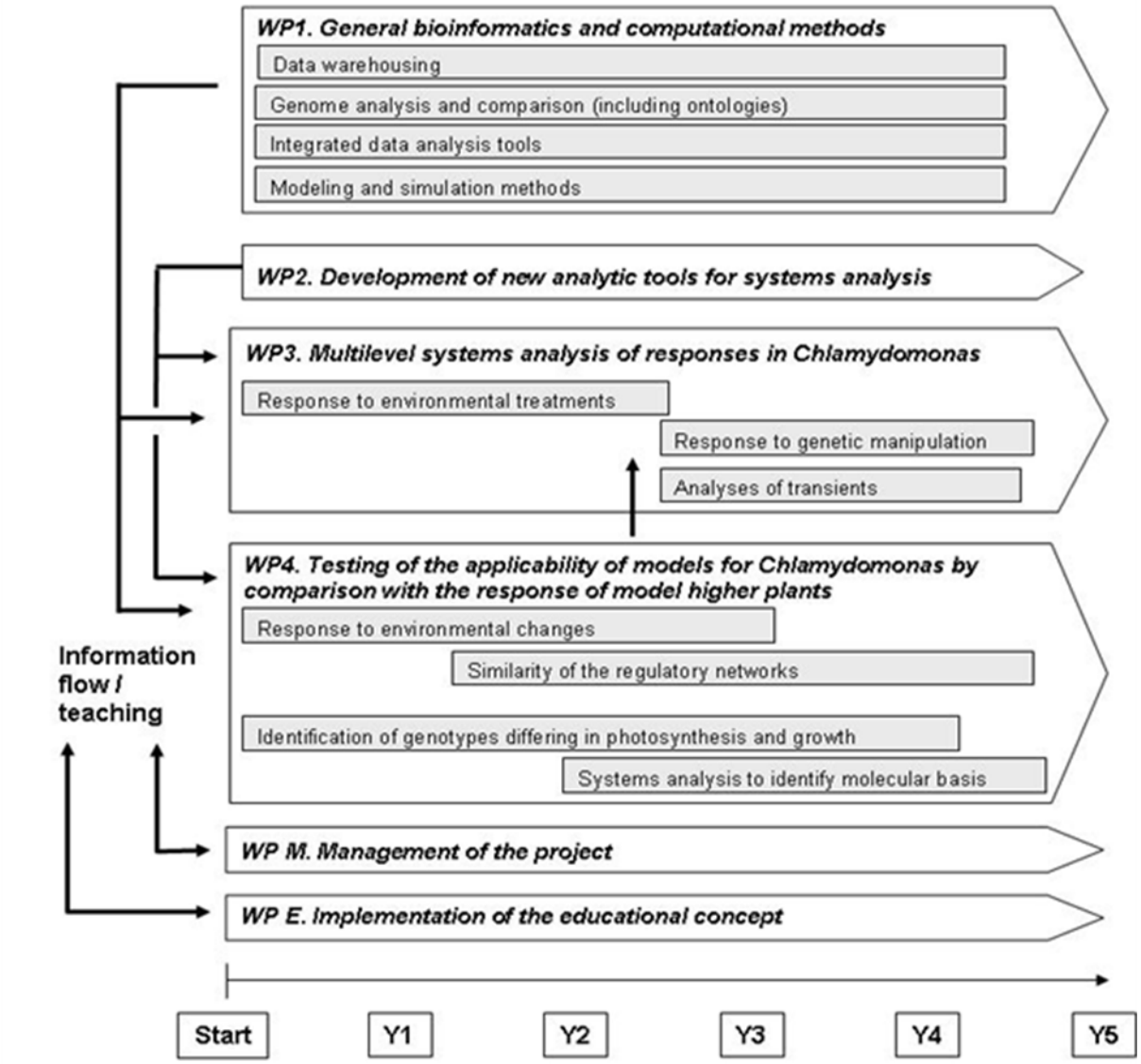
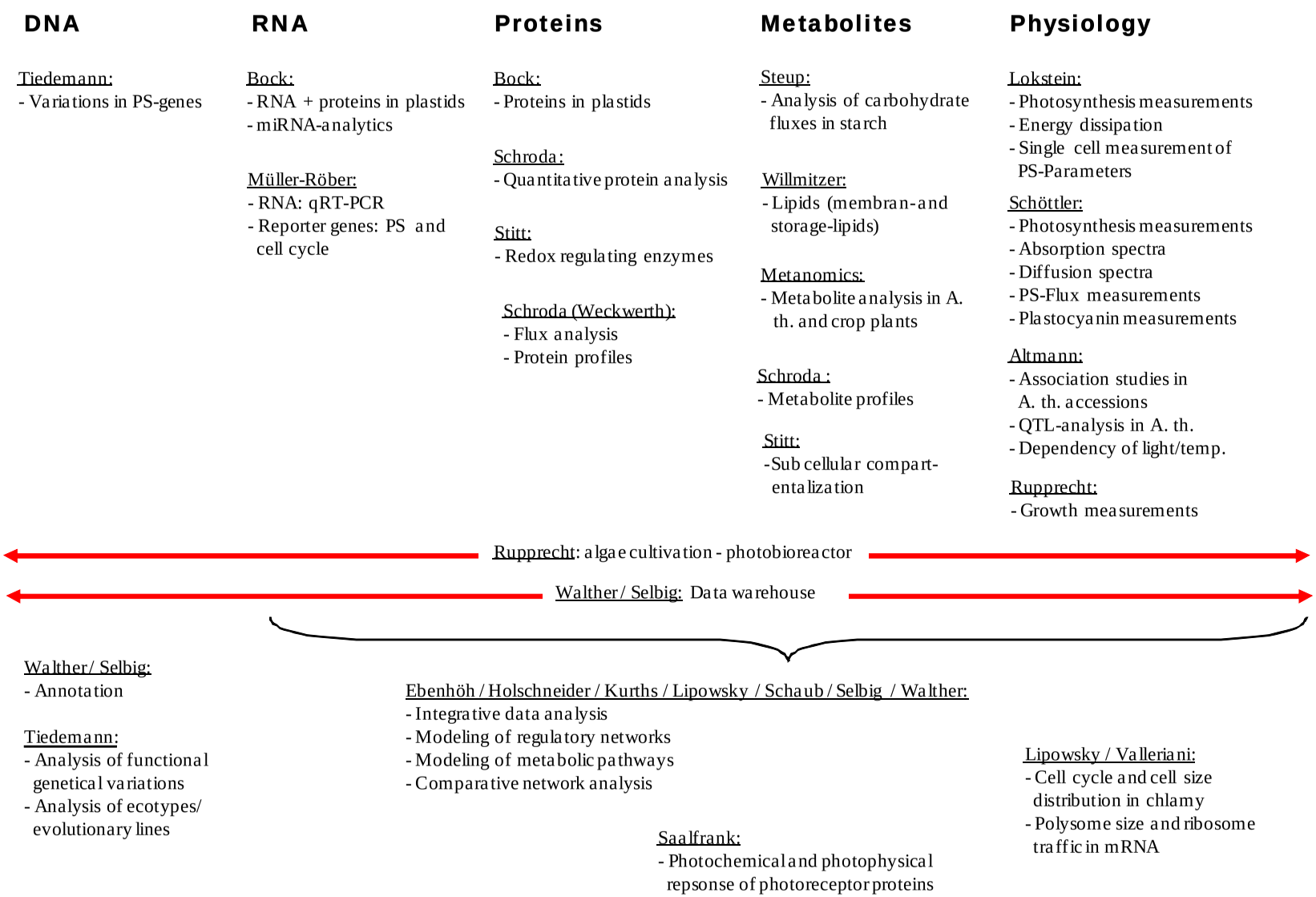


Figure 1: Workpackages and milestones

Figure 2: Assignment of PI to WPs



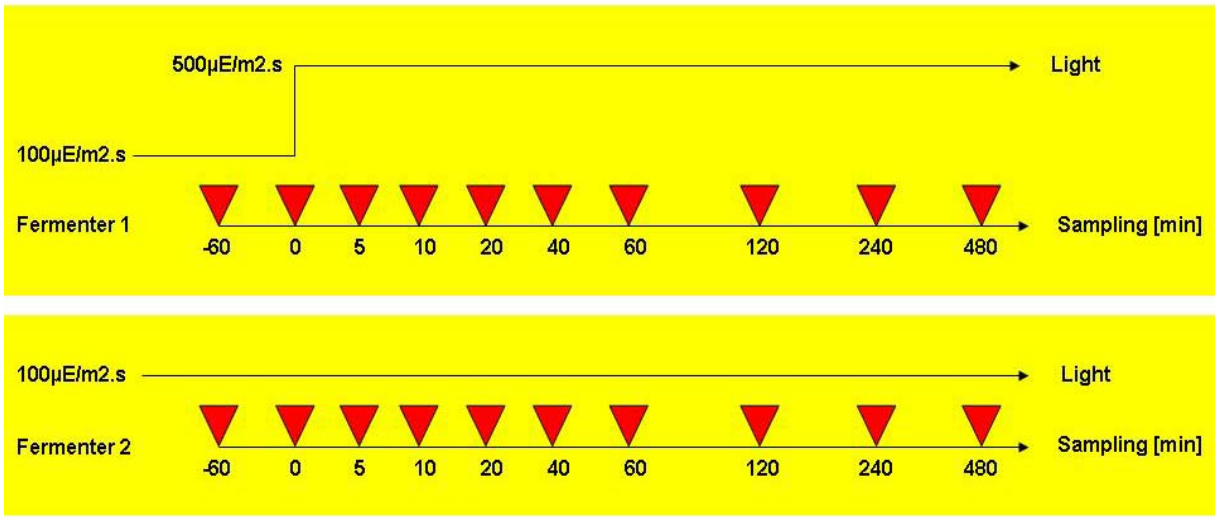
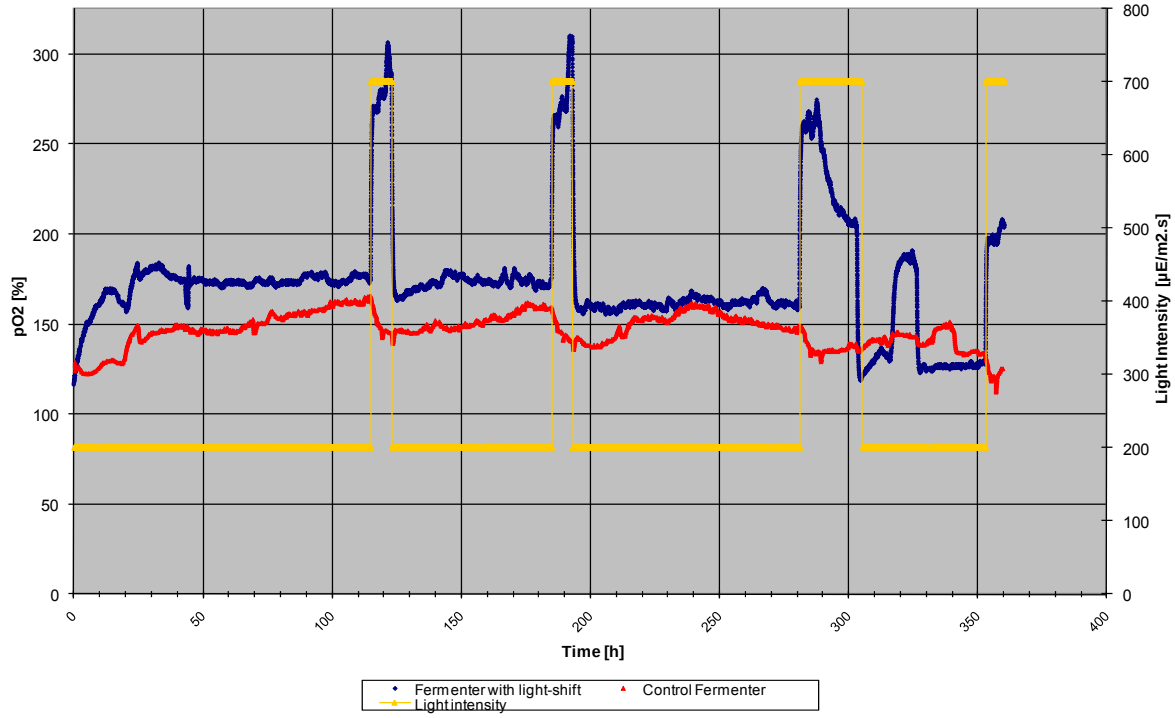


Figure 3: Sampling scheme



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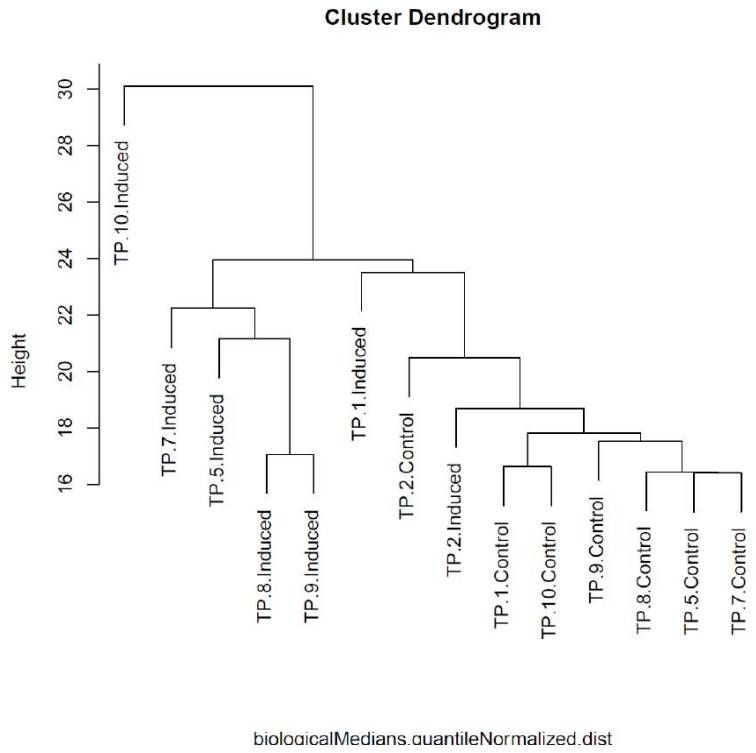

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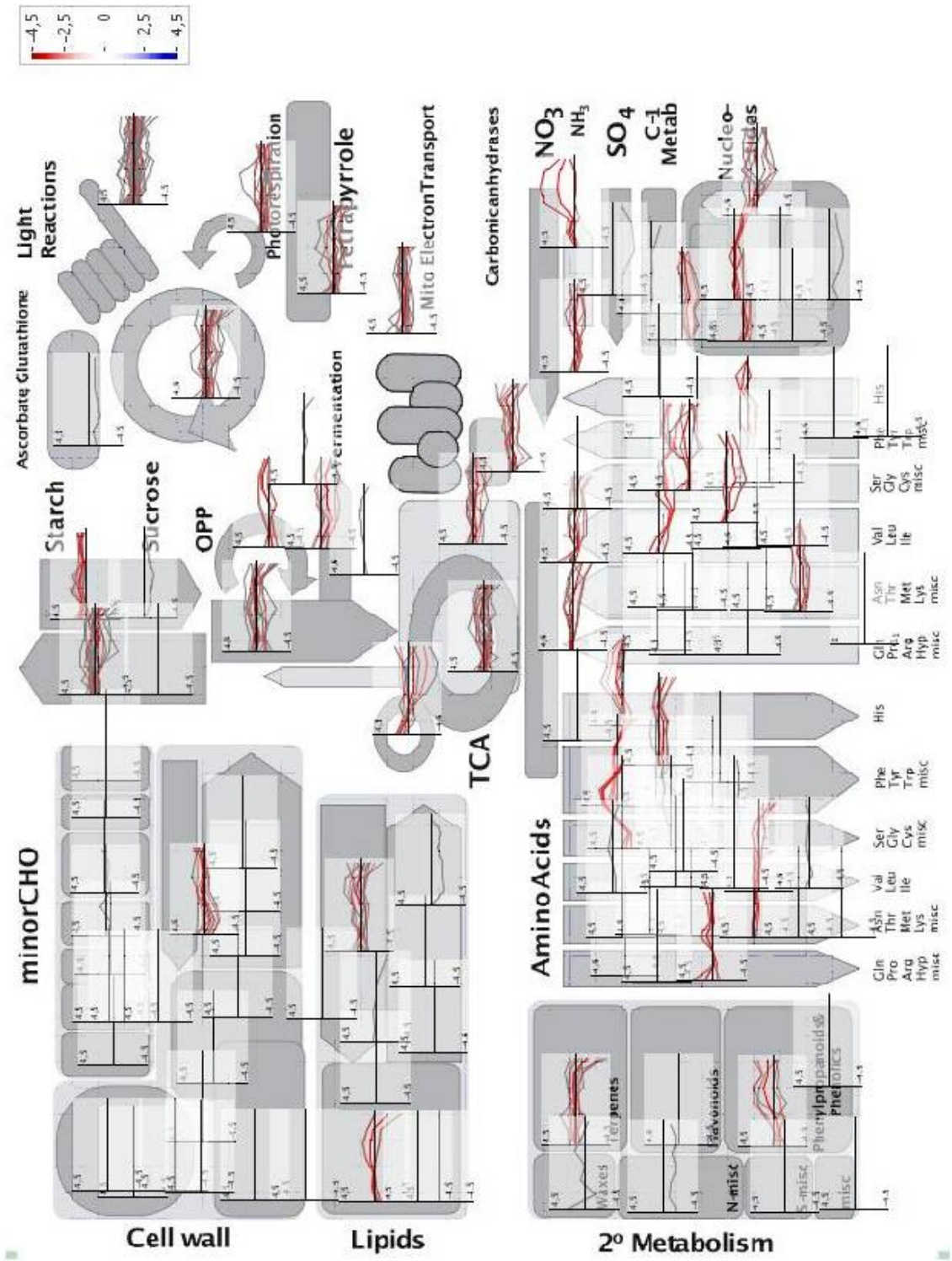

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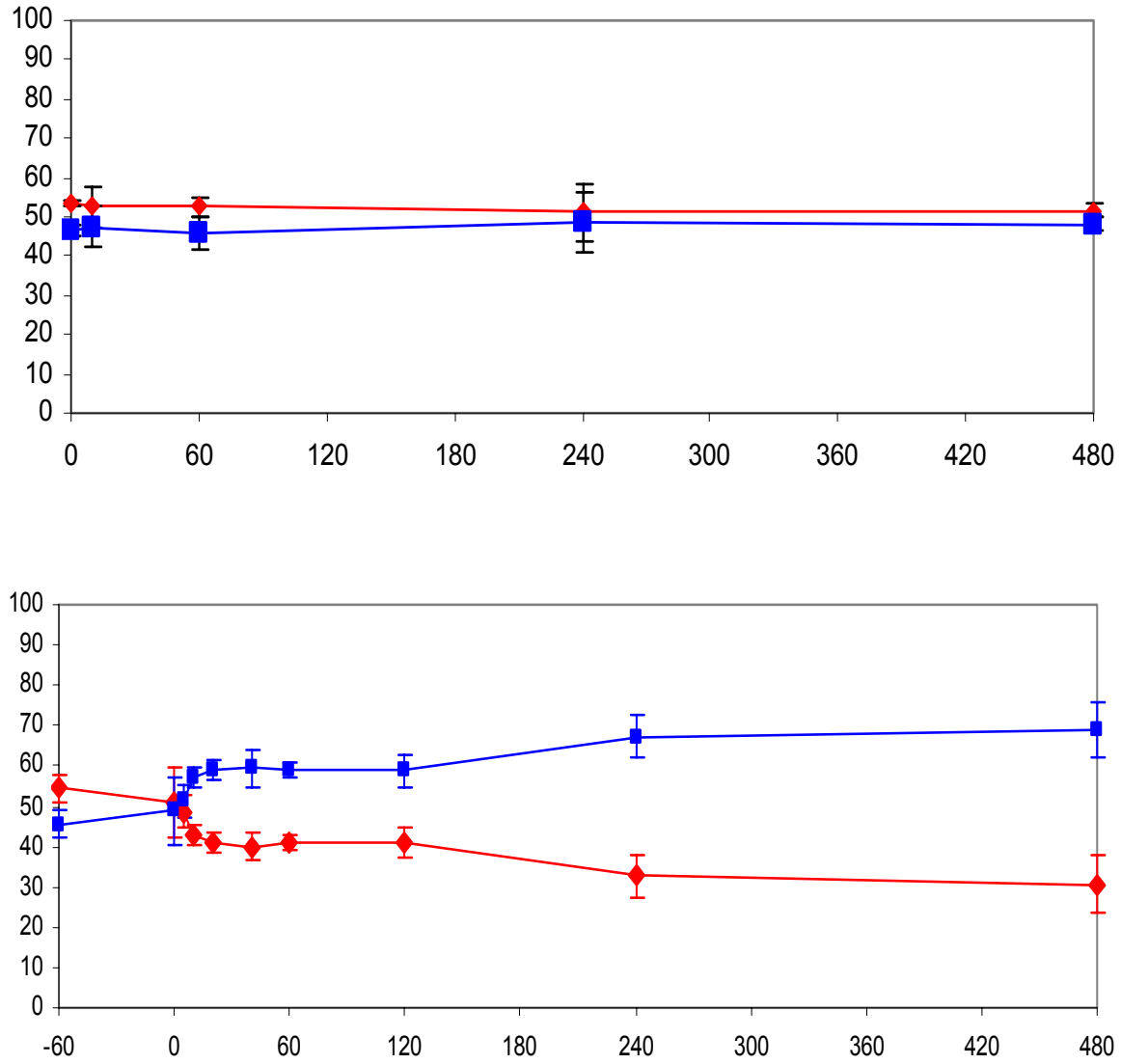
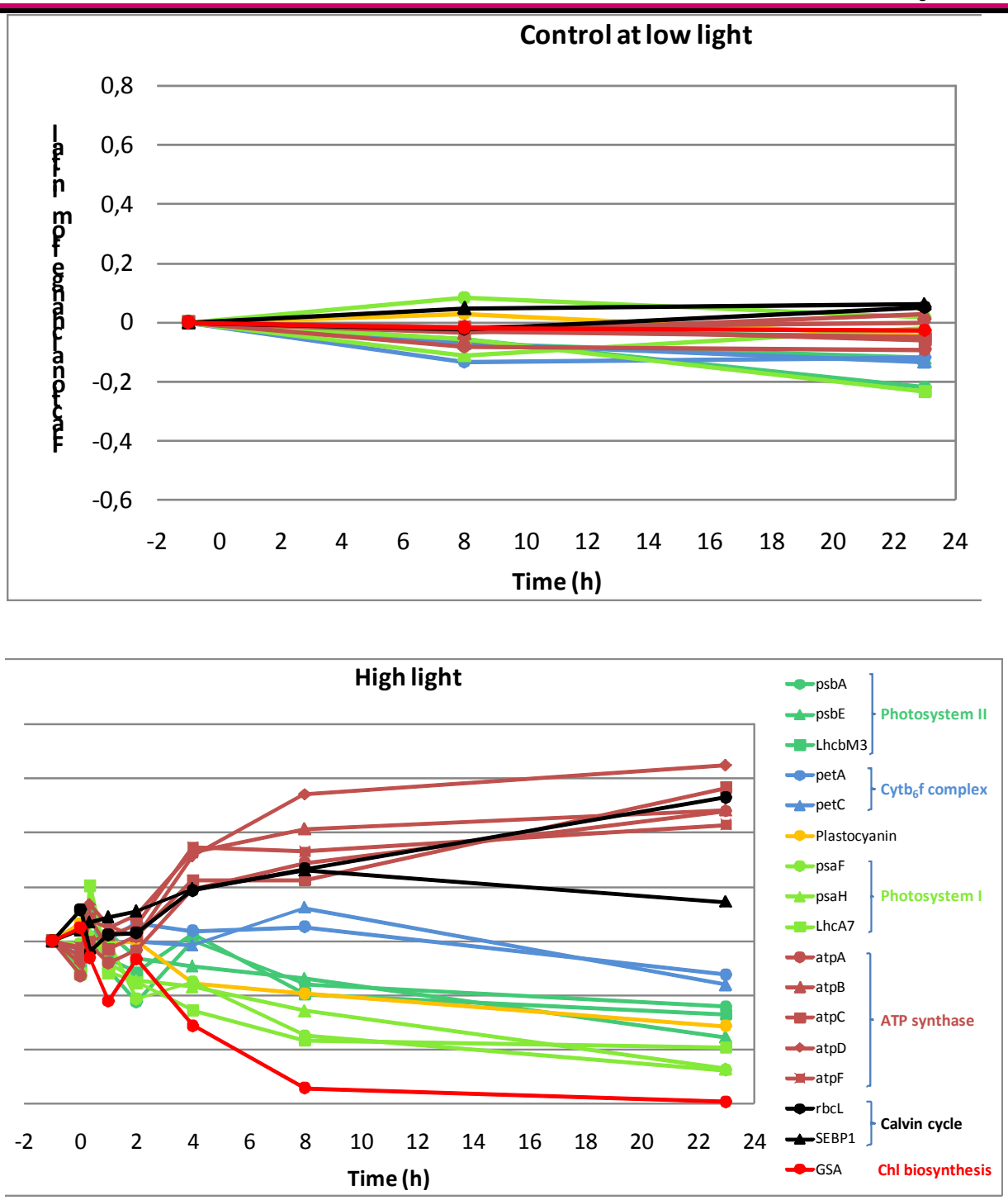


Figure 7: Polysome loadings of Chlamydomonas exposed to high light (right panel) or low light (left panel) conditions. Blue: polysomal fraction; red: non-polysomal rRNA fraction. The y-axis shows A_{254}, the x-axis the time in minutes, with 0 equalling the onset of the light shift.



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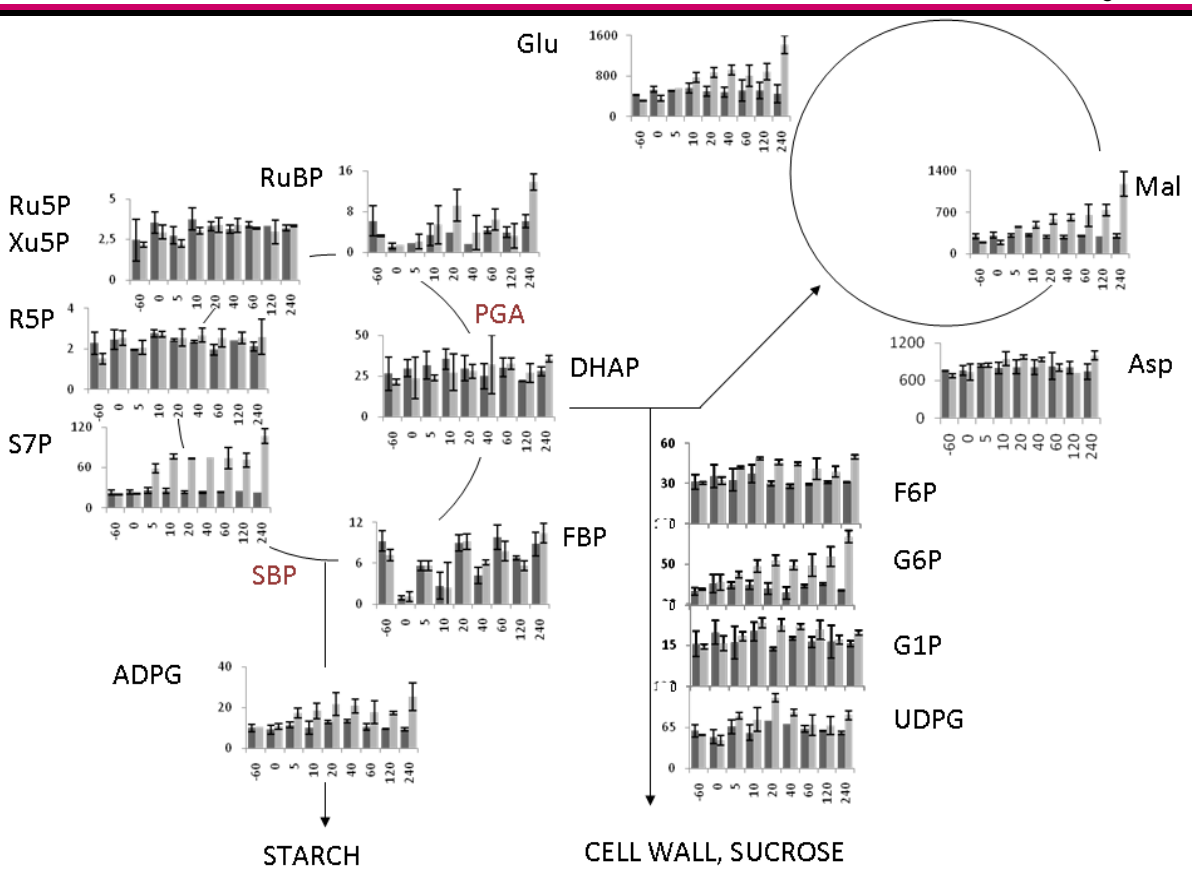

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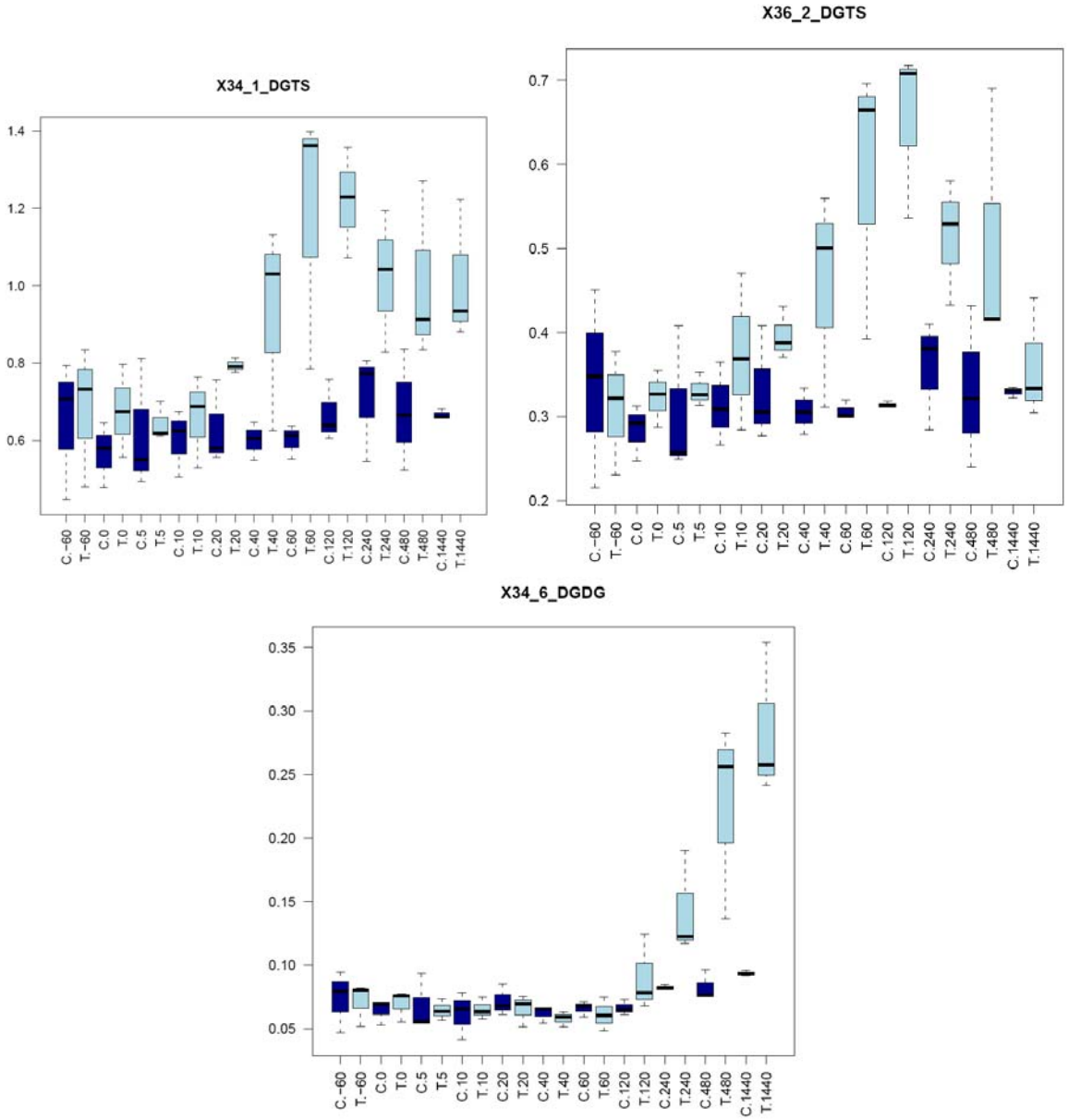

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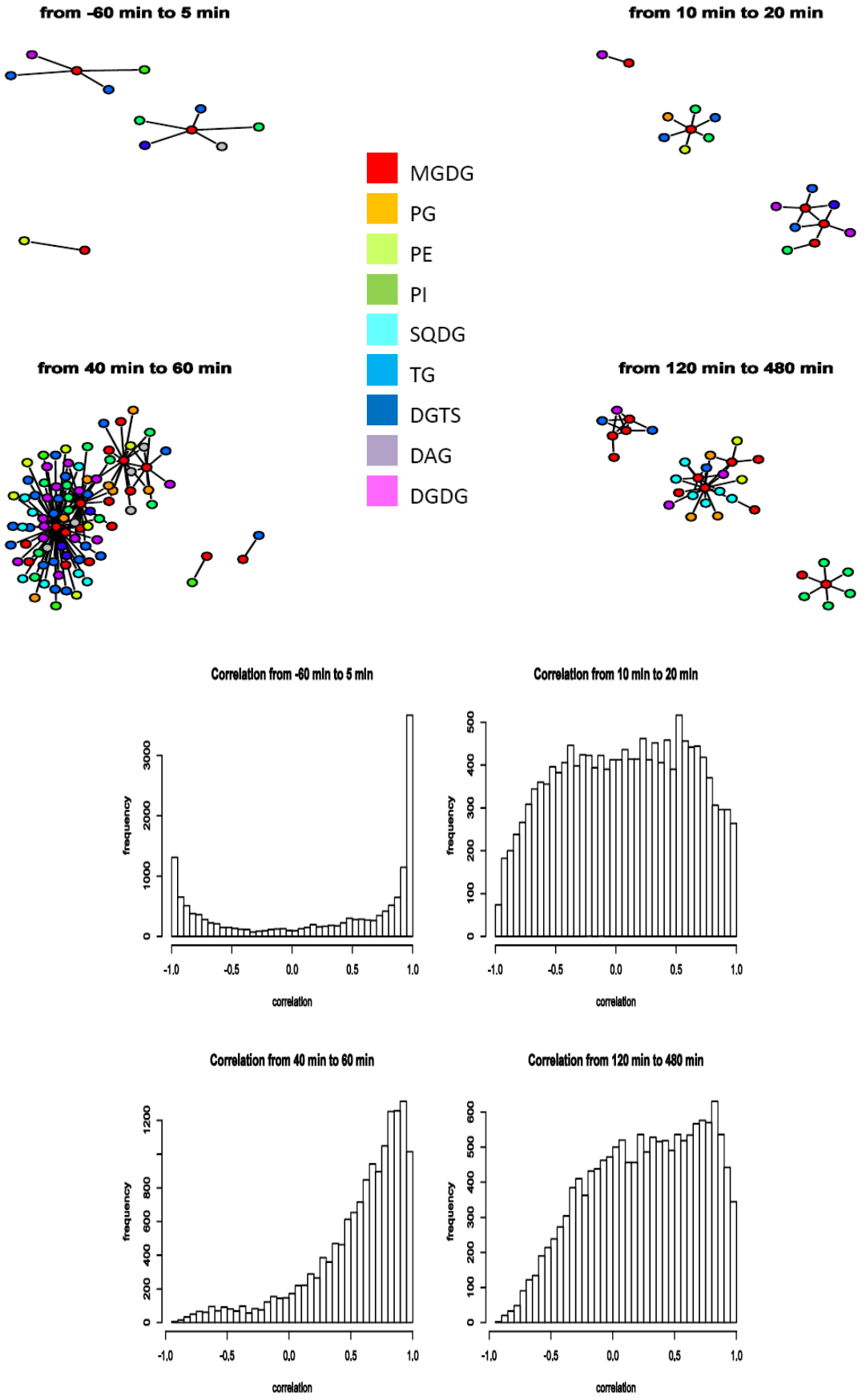

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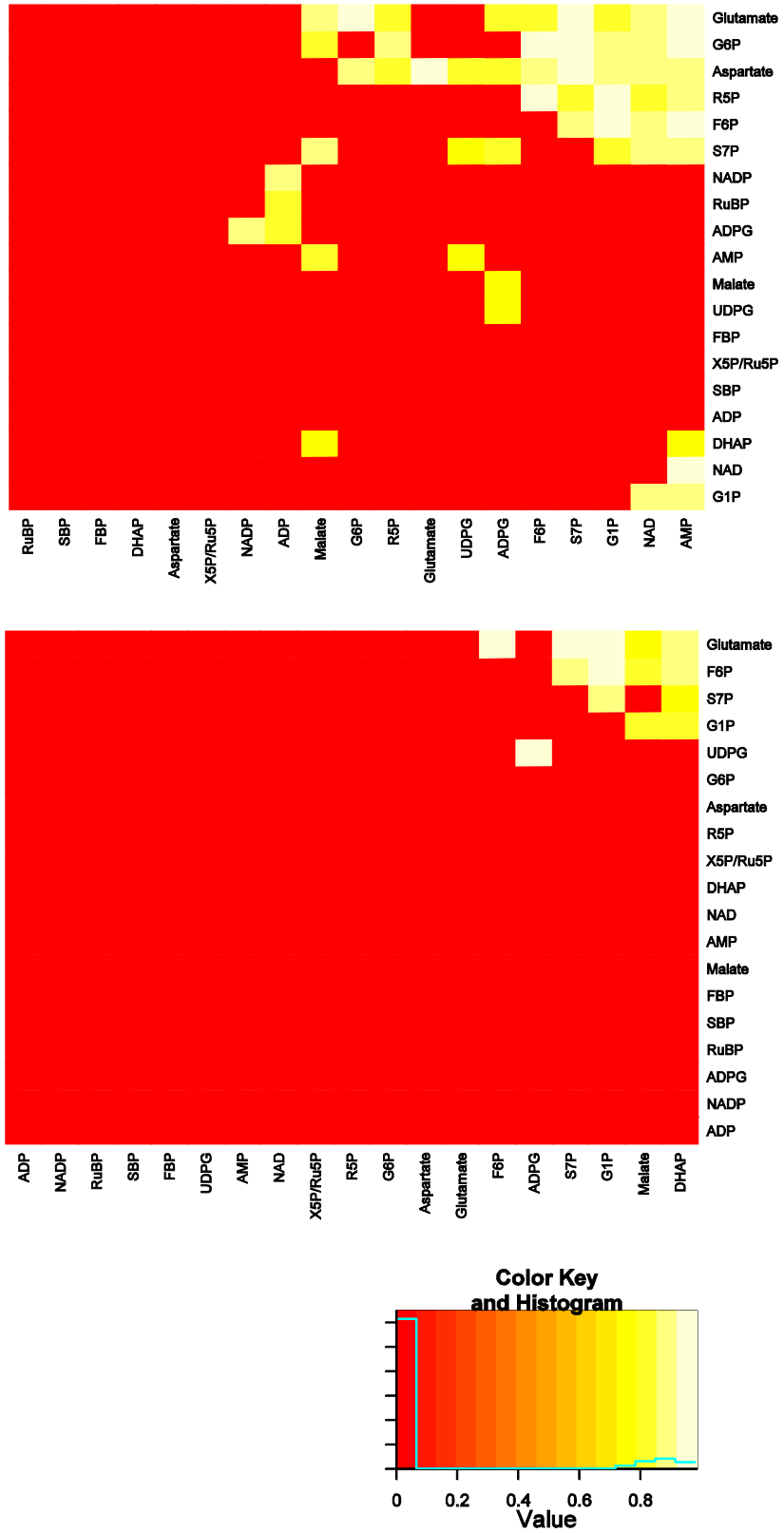

Figure 12: Zero-order (A) and first-order (B) partial correlations of the Calvin cycle intermediates from the high light treatment, and zero-order correlations for the control data set (C). p-value < 0.01 are coloured from yellow to white, red squares indicate no significant correlation (represented as 0)

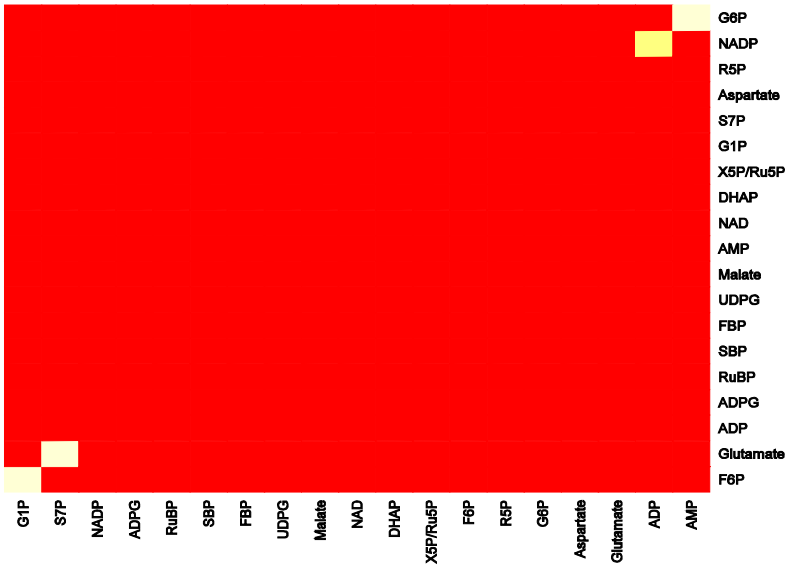

Figure 12: Zero-order (A) and first-order (B) partial correlations of the Calvin cycle intermediates from the high light treatment, and zero-order correlations for the control data set (C). p-value < 0.01 are coloured from yellow to white, red squares indicate no significant correlation (represented as 0)

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1982:	PhD (“summa cum laude") at the University of Munich, PhD thesis with Herbert Wagner on surface phase transitions.
1984 – 1986:	Research associate with Michael E. Fisher, Cornell University.
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ORGANISATION/ INSTITUTE:
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CURRICULUM VITAE:

1990:	Diploma Technical (Informatics), University of Darmstadt
1990 - 1993:	Researcher, Technical University of Darmstadt
1992:	PhD, (Informatics), Technical University of Darmstadt
1993 - 1995:	Research Associate at IRISA/INRIA at Rennes
1995:	Habilitation in Informatics, University of Rennes I, France
1995 – 1997:	Professor at the University of Angers (establishment of the research group FLUX dealing with the automatisation of reasoning from incomplete, contradictory, and evolutive information)
Since 1997:	Professor for knowledge processing and information systems at the University of Potsdam
1999:	Adjunct Professor at the School of Computing Science at Simon Fraser University, Canada
since 2006:	Adjunct Professor in the Institute for Integrated and Intelligent Systems at Griffiths University, Australia

RESEARCH INTERESTS IN KEYWORDS:
Theoretic foundations to the practical implementation of methods for reasoning from incomplete and/or inconsistent information, in particular Answer set programming

Dr. Mark Aurel Schöttler

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ORGANISATION/ INSTITUTE:
Max Planck Institute of Molecular Plant Physiology, Photosynthesis group (GoFORSYS)

CURRICULUM VITAE:

1994-2000:	Study of Biology, University of Münster, Germany
2000-2005:	Dissertation (PhD-thesis), University of Münster, Botanisches Institut
2005:	Post-Doc at the Max-Planck-Institute for Molecular Plant Physiology
2006:	Appointed as group leader of the “Photosynthesis group” (MPIMP)

RESEARCH INTERESTS IN KEYWORDS:
Photosynthesis, Chloroplast biogenesis, Spectroscopy

Dr. Michael Schroda

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ORGANISATION/ INSTITUTE:
Max Planck Institute of Molecular Plant Physiology, Plant molecular chaperone networks and stress (GoFORSYS)

CURRICULUM VITAE:

1988 - 1993: Study of Biology at the Philipps-Universität Marburg (scholar of the "Friedrich-Ebert-Stiftung" from 1990-1994
1991 - 1992: Graduate studies at the University of Tennessee, Knoxville, USA (funded by Fulbright, ISEP and Friedrich-Ebert-Stiftung)
1993 - 1994 Diploma thesis at the University of Helsinki in the laboratory of Dr. K. Lindström
1995 - 1999 Doctoral thesis at the Albert-Ludwigs-Universität Freiburg in the laboratory of Prof. Dr. C. F. Beck, PhD awarded (summa cum laude)
1999 - 2001: DAAD-postdoctoral fellowship at the Institut de Biologie Physico-Chimique, Paris
2001 - 2003: Assistant, Department of Plant Biochemistry, University of Freiburg
2003 - 2008: Junior Professor at the Department of Plant Biochemistry, University of Freiburg; positive evaluation in February 2007
July 2007 "Habilitation" at the Faculty of Biology, University of Freiburg; *Venia Legendi* in Biochemistry
October 2008: GoFORSYS group leader position at the Max-Planck-Institute of Molecular Plant Physiology in Potsdam-Golm
June 2009: *Venia Legendi* in Biochemistry at the University of Potsdam

RESEARCH INTERESTS IN KEYWORDS:
Protein identification and quantification based on mass spectrometry, stable isotope labelling and 15NH4Cl labeling, Metabolite profiling using GC-TOF-MS, Chromatin immunoprecipitation, Analysis of protein-protein interactions, Transgene expression and RNAi approaches in Chlamydomonas, Gene identification, Chlamydomonas

Prof. Dr. Joachim Selbig

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ORGANISATION/ INSTITUTE:
University of Potsdam, AG Bioinformatics
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CURRICULUM VITAE:

1969-1973:	Study in physics at University of Leipzig
1988:	PhD in computer science at Academy of Sciences in Berlin
1973-1991:	Research associate and group leader at Academy of Sciences, Central Institute of Cybernetics and Information Processes in Berlin
1992-2002:	Research associate and project leader at German National Research Center for Information Technology, Institute for Algorithms and Scientific Computing in Sankt Augustin
1996:	Visiting scientist at European Molecular Biology Laboratory in Heidelberg
since 2002:	Group leader at Max Planck Institute of Molecular Plant Physiology in Potsdam
since 2004:	Professor of Bioinformatics at University of Potsdam

RESEARCH INTERESTS IN KEYWORDS:
Bioinformatics, systems biology, machine learning, applied graph theory

Prof. Dr. Martin Steup

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ORGANISATION/ INSTITUTE:
University of Potsdam, Plant Physiology
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CURRICULUM VITAE:

1969-1972:	PhD. Study in Plant Physiology at the University of Göttingen (Prof. A. Pirson)
1973-1977:	Postdoctoral Fellow at the University of Göttingen
1975-1976:	Guest Researcher at the Brandeis University (Waltham, Ma. USA; Prof. Dr. M. Gibbs)
1977-1988:	Postdoctural Fellow and Professor at the University of Münster
1982:	Habilitation (Botanik)
1988-1991:	Professor at the University of Bochum
1992:	Professor at the University of Potsdam
since 1994:	Full Professor and Head of the Department of Plant Physiology at the University of Potsdam
1998-2003:	Head of the Institute of Biochemistry and Biology
since 1999:	PI “Sonderforschungsbereich 429“
2000-2002:	Vice Dean of the Faculty of Mathematics and Science
since 2007:	PI in GoFORSYS

RESEARCH INTERESTS IN KEYWORDS:
Plant Physiology and Molecular Plant Biology

Prof. Dr. Mark Stitt

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ORGANISATION/ INSTITUTE:
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CURRICULUM VITAE:

1975:	BA in Natural Sciences at the University of Cambridge, UK
1978:	PhD University of Cambridge UK, Botany department with Tom ap Rees
1978 – 1986:	PostDoc in the Institute of Physiological Chemistry at the LMU Munich, then in the Institute for Plant Biochemistry in the University of Göttingen
1986:	Associate Professor for Plant Biochemistry at University of Bayreuth
1991:	Full Professor and Director of the Botany Institute at the University of Heidelberg,
2000:	Director of the 2nd Department (Metabolic Networks) in the Max Planck Institute of Molecular Plant Physiology, Golm
2008:	Honorary Doctorate by the University of Umea, Sweden
2009:	Leopoldina National Academy of Science

RESEARCH INTERESTS IN KEYWORDS:
Plant metabolism, photosynthesis, functional genomics, systems biology

Prof. Dr. Ralph Tiedemann

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CURRICULUM VITAE:

1990:	Research Scientist, University of Iceland, Dept. of Genetics, Reykjavik
1990-1994:	PhD study in Zoology at Kiel University
1995:	Postdoctoral Fellow, Kiel University
1995-2004:	Assistant Professor (C1), Kiel University
1997:	Guest Researcher, Free University of Brussels (ULB)
2000:	Habilitation in Zoology
2001-2002:	Assistant Professor (C2), Kiel University
Since 2002:	Full Professor (C4) for Evolutional Biology/Systematic Zoology, Potsdam University
2004-2006:	Director of the Institute of Biochemistry and Biology, Potsdam University
2006-2008:	Dean of the Faculty of Mathematics and Natural Sciences, Potsdam University

RESEARCH INTERESTS IN KEYWORDS:
Evolutionary Genomics, Biodiversity, Speciation

Dr. Dirk Walther

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CURRICULUM VITAE:

1993:	Diploma in Biophysics, Humboldt University, Berlin, Germany
1996:	PhD Bioinformatics, EMBL, Heidelberg, Germany
1997-1999:	Postdoctoral Fellow, UCSF, San Francisco, CA, USA
1999-2004:	Sr. Scientist/Group Leader/ Director, Incyte Genomics, Palo Alto, CA, USA
2004-2005:	Director Research Informatics, XDx Inc., South San Francisco, CA, USA
Since 2005:	Group Leader Bioinformatics, MPIMP, Potsdam-Golm, Germany

RESEARCH INTERESTS IN KEYWORDS:
Bioinformatics, Computational Systems Biology

Prof. Dr. Lothar Willmitzer

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CURRICULUM VITAE:

1977:	Ph. D. (Dr. rer. nat.) Society of Biotechnological Research, Technical University Braunschweig
1979-1986:	Postdoc/Group Leader at Max Planck Institute for Plant Breeding Research,Cologne
1981:	Otto-Hahn-Medal of the Max-Planck-Society
1986:	Professor for Molecular Biology, FU Berlin and Scientific Director at the Institute of Genetic and Biological Research Ltd., Berlin
Since 1993:	Director at Max-Planck-Institute of Molecular Plant Physiology and Scientific Member of the Max-Planck-Society
1994:	Max Planck Research Award
Since 1995:	Honorarium Professor at University of Potsdam
1996 – 1999:	Founder of "PlantTec Biotechnologie GmbH Forschung und Entwicklung", Potsdam
1998:	Founder of "Metanomics GmbH und Co KG", Berlin, Germany
2002-2005:	Chairman of the Biology & Medical Section of the Scientific Council of the Max Planck Society

RESEARCH INTERESTS IN KEYWORDS:
Plant Biotechnology, Systems Biology, Molecular Plant Physiology