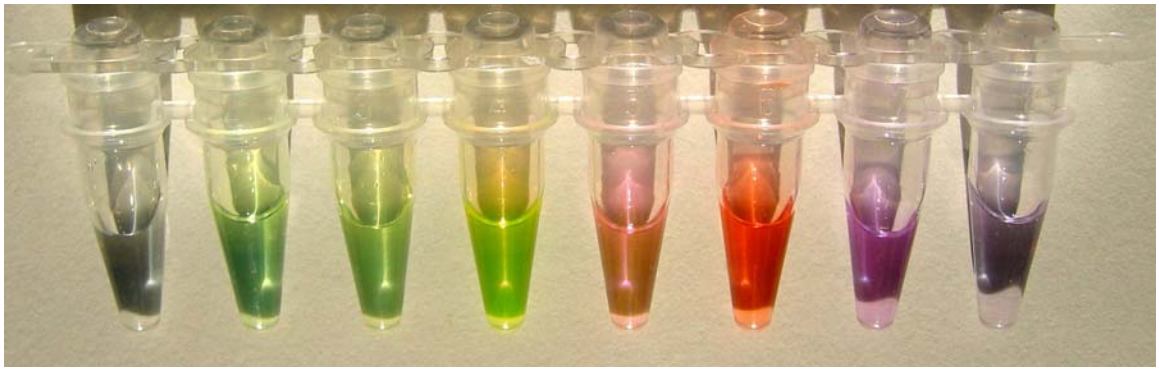
The Swammerdam Institute for Life Sciences

ANNUAL REPORT 2008



University of Amsterdam

FACULTY OF SCIENCE

1. Preface by the director

The Swammerdam Institute for Life Sciences: developments in 2008

2008 can be characterized as a year in which the various research groups of SILS further strengthened their research activities to enable competition at the national and international level. The highlights provided in the next chapters are an impressive summary of these achievements.
The SILS groups, participating in the Netherlands Institute for Systems Biology (NISB, established in 2006) reported very encouraging cooperation with the other partners at the Sciencepark.
Activities in the field of imaging in the Spinoza Center for Neuroimaging are ongoing; the masterplan for the set-up of the fMRI-facilities has been completed, and a large EFRO-grant proposal has been prepared in order to provide sufficient funds to support the acquisition and management of several fMRI-scanners.
With regards to valorization, 2008 was in year in which two new spin-off companies were formed. With the financial and administrative support of the UvA-holding, Photanol B.V. and CellaGenics B.V. were established. The former is based on the concept of photofermentation, developed by Klaas Hellingwerf in collaboration with Joost Teixeira de Mattos, while the latter, developed by Arie Otte, focuses on the expression of therapeutic proteins and cell growth.
2008 showed again an increase in especially BSc students for Psychobiology as well as for Biomedical Sciences. In order to manage the ever increasing educational needs, SILS has been granted in 2008 an additional yearly budget of 600 kEuro in order to support both the teaching activities in BSc Psychobiology and MSc Cognition, as well as research activities in that field.

2. Scientific Program

Research groups within the Swammerdam Institute for Life Sciences

The Living Cell	
Molecular Microbial Physiology	Prof.dr. K.J. Hellingwerf
Molecular Biology and Microbial Food Safety	Prof.dr. S. Brul
Structure and Functional Organisation of the Cell Nucleus	Prof.dr.R.. van Driel
Epigenetic Regulation of Gene Expression	Prof.dr. A.P. Otte
Molecular Cytology	Prof.dr. Th.W.J. Gadella
Plant Signalling	
Plant Physiology	Prof.dr. M.A. Haring
Plant-Pathogen Interaction	Prof.dr. B.J.C. Cornelissen
SILS Center for NeuroScience	
Animal Physiology and Cognitive Neuroscience	Prof.dr. C.M.A. Pennartz
Cellular and Systems Neurobiology	Prof.dr. W.J. Wadman
Hormonal Regulation of Signal Transduction in the Brain	Prof.dr. M. Joëls
Life Science Technologies	
Mass Spectrometry of Biomacromolecules	Prof.dr. C.G. de Koster
BioSystems Data Analysis	Prof.dr. A.K. Smilde
Micro Array Department and Integrated Bioinformatics Unit	Dr. T.M. Breit

Research Cluster

The Living Cell

Cells, the basic units of higher organisms and the competing individuals in microbiology, are the central topic of study of 'The Living Cell'. This cluster focuses on the chain of events all the way from modulation of gene expression, e.g. by signals coming from the environment, up to the resulting phenotype elicited by such signals. This process is studied in (cells of) micro-organisms, animals and plants, most often to resolve basic scientific issues, but also in a more applied context, in particular aiming to improve food and health. The mission of the cluster 'The Living Cell' is to understand life, in particular in its simplest form of a living cell, all the way from the molecular level, upwards via the complex biochemical and genetic networks that it encompasses, to the individual organism that can be successful in the struggle for survival. The information that this approach provides, the possibility of linking this information with metabolomics and proteomics data, combined with modelling to start simulating the processes under study, is contributing to our approach of Systems Biology. 'The Living Cell' is uniquely positioned to play a key role in this development.

Five chairs contribute to the research cluster 'The Living Cell': 'Molecular Microbial Physiology', 'Molecular Biology and Microbial Food Safety', 'Molecular Cytology', 'Epigenetic Regulation of Gene Expression', and 'Structural and Functional Organization of the Cell Nucleus'.

Molecular Microbial Physiology

Chairholder: Prof. dr. K.J. Hellingwerf

Prof. dr. J. Hugenholtz	Professor
Prof. dr. M.J. Teixeira de Mattos	Professor

Introduction

The general aim of our research team is to discover the properties that allow living (microbial) cells to catalyze a large array of concurrent chemical fluxes and information flows. From these processes and in particular their mutual interactions 'life', with its typical characteristics as adaptation to the environment, reproduction and evolution, has emerged. Microorganisms are particularly successful in this respect as can be concluded from the fact that they inhabit even the most extreme and variable ecosystems known to exist on this earth (and possibly even beyond), they can grow at very high rates and can even adapt/evolve genetically.
Our work focuses on various aspects of this process, like (i) the details of intra-molecular signal-generation in (photo) receptor proteins, (ii) signal transfer between subsequent components in a signal transduction chain, (iii) the regulatory function of modulated gene expression, and (iv) the functional integration of these processes in the physiology of a range of micro-organisms, relevant for food and health, etc. By combining theoretical (i.e. computational) and experimental approaches, insight is obtained into basic principles that underlie functional interactions in (information) flux-carrying macromolecular networks, and accordingly into a ***bio***chemical ***system*** that sustains microbial (i.e. cellular) ***life***.

Research Highlights

- The concept of photofermentation, developed by Klaas Hellingwerf in collaboration with Joost Teixeira de Mattos, has received positive attention nationally and internationally. This concept employs genetically modified cyanobacteria that use solar energy to drive CO2 reduction directly to useful end products, including biofuels. With financial and administrative support of the UvA-holding Photanol B.V. has been established. The investment by the UvA-holding allows for fulltime research activities for a technician, a PhD student and a postdoc researcher within the Mol Mic Phys group. As of January 1, 2009, the team will be completed. New ideas –all related to the Photanol concept- are being developed and research activities increase.
- As part of our studies on the variability of the efficiency of microbial respiration we have constructed a valuable collection of mutants that express the components of the respiratory chain in a variety of combinations. By comparative physiological studies with these mutants we have shown that the third terminal oxidase of *Escherichia coli* (cytochrome *bd*-II oxidase) does not contribute to respiratory energy conservation and serves solely as a redox sink. Our studies on the bio-energetics of yeast growth, carried out in collaboration with Dr. Gertien Smits of the Molecular Microbiology and Food Safety Group, suggest that also for lower eukaryotes like *Saccharomyces cerevisiae* the energetic variability of respiration is much larger than generally has been assumed.
- Our research aimed to characterize photoreceptor proteins has been brought to a new level by providing a systems-biology description of activation of the general stress response in *Bacillus subtilis* by blue light, mediated through the LOV-domain containing protein YtvA.

Other Highlights

The Molecular Microbial Physiology Group has been accepted as a partner of the national "Center for Photosynthesis Research", to allow participation on the programme: 'Towards BioSolar Cells'.

Future Prospects

- In our photofermentation research multiple metabolic pathways will be tested with respect to functionality in the cyanobacterium *Synechocystis*. Whenever relevant, initial tests of selected heterologous pathways will be performed in *Escherichia coli* MB43 in the absence and presence of oxygen. Pathways will be selected with specific emphasis on product recovery strategies.
- To deepen the systems-biology understanding of photoreceptor functioning in bacteria, we will extend our studies to a structure-driven site-directed mutagenesis study of the role of YtvA. In addition, jointly with our colleagues from Newcastle, we will try to reconstitute light-activation of the stressosome of *B. subtilis in vitro*. A synthetic-biology of designed hybrid 'photoregulators' will be used to study the functional role of diffusion processes in the yeast cytoplasm and nucleus.
- The work to provide a systems-biology description of both *Escherichia coli,* and of selected lactic acid bacteria, will be focusing on (i) the major redox fluxes in the cell related to energy conservation and (ii) a description of the dynamics of the reactions of glycolysis, respectively.

Key Publications

James Lee, Diana R. Tomchick, Chad A. Brautigam, Mischa Machius, Remco Kort, Klaas J. Hellingwerf, Kevin H. Gardner (2008). Changes at the PAS-A dimerization interface influence KinA histidine kinase function. Biochemistry 47: 4051-4064.

Santos, F., Wegkamp, A., de Vos, W.M., Smid, E.J., and Hugenholtz, J. 2008. High level folate production by the B12-producer *Lactobacillus reuteri* JCM1112. Applied and Environmental Microbiology 74: 3291-3294.

Schuurmans J.M., Rossell S.L., van Tuijl A., Bakker B.M., Hellingwerf K.J. and Teixeira de Mattos M.J. (2008). Effect of hxk2 deletion and HAP4 overexpression on fermentative capacity in *Saccharomyces cerevisiae.* FEMS Yeast Res. 8: 195-203.

PhD Theses

Boorsma, A. (2008, January 18). *Dissection of transcriptional regulation networks and prediction of genome functions in Saccharomyces cerevisiae*. Universiteit van Amsterdam. Prom./coprom.: prof.dr.K.J.Hellingwerf, dr.F.M.Klis & dr.H.J.Bussemaker

Schuurmans J.M. (2008, November 13). *The effect of altered expression of transcriptional regulators of catabolism on the transcription profile and physiology of Saccharomyces cerevisiae*. Universiteit van Amsterdam. Prof./coprom.: prof.dr.K.J.Hellingwerf & prof.dr.M.J.Teixeira de Mattos.

Academic publications (refereed)

Alexandre, M.T.A., Grondelle, R. van, Hellingwerf, K.J., Robert, B. & Kennis, J.T.M. (2008). Perturbation of the ground-state electronic structure of FMN by the conserved cysteine in phototropin LOV2 domains. *Phys. Chem. Chem. Phys., 10*(44), 6693-6702.

Bekaert, S., Storozhenko, S., Mehrshahi, P., Bennett, M.J., Lambert, W., Gregory III, J.F., Schubert, K., Hugenholtz, J., Van der Straeten, D. & Hanson, A.D. (2008). Folate biofortification in food plants. *Trends Plant Sci., 13*(1), 28-35.

Boorsma, A., Lu, X.-J., Zakrzewska, A., Klis, F.M. & Bussemaker, H.J. (2008). Inferring condition-specific modulation of transcription factor activity in yeast through regulon-based analysis of genomewide expression. *PLoS ONE, 3*(9), e3112.

Brighenti, F.L., Luppens, S.B.I., Delbem, A.C.B., Deng, D.M., Hoogenkamp, M.A., Gaetti-Jardim, E. jr., Dekker, H.L., Crielaard, W. & Cate, J.M. ten (2008). Effect of Psidium cattleianum leaf extract on Streptococcus mutans viability, protein expression and acid production. *CARIES RES, 42*(2), 148-154.

Brul, S., Mensonides, F.I.C., Hellingwerf, K.J. & Teixeira De Mattos, M.J. (2008). Microbial systems biology: New frontiers open to predictive microbiology. *Int. J. Food Microbiol., 128*(1), 16-21.

Brunner, J., Crielaard, W. & Winkelhoff, A.J. van (2008). Analysis of the capsular polysaccharide biosynthesis locus of Porphyromonas gingivalis and development of a K1-specific polymerase chain reaction-based serotyping assay. *J PERIODONTAL RES, 43*(6), 698-705.

Deng, D.M. & Crielaard, W. (2008). Microbiële genetica: Nieuwe mogelijkheden voor preventie en behandeling van (orale) infecties. *Nederlands Tijdschrift voor Tandheelkunde, 115*(2), 93-99.

Groot, P.W.J. de, Kraneveld, E.A., Yin, Q.Y., Dekker, H.L., Gross, U., Crielaard, W., Koster, C.G. de, Bader, O., Klis, F.M. & Weig, M. (2008). The cell wall of the human pathogen Candida glabrata: Differential incorporation of novel adhesin-like wall proteins. *EUKARYOTIC CELL, 7*(11), 1951-1964.

Keijser, B.J.F., Zaura, E., Huse, S.M., Vossen, J.M.B.M. van der, Schuren, F.H.J., Montijn, R.C., Cate, J.M. ten & Crielaard, W. (2008). Pyrosequencing analysis of the oral microflora of healthy adults. *Journal of Dental Research, 87*(11), 1016-1020.

Lee, J., Tomchick, D.R., Brautigam, C.A., Machius, M., Kort, R., Hellingwerf, K.J. & Gardner, K.H. (2008). Changes at the KinA PAS-A dimerization interface influence histidine kinase function. *Biochemistry, 47*(13), 4051-4064.

Luppens, S.B.I., Kara, D., Bandounas, L., Jonker, M.J., Wittink, F.R.A., Bruning, O., Breit, T.M., Cate, J.M. ten & Crielaard, W. (2008). Effect of Veillonella parvula on the antimicrobial resistance and gene expression of Streptococcus mutans grown in a dual-species biofilm. *ORAL MICROBIOL IMMUN, 23*(3), 183-189.

Pereira-Cenci, T., Del Bel Cury, A.A., Crielaard, W. & Cate, J.M. ten (2008). Development of Candida-associated denture stomatitis: New insights. *J. appl. oral sci., 16*(2), 86-94.

Pereira-Cenci, T., Deng, D.M., Kraneveld, E.A., Manders, E.M.M., Cury, A.A. Del Bel, Cate, J.M. ten & Crielaard, W. (2008). The effect of Streptococcus mutans and Candida glabrata on Candida albicans biofilms formed on different surfaces. *ARCH ORAL BIOL, 53*(8), 755-764.

Picon, A., Teixeira De Mattos, M.J. & Postma, P.W. (2008). Protein production by Escherichia coli wild-type and ΔptsG mutant strains with IPTG induction at the onset. *J. ind. microbiol. biotechnol., 35*(4), 213-218.

Postmus, J., Canelas, A.B., Bouwman, J., Bakker, B.M., Gulik, W. van, Teixeira De Mattos, M.J., Brul, S. & Smits, G.J. (2008). Quantitative analysis of the high temperature-induced glycolytic flux increase in Saccharomyces cerevisiae reveals dominant metabolic regulation. *J. Biol. Chem., 283*(35), 23524-23532.

Rupenyan, A., Stokkum, I.H.M. van, Arents, J.C., Grondelle, R. van, Hellingwerf, K. & Groot, M.L. (2008). Characterization of the primary photochemistry of proteorhodopsin with femtosecond spectroscopy. *Biophys. J., 94*(10), 4020-4030.

Santos, F., Wegkamp, A., Vos, W.M. de, Smid, E.J. & Hugenholtz, J. (2008). High-level folate production in fermented foods by the B12 producer Lactobacillus reuteri JCM1112. *Appl. Environ. Microbiol., 74*(10), 3291-3294.

Santos, F., Vera, J.L., Heijden, R. van der, Valdez, G., Vos, W.M. de, Sesma, F. & Hugenholtz, J. (2008). The complete coenzyme B12 biosynthesis gene cluster of Lactobacillus reuteri CRL1098. *MICROBIOL-SGM, 154*(1), 81-93.

Schuurmans, J.M., Rossell, S.L., Tuijl, A. van, Bakker, B.M., Hellingwerf, K.J. & Teixeira De Mattos, M.J. (2008). Effect of hxk2 deletion and HAP4 overexpression on fermentative capacity in Saccharomyces cerevisiae. *FEMS Yeast Res., 8*(2), 195-203.

Schuurmans, J.M., Boorsma, A., Lascaris, R., Hellingwerf, K.J. & Teixeira De Mattos, M.J. (2008). Physiological and transcriptional characterization of Saccharomyces cerevisiae strains with modified expression of catabolic regulators. *FEMS Yeast Res., 8*(1), 26-34.

Sieuwerts, S., Bok, F.A.M. de, Hugenholtz, J. & Hylckama Vlieg, J.E.T. van (2008). Unraveling microbial interactions in food fermentations: From classical to genomics approaches. *Appl. Environ. Microbiol., 74*(16), 4997-5007.

Sosinska, G.J., Groot, P.W.J. de, Teixeira De Mattos, M.J., Dekker, H.L., Koster, C.G. de, Hellingwerf, K.J. & Klis, F.M. (2008). Hypoxic conditions and iron restriction affect the cell-wall proteome of Candida albicans grown under vagina-simulative conditions. *MICROBIOL-SGM, 154*(2), 510-520.

Terefework, Z., Pham, C.L., Prosperi, A.C., Entius, M.M., Errami, A., Spanning, R.J.M. van, Zaura, E., Cate, J.M. ten & Crielaard, W. (2008). MLPA diagnostics of complex microbial communities: relative quantification of bacterial species in oral biofilms. *J. microbiol. methods, 75*(3), 558-565.

Toh, K.C., Stokkum, I.H.M. van, Hendriks, J., Alexandre, M.T.A., Arents, J.C., Avila Perez, M., Grondelle, R. van, Hellingwerf, K.J. & Kennis, J.T.M. (2008). On the signaling mechanism and the absence of photoreversibility in the AppA BLUF domain. *Biophys. J., 95*(1), 312-321.

Vreede, J., Hellingwerf, K.J. & Bolhuis, P.G. (2008). Helix formation is a dynamical bottleneck in the recovery reaction of Photoactive Yellow Protein. *PROTEINS, 72*(1), 136-149.

Vreede, J., Hellingwerf, K.J. & Crielaard, W. (2008). TraR auto-inducer enhances protein backbone fluctuations in DNA binding domain. *FEBS lett., 582*(5), 805-809.

Kuipers, O.P., Poolman, B. & Hugenholtz, J. (2008). 9th International Symposium on Lactic Acid Bacteria. *Appl. Environ. Microbiol., 74*(15), 4589-4589.

Membership editorial board

Hugenholtz, J. (Ed.). (2008). *Journal of Applied Microbiology*.
Hugenholtz, J. (Ed.). (2008). *Letters in Applied Microbiology*.
Hugenholtz, J. (Ed.). (2008). *Microbial Cell Factory*.

Invited lectures

Hellingwerf, K.J. (2008, August 14). *Alternative routes to biofuels.* Bielefeld, Germany, plenary lecture CeBiTec Symposium Solar Bio-Fuels.

Hellingwerf, K.J. (2008, May 06). *Challenges in Biotechnology: From biofuel to 'vitromeat'.* Buenos Aires, Argentina, University of Buenos Aires, host Prof.B.C.Nudel.

Hellingwerf, K.J. (2008, March 06). *Cyanobacteria for solar fuel.* Leiden, Leiden University, Dept.of Chemistry, Prof.H.de Groot.

Hellingwerf, K.J. (2008, September 05). *Evolutie (of: genetische stabiliteit).* Amsterdam, Meeting on the interface between chemistry and biology.

Hellingwerf, K.J. (2008, February 05). *Flavin-containing photoreceptors in phototrophic and chemotrophic bacteria.* Davis CA, USA, University of California, host Prof.D.S.Larsen.

Hellingwerf, K.J. (2008, January 14). *From the details of photoperception to molecular systems biology.* Amsterdam, AMOLF.

Hellingwerf, K.J. (2008, April 17). *Medium design for large-scale, serum-free, growth of myoblasts.* Matforsk, Norway, In vitro meat Symposium NFR.

Hellingwerf, K.J. (2008, April 11). *Molecular systems biology of single species biofilm formation.* Utrecht, Biofilm Symposium at Annual NVVM Meeting.

Hellingwerf, K.J. (2008, January 25). *Flavin-containing photoreceptors in phototrophic and chemotrophic bacteria.* Stillwater USA, Department of Biochemistry, Host: prof.dr.W.D.Hoff.

Hellingwerf, K.J. (2008, January 27). *Photosensory receptors and signal transduction.* Ventura CA, USA, Vth Gordon Conference.

Hellingwerf, K.J. (2008, February 23). *Photosignalling.* Amsterdam, Free University, host Dr.J.Dekker.

Hellingwerf, K.J. (2008, November 10). *UvA-VU samenwerking.* Amsterdam, HIMS Heidagen. Host: dr. S.Woutersen.

Hugenholtz, J. (2008, April 18). *Melkzuurbacterien voor diversificatie en verrijking van zuivelproducten.* Arnhem, Jubileumbijeenkomst Genootschap voor Melkkunde.

Hugenholtz, J. (2008, May 23). *Metabolic engineering and genomics for improving food fermentations.* Shanghai, China, lecture on University of Shanghai.

Hugenholtz, J. (2008, October 09). *Starter cultures for enrichment of fermented dairy products.* Athens, Greece, Greek Dairy Organisation.

Hugenholtz, J. (2008, April 02). *Technical biodiversity in mixed starter cultures.* Papendal, The Netherlands, NVVM Spring meeting.

Hugenholtz, J. (2008, May 19). *The Kluyver Centre for genomics of industrial fermentation.* Hangzhou, China, 1st Annual World Congress of Industrial Biotechnology (iBIO).

Hugenholtz, J. (2008, May 22). *The Kluyver Centre for genomics of industrial fermentation.* Wuxi, China, lecture on the University of Wuxi.

Hugenholtz, J. (2008, June 12). *Unique metabolic characteristics of lactic acid bacteria.* As, Norway, Norforsk Course Agricultural Univerity Norway.

Hugenholtz, J. (2008, August 14). *Zero growth product formation in industrial microorganisms.* San Diego, USA, Annual Meeting of the Society for Industrial Microorganisms.

Teixeira De Mattos, M.J. (2008, October 20). *A shortcut to biofuels.* Barcelona, Spain, Workshop on Biofuels-Expochimica.

Teixeira De Mattos, M.J. (2008, January 08). *Adaptive strategies by microbes and the energetic consequences.* Delft, The Netherlands, Advanced Course on Biotechn. and Fermentation Technology.

Teixeira De Mattos, M.J. (2008, April 11). *CO2 reduction by photofermentation.* Papendal, The Netherlands, Annual NVVM Meeting, session Biomass from Energy.

Teixeira De Mattos, M.J. (2008, May 18). *Light-driven CO2 reduction by a photofermentative chimera.* Hangzhou, China, 1st Annual World Congress of Industrial Biotechnology.

Teixeira De Mattos, M.J. (2008, May 18). *Opening lecture session on Breakthroughs for non-grain bioethanol and biodiesel production.* Hangzhou, China, 1st Annual World Congress of Industrial Biotechnology.

Teixeira De Mattos, M.J. (2008, February 01). *The photanol approach.* Amsterdam, The Netherlands, Workshop on clean solar fuels, KNAW.

Teixeira De Mattos, M.J. (2008, May 16). *Understanding the inside by measuring outside.* Beijng, China, University of Beijng, Biotechnology Department.

Molecular Biology and Microbial Food Safety

Chairholder: Prof. dr. S. Brul

Dr. J.C. van der Spek	Assistant Professor
Dr. G.J. Smits	Assistant Professor
Dr. F.M. Klis	Senior scientist (former Associate Professor)
Dr. B. Ter Kuile	Researcher Dutch Food & Drug Authority (VWA)
Dr. J.P.P.M. Smelt	Senior researcher (former Unilever scientist)

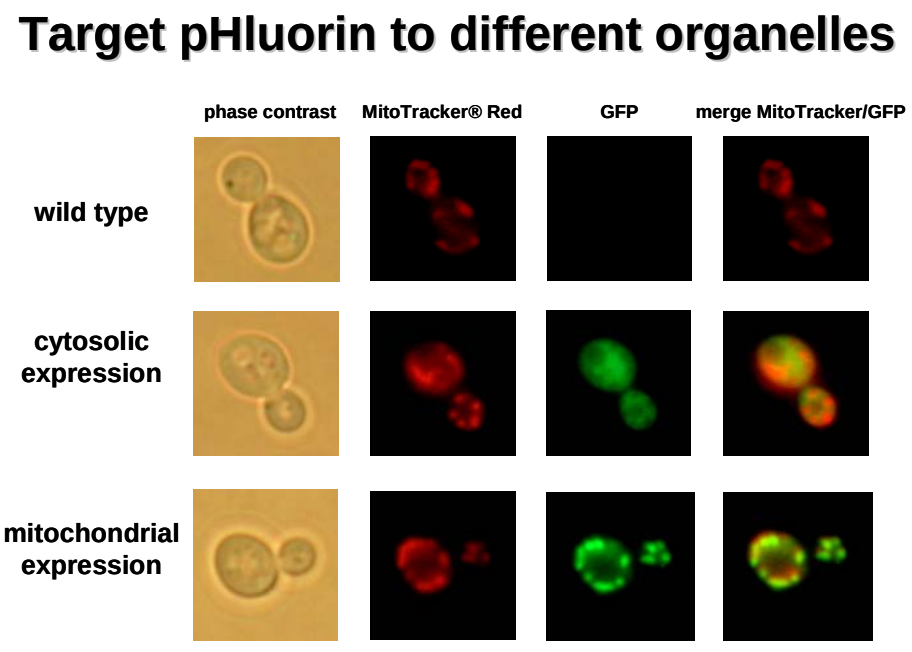
Introduction

Our group aims at understanding the stress response of micro-organisms in relation to infection and infection prevention. We focus on food and pharma related environments and apply functional genomics as well as systems biology approaches. Both in bacteria (primarily *Bacillus subtilis* and enterobacteriaceae) and fungi (primarily *Saccharomyces cerevisiae* and Candida spp.) we study responses to (weak) organic acid, antimicrobial agents and temperature stresses. Furthermore, we use the model yeast *Saccharomyces cerevisiae* and the multicellular organisms *Caenorhabditis elegans* to develop generic genome-wide analysis concepts and models for host-microbe interactions. The responses of the microbes are monitored under well controlled culturing conditions.

The group is a member of the Netherlands Institute for Systems Biology (NISB). We ask both 'bottom-up' as well as 'top-down' systems biology questions. The tools we apply include genome-wide micro-array analysis, proteomics, various advanced microscopy techniques and controlled cell culturing systems using fermentors and chemostats. Results are generally quantified and analysed using a number of modelling tools including in-house developed micro-array and functional data analysis software. We have major contacts with food safety as well as medical groups and contracts with the food and pharma industry focussing on the application of our research in practical settings. The research highlights are given thematically i.e. for the 'Food chain' and in the 'Medical field'.

Research Highlights

Illustration:



Legend: Expression of a pH sensitive green fluorescent protein (pHluorin) in the cytosol and the mitochondria of *Saccharomyces cerevisiae* cells. The green fluorescence is co-localised with the mitochondrial MitoTracker® Red stain. The cells were used to assess effects of various physiological

states and perturbations on $pH_{cytosol}$ and $pH_{mitochondrial\ matrix}$ and the derived energy status of the mitochondria.

Food chain

Bacterial spore formers

In 2008 we performed a 'systems' analysis of the response of spore-forming Bacilli against the most commonly used food preservative sorbic acid with an extensive micro-array analysis, validation and functional interpretation in vegetative cells(TerBeek et al., 2008a). A Unilever patent based on joint research with regard to the molecular mechanisms involved in weak organic acid resistance development in vegetative cells and outgrowing spores was published (TerBeek et al., 2008b). Additionally we succeeded in expressing the intracellular pH probe pHluorin in Bacillus cells. This was a joint effort between our group and the Unilever Research & Development lab. in Vlaardingen (TerBeek, Wijman et al., in preparation). At Unilever the system will be used to screen various antimicrobial compounds of natural origin for a putative synergistic action with THE classical food preservative sorbic acid. In 2008 we concluded our work with Unilever on high pressure effects on Bacilli (see Shen et al., 2009 Innov. Food Sci. Emerg. Technol. 10, 9-15).

Concomitantly we developed in Amsterdam together with experimental aid in the Vlaardingen lab. a model that describes ***B. subtilis*** spore germination and outgrowth at the single spore level (Smelt et al., 2008). In the area of analysing extreme thermal resistance of Bacillus spores a detection system for such strains was developed with Unilever and funding was secured to have it validated at the company.

More fundamental studies on the proteome of extreme thermal resistant spore formers versus the laboratory strain were set-up successfully. Here the approach followed with yeast was leveraged (below). Finally, we presented options to deploy the fundamental concepts developed in molecular systems biology of microbes (see below) in the area of food microbiology (Brul et al., 2008). Such approaches are now translated in a new project proposal with the Food and Drug Authority (VWA), the Top Institute Food and Nutrition (TIFN) and the Netherlands Institute for Systems Biology (NISB).

Spoilage Yeasts

In order to analyze the organisational level at which cells regulate their response to adverse environmental temperatures we have extended regulation analysis to include a temperature dependent component (Postmus et al., 2008). The data showed convincingly that regulation often occurs predominantly at the metabolic level and only in a secondary phase at a hierarchical level. Furthermore we observed that mitochondrial function critically changes in efficiency in the temperature domain of 30-38 degrees Celsius. After an initial increase in efficiency a sudden drop upon shifting from 37-38 ºC is commonly observed. The nature of both the temperature dependent increase and sudden decrease in efficiency is currently under investigation. Maintenance of a proper mitochondrial membrane potential and the prevention of the emergence of reactive oxygen species are important parameters in this.

We have thereto optimized for *S. cerevisiae* the measurement of the intracellular pH in the cytosol and the mitochondrial matrix. The approach using pHluorin expression in these cellular compartments has been published (Orij et al., 2009; see also the illustration embedded in this paragraph). Next to analysing the delta pH cytosol / mitochondrial matrix, we also deploy this tool in the analysis of the effect of weak-organic acids. The experimental set-up is furthermore leveraged in the study of bacterial spore formers (above) and we are pursuing similar work in the sorbic acid resistant food spoilage yeast *Zygosaccharomyces bailii* and the medically relevant *Candida glabrata* species (below).

In 2008 PhD student Andre Boorsma defended his thesis. He successfully established, using *Saccharomyces cersvisiae* as a prime model and working in between the Microbial Physiology group and our group, tools to functionally analyse microbial responses at the level of gene-expression. Frans Klis was his daily supervisor and co-promotor. His tools are now regularly used both for analysis of yeast behaviour as well as ***Bacillus subtilis.***

Antibiotic resistance

The studies on the development of antibiotic resistance in infectious organisms of concern to the food chain focussed initially on the acquisition of resistance by adaptation of the organism when exposed to sub-lethal concentrations. By now two post-doctoral fellows are working in

this field as well as a research technician from the Dutch Food and Consumer Product Safety Authority (VWA). One of the post-docs is supported by funds from the VWA and the other by a recently granted project of the ministry of Agriculture (LNV). In this latter project the VWA, the University Utrecht, the University of Amsterdam and the consultancy company Innotact collaborate. The physiological studies showed that *Pseudomonas putida* becomes resistant to fluoroquinolones after short exposure to low concentrations. The resulting resistant strain can still grow at levels that far exceed the concentration used to induce resistance. This finding has far-reaching implications for the use of fluoroquinolones in an agricultural setting. Together with the molecular expertise at our department we have started to unravel the molecular basis of this resistance to fluoroquinolones in. All data obtained are consistent with fluoroquinolone resistance being caused by a single mutation of the gyrase A gene, a key target of the antibiotic. The mechanisms of this genetic change are currently under investigation.
Another line of investigation is the development of resistance to amoxicillin in *E. coli*. This resistance builds up more gradually, but can reach high levels as well. As these data are compatible with a gradual modification of household pumps to antibiotic efflux pumps, the simultaneous exposure to other environmental stresses is explored. Acquisition of tetracycline resistance is solely dependent on the transfer of resistance genes from other strains, therefore experiments on tetracycline resistance use chemostats to monitor transfer of resistance genes under varying conditions.

Medical field

Medical Yeasts

Concerning medically important yeasts, the analysis of the dynamics of the cell wall proteome of *Candida albicans* in response to various infection-related stress conditions such as hypoxia, iron depletion and the presence of antifungal drugs is currently embedded in a new EU Marie-Curie Initial Stage Training Network (FINSysB). In this program, the use of systems biology approaches at all levels of the study of Candida infection will be emphasized. We have set up an *in vitro* model for mucosal infections to study the development of the invasive, filamentous form of Candida as a function of environmental pH, iron depletion, antifungal drug concentration, and hypoxic conditions (Sosinska et al., 2008). Together with the proteomics group of Chris de Koster we have established quantitative approaches to analyse the proteome of the yeast cell wall as well as yeast glycolytic enzymes. The proteomic data so obtained extend from a qualitative description of cellular proteins to their relative and absolute quantification through the use of ^{15}N labelling techniques. Similar mass spectrometric approaches are being developed for Bacillus (above).

Mitochondrial dysfunction and disease

Finally, in the field of analysing in a multicellular model stress response, we successfully expressed fluorescent proteins in mitochondria of muscle cells of *Caenorhabditis elegans* which gives us now a perfect tool to study mitochondrial morphology in cells exposed to toxic compounds such as antiviral agents. In this simple model for higher eukaryotes we are able to monitor at various levels the (side-) effects of antimicrobial compounds on the biogenesis and function of mitochondria. The functional analysis will be extended and an initial manuscript establishing the basic parameters of the system will be submitted early 2009. The approach has yielded significant interest from other parties including those aiming at the study of physiological effects of compounds commonly present in or added to food.

Other Highlights

Prof. dr. Stanley Brul is Distinguished Research Scholar of the University of Tasmania, Australia, Consultant Food Microbiology for Unilever, Editor of Innovative Food Science and Emerging Technologies and Member of the Faculty of 1000. In 2008 he received a Unilever author award.
In September 2008 Stanley Brul addressed in a key-note lecture in Aberdeen Food Micro 2008.

Dr. Frans Klis is Editor of Eukaryotic Cell, FEMS Yeast Research and Yeast.

Dr. Benno ter Kuile (VWA, UvA MBMFS) organised together with Prof. dr. Brul, Prof. dr. Zwietering (WUR) and Prof. dr.Arie Havelaar (RIVM, UU) a high profile international conference sponsored by the Dutch Food and Consumer Products Agency (VWA) and the European Food Safety Authority (EFSA) on Future Developments in Microbial Food Safety. The conference took place in the Netherlands in June 2008. Participants ranged from academia and industry to FDA and WHO.

Future Prospects

* In 2009 we will focus our work on stress response in model yeasts and *C. elegans* on the study of damage (repair) induced by reactive oxygen species. The basic principles will be further analysed in baker's yeast using an in situ approach where a hydrogen peroxide sensitive GFP will be used. The results will be validated in *C. elegans*.
The underpinning theoretical studies on microbial stress response analysis focuses on a genome wide analysis of the interaction between pH homeostasis and weak acid stress as well as on studying the causal relationship between growth rate and stress tolerance. In both areas the role that mitochondrial energy metabolism plays as well as its response to the generation of reactive oxygen species is key.

* In our medical yeast research line we will continue to use quantitative proteomics to study the adaptation of the cell wall proteome during the initial stages of mucosal infections and during infection-related stress conditions. A parallel project has been initiated, in which the cell wall proteome of C. albicans will be studied to identify suitable candidates for vaccine development. The research will be primarily carried out by the two new PhD students that have been appointed within the framework of the EU ITN Program FINSysB in collaboration with the De Koster group.

*Our *B. subtilis* studies will focus on a further unravelling of the mechanisms involved in sorbic acid stress signalling and resistance development. The data from transposon mutagenesis indicate the key role for a putative membrane localised response regulator in mediating the sorbic acid stress signal. The current activities are aimed at generating strains that overexpress this regulator to understand its role in cellular metabolism. Furthermore we aim at confirming the inferred membrane phospholipid biochemical adaptations in response to weak-acid stress with actual biochemical measurements. These are done in collaboration with the department of Genetic metabolic disorders at the Academic Medical Centre in Amsterdam.
In the framework of the consultancy at Unilever the studies on the measurement of the intracellular pH in *B. subtilis* will be pursued as a screening tool to identify antimicrobial compounds.
2008 saw post-doc Luc Hornstra move to a permanent position at a water microbiology research institute. We thus have applied for new funds to pursue our fundamental studies on the mechanisms operative in *Bacillus subtilis* spore germination. Together with Unilever we aim at complementing fundamental studies with support for applied work aiming at the prevention of spore outgrowth. The successfully established promoter trap library in *B. subtilis* (Hornstra et al., 2009 Int. J. Food Microbiol., in press) will be instrumental in both fields.

* Stress response research on antibiotic resistance acquisition was recently started in *Escherichia coli* and is aimed in 2009 at obtaining sufficient physiological and molecular data to be able to provide initial data on the speed of de novo antibiotic acquisition costs with respect to the use of fluoroquinolones.

Key Publications

Postmus, J., Canelas, A.B., Bouwman, J., Bakker, B.M., Gulik, W. van, Teixeira De Mattos, M.J., Brul, S. & Smits, G.J. (2008). Quantitative analysis of the high temperature-induced glycolytic flux increase in Saccharomyces cerevisiae reveals dominant metabolic regulation. *J. Biol. Chem., 283*(35), 23524-23532.

Beek, A. ter, Keijser, B.J.F., Boorsma, A., Zakrzewska, A., Orij, R., Smits, G.J. & Brul, S. (2008). Transcriptome analysis of sorbic acid-stressed Bacillus subtilis reveals a nutrient limitation response and indicates plasma membrane remodeling. *J. Bacteriol., 190*(5), 1751-1761.

PhD Theses

Boorsma, A. (2008, January 18). *Dissection of transcriptional regulation networks and prediction of gene functions in Sacchaomyces cerevisiae.* Universiteit van Amsterdam. Prom./coprom.: prof.dr. K.J. Hellingwerf, dr.F.M. Klis & dr.H.J. Bussemaker.

Patent

Beek, A.S. ter, Brul, S. & Vaart, M van (). Screening method for a preservative. no EP 1935984.

Academic publications (refereed)

Beek, A. ter, Keijser, B.J.F., Boorsma, A., Zakrzewska, A., Orij, R., Smits, G.J. & Brul, S. (2008). Transcriptome analysis of sorbic acid-stressed Bacillus subtilis reveals a nutrient limitation response and indicates plasma membrane remodeling. *J. Bacteriol., 190*(5), 1751-1761.

Boorsma, A., Lu, X.-J., Zakrzewska, A., Klis, F.M. & Bussemaker, H.J. (2008). Inferring condition-specific modulation of transcription factor activity in yeast through regulon-based analysis of genomewide expression. *PLoS ONE, 3*(9), e3112.

Brul, S., Kallemeijn, W. & Smits, G. (2008). Functional genomics for food microbiology: Molecular mechanisms of weak organic acid preservative adaptation in yeast. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 3*, 005.

Brul, S., Mensonides, F.I.C., Hellingwerf, K.J. & Teixeira De Mattos, M.J. (2008). Microbial systems biology: New frontiers open to predictive microbiology. *Int. J. Food Microbiol., 128*(1), 16-21.

Damveld, R.A., Franken, A., Arentshorst, M., Punt, P.J., Klis, F.M., Hondel, C.A.M.J.J. van den & Ram, A.F.J. (2008). A novel screening method for cell wall mutants in Aspergillus niger identifies UDP-galactopyranose mutase as an important protein in fungal cell wall biosynthesis. *GENETICS, 178*(2), 873-881.

Groot, P.W.J. de, Kraneveld, E.A., Yin, Q.Y., Dekker, H.L., Gross, U., Crielaard, W., Koster, C.G. de, Bader, O., Klis, F.M. & Weig, M. (2008). The cell wall of the human pathogen *Candida glabrata*: Differential incorporation of novel adhesin-like wall proteins. *EUKARYOTIC CELL, 7*(11), 1951-1964.

Groot, P.W.J. de & Klis, F.M. (2008). The conserved PA14 domain of cell wall-associated fungal adhesins governs their glycan-binding specificity. *Mol. Microbiol., 68*(3), 535-537.

Postmus, J., Canelas, A.B., Bouwman, J., Bakker, B.M., Gulik, W. van, Teixeira de Mattos, M.J., Brul, S. & Smits, G.J. (2008). Quantitative analysis of the high temperature-induced glycolytic flux increase in *Saccharomyces cerevisiae* reveals dominant metabolic regulation. *J. Biol. Chem., 283*(35), 23524-23532.

Smelt, J.P.P.M., Bos, A.P., Kort, R. & Brul, S. (2008). Modelling the effect of sub(lethal) heat treatment of *Bacillus subtilis* spores on germination rate and outgrowth to exponentially growing vegetative cells. *Int. J. Food Microbiol., 128*(1), 34-40.

Sosinska, G.J., Groot, P.W.J. de, Teixeira De Mattos, M.J., Dekker, H.L., Koster, C.G. de, Hellingwerf, K.J. & Klis, F.M. (2008). Hypoxic conditions and iron restriction affect the cell-wall proteome of *Candida albicans* grown under vagina-simulative conditions. *MICROBIOL-SGM, 154*(2), 510-520.

Yin, Q.Y., Groot, P.W.J. de, Koster, C.G. de & Klis, F.M. (2008). Mass spectrometry-based proteomics of fungal wall glycoproteins. *Trends Microbiol., 16*(1), 20-26.

Membership editorial board

Brul, S. (Ed.). (2008). *Innovative Food Science and Emerging Technologies*
Brul, S. (Ed.). (2008). *The Open Biotechnology Journal*
Klis, F.M. (Ed.). (2008). *Eukaryotic Cell*
Klis, F.M. (Ed.). (2008). *FEMS Yeast Research*
Klis, F.M. (Ed.). (2008). *Yeast*.

Invited lectures

Brul, S. (2008, September 01). *Applying systems biology approaches to microbial food preservation.* Aberdeen, Food Micro Congress.

Brul, S. (2008, April 21). *Systems biology of Bacillus subtilis: spore formation and germination.* Naples, Italy, European Spore Conference.

Klis, F.M. (2008, November 20). *Exploring the fungal wall proteome.* Gottingen, Germany, 3rd Symposium of the Gottingen Proteomics Forum.

Klis, F.M. (2008, December 13). *Exploring the cell wall proteome of Candida albicans.* Jena, Germany, Leibniz Inst.for natural product research and infection biology.

Klis, F.M. (2008, July 10). *Mass spectrometric explorations of the fungal wall proteome.* Marseille, France, Annual Main Meeting Soc.for Experimental Biol.

Structure and Functional Organisation of the Cell Nucleus

Chairholder: Prof.dr. R. van Driel

Dr. P.F. Fransz	Assistant Professor
Dr. M.E. Stam	Assistant Professor
Dr. P.J. Verschure	Assistant Professor

Introduction

The one-dimensional structure of the genome of an increasing number of eukaryotes has been fully sequenced. A major challenge is to understand how it functions in terms of orchestration of expression of the many thousands of genes it encodes. Gene expression in eukaryotes is controlled at three hierarchical levels. One is that of individual genes, involving *cis* regulatory elements and *trans*-acting factors. Information at the second level is stored as posttranslational modifications of histones, incorporation of histone variants and DNA methylation patterns. The third level is the compartmentalisation of the interphase cell nucleus. Gene regulation at this last level is tightly associated with the folding of the chromatin fibre in the nucleus.

Our aim is to unravel gene regulatory mechanisms that combine these three control levels. We concentrate on the dynamic structure of chromatin and the behaviour of the nuclear machineries involved in gene activation and silencing and DNA repair. We combine structural studies, often on living cells, with molecular biological, biochemical and other methodologies and with predictive modelling. We approach regulatory systems in the genome as networks of molecular components that interact in time and space. It is our ambition to be among the first to develop quantitative and predictive models of regulatory epigenetic systems.

Research Highlights

Maike Stam and colleagues were the first to use the 3C technology in plants to analyse the role of chromatin-chromatin interactions in the epigenetic regulation of gene expression in plants. Results revealed essential aspects of the molecular basis of paramutation in maize.

Paul Fransz and coworkers showed that a variety of external cues (e.g. low light levels) induce major changes in large-scale chromatin structure in Arabidopsis cells and that this effect is mediated by two proteins that act at the chromatin level: PHYB and HDA6.

Pernette Verschure and colleagues completed the first steps towards the engineering of a small artificial epigenetic system in mammalian cells. This allows the in vivo quantitative measurement of key variables and a systems biology type of approach of epigenetic control mechanisms. Analysis of the role of the epigenetic regulator MeCP2 showed an unexpected mix of gene inhibitory and activating properties.

Major steps have been made by Roel van Driel et al. in the implementation of systems biology approaches in the analysis of genome function. (i) Based on systematic measurements in living cells, a quantitative and predictive polymer model has been developed that describes the folding of the chromatin fibre inside the cell nucleus (cooperation Heermann and Bohn, Heidelberg). (ii) A detailed kinetic mathematical model has been made of the nucleotide excision DNA repair (NER) system, based on in vivo measurements on the kinetic properties of a number of NER proteins (cooperation Höfer and Von Bornstead, Heidelberg). Both models reveal astonishing emerging system properties and constitute the basis for follow-up research.

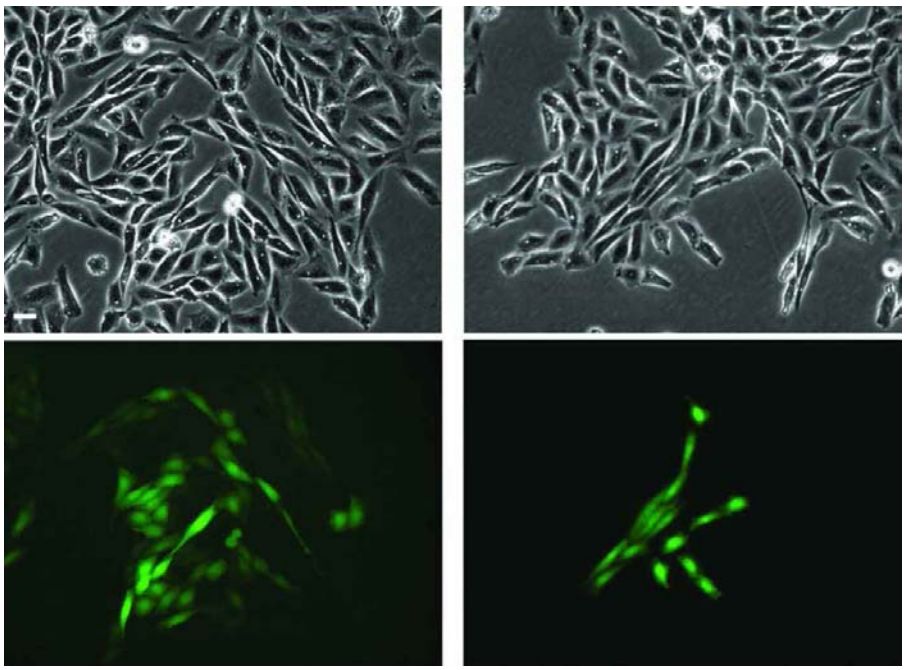
Other Highlights

Roel van Driel is, together with some colleagues, setting up a large-scale 10 years research program named Systems Biology to Combat Metabolic Syndrome (SBMS). It is a prototype project to explore the systematic use of systems biology as driver and integrator of research. This endeavour is strongly supported by the German Government.

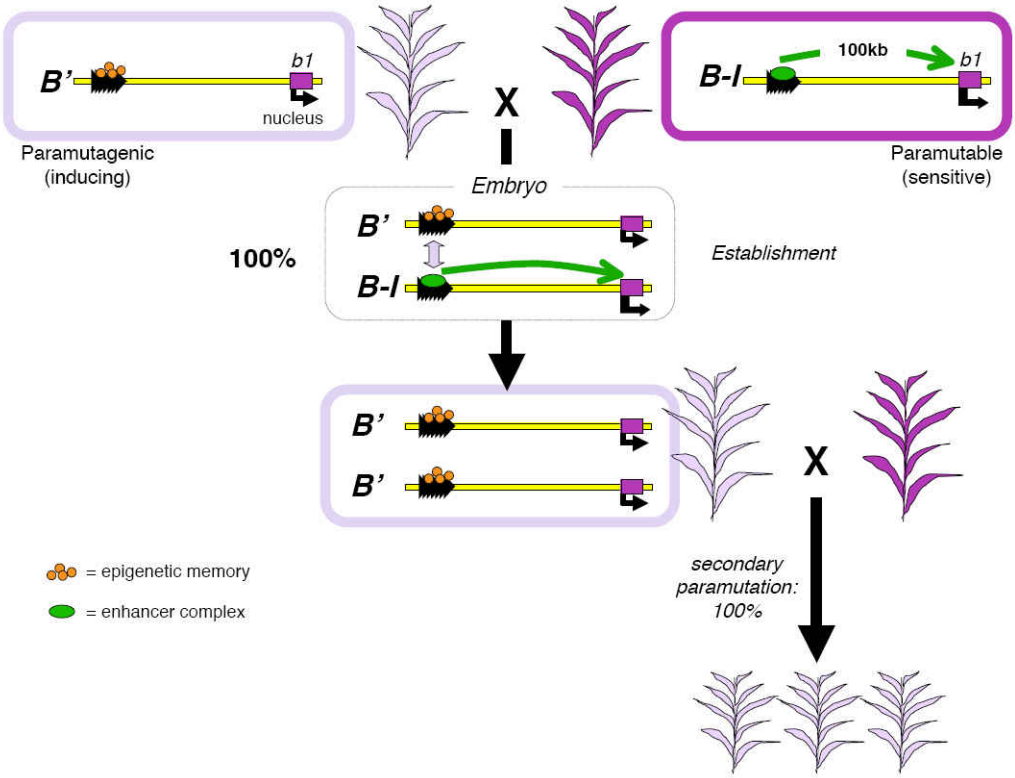
Roel van Driel has been advisor of a variety of systems biology research programs in Europe.

Future Prospects

- One or more new research lines will be initiated to investigate the role of long-distance *in cis* and *in trans* chromatin-chromatin interactions in relation to gene regulation, using the 3C technology and polymer modeling. Arabidopsis will be the model system of choice.
- The systematic studies on the role of DNA methylation, histone modifications and chromatin-chromatin interactions in the epigenetic regulation of the maize b1 locus will be continued.
- The engineered epigenetic system in mammalian cells will be further expanded to allow in vivo measurements.
- Our polymer model for chromatin folding inside the cell nucleus will be the basis for a project funded by FOM.
- If our ZonMW grant application is honoured, we will begin to explore several of the emerging properties of chromatin-associated processes in living mammalian cells that are revealed by the kinetic NER model.



Legend
Variegation of expression of a reporter gene (GFP) on an a small episome that is stably present at low copy numbers in cultured CHO cells (EU project EpiVector; Dr Federico Tessadori). All cells contain the episome, only small clusters of cells in a cell colony express the gene. (upper: phase contrast; lower: GFP fluorescence)



Legend
Cartoon showing the molecular basis of paramutation in maize (Dr M. Stam).

Key publications

Bruggeman, F.J., Oancea, I. & Driel, R. van (2008). Exploring the behavior of small eukaryotic gene networks. *J. Theor.Biol. 252*, 482-487

Solimando, L., Luijsterburg, M.S., Vechio, L., Vermeulen, W., Driel, R.van & Fakan, S. (2009). Spatial organization of nucleotide excision repair proteins after UV-induced DNA damage in the human cell nucleus. *J. Cell Sci. 122,* 83-91

PhD Theses

Haring, M. (2008, November 12). *Paramutation and chromatin dynamics in maize.* Universiteit van Amsterdam. Prom./coprom.: prof.dr. R. van Driel & dr. M. Stam.

Louwers, M.L.D. (2008, September 4). *Chromatin looping and epigenetic regulation at the maize b1 locus.* Universiteit van Amsterdam. Prom./coprom.: prof.dr. R. van Driel & dr. M. Stam.

Luijsterburg, M.S. (2008, September 12). *Dynamics of nucleotide excision repair complex assembly and disassembly in vivo.* Universiteit van Amsterdam. Prom./coprom.: prof.dr. R. van Driel.

Academic publications (refereed)

Alekseev, S., Luijsterburg, M.S., Pines, A. & Geverts, B. (2008). Cellular concentrations of DDB2 regulate dynamic binding of DDB1 at UV-induced DNA damage. *MOL CELL BIOL, 28*(24), 7402-7413.

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Fransz, P.F. (2008). Chromatin Domains and Function. In: Functional Organization of the Plant Nucleus. *Springer -Verlag Berlin Heidelberg New York*, 131-155.

Gladilin, E., Goetze, S., Mateos-Langerak, J., Driel, R. van, Eils, R. & Rohr, K. (2008). Shape normalization of 3D cell nuclei using elastic spherical mapping. *J. Microsc., 231*, 105-114.

Hoogstraten, D., Bergink, S., Verbiest, V.H.M., Luijsterburg, M.S. & Geverts, B. (2008). Versatile DNA damage detection by the global genome nucleotide excision repair protein XPC. *J. Cell Sci., 121*, 2850-2859.

Kanger, J.S., Subramaniam, V. & Driel, R. van (2008). Intracellular manipulation of chromatin using magnetic nanoparticles. *Chromosome Res., 16*, 511-522.

Louwers, M.L.D., Bader, R., Driel, R. van, Laat, W. & Stam, M. (2008). Tissue- and expression level-specific chromatin looping at maize b1 epialleles. *Plant Cell*.

Luijsterburg, M.S., White, M.F., Driel, R. van & Dame, R.T. (2008). The Major Architects of Chromatin: Architectural Proteins in Bacteria, Archaea and Eukaryotes. *Crit. Rev. Biochem. Mol., 43*, 393-418.

Solimando, L., Luijsterburg, M.S., Vecchio, L. del, Vermeulen, W., Driel, R. van & Fakan, S. (2008). Spatial organization of nucleotide excision repair proteins after UV-induced DNA damage in the human cell nucleus. *J CELL SCI, 122*(PT1), 83-91.

Verschure, P.J. (2008). Finger pointing: engineered zinc finger proteins allow precise modification and regulation of genes. *Chemistry & Biology, 15*, 1241-1242.

Yang, S., Illner, D., Teller, K., Solovei, I., Driel, R. van, Joffe, B., Cremer, T., Eils, R. & Rohr, K. (2008). Structural analysis of interphase X-chromatin based on statistical shape theory. *BIOCHIM BIOPHYS ACTA, 1783*, 2089-2099.

Membership editorial board

Fransz, P.F. (Ed.). (2008). *Chromosome Res.*.

Invited lectures

Driel, R. van (2008, October 03). *Biology is coming of age.* University of Gent, Gent, Belgium, University of Gent.

Driel, R. van (2008, August 21). *Choreography of chromatin-associated protein complexes.* Gothenburg, Sweden, ICSB2008.

Driel, R. van (2008, February 05). *How to package two meters of DNA with tens of thousands of genes inside a microns-size cell nucleus and let it work for you.* Amsterdam, KNAW Biophysics.

Driel, R. van (2008, March 07). *How to package two meters of DNA with tens of thousands of genes inside a microns-size cell nucleus and let it work for you.* Banff, Canada, Canadian Society for Biochemistry and Molecular Cell Biology.

Driel, R. van (2008, mei 09). *How to package two meters of DNA with tens of thousands of genes inside a microns-size cell nucleus and let it work for you.* Leiden, Leiden University.

Driel, R. van (2008, December 08). *In vivo assembly and functioning of a chromatin-associated complex: the choreography of DNA repair proteins as a paradigm.* Babraham Institute Cambridge, UK, Babraham Institute Cambridge.

Driel, R. van (2008, March 26). *Is biology coming of age…?* Jouy en Josas (Paris), France, Rivage Research School.

Driel, R. van (2008, October 21). *Proteins dancing on DNA to fix the damage: how it really works quick-quick-slow dynamics.* Leiden University, Leiden, Leiden University.

Driel, R. van (2008, September 09). *Systems biology to combat metabolic syndrome: a pan-European initiative.* Dusseldorf, Germany, EraSysBio meeting.

Driel, R. van (2008, June 17). *Systems biology to combat metabolic syndrome: the international SBMS initiative:.* Schiphol, ESF-GRASB meeting.

Driel, R. van (2008, October 21). *Thinking about DNA folding.* Leiden, Lorentz Center workshop on The physics of genome folding and function.

Fransz, P.F. (2008, December 08). *Chromatin compaction, dynamics and function in the plant nucleus.* Montpellier, France, Plenary lecture, Plant Epigenetics Workshop.

Fransz, P.F. (2008, September 21). *Genetic and epigenetic consequences of a paracentric inversion in the short arm of chromosome 4 of Arabidopsis.* Donja Stubica, Croatia, ESF Exploratory Workshop.

Fransz, P.F. (2008, September 21). *Heterochromatin decondensation in Arabidopsis triggered by light stress.* Donja Stubica, Croatia, ESF Exploratory Workshop.

Fransz, P.F. (2008, February 26). *Low light triggered heterochromatin dynamics in Arabidopsis.* University College Cork, Ireland, Seminar, Genetics & Biotechnology.

Fransz, P.F. (2008, February 18). *Stress-induced dynamics of heterochromatin domains in Arabidopsis thaliana.* Vienna, Austria, Seminar, Gregor Mendel Institute.

Stam, M. (2008, April 04). *b1 paramutation: the heritable transfer of epigenetic information in trans.* Lunteren, EPW meeting.

Verschure, P.J. (2008, September 26). *Dysregulation of gene expression in Huntington's disease: epigenetic control systems.* Naarden, Huntington's disease PBF workshop.

Verschure, P.J. (2008, April 24). *Epigenetic gene regulation in Huntington's disease.* AMC Amsterdam, NIH workshop.

Other results

Driel, R. van (2008). The physics of genome folding and function.

Epigenetic Regulation of Gene Expression

Chairholder: Prof.dr A.P. Otte

Dr.Ir.J.A. Verhees Assistant Professor

Introduction

It is our long term aim to understand aspects of epigenetic regulation of gene expression. Research is focused on genomic elements, STAR elements, as well as other, novel elements that have in common that they have a powerful, positive influence on promoter activity. These elements are employed to facilitate the production of therapeutic monoclonal antibodies in mammalian cell lines. Unfortunately, the increased protein expression levels have a negative influence on cell growth. Very high protein expression levels even force cells to stop growing at all, which is an undesirable phenomenon from a practical point of view. We attempt to understand this inverse relationship between protein expression levels and cell growth at a quantitative level. Furthermore, we develop inducible gene expression systems in which we can reversibly modulate protein expression levels and cell growth.

Research Highlights

We have found that STAR elements are initiation point for high levels of transcription. The STAR elements are also defined by a higher histone acetylation status. Together these results point to a model in which STAR elements provide a more 'open' chromatin state in which the gene that is flanked by the STAR elements also become more open for transcription.
In order to develop novel inducible expression systems in which we can reversibly modulate protein expression levels and cell growth, we devised a novel set of selection markers. These markers include the Zeocin resistance protein, as well as markers that restore the synthesis of essential metabolic components that normally lack from the cells. Application of these markers warrants both high proteins expression levels as well as a high degree of stability of protein expression over prolonged periods of time.

Other Highlight

Director of a novel biotechnology company, CellaGenics, a spin-off company, emerging from SILS, FNWI and the UvA Holding. CellaGenics works in the research area of expression of therapeutic proteins and cell growth.

Future Prospects

In the coming year we focus on further developing expression systems in which protein expression, growth rates of the cells and secretion of the proteins can be coordinately modulated to achieve an optimal expression platform for therapeutic proteins. As such we aim to investigate and modulate in a comprehensive way

(i) the role of STAR elements in expression and stability of protein expression;
(ii) an inverse relationship between cell growth and protein expression levels;
(iii) secretion of proteins.

Key Publication

Puschendorf, M., Terranova, R., Boutsma, E., Mao, X., Isono, K., Brykczynska, U., Kolb, C., Otte, A.P., Koseki, H., Orkin, S.H., Lohuizen, M. van & Peters, A.H. (2008). PRC1 and Suv39h specify parental asymmetry at constitutive heterochromatin in early mouse embryos. *Nature Genetics 40,* 411-420.

PhD Theses

Kwaks, T.H.J. (2008, February 14). *Employing epigenetics to augment protein expression in mammalian cels.* Universiteit van Amsterdam. Prom./coprom.: prof.dr. A.P. Otte.

Academic publications (refereed)

Endoh, M, Endo, T.A., Endoh, T., Fujimura, Y. & Otte, A.P. (2008). Polycomb group proteins Ring1A/B are functionally linked to the core transcriptional regulatory circuitry to maintain ES cell identity. *Development, 135*, 1513-1524.

Engelsen, I.B., Mannelqvist, M., Stefansson, I.M, Carter, S.L. & Otte, A.P. (2008). Low BMI-1 expression is associated with an activated BMI-1-driven signature, vascular invasion, and hormone receptor loss in endometrial carcinoma. *Brit. J. Cancer, 20*, 1662-1669.

Hoffmann, M.J., Engers, R., Florl, A.R., Otte, A.P., Muller, M. & Schulz, W.A. (2008). Expression changes in EZH2, but not in BMI-1, SIRT1, DNMT1 or DNMT3B are associated with DNA methylation changes in prostate cancer. *Cancer Biol. Ther., 6*, 1403-1412.

Puschendorf, M., Terranova, R., Boutsma, E., Mao, X., Isono, K., Brykczynska, U., Kolb, C., Otte, A.P., Koseki, H., Orkin, S.H., Lohuizen, M. van & Peters, A.H. (2008). PRC1 and Suv39h specify parental asymmetry at constitutive heterochromatin in early mouse embryos. *Nat. Genet., 40*, 411-420.

Terranova, R., Yokobayashi, S., Stadler, M.B., Otte, A.P. & Lohuizen, M. (2008). Polycomb Group Proteins Ezh2 and Rnf2 Direct Genomic Contraction and Imprinted Repression in Early Mouse Embryos. *Dev. Cell, 5*, 668-679.

Vékony, H., Raaphorst, F.M., Otte, A.P., Lohuizen, M. van, Leemans, C.R., Waal, I. van der & Bloemena, E. (2008). High expression of Polycomb group protein EZH2 predicts poor survival in salivary gland adenoid cystic carcinoma. *J.Clin.Pathol., 61*(6), 744-749.

Molecular Cytology

Chairholder: Prof.dr Th.W.J. Gadella

Dr. M. Müller	Associate Professor
Dr. T. den Blaauwen	Assistant Professor
Dr. Ir. J. Goedhart	Assistant Professor
Dr. E. M. M. Manders	Assistant Professor

Introduction

Molecular Cytology is the study of the dynamic architecture of living cells.
Our central theme is 'Self-organization and signalling in living cells'. Self-organization is the intrinsic property of matter to organize itself in a (dynamic) structure, whereas signalling implies the activity of gene-products to control a local activity which can alter the local cellular architecture (e.g. driving morphogenesis). In order to achieve a certain 3D architecture in cells, these two important mechanisms work in concert. At Molecular Cytology both mechanisms are studied with emphasis on membrane-related architecture of living cells using advanced microscopy tools.

The main research areas are:
1) Spatial organization of sub-cellular signalling (group leader prof.dr Dorus Gadella & dr. Joachim Goedhart). We want to understand how cells can achieve and maintain a local signal in order to drive morphogenesis, to define new cytoskeletal anchorage or vesicle-docking sites. We focus on signal flow across and in the plane of the membrane of living mammalian cells starting from, histamine/P2Y receptors, G-alfaQ to PLC activation triggering downstream calcium and kinase signalling.
2) Molecular mechanisms of bacterial proliferation (group leader dr Tanneke den Blaauwen). Two dynamic self-assembling membrane-bound protein complexes (hyperstructures) are involved in the elongation and division of the bacterial cell. The complexes extend the lateral cell envelope and produce complete new cell poles. The actin homologue MreB and the tubulin homologue FtsZ recruit these complexes, respectively. The identity, function and dynamics of the proteins in these complexes are studied.
3) Self organisation in complex (biological) systems (group leader dr. Michiel Müller). We aim to understand the self-organizational properties of biomembranes in functional domains crucial in cellular signalling. We also study the mechanism of lipid droplet formation and breakdown, and aim to find cell morphological parameters linked to the (mal)function of lipid droplets, with implications for diabetes and atherosclerosis. To this end we apply and develop quantitative microscopy reporting on the chemical composition and physical state.

These research themes heavily depend on advanced microscopy technology organized within the Centre for Advanced Microscopy (CAM, 2004). The goal of CAM is to boost Life Sciences research using & developing (optical) microscopy techniques. Current most prominent developments are Coherent Anti-Stokes Raman (CARS) Microscopy (dr Michiel Müller), Third-Harmonic Generation (THG) Microscopy (dr Michiel Müller), Controlled Light Exposure Microscopy (CLEM) (dr Erik Manders) and Spinning disk & Total Internal Reflection (TIR) - Fluorescence Lifetime Imaging Microscopy (FLIM) (prof.dr Dorus Gadella).

Research Highlights

•The Controlled Light Exposure Microscopy (CLEM) technology for strong reduction of phototoxicity and photobleaching was published (Hoebe et al (2007), Nature Biotechnology).

• We have achieved for the first time imaging of cell type and cell location-dependent lipid droplet chemical composition and morphology in living cells using CARS microscopy (Rinia et al., submitted)
• We have made a FRET-based reporter system to monitor agonist-induced GalphaQ heterotrimer-activation in living mammalian cells. Using this reporter we elucidated for the first time that GalphaQ does not dissociate but undergoes a conformation change upon activation (Adjobo-Hermans et al, submitted)
•In contrast to what was generally believed, we show that MreB is not involved in genome segregation in E. coli, but that it is involved in the recruitment of a protein complex (elongase) that directs lateral cell envelope synthesis (Karzmarek et al., 2007). We also show that the peptidoglycan precursor synthases MraY and MurG are part of this complex (Mohammadi et al., 2007).

Other Highlights

• Dorus Gadella was elected as president of the Netherlands Society for Microscopy
• An EU SME-grant was awarded to Tanneke den Blaauwen in collaboration with 12 European groups working on the development of antibiotic screening assays and the development of new antibiotics that will inhibit bacterial cell division.

Future Prospects

• We aim to publish a novel FLIM-based screening method for selection of super fluorescent protein variants with the identification of the most efficient fluorescent protein variant ever described.
• We aim to publish about the mathematics behind CLEM and a major improvement of the confocal CLEM technology and we aim to make the first wide-field CLEM images.
•We aim to publish the first paper that describes a reliable FRET-system to study the interaction of rare proteins in bacteria. Furthermore we aim to deepen our understanding of the bacterial divisome and elongation complexes including the analysis of the GTP-binding pocket of FtsZ, a mutagenesis analysis and first crystal structure of FtsQ, the in situ labelling of peptidoglycan synthesis and the analysis of the localization of PBP5 in *E. coli*.
•We aim to publish a paper describing the characterization of p63RhoGEF (a novel novel effector of Galphaq) in single living cells. The dynamics of the interaction of p63RhoGEF with Galphaq will be examined as well.

Key Publications

Dhonukshe, P., Grigoriev, I., Fischer, R., Tominaga, M., Robinson, D.G., HaĬek J., Paciorek, T., PetráĬek, J., Seifertová, Tejos, R., Meisel, L.A., E., Zaz□ímalová, E., Gadella Jr, T.W.J., Stierhof, Y.-D., Ueda T., Oiwa K., Akhmanova, A., Brock, R., Spang, A., Friml, J. (2008). Auxin transport inhibitors impair vesicle motility and actin cytoskeleton dynamics in diverse eukaryotes. Proc. Nat. Acad. Sci. USA 105, 4489-1194.

Thole, J.M., Vermeer, J.E.M., Zhang, Y., Gadella, Th.W.J. & Nielsen, E. (2008). Root hair defective4 encodes a phosphatidylinositol-4-phosphate phosphatase required for proper root hair development in Arabidopsis thaliana. The Plant Cell 20, 381-395.

PhD Theses

Adjobo Hermans, M.J.W. (2008, January 18). *Visualizing G protein signaling in living cells.* Universiteit van Amsterdam . Prom./coprom.: prof. dr. Th.W.J. Gadella & dr.ir. J. Goedhart.

Verhoeven, G.S. (2008, December 02). *Force generation in dividing E. coli cells: A handles-on approach using optical tweezers.* Universiteit Leiden . Prom./coprom.: prof.dr. M. Dogterom & dr. T. den Blaauwen.

Academic publications (refereed)

Adjobo-Hermans, M.J.W., Goedhart, J. & Gadella, Th.W.J. (2008). Regulation of PLC{beta}1a membrane anchoring by its substrate phosphatidylinositol (4,5)-bisphosphate. *J. Cell Sci., 121*, 3770-3777.

Blaauwen, T. den, De Pedro, M.A., Nguyen- Distèche, M. & Ayala, J.A. (2008). Morphogenesis of the rod shaped sacculus. *FEMS Microbiol. Rev., 32*, 321-344.

Dhonukshe, P.B., Grigoriev, I., Fischer, R. & Tominaga, M. (2008). Auxin transport inhibitors impair vesicle motility and actin cytoskeleton dynamics in diverse eukaryotes. *PNAS, 105*(11), 4489-4494.

Does, H.C. van der, Duyvesteijn, R.G.E., Goltstein, P.M., Schie, C.C.N. van, Manders, E.M.M., Cornelissen, B.J.C. & Rep, M. (2008). Expression of effector gene SIX1 of Fusarium oxysporum requires living plant cells. *Fungal Genet. Biol., 45*(9), 1257-1264.

Ent, F. van den, Vinkenvleugel, T.M.F., Ind, A., West, P., Veprintsev, D., Nanninga, N., Blaauwen, T. den & Löwe, J. (2008). Structural and Mutational analysis of cell division protein FtsQ. *Mol. Microbiol., 68*(1), 110-123.

Hoebe, R.A., Voort, H.T.M. van der, Stap, J., Noorden, C.J.F. van & Manders, E.M.M. (2008). Quantitative determination of the reduction of phototoxicity and photobleaching by controlled light exposure microscopy. *J. Microsc., 231*(1), 9-20.

Kremers, G.J., Munster, E.B. van, Goedhart, J. & Gadella, Th.W.J. (2008). Quantitative lifetime unmixing of multiexponentially decaying fluorophores using single-frequency fluorescence lifetime imaging microscopy. *Biophys J., 95*(1), 378-389.

Läppchen, T., Pinas, V., Hartog, A.F., Koomen, G.J., Schaffner, C., Andreu, J.M., Trambaiolo, D., Löwe, J., Juhem, A., Popov, A.V. & Blaauwen, T. den (2008). Probing FtsZ and Tubulin with C8-substituted GTP Analogues Reveals Differences in their Nucleotide Binding Sites. *Chem. Biol., 15*, 189-199.

Oomen, L.C.J.M., Sacher, R., Brocks, H.H.J., Zwier, J.M., Brakenhoff, G.J. & Jalink, K. (2008). Immersion oil for high-resolution live-cell imaging at 37◦C: optical and physical characteristics. *J. Microsc., 232*(2), 353-361 (9).

Pereira-Cenci, T., Deng, D.M., Kraneveld, E.A., Manders, E.M.M., Cury, A.A. Del Bel, Cate, J.M. ten & Crielaard, W. (2008). The effect of Streptococcus mutans and Candida glabrata on Candida albicans biofilms formed on different surfaces. *ARCH ORAL BIOL, 53*(8), 755-764.

Rinia, H.A., Burger, K.N., Bonn, M. & Müller, M. (2008). Quantitative label-free imaging of lipid composition and packing of individual cellular lipid droplets using multiplex CARS microscopy. *Biophys J., 95*(10), 4908-4914.

Rino, J., Desterro, J.M.P., Pacheco, T.R., Gadella, Th.W.J. & Carmo-Fonseca, M. (2008). Splicing Factors SF1 and U2AF Associate in Extraspliceosomal Complexes. *Mol. Cell. Biol., 28*(9), 3045-3057.

Schafer, D., Squier, J.A., Maarseveen, J.H. van, Bonn, D., Bonn, M. & Müller, M. (2008). In situ quantitative measurement of concentration profiles in a microreactor with submicron resolution using multiplex CARS microscopy. *J.Am.Chem.Soc., 3*(130), 11592-11593.

Schenning, M., Goedhart, J., Gadella, Th.W.J., Wirtz, K.W.A. & Snoek, G.T. (2008). The anti-apoptotic activity associated with phosphatidylinositol transfer protein alpha activates the MAPK and Akt/ PKB pathway. Biochim.Biophys.Acta*, 1783*, 1700-1706.

Sovago, M., Campen, R.K., Wurpel, G.W.H., Müller, M., Bakker, H.J. & Bonn, M. (2008). Vibrational response of hydrogen-bonded interfacial water is dominated by intramolecular coupling. *Phys. Rev. Lett., 100*(17), 173901.

Thole, J.M., Vermeer, J.E.M., Zhang, Y., Gadella, Th.W.J. & Nielsen, E. (2008). Root hair defective4 encodes a phosphatidylinositol-4-phosphate phosphatase required for proper root hair development in Arabidopsis thaliana. *The Plant Cell, 20*(2), 381-395.

Zwier, J.M., Oomen, L.C.J.M., Brocks, L., Jalink, K. & Brakenhoff, G.J. (2008). Quantitative image correction and calibration for confocal fluorescence microscopy using thin reference laye and SIPchart based calibration procedureQuantitative image correction and calibration for confocal fluorescence microscopy using thin reference laye and SIPchart based calibration procedure. *J. Microsc., 231*(11), 59-69.

Goedhart, J. & Gadella, Th.W.J. (2008). Fluorescence resonance energy transfer imaging of PKC signalling in living cells using genetically encoded fluorescent probes. *Journal of the Royal Society Interface, 9*(1), S27-S34.

Verbeek, D.S., Goedhart, J., Bruinsma, L., Sinke, R.J. & Reits, E.A. (2008). PKC{gamma} mutations in spinocerebellar ataxia type 14 affect C1 domain accessibility and kinase activity leading to aberrant MAPK signaling. *J. Cell Sci., 121*, 2339-2349.

Book chapter

Brakenhoff, G.J. & Zwier, J.M. (2008). Characterization and Calibration in Wide Field and Sectioned Fluorescence Microscopy SIPcharts. In *Standardization and Quality Assurance in Fluorescence Measurements II* (Springer Series on Fluorescence, Volume 6) (pp. 25-54). Berlin: Springer.

Invited lectures

Adjobo-Hermans, M.J.W., Goedhart, J., Weeren, L. van & Gadella, Th.W.J. (2008, November 10). *Visualizing G protein signalling in living cells.* Lunteren., SEN-prize lecture in the Fall meeting of the Netherlands Society of Microscopy.

Adjobo-Hermans, M.J.W., Tsur, A., Weeren, L. van, Goedhart, J. & Gadella, Th.W.J. (2008, December 12). *Visualizing Gq activity in single living cells under native receptor expression conditions.* Solvay Weesp, GPCR day meeting.

Blaauwen, T. den (2008, June 28). *Cell division protein FtsZ as new antibiotic target.* Notre Dame IN USA, Novel antibiotics, Old and New targets.

Blaauwen, T. den & Läppchen, T. (2008, December 11). *Cell division protein FtsZ as new antibiotic target.* London, England, Re-emerging infectious diseases.

Blaauwen, T. den (2008, May 21). *Growth and cell division of Escherichia coli.* Liège, Belgigue, The EURINTAFAR project, and The “Proteins.

Blaauwen, T. den (2008, March 17). *Growth and division of Escherichia coli.* Marburg, Duitsland, Marburg.

Blaauwen, T. den, Verheul, J. & Berg van Saparoea, B. van den (2008, October 21). *Localization and interactions of peptidoglycan synthesis enzymes MraY and PBP1A in Escherichia coli.* Bled, Slovenia, EUR-INTAFAR 6th general assembly.

Blaauwen, T. den (2008, October 25). *New concepts for the organization of the protein Scaffold underlying the cell wall.* Washington DC, USA, 48th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC).

Blaauwen, T. den (2008, April 13). *Who are synthesizing preseptal peptidoglycan?* Harzé, Belgique, 5nd general EUR-INTAFAR assembly.

Brakenhoff, G.J. (2008, April 19). *Confocal fluorescence microscopy: characterization of imaging and calibration using thin uniform fluorescence layers, SIPcharts.* Erice–Sicily, International School of Biophysics «Antonio Borsellino», Multidimensional optical fluorescence microscopy towards nanoscopy.

Brakenhoff, G.J., Oomen, L.C.J.M., Brocks, L., Jalink, K. & Zwier, J.M. (2008, April 15). *Quantitative image calibration for confocal fluorescence microscopy using thin reference layers and SIPchart based calibration procedures.* . Awaji Island, Japan, Focus on Microscopy 2008.

Gadella, Th.W.J. (2008, August 08). *(GFP-based) fluorescence microscopy in cell biology.* Nijmegen, ADONIS microscopy course.

Gadella, Th.W.J. (2008, November 11). *Fluorescence Resonance Energy Transfer Microscopy.* Amsterdam, Nodperceptrion workshop.

Gadella, Th.W.J. (2008, October 07). *Fluorescence Resonance Energy Transfer Microscopy.* Groningen, B-Basic Advanced Course on Visualization of Cellular Processes (ACVCP course).

Gadella, Th.W.J. (2008, April 23). *FRET-imaging of G-protein activation and downstream induced signalling.* London, UK, Microscience conference 2008.

Gadella, Th.W.J. (2008, August 24). *Imaging of signalling across the plasma membrane.* Gdansk, Poland., XIII international congress of histochemistry and cytochemistry.

Gadella, Th.W.J. (2008, november 21). *Measuring molecular processes in living cells.* Amsterdam., Netherlands Institite for Systems Biology Plenary meetiing.

Gadella, Th.W.J. (2008, October 07). *Multimode Fluorescence lifetime imaging microscopy (FLIM).* Groningen., B-Basic Advanced Course on Visualization of Cellular Processes (ACVCP course).

Gadella, Th.W.J. & Adjobo-Hermans, M.J.W. (2008, January 25). *Multimode Microscopy of signalling across the plasma membrane.* Würzburg, Germany., Würzburg University.

Gadella, Th.W.J. (2008, October 07). *Multiparameter imaging of signalling across the plasma membrane.* Groningen, B-Basic Advanced Course on Visualization of Cellular Processes (ACVCP course).

Gadella, Th.W.J. (2008, September 30). *Visualization of signalling across the plasma membrane in living cells using genetic encoded fluorescent sensors and multimode microscopy.* Paris, France., European Optical Society conference 2008.

Gadella, Th.W.J. (2008, April 23). *Visualization of signalling across the plasma membrane in living cells using genetic encoded fluorescent sensors and multimode microscopy.* Cambride Research Institute, Cambridge UK, Theodor Förster Lecture Series.

Gadella, Th.W.J. & Adjobo-Hermans, M.J.W. (2008, March 3). *Visualizing Gq mediated signalling in single living cells.* Free University, Amsterdam, seminar.

Goedhart, J., Elsenaar, I., Adjobo-Hermans, M.J.W., Weeren, L. van & Gadella, Th.W.J. (2008, November 10). *Quantitative co-expression of multiple proteins in eukaryotic cells.* Lunteren, Fall meeting of the Netherlands Society of Microscopy.

Hink, M.A. (2008, September 17). *Fluorescence fluctuation analysis of signaling complexes.* Amsterdam., SILS Research day.

Hink, M.A. (2008, October 09). *Fluorescence fluctuation spectroscopy.* Groningen, B-Basic Advanced Course on Visualization of Cellular Processes (ACVCP course).

Hink, M.A. (2008, September 23). *Fluorescence fluctuation spectroscopy.* Wageningen, FEBS Advanced Practical Course Microspectroscopy: Monitoring Molecular Interactions in Living Cells.

Hink, M.A. (2008, October 09). *Quantification of MAPK complexes in yeast pheromone signaling using advanced fluorescence techniques.* Groningen, B-Basic Advanced Course on Visualization of Cellular Processes (ACVCP course), Groningen.

Hink, M.A. (2008, November 11). *Study molecular dynamics by FCS and FRAP.* Amsterdam, Nodperception workshop.

Hoebe, R.A., Oven, C.H. van, Noorden, C.J.F. van & Manders, E.M.M. (2008, april 15). *Noise reduction in controlled light exposure microscopy (CLEM).* Osaka, Japan, Focus on Microscopy 2008.

Manders, E.M.M., Zoon, P.D., Vos, W. de & Hoebe, R. (2008, September 03). *Controlled Light Exposure Microscopy (CLEM) for prolonged live-cell imaging and strongly reduced photobleaching.* Aachen, Germany, EMC 2008, European Microscopy Society.

Manders, E.M.M., Zoon, P.D., Vos, W. de & Hoebe, R. (2008, August 06). *Controlled Light Exposure Microscopy (CLEM) for prolonged live-cell imaging and strongly reduced photobleaching.* Albuquerque, NM, USA, Microscopy & Analysis 2008, The Microscopy Society of America.

Manders, E.M.M. (2008, September 23). *Controlled Light Exposure Microscopy (CLEM) for prolonged live-cell imaging.* Bonn, Germany, High Speed and High Resolution Optical Microscopy.

Manders, E.M.M., Zoon, P.D., Vos, W. de & Hoebe, R. (2008, April 15). *Controlled Light Exposure Microscopy (CLEM) for prolonged live-cell imaging.* Osaka, Japan, Focus on Microscopy 2008.

Manders, E.M.M. (2008, May 21). *New strategies in microscopy.* Amsterdam, Guest lecture Nikon Europe.

Manders, E.M.M. (2008, March 13). *Strong reduction of Phototoxicity and Photobleaching by CLEM: Controlled Light Exposure Microscopy.* Marburg, Germany, German Society for Cell Biology.

Other results

Adjobo-Hermans, M.J.W. (2008, November 10). SEN (Stichting ter bevordering van de elektronenmicroscopie in Nederland) Prize 2008. Document, a replica of a Van Leeuwenhoek microscope + an amount of € 2000, Amsterdam.

Research Cluster

Plant Signalling

Plants are the world's primary source for food and feed, for raw materials for industry, and they provide the oxygen we breathe. Although the health of plants is constantly challenged in both natural and agronomic ecosystems, plants can master most challenges. The Swammerdam Institute for Life Sciences is aiming to unravel the molecular mechanisms of resistance against pathogens and insects, and to study the cellular signal transduction pathways controlling stress responses in general. Knowledge of the genetics and biochemistry of these processes can be translated into improvement of agricultural crops and plant protection schemes.

Two chairs contribute to the research cluster 'Plant Signalling': 'Plant Physiology' and 'Plant-pathogen Interactions', while the chairs "Molecular Cytology" and "Nuclear organization" study related topics on plant model systems

Plant Physiology

Chairholder: Prof. dr. M.A. Haring

Dr.Ir.R.C. Schuurink	Associate Professor
Dr. T. Munnik	Associate Professor
Dr. C. Testerink	Assistant Professor

Figure 1 Legend: Restoration of anthocyanin production in *Petunia hybrida* flowers by transient overexpression of a MYB transcription factor. This can be used as a visible marker when transiently overexpressing benzenoid biosynthesis related genes or promoter-reporter constructs (picture by Alex van Moerkercke).

Introduction

Our research on phospholipid signaling is centered around the lipid second messenger, phosphatidic acid (PA), which is produced via activation of phospholipase D (PLD) or indirectly, via the combined action of phospholipase C (PLC) and diacylglycerol kinase (DGK). Teun Munnik and his co-workers use knockout lines of individual PLC, DGK and PLD genes in *Arabidopsis* plants, to elucidate their role in plant stress signaling and development. How PA exerts its effects is still unknown, mainly due to the lack of characterized target proteins. We identified several protein kinases that directly and

specifically bind PA, including CTR1, a key regulator of the signaling pathway controlled by the stress hormone ethylene. One of the key players in the response of plants to many stress conditions is the lipid second messenger phosphatidic acid (PA). The research interest of Christa Testerink is to elucidate how phosphatidic acid modulates protein function and downstream plant responses.
Another goal of our research is to identify key information cascades that control pollen tube growth, and to understand how these networks link to the biomechanics that drive cell elongation. Our most recent work shows that transcellular hydrodynamic flux drives pollen tube growth and modulates the rates of exocytosis and endocytosis (see figure 2).
Robert Schuurink and his co-workers investigate the biosynthesis, regulation and biological role of plant volatiles. *Petunia hybrida* is our model of choice to study volatile benzenoid and phenylpropanoid synthesis, emission and regulation in flowers. These volatiles are synthesized predominantly in the corolla limb and emission is highly regulated: emission exhibitis a circadian rhythm, emission starts when corolla development is complete, emission ceases upon pollination and subsequent senescence. For plant-insect interaction studies we use tomato as a model system. We focus on terpene synthases expressed in trichomes and have identified several terpenoids that repel or attract whiteflies. Our aim is to engineer the production of these terpenoids in tomato trichomes in such a way that they become repellant for pest insects. Finally, we use Arabidopsis for transcriptomics and forward genetic screens to identify genes important in the response to the wound-induced C6-volatile *E*-2-hexenal, a reactive electrophile molecule.

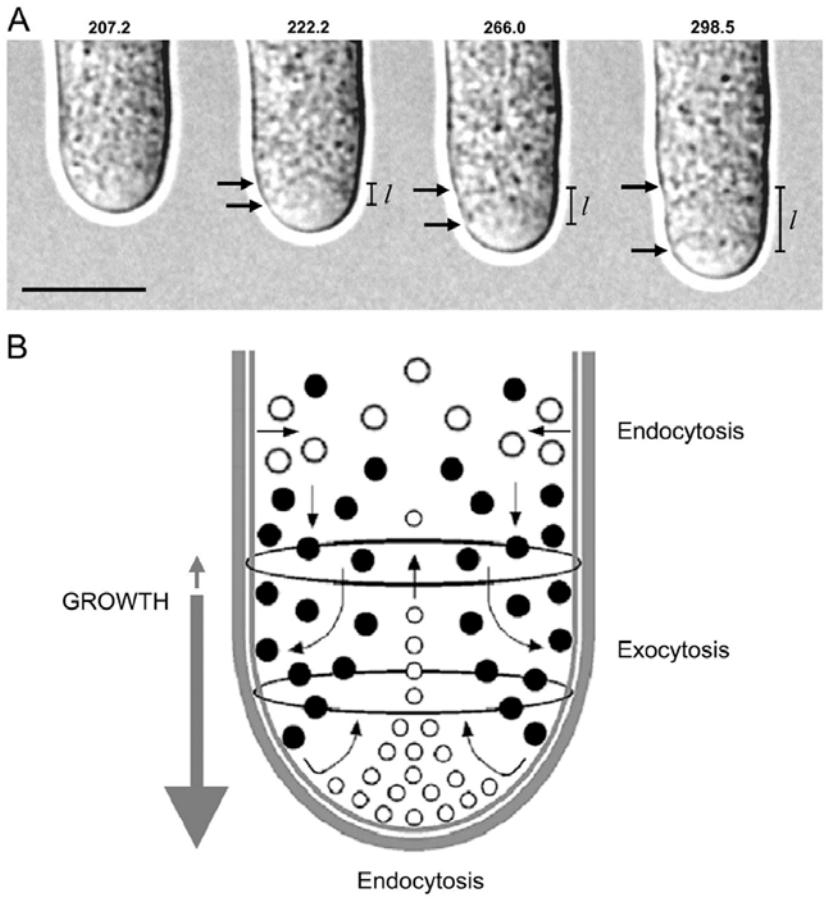


Figure 2 Legend: Cell surface dynamics reflect vesicle incorporation and retrieval at the plasma membrane in the apical region of growing pollen tubes. (A) Tobacco pollen tube undergoing a very strong growth pulse and a corresponding decrease in tube diameter. The region of newly incorporated growth is revealed as a hinge between the cap of the apical dome and the distal pollen tube. The length of pollen tube growth is equal to the length of expansion of the hinge region. Bar = 10 μm. (B) Schematic illustration of vesicle dynamics during pollen tube growth. Endocytic retrieval of excess plasma membrane occurs along the apical dome. Exocytosis occurs in the adjacent region. Coated vesicle endocytosis occurs along the distal shank of the pollen tube.

Research Highlights

Earlier, we have provided evidence for the role of PA in plant defense using elicitor-challenged cell suspensions of tomato, parsley and alfalfa. Currently, we are addressing PA's role in the model system *Arabidopsis thaliana* by insertion lines of selected lipid signaling genes. One DGK gene was found to be required for full resistance against virulent *Pseudomonas* and *H. parasitica*, while two PLD genes were found to be involved in resistance against avirulent *Pseudomonas* strains. The DGK mutant is affected in PR1 gene expression, which seems to be independent of salicylic acid. Furthermore a PLC mutant with aberrant root architecture was found, suggesting a link with the phytohormone auxin. Preliminary results indicate that various DGKs and PLCs are involved in the plants PA response to cold, while 2 PLD mutants were found to be affected in their salt tolerance. Several protein kinases, including two SNF-1 related protein kinases (SnRKs) implicated in osmotic stress, were found to bind PA. We have further characterized their function in plant stress responses and have found that they contribute to salt stress tolerance. Interestingly, only a select subgroup of the SnRKs can bind phosphatidic acid, while others show no affinity for lipids. Progress has also been made in the elucidation of the role of PA in ethylene signaling. In collaboration with the group of Dr. Ton Peeters (UU), we have determined that several lipid signaling mutants that are impaired in ethylene responses.
In collaboration with the lab of Takashi Aoyama (Kyoto, Japan), we found that Arabidopsis thaliana PIP5K3 (for Phosphatidylinositol Phosphate 5-Kinase 3) encodes a phosphatidylinositol 4-phosphate 5-kinase, a key enzyme producing PtdIns(4,5)P_2, that is preferentially expressed in growing root hairs. T-DNA insertion mutations that substantially reduced the expression of PIP5K3 caused significantly shorter root hairs than in the wild type. By contrast, overexpression caused longer root hairs and multiple protruding sites on a single trichoblast (see figure 3). A yellow fluorescent protein (YFP) fusion of PIP5K3, driven by the PIP5K3 promoter, complemented the short-root-hair phenotype. PIP5K3-YFP localized to the plasma membrane and cytoplasmic space of elongating root hair apices, to growing root hair bulges, and, notably, to sites about to form root hair bulges. The signal was greatest in rapidly growing root hairs and quickly disappeared when elongation ceased. These results provide evidence that PIP5K3 is involved in localizing PtdIns(4,5)P_2 to the elongating root hair apex and is a key regulator of the machinery that initiates and promotes root hair tip growth.
This year we mapped the gene that had been disrupted in the *E*-2-hexenal resistant mutant *her2* and localised its product to the mitochondria, where the GABA transaminase that we previously mapped in the *her1* mutant is also present. We also analysed the promoters of *E*-2-hexenal responsive genes and selected two motifs that are putatively important for this transcriptional regulation. In Petunia we firmly established the role of the β-oxidative pathway in the production of benzoic acid by knocking down a 3-ketoacyl-CoA thiolase, PhKAT1. This PhKAT1 is localised in the peroxisomes where it is elemental for the formation of benzoyl-CoA. Silencing of *PhKAT1* resulted in a major reduction in benzoic acid and benzenoid formation, leaving the production of other phenylpropanoid-related volatiles unaffected. In the tomato insect interaction project we cloned several terpene synthase from the trichomes of wild species and one cultivated tomato, using the Massive Parallel Sequence technology of 454 Life Sciences (GS-flex) in collaboration with Keygene (Wageningen). The enzymatic function of these terpene synthases has been determined based on the activity of recombinant proteins. Transgenic tomato lines were created expressing the most interesting terpene synthase and several precursor biosynthetic genes.

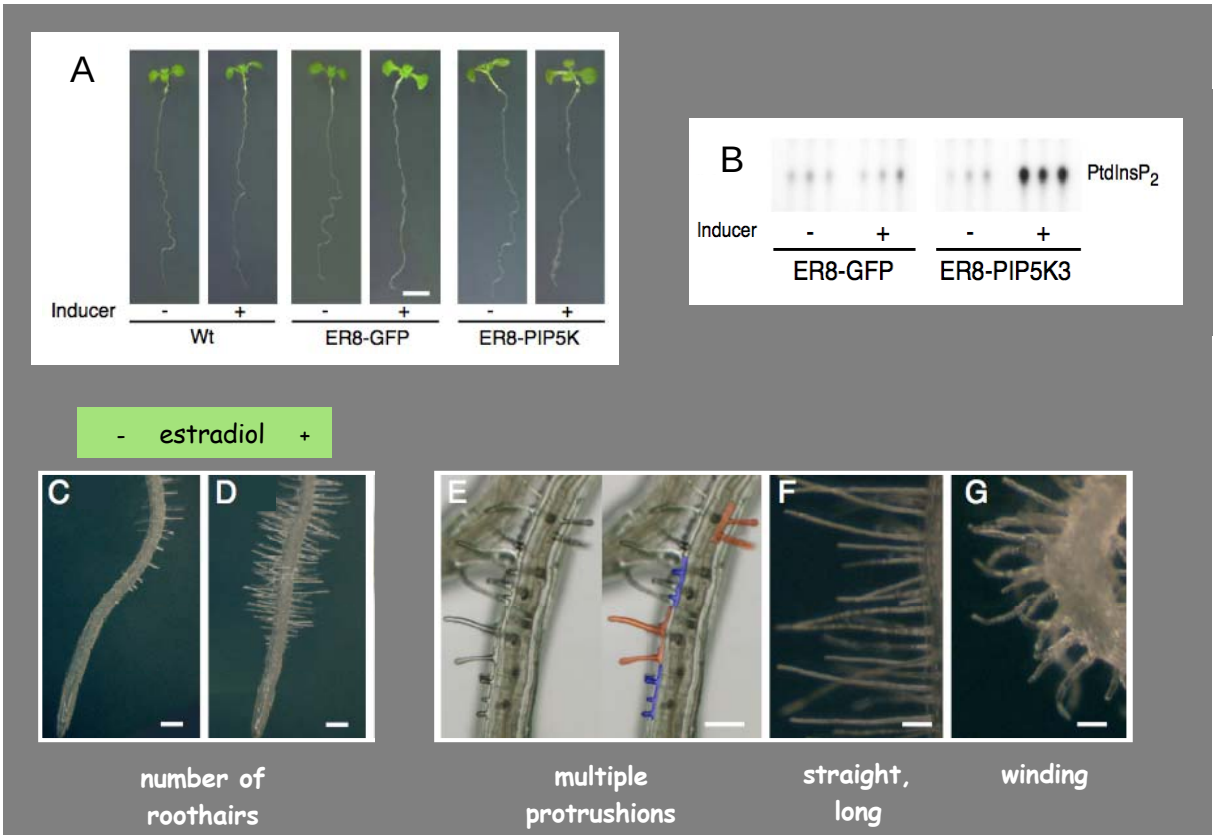


Figure 3 Legend: Phenotypes caused by inducible overexpression of a *PIP kinase* gene, *PIP5K3,* in Arabidopsis roots. (A) Wild-type (Wt) and transgenic seedlings with estrogen-inducible *PIP5K3* (*ER8-PIP5K3*) or *GFP* (*ER8-GFP*) genes were grown on medium supplemented with (+) or without (-) 10 μM β-estradiol. (A) Phenotypic changes, (B) PtdIns(4,5)P_2 levels of O/N $^{32}P_i$-labelled seedlings, (C) and (D) Close-up pictures of *ER8-PIP5K3* roots that were untreated (C) or treated (D) with β-estradiol. (E-G) Close-up pictures of the *ER8-PIP5K3* root surface that were treated with β-estradiol. In the right half of (E), cells with multiple protruding sites are highlighted with colors so that each cell can be clearly identified. Straight long root hairs and winding root hairs observed in different *ER8-PIP5K3* lines are shown in (F) and (G), respectively. Bars = 0.2 mm in (A), 0.1 mm in (E-G).

Other Highlights

NWO-ALW: The role of E-2-hexenal and GABA in plant stress responses
Ph.D student for 4 years for Robert Schuurink

NWO-ALW and TTI-GG: Novel tomatoes that counteract herbivore suppression of plant defences
Ph. D student for Merijn Kant (IBED) for 4 years, Postdoc for 3 years for R. Schuurink

Future Prospects

For more detailed visualization of lipid domains in the cell, we have constructed biosensors specific for PtdIns3P, PtdIns4P, PtdIns(4,5)P_2 and DAG, while those for PA are being developed. This will allow us to further characterize the role and dynamics of PA and phosphoinositides (PPIs) during plant development and stress responses. A functional genomics approach has been started to determine how subcellular phospholipid pools are being generated and maintained by screening T-DNA insertion mutants in lipid kinases, phosphatases and hydrolases. We aim to identify the PA-binding site within several different protein kinases, including the SnRKs, AGC protein kinases and CTR1. Systematic and quantitative manipulation of PA levels in both suspension cells and plants should reveal the in

vivo responses to a “PA burst”. We will focus in particular on the role of PA in salt tolerance and plant development.
In the coming year(s) we will continue characterizing terpene synthases from tomato trichomes and creating transgenic tomatoes overexpressing these genes in trichomes to investigate their role in tomato-whitefly interactions. We will try to determine the function of the HER2 gene product and investigate the role of the promoter motifs in the *E*-2-hexenal response of Arabidopsis. For Petunia we will focus finding the transcription factor that binds to the putative enhancer in the *ODORANT1* promoter using the yeast-one-hybrid technology.

Key Publications

Kusano H, Testerink **C**, Vermeer JE, Tsuge T, Shimada H, Oka A, Munnik T, Aoyama T. (2008) The Arabidopsis Phosphatidylinositol Phosphate 5-Kinase PIP5K3 is a key regulator of root hair tip growth. *Plant Cell*, 20, 367-380.

Mirabella R, Rauwerda H, Struys EA, Jakobs C, Triantaphylidès C, Haring MA, Schuurink RC. (2008) The Arabidopsis her1 mutant implicates GABA in E-2-hexenal responsiveness. *Plant J*. 53, 197-213.

PhD Theses

Schooten, B. van (2008, September 19). *Dissecting Arabidopsis phospholipid signaling using reverse genetics.* Universiteit van Amsterdam . Prom./coprom.: prof.dr. M.A. Haring & dr. T. Munnik.

Patent

Ament, K., Schuurink, R.C., Haring, M.A. & Both, M. de (). Farnesene synthase. no P29486US00.

Academic publications (refereed)

Kant, M.R., Sabelis, M.W., Haring, M.A. & Schuurink, R.C. (2008). Intraspecific variation in a generalist herbivore accounts for differential induction and impact of host plant defences. *Proc. R. Soc. Lond. Ser. B-Biol. Sci., 275*(1633), 443-452.

Mirabella, R., Rauwerda, H., Struys, E.A., Jakobs, C., Triantaphylidès, C., Haring, M.A. & Schuurink, R.C. (2008). The Arabidopsis her1 mutant implicates GABA in E-2-hexenal responsiveness. *Plant J., 53*(2), 197-213.

Testerink, C., Larsen, P.B., McLoughlin, F., Does, D. van der, Himbergen, J.A.J. van & Munnik, T. (2008). PA, a stress-induced short cut to switch-on ethylene signalling by switching-off CTR1? *Plant signaling and behavior, 3*(9), 681-683.

Verweij, W., Spelt, C., Di Sansebastiano, G.P. & Vermeer, J.E.M. (2008). An H+ P-ATPase on the tonoplast determines vacuolar pH and flower colour. *NAT CELL BIOL, 10*, 1456-1462.

Does, H.C. van der, Duyvesteijn, R.G.E., Goltstein, P.M., Schie, C.C.N. van, Manders, E.M.M., Cornelissen, B.J.C. & Rep, M. (2008).
Expression of effector gene SIX1 of Fusarium oxysporum requires living plant cells. *Fungal Genet. Biol., 45*(9), 1257-1264.

Zonia, L. & Munnik, T. (2008). Still life: Pollen tube growth observed in millisecond resolution. *Plant Signaling & Behavior, 3*(10), 836-838.

Zonia, L. & Munnik, T. (2008). Vesicle trafficking dynamics and visualization of zones of exocytosis and endocytosis in tobacco pollen tubes. *Journal of Experimental Botany, 59*(4), 861-873.

Gonorazky, G., Laxalt, A.M., Testerink, C., Munnik, T. & Canal, L. de la (2008). Phosphatidylinositol 4-phosphate accumulates extracellularly upon xylanase treatment in tomato cell suspensions. *PLANT CELL ENVIRON, 31*(8), 1051-1062.

Kusano, H., Testerink, C., Vermeer, J.E.M., Tsuge, T., Shimada, H., Oka, A., Munnik, T. & Aoyama, T. (2008). The Arabidopsis phosphatidylinositol phosphate 5-kinase PIP5K3 is a key regulator of root hair tip growth. *Plant Cell, 20*, 367-380.

Membership editorial board

Munnik, T. (Ed.). (2008). *Plant signaling and behavior*.
Munnik, T. (Ed.). (2008). *Planta*.
Munnik, T. (Ed.). (2008). *The Open Plant Science Journal*.

Invited lectures

Bleeker, P.M., Diergaarde, P.J., Ament, K., Haring, M.A., Both, M. de & Schuurink, R.C. (2008,August 17). *Volatile Chemical cues involved in tomato-whitefly interactions.* State College, Pennsylvania, USA, International society of chemical Ecology.

Haring, M.A. & Kroon, M.T. (2008, June 27). *A search for flavour-related transcription factors.* Wageningen NL, CBSG progress meeting.

Haring, M.A. (2008, June 19). *A tale of fruits and flowers: the role of ODORANT1 in Petunia and Tomato.* University of Verona, Dept Science and Technology Verona, Italy, University of Verona.

Haring, M.A. (2008, October 15). *Dissecting biochemistry and regulation of benzenoid production in petunia flowers.* Koln, Germany, The 5th SOL Genome Workshop.

Haring, M.A. (2008, November 11). *Forum discussion Cisgenesis.* Den Haag, NL, Senate of Dutch Parliament.

Haring, M.A. (2008, March 15). *Forum discussion documentary "Gen zoekt boer".* De Balie, Amsterdam NL, De Balie.

Haring, M.A. (2008, June 02). *Forum discussion Genetic modification of crops.* Assen, NL, Publiek debat gemeente Assen.

Haring, M.A. & Lammerts van Bueren, E. (2008, June 20). *Genetic Modification hidden in novel breeding techniques.* Modena, Italy, Organic World Conference IFOAM.

Haring, M.A. (2008, december 10). *GGO's, waar ligt de grens in de wetenschap?* Lelystad, NL, Studiedag Centrum voor Biologisch Landbouw, Biologica.

Haring, M.A. (2008, November 20). *Hoezo GGO? Plantenveredeling en biodiversiteit in een breed perspectief.* Hogeschool Gent, Belgium, Hogeschool Gent.

Haring, M.A. (2008, November 03). *Life Science for the future.* Science Park Amsterdam NL, Common purpose seminar.

Haring, M.A., Rep, M. & Schuurink, R.C. (2008, March 13). *Mastercourse Plantentaal.* Amsterdam, NL, Course for VWO teachers.

Haring, M.A. (2008, February 11). *Pflanzliche Duftstoffe: Signale für Pflanzen und Tiere.* Bad Vilbel, Germany, Landbauschule Dottenfelderhof.

Haring, M.A. (2008, January 09). *Plant Signaling Paradigms.* Amsterdam, NL, SILS Research day.

Haring, M.A. (2008, February 29). *Verantwoord modificeren: wetenschappelijke aspecten.* Rosmalen, NL, ZLTO Ondernemersdag.

Haring, M.A. (2008, January 18). *Waarom bloemen geuren...* Lunteren, NL, NIBI docentendag.

Haring, M.A. (2008, September 18). *Wetenschappelijke aspecten van genetische modificatie van planten.* Smallingerland, NL, Publiek debat Smallingerland, NL.

Haring, M.A. (2008, December 04). *Zelfverdediging voor planten.* Amsterdam, NL, Academisch Club.

Haring, M.A. (2008, June 07). *Zelfverdediging voor planten.* Amsterdam, NL, Universiteitsdag UvA.

Munnik, T. (2008, October 22). *Lipid signalling in plant Stress and development.* Uppsala, Sweden, Faculty Symposium Swedish University of Agricultural Sciences (SLU), Uppsala BioCenter.

Munnik, T. (2008, April 16). *Phospholipid signalling events during plant-microbe interactions.* Bad Honnef, Germany, The German research council (DFG) Priority Program 'Microbial Reprogramming of Plant Cell Development'.

Munnik, T. (2008, May 14). *Phospholipid Signalling in and Cytoskeletal control.* Vranovska Ves, Moravia, Czech Republic., Annual meeting of the European Cytoskeleton Club.

Munnik, T. (2008, October 21). *Phospholipid Signalling in Plant.* Stockholm, Sweden, Depart. of Botany, Stockholm University.

Munnik, T. (2008, November 24). *Phospholipid Signalling in Plant Stress and Development.* Kolkata (Calcutta), India, International Symposium in Commemoration of 150th Birth Anniversary of Sir J.C.Bose and the Birth Centenary of Prof. S.M. Sircar: "A Journey from Plant Physiology to Plant Biology".

Munnik, T. (2008, March 10). *Phospholipid Signalling in Plant Stress and Development.* Louvain-la-Neuve, Belgium, Invited seminar Université catholique de Louvain.

Munnik, T. (2008, July 24). *Phospholipid signalling in plant stress and development.* Bordeaux, France, International Symposium on Plant Lipids (ISPL).

Munnik, T. (2008, January 07). *Phospholipid signalling in Plant Stress.* Tübingen, Germany, Invited seminar University of Tübingen.

Munnik, T. (2008, April 10). *Phospholipid-signalling events during abiotic stress.* Matera, Italy, COST Meeting.

Munnik, T. (2008, February 15). *PLD Signalling in tomato.* Prague, Czech Republic, Institute for Experimental Botany.

Munnik, T. (2008, September 08). *Visualizing osmotic stress-induced phospholipid signals.* Big Sky Resort, Big Sky, Montana, Gordon Research Conference (GRC) on Salt & Water Stress in Plants.

Schuurink, R.C. (2008, June 12). *Floral scent production by Petunia.* Nijmegen, NL, SOL fruits and flowers symposium.

Schuurink, R.C., Bleeker, P.M., Diergaarde, P.J., Ament, K., Both, M. de & Haring, M.A. (2008, October 14). *Volatile Chemical cues involved in tomato-whitefly interactions.* Cologne, Germany, The 5th Solanaceae Genome workshop.

Testerink, C. (2008, June 13). *Lipid regulation of protein kinase function in plant development and stress responses.* Gif-sur-Yvette, France, CNRS-ISV.

Testerink, C. (2008, December 08). *Lipid signals direct development and stress responses in plants.* Veldhoven, NWO-CW meeting.

Vermeer, J.E.M. (2008, June 21). *Phosphoinositides in plant cell growth and membrane trafficking.* Island of Spetses, Greece, FEBS Workshop: Lipids as Regulators of Cell Function.

Other results

Schuurink, R.C. & Kant, M.R. (2008). Novel tomatoes that counteract herbivore suppression of plant defences. Prize / Grant.

Schuurink, R.C. (2008). The role of E-2-hexenal and GABA in plant stress responses. Prize / Grant.

Plant-pathogen Interactions

Chairholder: Prof. dr. B.J.C. Cornelissen

Dr. Ing. F.L.W.Takken	Assistant Professor
Dr. M. Rep	Assistant Professor

Introduction

Plant-pathogen interactions result either in colonisation of the plant or in a resistance response of the plant that prevents pathogen ingress. In the latter case one speaks of resistance, in the former of susceptibility of the plant. For in-depth research on the molecular basis of susceptibility and resistance we mainly focus on the interaction between the fungus *Fusarium oxysporum* and tomato (*Solanum esculentum*). We also study tomato resistance (R) proteins against other pathogens and for some aspects, the model plant *Arabidopsis thaliana* is included. Our specific interest is focussed on basal and induced defence mechanisms of the host and on virulence and avirulence factors of the pathogen.
Disease resistance of tomato to races of *F. oxysporum* producing avirulence factor 2 (Avr2), is mediated by the R protein I-2. Our working hypothesis is that upon recognition of a matching avirulence factor, an R protein changes conformation. This allows the R protein to form a multimeric protein complex, or activate a pre-existing protein complex, that subsequently activates a defence signalling cascade.
The ability of a pathogen to infect and colonise its host depends on 'general' pathogenicity genes as well as on specific, secreted 'effector' proteins. Secreted proteins can also be 'avirulence factors' when they are recognized in the host plant by a matching resistance gene, thereby triggering disease resistance.
Our research aims at 1) the identification and dissection of the protein complex(-es) involved in R protein mediated resistance. This work includes the functional analysis of individual complex-components and regulation of the downstream signalling components. 2) uncovering the role of pathogenicity genes and effector proteins (including avirulence factors) of *F. oxysporum* and identification of their targets in tomato.

Research Highlights

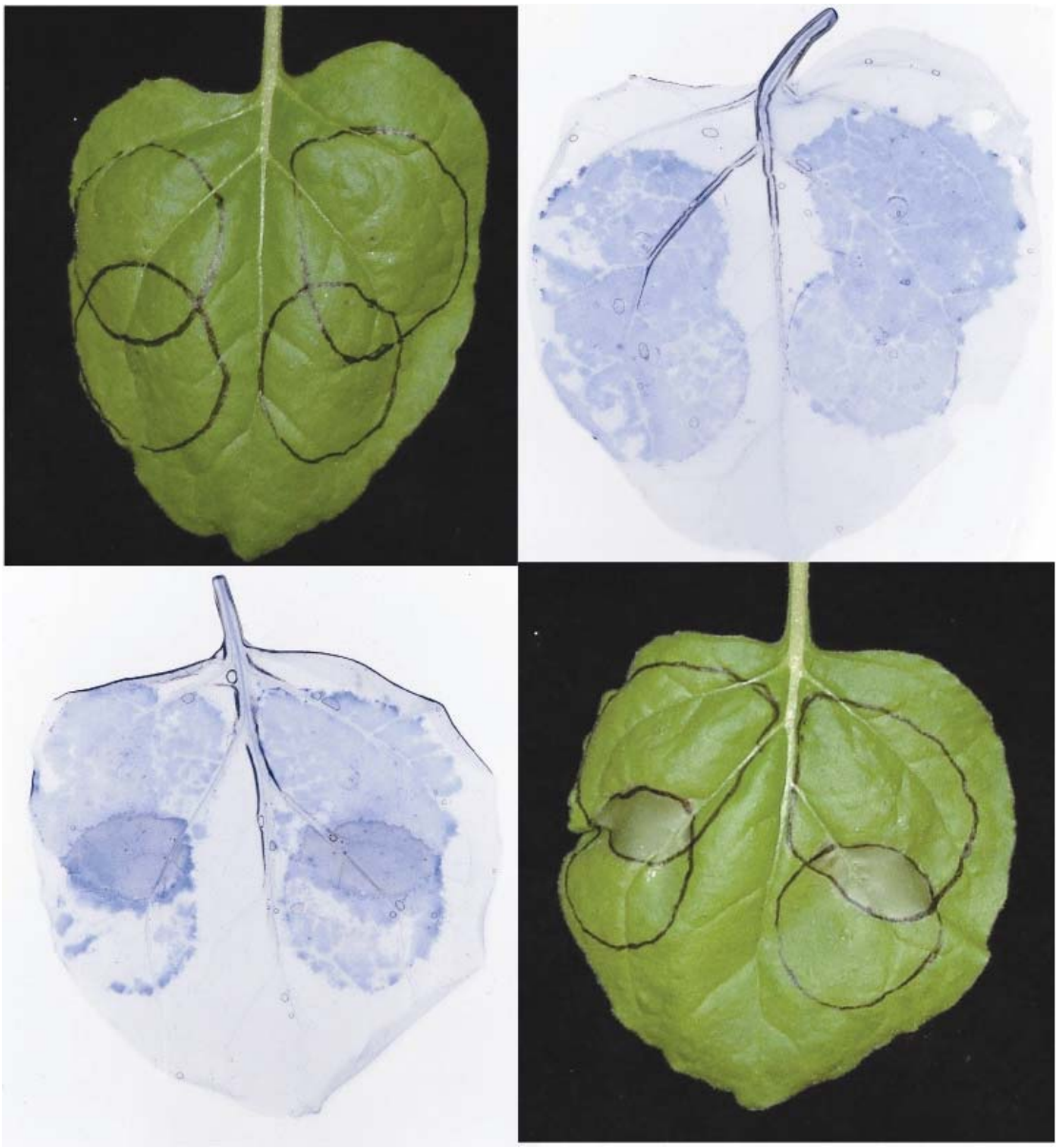
- We extended the catalogue of *F. oxysporum* proteins secreted in xylem of tomato (effectors or Six proteins) to eleven, three of which correspond to avirulence factors and at least seven of which affect virulence of the fungus and/or resistance reactions of tomato.
- The transcription factor Sge1 (formerly called 5G2) is required for the expression of at least some of the effector (*SIX*) genes as well as induction of their expression in response to exposure to living plant cells. This confirms our hypothesis that Sge1 is a master-switch for initiation of a 'pathogenicity programme' in *Fusarium oxysporum*, similar to the role of homologs of Sge1 in morphological switches in fungal pathogens of humans.
Mutation of the transcriptional repressor CreA restores pathogenicity in the absence of Frp1, confirming our suspicion that CreA and Frp1 act in opposite manners on genes required for pathogenicity, like genes for cell wall degrading enzymes.
- Most plant disease resistance (R) contain a nucleotide-binding domain, NB-ARC, which is also found in metazoan proteins Apaf-1 and CED-4. In the last year we have performed a detailed structure-function analysis of this domains in our model R proteins I-2 and Mi-1, resulting in a better understanding of the function of the various sub-domains and their conserved residues in autoinhibition and activation of these proteins.
Affinity purification using tagged Mi-1 proteins revealed *in planta* interactions with Hsp90, PP5, Sgt1 and Hsp17. Silencing of these genes was found to (partly) abolish Mi-1 and I-2 function, which appears to be caused by destabilisation of R protein accumulation.
Analysis of Arabidopsis plants in which SUMO (small ubiquitin-like modifier) isoforms were either silenced, knocked-out or over-expressed revealed that SUMO has an important function in plant development and defence.

Other Highlights

A TTI-GG (Green Genetics) program, headed by the plant biotech company KeyGene, has been awarded, allowing us to use our established experience in proteomics to explore the interaction between a plant and an insect (aphid) over the next 4 years.
Frank Takken has become subproject leader of the “resistance mechanism” project in Bioexploit, an Integrated Project supported by the European Commission through the 6th framework programme. The aim of this project is to force a break-through by developing efficient and rational breeding strategies using genomics and post-genomics tools to exploit natural host plant resistance.
Our partnership within the Centre for Biosystems Genomics has been renewed. The CBSG2012 consortium is a unique public-private partnership in plant genomics involving universities, research institutes, (inter)national companies and branch organisations active in potato, tomato and brassica research and exploitation. In 2008, CBSG2012 entered its second five year phase.

Future Prospects

The role of Sge1 in the switch of *F. oxysporum* to invasive growth will be further explored by investigating the effect of Sge1 on the secreted protein repertoire and cell wall composition by high throughput proteomics. Also, we will explore further the role of *F. oxysporum* effectors (Six proteins) in virulence of the fungus and suppression of defence in tomato.
We will also continue our nucleotide binding studies on I-2, Rx and Mi-1 to relate nucleotide binding to intra- and intermolecular interactions. Furthermore, the function of I-2 interacting proteins will be studied in relation to disease resistance mediated by *I-2, Rx* and *Mi-1* in stably silenced transgenic plants. Furthermore, we will focus on the function of Fusarium secreted proteins such as Avr2 and asses their involvement in plant disease resistance and susceptibility. Finally, in collaboration with Harrold van den Burg we will further analyse the SUMO lines to unravel the molecular basis of the observed phenotypes.



Legenda figure:
Transcomplementation assay with the tomato Mi-1.2 resistance protein. Nicotiana benthamiana leaves were agroinfiltrated to express the N-terminal (top circle) or C-terminal part of Mi-1.2 (bottom circle).

PhD Theses

Does, H.C. van der (2008, October 29). *Secrets in xylem: function, gene expression and processing of Six1, a Fusarium oxysporum effector protein encoded on a mobile pathogenicity chromosome.* UvA Universiteit van Amsterdam (170 pag.) (Amsterdam). Prom./coprom.: prof.dr. B.J.C. Cornelissen & dr. M. Rep.

Ooijen, G. van (2008, September 03). *Structure and function of tomato disease resistance proteins.* UvA Universiteit van Amsterdam (133 pag.). Prom./coprom.: prof.dr. B.J.C. Cornelissen & dr.ing. F.L.W. Takken.

Academic publications (refereed)

Burg, H.A. van den, Tsitsigiannis, D.I., Rowland, O., Lo, J., Rallapalli, G., MacLean, D., Takken, F.L.W. & Jones, J.D.G. (2008). The F-box protein ACRE189/ACIF1 regulates cell death and defense responses activated during pathogen recognition in tobacco and tomato. *Plant Cell, 20*(3), 697-719.

Does, H.C. van der, Lievens, B., Claes, L., Houterman, P.M., Cornelissen, B.J.C. & Rep, M. (2008). The presence of a virulence locus discriminates Fusarium oxysporum isolates causing tomato wilt from other isolates. *Environ. Microbiol., 10*(6), 1475-1485.

Does, H.C. van der, Duyvesteijn, R.G.E., Goltstein, P.M., Schie, C.C.N. van, Manders, E.M.M., Cornelissen, B.J.C. & Rep, M. (2008).
Expression of effector gene SIX1 of Fusarium oxysporum requires living plant cells. *Fungal Genet. Biol., 45*(9), 1257-1264.

Houterman, P.M., Cornelissen, B.J.C. & Rep, M. (2008). Suppression of plant resistance gene-based immunity by a fungal effector. *PLOS PATHOG, 4*(5), e1000061.

Kant, M.R., Sabelis, M.W., Haring, M.A. & Schuurink, R.C. (2008). Intraspecific variation in a generalist herbivore accounts for differential induction and impact of host plant defences. *Proc. R. Soc. Lond. Ser. B-Biol. Sci., 275*(1633), 443-452.

Lievens, B., Rep, M. & Thomma, B. (2008). Recent developments in the molecular discrimination of formae speciales of Fusarium oxysporum. (review). *Pest Manag Sci, 64*, 781-788.

Ooijen, G. van, Mayr, G., Kasiem, M.M.A., Albrecht, M., Cornelissen, B.J.C. & Takken, F.L.W. (2008). Structure–function analysis of the NB-ARC domain of plant disease resistance proteins. *Journal of Experimental Botany, 59*(6), 1383-1397.

Ooijen, G. van, Mayr, G., Albrecht, M., Cornelissen, B.J.C. & Takken, F.L.W. (2008). Transcomplementation, but not physical association of the CC-NB-ARC and LRR domains of tomato R protein Mi-1.2 is altered by mutations in the ARC2 subdomain. *Molecular Plant, 1*(3), 401-410.

Tameling, W.I.L. & Takken, F.L.W. (2008). Resistance proteins: scouts of the plant innate immune system. *European Journal of Plant Pathology, 121*(3), 243-255.

Book chapter

Tameling, W.I.L. & Takken, F.L.W. (2008). Sustainable disease management in a european context. In D.B. Colling, L. Munk & B.M. Cooke (Eds.), *Sustainable disease management in a european context* (D.B. Colling, L. Munk en B.M. Cooke) (pp. 243-256). Springer.

Invited lectures

Lukasik, E. (2008, June 18). *Inter- and intra-molecular interactions in R proteins.* Wageningen, the Netherlands, EPS/Bioexploit Summerschool “On the evolution of plant-pathogen interactions: from principles to practices.

Rep, M. (2008, May 03). *Fusarium wilt diseases - building a molecular picture of pathogen virulence and host resistance.* Canberra (group Jeff Ellis) and Brisbane (group John Manners), Visiting McMaster fellow in Australia.

Rep, M. (2008, September 22). *Molecular arms race between a xylem colonizing fungus and its plant host.* Cologne, Germany, International Symposium of the SFB 670 on Structure, Function and Evolution of Innate Immunity.

Rep, M. (2008, January 25). *The evolution of host- and cultivar-specific virulence in Fusarium oxysporum.* Science Faculty of the University of Cordoba, Spain, Seminar series.

Takken, F.L.W. (2008, February 10). *Function of the Nucleotide Binding Domain for R Proteins.* Resort in Keystone, Colorado, USA, Keystone Symposium on Plant Innate Immunity.

Takken, F.L.W. (2008, May 01). *Molecular aspects of I-2 mediated resistance to Fusarium oxysporum.* Koln, Germany, Max-Planck-Institut für Züchtungsforschung.

Takken, F.L.W. (2008, September 09). *Resistance proteins: scouts of the plant innate immune system.* Valencia, Spain, 18th EUCARPIA congress, "Modern Variety Breeding for Present and Future Needs.

Takken, F.L.W. (2008, June 18). *Resistance proteins: scouts of the plant innate immune system.* Wageningen, the Netherlands, EPS/Bioexploit Summerschool “On the evolution of plant-pathogen interactions: from principles to practices.

Takken, F.L.W. (2008, June 25). *Resistance proteins: scouts of the plant innate immune system.* Presqu'ile de Giens (Côte d'Azur), France, 4th EPSO Conference.

Takken, F.L.W. (2008, September 22). *Structure-function analysis of plant NB-LRR disease resistance proteins at the SFB 670.* Max-Planck-Institut für Züchtungsforschung Koln, Germany, Structure Function and Evolution of Innate immunity.

Research Cluster

SILS – Center for NeuroScience

The human brain might well be the most complex control system on earth. It consists of billions of nerve cells that are connected to each other in circuits of dazzling complexity. Moreover each individual nerve cell is capable of processing information that it receives from thousands of companions, finally resulting in a very complex and precisely fine-tuned response pattern. At the Swammerdam Institute for Life Sciences the brain is studied at the level of the molecule, the cell, the network and the organism. Although still many questions are unanswered about the structure of the nerve cell, and the way transmission of signals takes place, the real challenge is to bridge the gap between the various levels of integration. In particular, real breakthroughs are expected at the level of networks. How are they organized, and how can they be influenced? Various electrophysiological techniques help the researchers of the institute to answer these questions by allowing them to observe individual nerve cells and groups of nerve cells in specific parts of the brains. Such techniques will provide insight in processes taking place during learning, but also in diseases like epilepsy and in responses to stress.

Three chairs contribute to the research cluster 'SILS Center for NeuroScience': 'Animal Physiology and Cognitive Neuroscience', 'Cellular and Systems Neurobiology' and 'Hormonal Regulation of Signal Transduction in the Brain'.

Animal Physiology and Cognitive Neuroscience

Chairholder: Prof. dr. C.M.A. Pennartz

Dr. W.E.J.M. Ghijsen	Assistant Professor
Dr. F.P. Battaglia	Assistant Professor
Dr. S.M. Daselaar	Assistant Professor

Introduction

The group's global research aim is to elucidate how neuronal networks distributed across the prefrontal cortex, sensory neocortex, hippocampus and ventral striatum, cooperate in a number of cognitive processes, including learning and memory consolidation, attention and multisensory integration. This aim is pursued using a variety of techniques and at various aggregate levels, ranging from the subcellular to systems and behavioral levels. Most of the research focuses on the level of systems physiology. General research topics include: **The consolidation of memorized information of recent experiences. A very promising candidate mechanism for mediating this process is spontaneous "off-line" reactivation of stored information. After an initial experience which is marked by highly specific firing patterns in brain structures involved in memory, a replay of these firing patterns can be observed, with preservation of temporally specific features such as the order in which brain cells fire. In particular, we pursue the relevance of this phenomenon for memory consolidation, and how the replay is being orchestrated amongst different brain areas, such as the hippocampus and nucleus accumbens. Technically, this project is carried out in animals by performing ensemble recordings using ´tetrode arrays´, and in humans by fMRI (functional magnetic resonance imaging) and TMS (transcranial magnetic stimulation) techniques. In addition, fMRI and TMS are used to study how brain systems interact during

encoding, storage and retrieval of information. Translational research from animal to humans (and back) is considered a general aim of the group.
**We are also studying memory consolidation problem from theoretical and computational viewpoints. We are developing new computational models of memory consolidation and the formation of semantic memories, by making use of concepts from computational linguistics and Bayesian inference.
**Population coding of motivationally driven changes in the valuation of sensory cues and contexts, and in attention. In two electrophysiological projects, we study how neuronal groups in a frontal brain structure engage in the formation of representations of reward, and how networks of cells collectively learn to generate predictions about upcoming rewards, based on sensory cues that precede reward delivery. We investigate which neurotransmitters and receptors influence the formation of neural representations of reward predictions. In addition, neural correlates of attention and flexible shifting of attention are studied with ensemble recording techniques.
**An important goal in studying the motivational basis of action selection is to understand how the output of the ventral striatum to motor structures is constructed and regulated at the mechanistic level. The formation of striatal output involves the release of amino acid and neuropeptide transmitters. Using brain slices of the striatum and patch-clamp techniques we investigate how variation in presynaptic stimulation patterns leads to differentiation in release between amino acids and neuropeptides, and how these processes affect striatal output. Subcellular mechanisms will be investigated by measuring effects of presynaptic neuropeptide receptor activation/inactivation on fast amino acid release in purified nerve terminals.
**The investigation of interrelationships between genes, learning and memory capacities as measured in behavior, and the systems physiology which forms the interface between gene expression and overt behavior. These interrelationships are being studied in the context of instrumental conditioning (learning to perform goal-directed, voluntary actions), spatial navigation and conditioned place preference in genetically varying, recombinant mouse strains and targeted knockout mice, e.g. regionally restricted NMDA receptor deletions in hippocampus. This research line has been supplemented with clinically relevant mouse models, e.g. of mental retardation (FMR-1 mice).
**An important research aim is to investigate how neural assemblies in the brain cooperate to generate conscious or unconscious multi-sensory representations, and how sensory inputs from different modalities are combined to achieve such integrated representations. This goal is being pursued by 2-photon-imaging, fMRI, ensemble recording techniques and neurocomputational approaches.

Research Highlights

- Joint ensemble recordings have been made from two brain structures simultaneously. These recordings are being made in a study on replay, which can be observed when rats go to sleep and rest after an intensive period of reward-seeking behavior. We found that two connected brain structures, the hippocampus and the nucleus accumbens, reactivate coherently (i.e., together in time) during off-line processing. Our most fundamental and novel discovery here is that the Hippocampus “leads” over the striatum in the replay of behavioral information during deep sleep.
- In examining the neural basis of reinforcement learning and attention switching, we found that the rat orbitofrontal cortex encodes information about the reward probability and reward magnitude an animal expects after having perceived an olfactory cue associated with the reward. We discovered oscillatory activity in the theta-range (4-12 Hz) to which spikes are phase-locked, a phenomenon that directly correlates to Reward expectancy. Finally, we studied the dynamics of medial prefrontal ensemble firing patterns when rats are exposed to attentional distracters and engage in attentional switching.
- In a post-doc project we investigate the role of opioid neuropeptides and the “pain/capsaicin” receptor TRPV1 in synaptic communication inside the nucleus accumbens. In dual whole-cell patch clamp electrophysiology, we regularly found connected pairs of medium-sized spiny neurons and interneurons. Opioids are important

substances not only regulating normal information trafficking inside the nucleus, but have been implicated in drug addiction; a novel route towards therapy is to investigate the possible modulatory role of pain/capsaicin receptors in opioid transmission.

- We carried out an extensive set of experiments involving tetrode ensemble recordings in the hippocampus of control and NMDA knockout mice, in a series of tasks, involving food search in a star-shape maze and running in a circular task, with the purpose of analyzing the activity of hippocampal place cells when the NMDA receptor, crucial for synaptic plasticity, is functionally impaired. A first series of recordings was successfully conducted in mice with hippocampal NMDA-receptor deletions. This study sheds new light on the role of these receptors in the formation of spatial representations and in the dynamics of hippocampal firing and plasticity. Two additional mutant mouse lines are being recorded.
- We finished data analysis of two new fMRI experiments. The first experiment investigated the effects of retrieval delays on memory-related brain activation. The rationale for this study was that successful retrieval decisions are less demanding than unsuccessful decisions. Results showed three different delay patterns within regions associated with successful long-term memory retrieval. The first set of regions showed decreasing activity with increasing delays, the second set showed increasing activity, and the third set showed a V-shaped pattern, decreased for short delays but increased for long delays. These findings shed more light on the neural correlates of long-term memory and on the roles of different retrieval regions. The second study examined the overlap between in brain region supporting memory retrieval and those supporting mental imagery. Results showed that many of the brain regions activated during memory retrieval are also activated during mental imagery. These findings indicate that these regions are not involved in primary memory processes involved in activating memory traces, but rather support secondary processes that are involved in constructing mental representations of events following trace activation.
- We defined and studied a computational model of memory consolidation, inspired by concepts from computational linguistics, in which semantic memories are considered as neural traces that encode relationships between items, expressed in tree-like graphs, and in which offline reactivation of recent memories acts as a trigger of Monte-Carlo-like training of the correspondent generative model. Simulations show it can reproduce several important features of memory systems, including consolidation and generalization across different contexts. We also modelled the development of neural networks in the entorhinal cortex of rats, producing the "grid-cells" phenomenon: place cells activating a multiple places in an environment, neatly arranged in a triangular grid.
- A 2-photon imaging setup was used to visualize the spatially ordered structure of neuronal population activity in the living mouse brain. We are examining the effects of appetitive conditioning on visual processing in area V1 by pairing moving grid patterns with different outcomes (reward or no reward).

Other Highlights

1. A VENI grant on the neuroscience of decision-making was awarded to Dr. Tobias Kalenscher on behalf of ALW (Earth & Life Sciences division of NWO).

2. On Nov 26th the paper by Huijbers et al., entitled: "When learning and remembering compete: a functional MRI study" was accepted in PLoS Biology, a high-impact journal with broad coverage of the Biological Sciences. This paper received much attention from both the national and international media.

3. A paper by Lansink, Goltstein, Lankelma, Joosten, McNaughton and Pennartz was published in J. Neuroscience, and received a highly positive review in this Journal. This paper also received a lot of attention from the national media.

4. Cyriel Pennartz was appointed Associate Editor for the European Journal of Neuroscience, which has broad and world-wide coverage of the Neurosciences.

5. Application was filed for a patent on a mouse microdrive for multi-electrode recordings. This “Lantern” is an ultra-light microdrive for multi-tetrode recording in freely moving mice and other small animals.

Future Prospects

- We aim to disrupt off-line learning and memory consolidation by transcranial magnetic stimulation in humans, and by electrical intervention of hippocampal processing in rats.
- The role of neuropeptides and the vanilloid receptor (TRPV1) in intra-striatal synaptic communication will be further investigated in relation to drug addiction, using cocaine-induced behavioral sensitization..
- The *in* vivo 2-photon imaging technique, combined with bulk labelling of neurons with Calcium-indicator dyes *in* vivo, will permit us to study multimodal interactions in the population dynamics of sensory neurons in the rat neocortex
- Furthermore, the role of Orbitofrontal and Ventral striatal gamma oscillations in mediating reward-expectancy coding and odor discrimination will be elucidated.
- We aim to make further ensemble recordings from mutant mouse brains, yielding indications about the neural mechanisms of spatial memory, self-localization, short- and long-term consolidation. Recordings from several genetically modified mouse lines will be initiated (e.g. mental retardation model, FMR-1 gene).
- We aim to complete the ensemble recordings from mice with hippocampal NMDA-receptor deletions performing for the star-maze task. This project will also be the test-bed for the development of a wireless electrophysiology recording system, funded by a STW grant. New mutant mouse lines will be recorded to probe the molecular mechanisms governing hippocampal memory consolidation processes and spatial representation.
- We plan a new series of experiments investigating the interaction between the hippocampus and prefrontal cortex during sleep, by using Local Field Potential and Current Source Density Analysis methods.
- We are currently following up on our recent fMRI experiments using transcranial magnetic stimulation, which allows us to temporarily disrupt the brain regions that were active in the fMRI experiments. We are also examining the effects of physiological variables, such as respiration and heart rat, on the fMRI signal and their relation with cognitive performance.
- The question of how neural assemblies in the brain cooperate to generate multi-sensory representations will be pursued using ensemble recording techniques applied to several neocortical and hippocampal recording areas simultaneously.
- Novel experiments will target how brain systems (in particular, the hippocampus) represent data on external agents relative to the representation of the organism´s own state. We will also study how information from different sensory modalities is being integrated along the sensory neocortical-to-hippocampal hierarchy.

Key Publications

Lansink, C.S., Goltstein, P., Lankelma, J., Joosten, R.J.N.M.A., McNaughton, B.L. and Pennartz, C.M.A. (2008). Preferential reactivation of motivationally valuable information in the ventral striatum. *J. Neurosci.* 28: 6372-6382.

Van Duuren E., Lankelma J. and Pennartz C.M.A. (2008). Population coding of reward magnitude in the orbitofrontal cortex of the rat. *J. Neurosci.* 28: 8591-8604.

Huijbers W., Pennartz C.M.A., Cabeza, R. and Daselaar, S.M. (2008). When remembering competes with learning. *PLoS Biology* 7: e1000011.

PhD Theses

Lansink, C.S. (2008, October 28).
The ventral striatum in goal-directed behavior and sleep: intrinsic network dynamics, motivational information and relation with the hippocampus. Universiteit van Amsterdam (191 pag.) Prom./coprom.: prof.dr. C.M.A. Pennartz.

Duuren, E. van (2008, September 16).
Neural representation of reward information: coding by single cells and populations in rat orbitofrontal cortex. Universiteit van Amsterdam (171 pag.) Prom./coprom.: prof.dr. C.M.A. Pennartz.

Topala, C.N. (2008, August 21).
Current insights into the physiology of the epithelial calcium and magnesium channels. Radboud Universiteit Nijmegen (184 pag.). Prom./coprom.: prof.dr. R.J.M. Bindels & dr. J.G.J. Hoenderop.

Academic publications (refereed)

Aronica, E., Boer, K., Becker, A., Redeker, S., Rijen, P.C. van, Wittink, F., Breit, T., Spliet, W.G.M., Wadman, W.J., Lopes da Silva, F.H., Troost, D. & Gorter, J.A. (2008). Gene expression profile analysis of epilepsy-associated gangliogliomas. *Neuroscience, 151*(1), 272-292.

Battaglia, F.P., Kalenscher, T., Cabral, H., Winkel, J., Bos, J., Manuputy, R., Lieshout, T., Pinkse, F., Beukers, H. & Pennartz, C.M.A. (2008). The Lantern: An ultra-light micro-drive for multi-tetrode recordings in mice and other small animals. *J. Neurosci. Methods*.

Daselaar, S.M., Rice, H.J, Greenberg, D.L., Cabeza, R., LaBar, K.S. & Rudin, D.C. (2008). The spatiotemporal dynamics of autobiographical memory: Neural correlates of recall, emotional intensity, and reliving. *CEREB CORTEX, 18*(1), 217-229.

Davis, S.W., Dennis, N.A., Daselaar, S.M., Fleck, M.S. & Cabeza, R. (2008). Que PASA? The Posterior Anterior Shift in Aging. *CEREBRAL CORTEX, 18*(5), 1201-1209.

Duuren, E. van, Lankelma, J. & Pennartz, C.M.A. (2008). Population coding of reward magnitude in the orbitofrontal cortex of the rat. *J. neurosci., 28*(34), 8590-8603.

Heimel, J.A., Hermans, J.M., Sommeijer, J.-P., Brussaard, A.B., Borst, J.G., Elgersma, Y., Galjart, N., Horst, G.T. van der, Levelt, C.N., Pennartz, C.M., Smit, A.B., Spruijt, B.M., Verhage, M. & Zeeuw, C.I. de (2008). Genetic control of experience-dependent plasticity in the visual cortex. *GENES BRAIN BEHAV, 7*(8), 915-923.

Ito, R., Robbins, T.W., Pennartz, C.M. & Everitt, B.J. (2008). Functional interaction between the hippocampus and nucleus accumbens shell is necessary for the acquisition of appetitive spatial context conditioning. *J. Neurosci., 28*(27), 6950-6959.

Kalenscher, T. & Pennartz, C.M.A. (2008). Is a bird in the hand worth two in the future? The neuroeconomics of intertemporal decision-making. *Progress in Neurobiology, 84*(3), 284-315.

Kretzschmar, C., Kalenscher, T., Güntürkün, O. & Kaernbach, C. (2008). Echoic memory in pigeons. *BEHAV PROCESS, 79*(2), 105-110.

Lansink, C.S., Goltstein, P.M., Lankelma, J.V., Joosten, R.N.J.M.A., McNaughton, B.L. & Pennartz, C.M.A. (2008). Preferential reactivation of motivationally relevant information in the ventral striatum. *J. Neurosci., 28*(25), 6372-6382.

Lopes da Silva, F.H. (2008). The impact of EEG/MEG signal processing and modeling in the diagnostic and management of epilepsy. *IEEE Reviews in Biomedical Engineering, 1*, 143-156.

Munck, J.C. de, Goncalves, S.I., Faes, T.J.C., Kuijer, J.P.A., Pouwels, P.J.W., Heethaar, R.M. & Lopes da Silva, F.H. (2008). A study of the brain's resting state based on alpha band power, heart rate and fMRI. *NEUROIMAGE, 42*(1), 112-121.

Nordquist, R.E., Vanderschuren, L.J.M.J., Jonker, A.J., Bergsma, M., Vries de, T.J., Pennartz, C.M.A. & Voorn, P. (2008). Expression of amphetamine sensitization is associated with recruitment of a reactive neuronal population in the nucleus accumbens core. *PSYCHOPHARMACOLOGY, 198*(1), 113-126.

Pfurtscheller, G., Scherer, R., Müller-Putz, G.R. & Lopes da Silva, F.H. (2008). Short-lived brain state after cued motor imagery in naive subjects. *Eur. J. Neurosci., 28*(7), 1419-1426.

Tobler, P.N., Kalis, A. & Kalenscher, T. (2008). The role of moral utility in decision making: An interdisciplinary framework. *Cognitive, Affective & Behavioral Neuroscience, 8*(4), 390-401.

Kalenscher, T. & Tobler, P.N. (2008). Introduction. *Cognitive, Affective & Behavioral Neuroscience, 8*(4), 345-347.

Book Chapters

Battaglia, F.P., Peyrache, A., Khamassi, M. & Wiener, S.I. (2008). Spatial decisions and neuronal activity in hippocampal projection zones in prefrontal cortex and striatum. In S.J.Y. Mizumori (Ed.), *Hippocampal place fields: Relevance to learning and memory* (pp. 289-309). Oxford: Oxford University Press.

Kalenscher, T. & Tobler, P.N. (2008). Comparing Risky and Inter-Temporal Decisions; Views from Psychology, Ecology and Microeconomics. In K.P. Hoffmann (Ed.), *Psychology of Decision Making in Economics, Business and Finance* (pp. 111-135). New York: Nova Science Publisher.

Invited lectures

Battaglia, F.P. (2008, June 05). *Dynamics of hippocampal code.* Spitsbergen, Noorwegen, Fridtjof Nansen Conference on Neural Networks and Behaviour.

Battaglia, F.P. (2008, April 01). *Hippocampal physiology, and the place cells system.* Oeiras, Portugal, School on "Hippocampus and Navigation" Instituto Gulbenkian de Ciencia Oeiras.

Battaglia, F.P. (2008, May 16). *Network Synchronization: from dynamical systems to neuroscience.* Leiden University.

Daselaar, S.M. (2008, April 24). *Building memories off-line: the relation between reactivation and memory consolidation.* 2008 Funcomeet, Rudolf Magnus Institute for Neuroscience, Utrecht University Medical Center.

Daselaar, S.M. (2008, February 28). *Building memories off-line: the relation between reactivation and memory consolidation.* Amsterdam, CSCA Symposium.

Daselaar, S.M. (2008, February 01). *Conscious resting state activity and human memory consolidation.* Leiden, Onderzoeksschool EPOS, Leiden University.

Daselaar, S.M. (2008, December 10). *Effects of Healthy Aging on hippocampal and rhinal memory functions.* Saarbrucken, Germany, Institutskolloquium der Fachrichtung Psychologie.

Daselaar, S.M. (2008, June 12). *Role of the default mode network in learning and remembering.* Leiden, Symposium, Colloquia Main Series, Leiden Institute for Brain and Cognition.

Kalenscher, T. (2008, October 14). *Intransitive preferences in the brain.* Magdeburg, Duitsland, Invited by Dr. Michael Brosch at the Leibniz Institute for Neurobiology in Magdeburg.

Kalenscher, T. (2008, July 16). *Intransitive preferences in the brain.* Vermont USA, 'Attention & Performance 13' Meeting in Vermont.

Pennartz, C.M.A. (2008, May 15). *Memory and the brain.* Rotterdam, Dutch Science Alliance for integration Scientific research with higher educational institutions in the Netherlands.

Pennartz, C.M.A. (2008, May 26). *Memory processes in the brain – neural networks caught in the act.* Utrecht, Annual Symposium of the Utrecht Society of Students in Biology.

Pennartz, C.M.A. (2008, October 02). *Memory, emotion and sleep.* Utrecht, Public Brain Awareness Day, Dutch Brain Foundation.

Pennartz, C.M.A. (2008, October 23). *Memory, synaptic plasticity and sleep.* Rotterdam, Higher education for the elderly Course, Erasmus Medical Center.

Pennartz, C.M.A. (2008, March 05). *Neural coding and information storage in systems for emotion & memory.* Beerse, Belgium, Neuroscience division of Johnson & Johnson.

Pennartz, C.M.A. (2008, December 01). *Population coding of reward information in the rat orbitofrontal cortex.* Leiden, Marius Tausk Symposium, University of Leiden.

Pennartz, C.M.A. (2008, May 30). *Population coding of reward information.* Amsterdam, International Symposium organized by the Dutch Royal Academy of Sciences, Amsterdam.

Pennartz, C.M.A. (2008, November 01). *Reward prediction and reward memory in the mesolimbic system: cognitive neurophysiology in relation to robotics and machine learning.* Barcelona, Spain, Summer School at University Pompeu Fabra.

Pennartz, C.M.A. (2008, July 14). *Sleep, off-line reactivation and memory consolidation.* Geneva, International Symposium at the FENS forum meeting in Geneva, Switzerland (Federation of European Neuroscience Societies).

Cellular and Systems Neurobiology

Chairholder: Prof.dr W.J. Wadman

Dr. J. A. van Hooft	Assistant Professor
Dr. J. A. Gorter	Assistant Professor
Dr. T. R. Werkman	Assistant Professor
Dr. N. L. M. Cappaert	Assistant Professor

Introduction

Excitability is still the most prominent property of the nervous system. How ion-channels are organized and quantitatively balanced in the neuronal membrane, how they lead to neuron specific firing patterns and how these can be modulated at different times scales (plasticity) belong to the most exciting problems in neuroscience that can now be solved in a multidisciplinary approach. Neurons communicate with each other through a variety of synapses. To provide minimal functionality neurons need to be combined in small circuits. We have organized our research around a few well defined topics in the realm of neuronal excitability. Our core approach is functional electrophysiological one (from patch-clamping to *in vivo*). State-of-the-art optical techniques (Ca-imaging, Voltage Sensitive Dyes) and various multi-contact electrode recordings allow the analysis of population activity. When needed, collaborations provide anatomical, immuno histochemical, molecular, genetic and behavioural expertise.

The first of our three major research lines studies the fundamental properties of the 5-HT3 receptor and tries to understand its functional role in local circuits and development. Molecular techniques produced mice in which the 5-HT3 receptor expressing neurons are labelled with GFP and can be studied efficiently. This has opened a wide range of possibilities to investigate the role of this receptor in functionally connected neurons and also its highly specific role in cortical column formation.

The second research line studies epilepsy e.g. seizure generation, epileptogenesis (micro-array technology) and pharmacoresistance. The latter topic we approach from two sides: a) (non-)penetration of druge sia the blood-brain-barrier and b) modification of drug targets, mainly sodium channels. These studies are of high clinical relevance and we strengthen them through a side appointment at the Academic Hospital in Ghent and intense collaboration with the epilepsy center in Heemstede (SEIN). The therapeutic potential of deep brain stimulation is investigated in patients and in animal models.

The third research line concentrates on specific pharmacological modulation of neuronal circuits. A new line that focuses on the role of the endocannabinoids system has been started. We support the activities of a spin-off company Sensocom.

Most of our experiments are supported by computer modeling, focusing on single cell excitability in relation to the direct chemical surrounding of the neuron as well as on the adaptive strategies for excitability that optimize the working range of active neurons. In the latter case we try to extrapolate the consequences of single cell strategies to larger neuronal networks. The combination of theoretical and experimental work has proven to be very fruitful in the scientific setting of SILS and the FNWI.

Research Highlights

Within the research line on epilepsy projects, both dealing with potential sources of pharmaco resistance came to a successful finish this year. The long standing study that concentrated on the role of the sodium channel in epilepsy and in pharmacoresistance has completed a large study where the responses of different sodium channel subunit types to the standard collection of anti-epileptic drugs was investigated with state-of-the-art patch clamp techniques. We observed considerable differences that open possibilities for therapeutic strategies. A new project subsidised by NEF has started to this aim. In a second project the role of the blood brain barrier, which under normal conditions forms an almost impassable barrier to the brain was investigated. Special proteins remove unwanted foreign objects that leak through the barrier and a lot of pharmaceuticals share this fate. However, in particularly during epilepsy, large leakage of the BBB may occur in particular during and after seizures. On the other hand such events also up regulate the protein with the barrier function. Erwin van Vliet carefully investigated this delicate balance and also manipulated the proteins involved in order to understand their role. The challenge was also to find differences in transport into the brain for classical and new anti-epileptic drugs, which has potential therapeutic value. After successful defence of this thesis Erwin continued as a post-doc on this project.

The studies on homeostatic scaling of brain excitability were restarted with three new project lines: one that aims at revealing the molecular mechanisms behind the increase/decrease of HCN expression (in collaboration with prof. Tallie Baram in Irvine, CA) funded by NEF, one that aims at theoretical understanding on how such a mechanism can be incorporated in large networks without serious negative consequences for stability, learning and memory in such networks and one project that aims at applying this knowledge in therapeutic strategies in particular involving deep brain stimulation. The link with the clinic in Ghent proved to be very useful in this sense.

The use of Voltage Sensitive Dyes for parallel recording from large numbers of sites in (neuronal slice) networks has finally surpassed the level of technical developments and a first study that proves its use as a technique to understand functional connectivity between larger brain structures was published. We predict that this technique will be incorporated in many other research projects as it is the best way investigate synchronization in large neuronal populations (epilepsy) as pilots show that it is quite possible to identify the cortical columnar organization (5-HT3 project) and as it might be the best way to determine the spatial extend of functional stimulation.

Finally the series of studies that used modelling, mainly at the cellular level, to understand the generation of epileptic seizures, spreading depression and the role of ion homeostasis in these events, was completed and finished with theses defences. In a new project on computational modelling we will investigate how the cellular knowledge can best be expanded to understand network behaviour.

Other Highlights

Yaov Noam received the Unilever research prize for this experimental work.
Taco Werkman acquired and started a large research grant in the context of the Top Institute Pharma and in collaboration with Solvay Pharmaceuticals.
Hans van Hooft acquired an ALW grant to further investigate the role of the 5-HT3 receptor in columnar cortical development.
Jan Gorter acquired a NEF grant to continue the studies on the role of inflammation in epileptogenesis.

Future Prospects
The almost complete renewal of the AIO crew in our group has lead to a considerable redefinition of the project lines, in light of current international developments. As all lines were very successful in acquiring external funding there was no reason to limit our efforts; the refocus on basis mechanisms of phenomena with strong clinical relevance (epileptogenesis, pharmaco resistance Deep Brain Stimulation, Cortical development,

Cannabinoid modulation) will therefore be continued with fresh spirits. The fact that we are strongly supported by at least three industrial partners underscores the societal relevance of the questions we are dealing with.

Key Publications

Somjen GG, Kager H, Wadman WJ., Computer simulations of neuron-glia interactions mediated by ion flux. J Comput Neurosci, 2008, 25(2):349-65.

PhD Theses

Keller, A.M. (2008, May 23). T*he role of CD70 / CD27 interactions at the dendritic cell / T cell interface.* Universiteit van Amsterdam. Prom./coprom.: prof.dr. J. Borst.

Langerak, P. (2008, February 01). *DNA damage tolerance: from error free UV-damage bypass to mutagenesis of immunoglobulin genes.* Universiteit van Amsterdam. Prom./coprom.: prof.dr. J. Borst & dr. H. Jacobs.

Academic publicatios (refereed)

Aronica, E., Boer, K., Becker, A., Redeker, S., Rijen, P.C. van, Wittink, F., Breit, T., Spliet, W.G.M., Wadman, W.J., Lopes da Silva, F.H., Troost, D. & Gorter, J.A. (2008). Gene expression profile analysis of epilepsy-associated gangliogliomas. *Neuroscience, 151*(1), 272-292.

Boer, K., Troost, D., Spliet, W.G.M., Rijen, P.C. van, Gorter, J.A. & Aronica, E. (2008). Cellular distribution of vascular endothelial growth factor A (VEGFA) and B (VEGFB) and VEGF receptors 1 and 2 in focal cortical dysplasia type IIB. *Acta Neuropathol., 115*(6), 683-696.

Boer, K., Troost, D., Timmermans, W., Gorter, J.A., Spliet, W.G.M., Nellist, M., Jansen, F. & Aronica, E. (2008). Cellular localization of metabotropic glutamate receptors in cortical tubers and subependymal giant cell tumors of tuberous sclerosis complex. *Neuroscience, 156*(1), 203-215.

Inta, D., Alfonso, J., Engelhardt, J. von, Kreuzberg, M.M., Meyer, A.H., Hooft, J.A. van & Monyer, H. (2008). Neurogenesis and widespread forebrain migration of distinct GABAergic neurons from the postnatal subventricular zone. *PNAS, 105*(52), 20994-20999.

Noam, Y., Wadman, W.J. & Hooft, J.A. van (2008). On the voltage-dependent Ca2+ block of serotonin 5-HT3 receptors: A critical role of intracellular phosphates. *The Journal of Physiology, 586*(15), 3629-3638.

Somjen, G.G., Kager, J. & Wadman, W.J. (2008). Calcium sensitive non-selective cation current promotes seizure-like discharges and spreading depression in a model neuron. *J. Comput. Neurosci.*.

Somjen, G.G., Kager, H. & Wadman, W.J. (2008). Computer simulations of neuron-glia interactions mediated by ion flux. *J. Comput. Neurosci., 25*(2), 349-365.

Vliet, E.A. van, Schaik, R. van, Edelbroek, P.M., Lopes da Silva, F.H., Wadman, W.J. & Gorter, J.A. (2008). Development of tolerance to levetiracetam in rats with chronic epilepsy. *Epilepsia, 49*(7), 1151-1159.

Hormonal Regulation of Signal Transduction in the Brain

Chairholder: Prof.dr M. Joëls

Dr. P.J. Lucassen	Associate Professor
Dr. H. Krugers	Assistant Professor
Dr. H. Karst	Researcher

Introduction

The main aim of our research is to delineate how stress hormones affect the function of rodent brain cells and how this can explain behavioural adaptation. The emphasis of our research is on the cellular and network processes that are altered by stress hormones like corticosterone, in interaction with other stress-released modulators such as noradrenaline. To investigate this we use electrophysiological recording techniques, mostly in vitro. In collaboration with others this is combined with methods that aim to resolve the underlying molecular mechanism. This is highly relevant, as corticosteroid hormones act via their nuclear receptors as regulators of transcriptional activity. Recently, however, we discovered that corticosteroid hormones also change neuronal function in a rapid non-genomic manner. It is the combined rapid (non-genomic) and delayed (genomic) pathways which make corticosteroid hormones such powerful tools, changing brain function over a prolonged period of time after stress. In addition to examining neuronal cell function and the underlying molecular mechanism, we also investigate the effect of stress on the generation, morphology and turn-over of brain cells. The theories that come forward from the experimental work in animals are tested also in the human brain, in collaboration with other research labs applying neuropsychological and neuroimaging methods.

While most of the research focuses on the effect of a single exposure to stress, we are also interested in long-term consequences of stress. We particularly focus on the long-term consequences of stressful situations early in life. The latter can pertain to maternal deprivation of neonatal rat pups or natural variation of maternal care.
A separate research line concerns the role of structural plasticity and cell cycle factors in neurodegenerative diseases, like Alzheimer's disease. Here we use human postmortem brain tissue as well as experimental animal models for neurodegenerative diseases (usually genetically modified mutants) and examine changes in neurogenesis, morphology, electrical properties and behavior at various ages.

Research Highlights

In hippocampal cells corticosterone can rapidly and reversibly change the spontaneous release probability of glutamate containing vesicles. We now established that similar effects occur in other limbic areas such as the dentate gyrus and the basolateral amygdala. In the latter area, however, the effects seem more persistent, so that acute stressors may have a relatively long-lasting impact on amygdalar excitability.
Over the past year we further examined the impact of early life stress on hippocampal structure and function later in life. We demonstrated that dendritic complexity and synaptic potentiation in non-stress conditions are diminished in male offspring from low licking-grooming (LG) compared to high LG mothers, both in the CA1 region and dentate gyrus. Surprisingly, though, the synaptic plasticity phenotype reversed by rapid effects of stress hormones (-adrenoceptor agonists or corticosterone, in the dentate) but also by slow gene-mediated corticosteroid actions (in CA1). This was also reflected in learning behavior: Under mild stress conditions high LG offspring outperformed low LG animals, but under high stress conditions learning was improved in low versus high LG offspring. This suggests that early life conditions may program the brain to perform optimally under comparable conditions later in life.
At least some effects of early life stress are sex-dependent as was demonstrated in rats that were deprived from maternal care for 24 hrs at day 3 after birth. At 3 weeks of age, male rats showed higher levels of neurogenesis (compared to non-deprived rats), whereas in females a profound decrease in neurogenesis was observed.

Other Highlights

Harm Krugers received a grant from the HersenStichting Nederland and a KNAW China exchange project grant.

Paul Lucassen received financial support from the HersenStichting Nederland, from TNO, and a grant from the ISAO (PhD project). He was appointed guest professor at Wuhan University, China, and further served as a member of the editorial board of Frontiers in Neurogenesis and as member of the scientific committee of the Internationale Stichting Alzheimer Onderzoek.

Members of the group served as referee for many international journals (including top-tier journals like Science and Nature Neuroscience) and funding agencies; Marian Joëls served on the Editorial Board of Stress, Neural Plasticity, the open access journal Frontiers in Behavioral Neuroscience and was guest-editor for a special issue of the European Journal of Pharmacology. She further received support from Corcept Inc.

Marian Joëls was (re-)elected as member (vice-chairman) of the Board of the KNAW Science Division; accepted a second (3-years) term on the Board of the Division Earth and Life Sciences ALW/NWO; acted as Departmental Head Earth and Life Sciences UvA; served as chairman of the Dutch Neurofederation; and is vice-chairman of the organizing committee for the FENS Forum 2010 meeting.

Future Prospects

In the upcoming period we will further explore the functional relevance of rapid non-genomic corticosteroid effects. In rats we are now testing if the effects via membrane mineralocorticoid receptors are the only means for hippocampal neurons to functionally follow an ultradian pattern. We will also examine the influence of corticosteroid actions in the human brain. We hypothesize that elevated cortisol levels at the time of encoding promote memory processes via mineralocorticoid receptors, while elevations in hormone level taking place several hours before encoding of information hamper subsequent memory formation via glucocorticoid receptors.
With respect to early life stress we will refine the present model (in which the behavior of the mother to the entire litter is used as variable) to the single-pup level, testing the idea that the amount of care each individual pup receives predicts the ability to induce synaptic plasticity later in life, through a mechanism involving epigenetic programming. We will also follow up earlier experiments on the effect of chronic stress on neurogenesis and determine critical windows in time for successful intervention.
In the human brain, we will examine the distribution and regulation of hippocampal glucocorticoid receptors, in healthy and diseased subjects. In post-mortem Alzheimer brain, we will further examine to what extent microglia or astroglia contributes to the proliferative changes seen in relation to amyloid plaque formation.

Key Publication

Joëls M., Karst H., Rijk, R.de & Kloet, E. R.de (2008). The coming out of the brain mineralocorticoid receptor. *Trends in Neurosci.* 31: 1-7.

PhD Theses

Gemert, N.G. van (2008, April 25). *Effects of stress and corticosterone on the hippocampus: linking gene transcription to physiology.* Universiteit van Amsterdam (144 pag.) Prom./coprom.: prof.dr. M. Joëls.

Wiegert, O. (2008, March 06). *Stress and plasticity relevance of timing and AMPA receptors.* Universiteit van Amsterdam (120 pag.). Prom./coprom.: prof.dr. M. Joëls & dr. H. Krugers.

Academic publications (refereed)

Alfarez, D.N., Karst, H., Velzing, E.H., Joëls, M. & Krugers, H.J. (2008). Opposite effects of glucocorticoid receptor activation on hippocampal CA1 dendritic complexity in chronically stressed and handled animals. *Hippocampus, 18*(1), 20-28.

Boekhoorn, K., Sarabdjitsingh, A., Kommerie, H., Punder, K. de, Schouten, T., Lucassen, P.J. & Vreugdenhil, E. (2008). Doublecortin (DCX) and doublecortin-like (DCL) are differentially expressed in the early but not late stages of murine neocortical development. *The Journal of comparative neurology, 507*(4), 1639-1652.

Champagne, D.L., Bagot, R.C., Hasselt, F. van, Ramakers, G., Meany, M.J., Kloet, E.R. de, Joëls, M. & Krugers, H. (2008). Maternal care and hippocampal plasticity: evidence for experience-dependent structural plasticity, altered synaptic functioning, and differential responsiveness to glucocorticoids and stress. *J. Neurosci., 28*(23), 6037-6045.

Heuts, B. & Brunt, T. (2008). Voracious male spiders that kill adult females of their own species (genera Walckenaeria, Diplostyla, Neriene, Meta, Araneae). *Spined Nieuwsbrief Spinnenwerkgroep Nederland, 24*, 2-8.

Heuts, B.A. & Brunt, T.M. (2008). Undirectional and transitive predatory relationships of spider species in One-on-One encouters. (Arachnida:Araneae). *Nieuwsbrief Spinned*, 18-23.

Joëls, M. (2008). The concept of allostasis and allostatic load. *EUR J PHARMACOL, 7*, 173.

Joëls, M. (2008). Functional actions of corticosteroids in the hippocampus. *EUR J PHARMACOL, 583*(2-3), 312-321.

Joëls, M. (2008). Hoe stress werkt in ons lichaam. *PHAXX*, 8-9.

Joëls, M., Karst, H., DeRijk, R. & Kloet, E.R. de (2008). The coming out of the brain mineralocorticoid receptor. *Trends Neurosci., 31*(1), 1-7.

Kloet, E.R. de, Karst, H. & Joëls, M. (2008). Corticosteroid hormones in the central stress response: Quick-and-slow. *Frontiers in Neuroendocrinology, 29*(2), 268-272.

Liebmann, L., Karst, H., Sidiropoulou, K., Gemert, N. van, Meijer, O.C., Poirazi, P. & Joëls, M. (2008). Differential effects of corticosterone on the slow afterhyperpolarization in the basolateral amygdala and CA1 region: Possible role of calcium channel subunits. *J NEUROPHYSIOL, 99*(2), 958-968.

Marlatt, M.W., Lucassen, P.J., Perry, G., Smith, M.A. & Zhu, X. (2008). Alzheimer's disease: Cerebrovascular dysfunction, oxidative stress, and advanced clinical therapies. *J. Alzheimer's dis., 15*(2), 199-210.

Olijslagers, J.E., Kloet, E.R. de, Elgersma, Y., Woerden, G.M. van, Joëls, M. & Karst, H. (2008). Rapid changes in hippocampal CA1 pyramidal cell function via pre- as well as postsynaptic membrane mineralocorticoid receptors. *Eur. J. Neurosci., 27*(10), 2542-2550.

Simic, G, Mladinov, M., Jovanov-Milosevic, N., Islam, A., Pajtak, A., Sertic, J., Lucassen, P.J., Hof, P.R. & Kruslin, B. (2008). Abnormal motoneuron migration, differentiation, and axon outgrowth in spinal muscular atrophy. *ACTA NEUROPATHOL, 115*, 313-326.

Thompson, A., Boekhoorn, K., Dam, A.M. & Lucassen, P.J. (2008). Changes in adult neurogenesis in neurodegenerative diseases: Cause or consequence? *GENES BRAIN BEHAV, 7*(s1), 28-42.

Joëls, M. (2008). Preface. *European Journal of Pharmacology, 583*(2-3), 173-173.

Lucassen, P.J., Verbeek, E.C. & Oomen, C.A. (2008). Wat doen die nieuwe hersencellen daar? Neurogenese en celdood in volwassen hersenen. *Blind! Elektronisch Tijdschrift voor Interdisciplinaire Studies*.

Book chapters

Lucassen, P.J., Oomen, C., Dam, A.-M. van & Czéh, B. (2008). Regulation of hippocampal neurogenesis by systemic factors including stress, glucocorticoids, sleep, and inflammation. In F.H. Gage, G. Kempermann & H. Song (Eds.), *Adult neurogenesis* (Cold Spring Harbor monograph series, 52) (pp. 363-396). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Krugers, H. (2008). Stress en plasticiteit van de hersenen: effecten op functie en structuur. In Derix Lafosse Van der Meulen (Ed.), *Neuroplasticiteit*. Amsterdam: Boom.

Invited lectures

Joëls, M. (2008, February 20). *Corticosteroid effects in hippocampus: Importance of history and cellular context.* New Jersey, USA, seminar in Lundbeck.

Joëls, M. (2008, November 13). *Corticosteroid influences on limbic neurons, from minutes to months.* Arlington, USA, Brain Research Conference on Stress, Coping and Disease.

Joëls, M. (2008, March 03). *Corticosteroids and hippocampal function: Temporal and regional differentiation.* Bochum, Ruhr Universität, Germany.

Joëls, M. (2008, June 08). *Corticosteroids and hippocampal function: Temporal and regional differentiation.* Trier-Leiden Summer School, Leiden.

Joëls, M. (2008, February 06). *Effects of Stress on Synaptic Plasticity in Hippocampus.* Seminar at Abbott, Manheim, Germany.

Joëls, M. (2008, July 10). *Interactive noradrenergic and corticosteroid action during stressful memory formation.* 3rd Stress, Learning & Plasticity Meeting, Villars, Switserland.

Joëls, M. (2008, May 22). *Is stress altijd slecht voor je hersenen?* Amsterdam, De Alumni Bijeenkomst Universiteit van Amsterdam.

Joëls, M. (2008, December 19). *Is stress bad?* Amsterdam, API Instituut.

Joëls, M. (2008, June 07). *Is stress slecht?* Amsterdam, Annual UvA Universiteitsdag.

Joëls, M. (2008, March 11). *Mechanisms of stress effects on learning: Importance of context.* Dublin, Ierland, HFSP symposium.

Joëls, M. (2008, December 09). *Rapid and slow effects of corticosteroid hormones on limbic cell function.* Zürich, Zwitserland, ETH.

Joëls, M. (2008, February 16). *Stress, emoties en geheugen.* Amsterdam, NV&V Annual Symposium.

Joëls, M. (2008, November 20). *The paso doble of the mineralocorticoid receptor.* Utrecht, seminar at Benelux Meeting on Nuclear Receptors.

Krugers, H. (2008, March 14). *Corticosteroid modulation of AMPA receptor function.* Shanghai, China, The Institute for Neuroscience.

Krugers, H. (2008, February 06). *Corticosteroid modulation of AMPA receptor function.* Leiden, seminar at Medical Pharmacology in Leiden.

Krugers, H. (2008, July 11). *Glucocorticoid receptor activation promotes AMPA receptor trafficking and hippocampal learning.* Villars, Zwitserland, 3rd Stress, learning and Plasticity meeting.

Krugers, H. (2008, September 29). *Glucocorticoid receptor activation promotes AMPA receptor trafficking and hippocampal learning.* Leiden, Marius Tausk Symposium.

Krugers, H. (2008, October 02). *Zorg voor geheugen.* Utrecht, seminar at the Publieksdag Hersenstichting Nederland.

Lucassen, P.J. (2008, April 21). *Alzheimer Mouse models; where have we come from and are we there yet ?* Amsterdam, Netherlands Institute for Neuroscience.

Lucassen, P.J. (2008, February 29). *Can we determine neurogenesis in the human brain.* Amsterdam, Stem cell meeting at the Netherland Institute for Neuroscience.

Lucassen, P.J. (2008, June 05). *Doublecortin proteins in human brain.* Doorwerth, EndoNeuroPsycho meeting.

Lucassen, P.J. (2008, December 03). *Expression of doublecortin and doublecortin-like during early cortical development and in human brain.* Nijmegen, NCMLS, University of Nijmegen.

Lucassen, P.J. (2008, January 21). *Neurogenesis and hippocampal function in Alzheimer's disease.* Wuhan, China, Wuhan Institute for Neuroscience and Neuroengineering (WINN), South-center University for Nationalities.

Lucassen, P.J. (2008, September 01). *Neurogenesis in relation to aging and dementia.* Barcelona, Spanje, ECNP meeting.

Lucassen, P.J. (2008, April 14). *Neurogenesis in relation to dementia.* Paris, France, Bilateral Paris-Amsterdam NEURAD meeting.

Lucassen, P.J. (2008, February 22). *Neurogenesis in relation to stress, depression and dementia.* Amsterdam, Amsterdam-Groningen bilateral meeting.

Lucassen, P.J. (2008, January 22). *Neurogenesis, Stress and Depression.* Wuhan, China, Tongji Medical College of Huazhong University of Science and Technology.

Lucassen, P.J. (2008, maart 10). *Nieuwe ontwikkelingen in het hersenonderzoek naar stress en stam cellen.* Castricum, voordracht gehouden in het kader van framework of the Dutch Brain Awareness week at the Jac.P. Thijsse college.

Lucassen, P.J. (2008, March 28). *Structural hippocampal plasticity in relation to dementia.* Gottingen, Duitsland, Bilateral Amsterdam-Gottingen NEURAD meeting, Dept Psychiatry, Gottingen University.

Lucassen, P.J. (2008, September 18). *Structural plasticity during early development and in the Alzheimer brain.* Leuven, België, 2nd NEURAD Summer School.

Lucassen, P.J. (2008, November 13). *Update on Alzheimer's disease.* Washington, USA, EURON PhD Workshop on "Animal models to mimic and understand Alzheimer's disease etiology" at the Society for Neuroscience premeeting.

Lucassen, P.J. (2008, mei 21). *Voor- en nadelen van een demente muis.* Amsterdam, voordracht bij ISAO meeting at the Netherlands Institute for Neuroscience.

Lucassen, P.J. (2008, April 03). *Zit stress tussen de oren ?* Amsterdam, seminar at 'het proefstuderen'.

Lucassen, P.J. (2008, March 14). *"Effects of perinatal stress exposure on behaviour, endocrinology and adult neurogenesis in 2 rat lines that differ in stress coping style and anxiety related behaviour.* Tübingen, Germany, VW Statussymposium.

Research Cluster

Life Science Technologies

The Swammerdams' Life Science Technologies Cluster carries out fundamental biological research and makes a wide range of techniques available to the SILS researchers. Advanced microscopy enables us to bridge the gap between the level of single molecules and the level of aggregates of molecules in single/multiple cells, and to make the life processes visible in time (4D imaging). The institute works with the newest modes of advanced optical microscopy, for instance FRET-microscopy, enabling the measurement of distances between molecules in a range of one to eight nanometres in intact living cells.

Micro-array technology simultaneously analyses all genes of a particular organism, in a particular cell type or under specific growth or stress conditions or other internal or external stimuli. The Micro-Array Department (MAD), a semi commercial facility, produces micro-arrays for the spotted-array technology platform, for example for the genes of man, mouse, yeast, tomato, petunia plants, etc. and makes GeneChips from Affymetrix available. The MAD is accessible to all academic, as well as industrial research groups, and can be found on the internet at www.microarray.nl. The large stream of data, produced by micro array technology, needs to be processed and analysed by means of bioinformatics, for which a bioinformatics support group has been established.

Mass spectrometry is essential to characterize proteins and to analyze the relationship between the structure and function of a protein (proteomics). Mass spectrometry is a key technology at SILS in which we have a long history and substantial expertise. An important part of the research involves the identification, quantification and functional characterization of proteins. Bio-informatics also plays an important role in this by efficiently analysing the data streams. Bioinformatics and data analysis are essential to collect, compare and integrate all available data, and to present them to the researchers in a way that the information is summarised and visualises the underlying biological processes.

Three chairs contribute to the research cluster 'Life Science Technologies': 'Mass Spectrometry of Biomacromolecules', 'Micro Array Department and Integrative Bioinformatics Unit'and 'Biosystems Data Analysis'.

Mass Spectrometry of Biomacromolecules

Chairholder: Prof.dr. C.G. de Koster

Dr. L. de Jong	Associate Professor
Dr. L.J. de Koning	Assistant Professor

Introduction

Future progress in the life sciences will heavily depend on the integration of chemistry, physics, mathematics, (bio)informatics and biology. Our group combines mass spectrometry with biomolecular and organic chemistry. We focus on three research themes that are carried out in close collaboration with other groups within and beyond the Swammerdam Institute for Life Sciences (SILS) of the University of Amsterdam, i.e., (i) systematic analysis of protein-protein interactions, (ii) post-transcriptional regulation of gene expression, and (iii) host-fungal pathogen interactions. We are developing innovative, mass spectrometry-based experimental approaches that are designed for these research areas, but which are also more widely applicable.

Research Highlights

The Gel/Gas/Phr family of fungal beta(1,3)-glucanosyltransferases plays an important role in cell wall biogenesis by processing the main component beta(1,3)-glucan. Two subfamilies are distinguished depending on the presence or absence of a C-terminal cysteine-rich domain, denoted "Cys-box." The N-terminal domain (NtD) contains the catalytic residues for transglycosidase activity and is separated from the Cys-box by a linker region. To obtain a better understanding of the structure and function of the Cys-box-containing subfamily, we identified together with the Popolo group (Università degli Studi di Milano) the disulfide bonds in Gas2p from *Saccharomyces cerevisiae* by an improved mass spectrometric methodology. We mapped two separate intra-domain clusters of three and four disulfide bridges. One of the bonds in the first cluster connects a central Cys residue of the NtD with a single conserved Cys residue in the linker. It is shown that this disulfide bond has a crucial role in folding as it may stabilize the NtD and facilitate its interaction with the C-terminal portion of a Gas protein.

A mass spectrometric method is developed to identify and quantify several hundreds of newly synthesized proteins in *Escherichia coli* upon pulse labeling cells with the methionine analogue azido homoalanine (AZHAL). For the first 30 minutes after inoculation, a methionine-auxotrophic strain grows equally well on azhal as on methionine. Upon a pulse of 15 minutes and digestion of total protein, azhal-labeled peptides are isolated by a retention-time shift between two reversed phase chromatographic runs. The retention time shift is induced by a reaction selective for the azido group in labeled peptides using tris-(2-carboxy-ethyl)-phosphine. Selectively modified peptides are identified by LC-tandem MS. We identified 527 newly synthesized proteins. These proteins are representative of all major Gene Ontology categories.

Future Prospects

Focal points in our MS research program are as mentioned above (i) systematic analysis of protein-protein interactions, (ii) post-transcriptional regulation of gene expression, and (iii) host-fungal pathogen interactions. In program (i) we will further explore the use of our BAMG cross-linker to map protein-protein interactions in complex biomatrices. The development of accurate LC-FT-ICR-MS and novel software in our second research line opens new ways to study tertiary structure of multi-domain proteins and topology of protein complexes. With the Popolo group we will continue the study the structure and function cell wall □(1,3)-glucanases. (ii) In collaboration with Prof. dr. K.J. Hellingwerf and Prof. dr. M.J. Teixeira de Mattos we will use our mass spectrometric AZHAL pulse labeling method to unravel the regulatory circuit underlying the transition of aerobic to (semi)-anaerobic metabolism in *E. coli*. We will also explore AZHAL labeling of proteins in other micro organisms. Parallel to our diagonal chromatography approach we will develop selective and sensitive methods for sequestration of AZHAL containing peptides in total *E. coli* cell lysates and of BAMG cross-linked peptides in complex biomatrices. Enrichment of AZHAL peptides will lead to higher protein coverage in proteome wide pulse labeling studies. (iii) The MS group will further explore the question how mass spectrometry in combination with novel purification strategies and bioinformatics tools can provide detailed quantitative structural and functional information about cell wall proteins of *Candida albicans* and other fungi. In the framework of the EC FINSysB project we will focus on the quantitative cell wall protein composition of *Candida albicans* to identify new leads for novel anti-*Candida* vaccines, drugs and diagnostic markers. Furthermore, we will extent research line (iii) in collaboration with Prof. dr. S. Brul to the functional characterization of spore coat proteins of *B. subtilus.* We will continue the productive collaborations with the groups of the SILS-plant cluster and our external national and international partners.

Key Publications

Popolo, L., Ragni, E., Carotti, C., Palomares, O., Aardema, R., Back, J.W., Dekker, H.L., Koning, L.J. de, Jong, L. de & Koster, C.G. de (2008). Disulfide bond structure and domain organization of yeast beta(1,3)-glucanosyltransferases involved in cell wall biogenesis. *J. Biol. Chem., 283*(27), 18553-18565.

Yin, Q.Y., Groot, P.W.J. de, Koster, C.G. de & Klis, F.M. (2008). Mass spectrometry-based proteomics of fungal wall glycoproteins. *Trends Microbiol., 16*(1), 20-26.

PhD Theses

Yin, Q. (2008, January 11). *Exploring the fungal wall proteome by mass spectrometry.* Universiteit van Amsterdam . Prom./coprom.: prof.dr. C.G. de Koster, dr. F.M. Klis & dr. L. de Jong.
Loon, A. van (2008, January 15). *Color changes and chemical reactivity in seventeeth-century oil paintings.* Universiteit van Amsterdam. Prom./coprom.: prof.dr. J.J. Boon.

Academic publications (refereed)

Aerts, J.M., Breemen, M.J. van, Bussink, A.P., Ghauharali, K., Sprenger, R., Boot, R.G., Groener, J.E., Hollak, C.E., Maas, M., Smit, S., Hoefsloot, H.C., Smilde, A.K., Vissers, J.P.C., Jong, S. de, Speijer, D. & Koster, C.G. de (2008). Biomarkers for lysosomal storage disorders: Identification and application as exemplified by chitotriosidase in Gaucher disease. *Acta Paediatrica, 97*(s457), 7-14.

Bleijlevens, B., Breemen, M.J. van, Donker-Koopman, W.E., Koster, C.G. de & Aerts, J.M.F.G. (2008). Detection of mutant protein in complex biological samples: Glucocerebrosidase mutations in Gaucher's disease. *Analytical Biochemistry, 372*(1), 52-61.

Bloois, E. van, Dekker, H.L., Houben, E.N.G., Urbanus, M.L., Koster, C.G. de, Gier, J.W. de & Luirink, J. (2008). Detection of cross-links between FtsH, YidC, HflK/C suggests a linked role for these proteins in quality control upon insertion of bacterial inner membrane proteins. *FEBS lett., 582*(10), 1419-1424.

Bolton, M.D., Esse, H.P. van, Vossen, J.H., Jonge, R. de, Sterglopoulos, I., Stulemeijer, I.J.E., Berg, G.C.M. van den, Borrás-Hidalgo, O., Dekker, H.L., Koster, C.G. de, Wit, P.J.G.M. de, Joosten, M.H.A.J. & Thomma, B.P.H.J. (2008). The novel Cladosporium fulvum lysin motif effector Ecp6 is a virulence factor with orthologues in other fungal species. *Mol. Microbiol., 69*(1), 119-136.

Brighenti, F.L., Luppens, S.B.I., Delbem, A.C.B., Deng, D.M., Hoogenkamp, M.A., Gaetti-Jardim, E. jr., Dekker, H.L., Crielaard, W. & Cate, J.M. ten (2008). Effect of Psidium cattleianum leaf extract on Streptococcus mutans viability, protein expression and acid production. *CARIES RES, 42*(2), 148-154.

Groot, P.W.J. de, Kraneveld, E.A., Yin, Q.Y., Dekker, H.L., Gross, U., Crielaard, W., Koster, C.G. de, Bader, O., Klis, F.M. & Weig, M. (2008). The cell wall of the human pathogen Candida glabrata: Differential incorporation of novel adhesin-like wall proteins. *EUKARYOTIC CELL, 7*(11), 1951-1964.

Groot, P.W.J. de & Klis, F.M. (2008). The conserved PA14 domain of cell wall-associated fungal adhesins governs their glycan-binding specificity. *Mol. Microbiol., 68*(3), 535-537.

Peters, R., Tonoli, D., Duin, M. van, Mommers, J., Mengerink, Y., Wilbers, A.T.M., Benthem, R. van, Koster, C. de, Schoenmakers, P.J. & Wal, S. van der (2008). Low-molecular-weight model study of peroxide cross-linking of ethylene-propylene (-diene)

rubber using gas chromatography and mass spectrometry: I. Combination reactions of alkanes. *J. Chromatogr. A, 1201*(2), 141-150.

Peters, R., Duin, M. van, Tonoli, D., Kwakkenbos, G., Mengerink, Y., Benthem, R.A.T.M. van, Koster, C.G. de, Schoenmakers, P.J. & Wal, S. van der (2008). Low-molecular-weight model study of peroxide cross-linking of ethylene–propylene–diene rubber using gas chromatography and mass spectrometry: II. Addition and combination reactions. *J. Chromatogr. A, 1201*(2), 151-160.

Popolo, L., Ragni, E., Carotti, C., Palomares, O., Aardema, R., Back, J.W., Dekker, H.L., Koning, L.J. de, Jong, L. de & Koster, C.G. de (2008). Disulfide bond structure and domain organization of yeast beta(1,3)-glucanosyltransferases involved in cell wall biogenesis. *J. Biol. Chem., 283*(27), 18553-18565.

Sosinska, G.J., Groot, P.W.J. de, Teixeira De Mattos, M.J., Dekker, H.L., Koster, C.G. de, Hellingwerf, K.J. & Klis, F.M. (2008). Hypoxic conditions and iron restriction affect the cell-wall proteome of Candida albicans grown under vagina-simulative conditions. *MICROBIOL-SGM, 154*(2), 510-520.

Yin, Q.Y., Groot, P.W.J. de, Koster, C.G. de & Klis, F.M. (2008). Mass spectrometry-based proteomics of fungal wall glycoproteins. *Trends Microbiol., 16*(1), 20-26.

Invited lectures

Kramer, G. (2008, November 03). *Enrichment of azido homoalanine labeled peptides with diagonal chromatography.* Lunteren, The Netherlands, NWO/CW Analytische Scheikunde.

Kramer, G. (2008, December 08). *Identification of newly synthesized protein in E.coli.* Veldhoven, The Netherlands, NWO/CW Studiegroep.

Biosystems Data Analysis

Chairholder: Prof.dr. A.K. Smilde

Dr. H.C.J..Hoefsloot	Associate Professor
Dr. J.A. Westerhuis	Assistant Professor
Prof. dr. A.H.C.van Kampen	Professor (0.2 fte)

Introduction

General goal: Developing and validating methods for organizing, summarizing and visualizing complex biological data.

Specific: The research is divided in two closely related themes: bioinformatics and biostatistics.

Application areas: We apply our methods in diverse areas of systems biology focussing mainly on microbiology, nutrition and medical biology.

Bioinformatics

Challenges in systems biology, biomedical research and e-bioscience include the organization, representation, integration and presentation of knowledge and experimental data. An information management framework (BioExpert) dedicated to the biomedical domain will provide mechanisms to address these challenges. BioExpert uses novel approaches for information representation that are based on Semantic Web technology standards (RDF, OWL and SKOS) while graphical concept maps are used to organize and present pieces of information to the researcher or clinician. The concept maps may describe systems at a multi-scale level (in time and space), which may provide input for data-driven and mechanistic models. Fundamentally, the use of RDF/OWL/SKOS ensures the benefits provided by the emerging standard of linking, for example, experimental data, public biological databases and statistical models on the Web through the common description of resources in RDF.
BioExpert can be used to describe any specific domain (e.g., pathway, organelle, cell, organ, organism) and provides the user with overview and insight in this domain.

Biostatistics

In systems biology an abundance of omics data is generated. These data have to be analyzed to infer properties of the biological system. These analyses are done by making models and fit the data to the models. The estimated model parameters serve then as the vehicle for understanding the underlying phenomena of the system. The models used can range from purely data driven models (multiway component analysis and extensions) to mechanistic models (e.g. sets of differential equations) and combinations of those models; so called grey models. These grey models form the bridge between the two themes: biological *a priori* knowledge can be formalized in concept maps and thus incorporated into the grey models. Mostly, proteomics and metabolomics data are being analyzed.

Research Highlights

Bioinformatics

The first publication about the BioExpert framework (www.bioexpert.nl) and the peroxisome knowledge base that was constructed within this framework was published in Bioinformatics (Willemsen, 2008) and presented at the European Conference of Computational Biology in Italy. We made significant progress with the development of novel approaches for the representation of information. We incorporated the Simple Knowledge Organization System (SKOS) in BioExpert to manage complex terminologies used in highly specific expert domains. We used SKOS to develop a first version of a Peroxisome vocabulary that will be used as a basis to construct graphical concept maps. In addition, we started the development of a concept map editor as a plugin to Protege.

Biostatistics

Association networks are convenient methods to compress and visualize complex information. Through a collaboration with the LUMC (Pijl, Roelfsema) we obtained frequently measured time-resolved hormone data from obese women before and after treatment with a dopamine agonist (bromocrptine). From the abundance of data we were able to extract and visualize the essential differences in hormone synchronicity. Striking difference of the before and after treatment network are i) the loss of connectivity after treatment and ii) the disruption of the connection between two important hormone axis prolactine-TSH and ACTH-cortisol. These aspects are currently discussed with the medical biologists involved.

We have introduced *nutrikinetics* as an integration of metabolomics and pharmacokinetics as a concept to segment a human study population with different metabolic phenotypes following a nutritional intervention (see Figure 1). The approach facilitates an unbiased analysis of the time-response of body fluid metabolites from crossover designed intervention trials without any prior knowledge of the underlying metabolic pathways. The method is explained for the case of a human intervention study in which the nutrikinetics of polyphenol-rich black tea consumption was investigated in urine over a period of 48 hours. First, discrimination analysis was applied to the urinary ^{1}H-NMR profiles to select the most differentiating biomarkers between the treated and placebo samples. Then, a one-compartment nutrikinetic model with 1st-order kinetics was fitted to the time courses of these selected biomarkers. In the nutrikinetic model used here, the crossover structure in the data was fully exploited by fitting the data from both the treatment period and the placebo period simultaneously. As an example 1,3-dihydroxyphenol-2-*O*-sulphate, derived from microbial fermentation of polyphenols in the gut, was used in the model fitting. Variations in urinary excretion of these biomarkers between the subjects due to the intervention were observed, and facilitate segmentation of sub-populations with different gut-microbial phenotypes. In the top figure the ^{1}H-NMR peak of 1,3-dihydroxyphenol-2-*O*-sulphate is shows after placebo and tea consumption. The middle plot reveals the variation in 1,3-dihydroxyphenol-2-*O*-sulphate over the study population. The bottom plot reveals the results for the one-compartment 1st order model are presented. The lag time between tea intake and first increase of 1,3-dihydroxyphenol-2-*O*-sulphate in urine (~5h) is clearly visible indicating the gut activity in polyphenol degradation.

Other Highlights

Van Kampen heads the AMC Bioinformatics Laboratory and holds a position of associate professor (UHD) and principal investigator at the AMC. Van Kampen is Scientific Director of the Netherlands Bioinformatics Centre.

Future Prospects

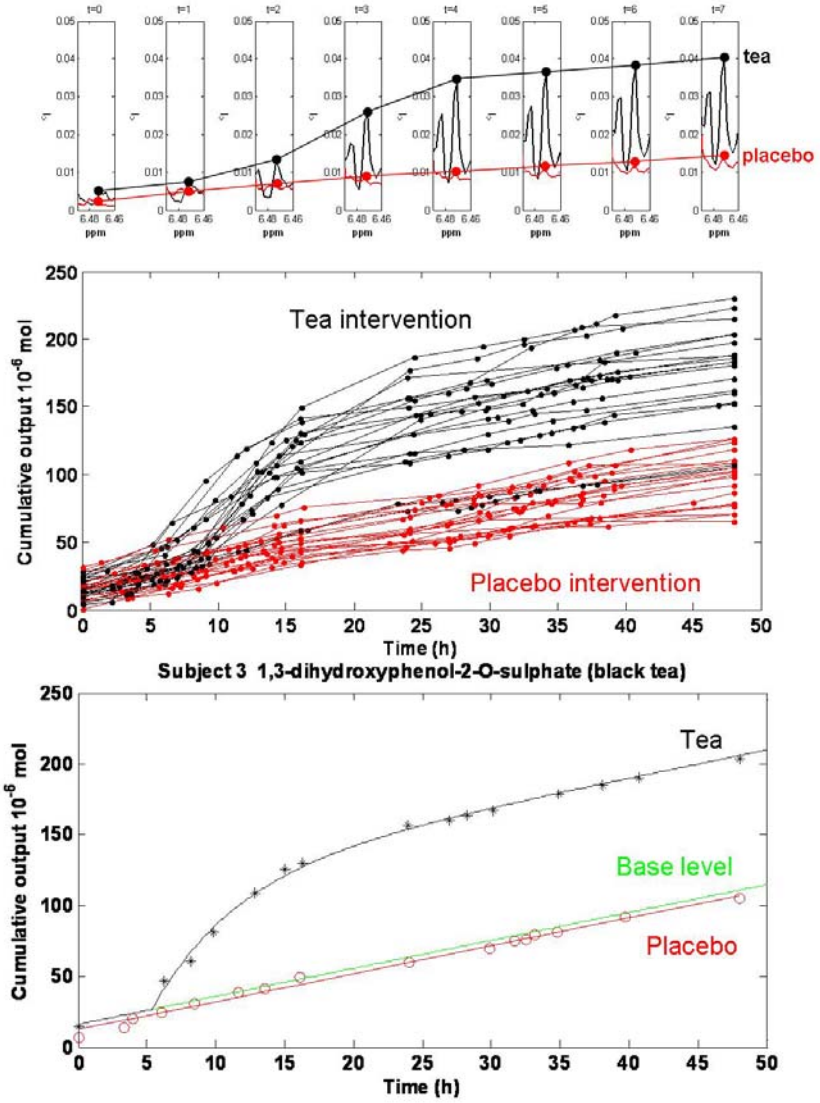
Bioinformatics

The BioExpert framework will be further extended an applied to biological system. We will further improve our approach to represent information by RDF/SKOS/OWL, which will directly be implemented as part of the Peroxisome knowledge base (www.peroxisomekb.nl). A first version of a concept map editor will be finished that allows the build concept maps from a SKOS vocabulary and to link individual concepts to external resources. Together with the Netherlands Consortium for Systems Biology we will apply the BioExpert framework to support systems biology. As part of this we will extend BioExpert to allow the incorporation of (SBML-based) mathematical models. To further establish an integration between Bioinformatics and Biostatistics within the department we will develop approaches to incorporate and represent statistical grey-models within the BioExpert framework.

Biostatistics

The work and association networks will be continued and expanded in three directions. First. a challenging topic is whether the dynamic data is rich enough to also infer directionality and causality in the networks. If so, then this type of data can be used for inferring regulatory information and may hint to mechanistic principles. Secondly, through collaboration within the Netherlands Metabolomics Center more frequently measured compounds (e.g. lipids, fatty acids) will become available which can also be put in the same framework. Thirdly, incorporation of physiological knowledge in such networks through grey models is foreseen.

In the nutrikinetics, new data will become available regarding the gut microbiota and exogenous plasma metabolites. The data allows *in-vivo* compartmental analysis of potentially bioactive molecules (and their metabolites) along the human gastrointestinal tract (i.e. gut, blood circulation, liver, and kidney). Together with the already collected data, the new nutrikinetic data will be used to obtain a good phenotypic segmentation of the subjects, where differences between the segments can be understood from the differences in gut microbial composition. This will give insight into mechanisms of probiotic behavior, and the role of the gut micro flora on the adsorption, disposition, metabolism, and nutrikinetics (ADMN) of potentially bioactive nutrients across humans. Besides mechanistically knowledge, we will also gain knowledge about the ADMN of nutrients in relation to their nutritional source and the applied food matrix.



Key Publications

Willemsen, A.M., Jansen, G.A., Komen, J.C., Hooff, S. van, Waterham, H.R., Brites, P.M.T., Wanders, R.J.A. & Kampen, A.H.C. van (2008). Organization and integration of biomedical knowledge with concept maps for key peroxisomal pathways. *Bioinformatics, 24*(16), i21-i27.

Velzen, E.J.J. van, Westerhuis, J.A., Duynhoven, J.P.M. van, Dorsten, F.A. van, Hoefsloot, H.C.J., Jacobs, D.M., Smit, S., Draijer, R., Kroner, C.I. & Smilde, A.K. (2008). Multilevel data analysis of a crossover designed human nutritional intervention study. *J. Proteome Res., 7*(10), 4483-4491.

PhD Theses

Berg, R.A. van den (2008, September 24). *Crossing borders between biology and data analysis.* UvA Universiteit van Amsterdam (Amsterdam). Prom./coprom.: prof.dr. A.K. Smilde.

Stanimirovic, O. (2008, December 04). *Optimal sensor placement and timing. Where and when to measure.* UvA Universiteit van Amsterdam (Amsterdam). Prom./coprom.: prof.dr. A.K. Smilde & dr.ir. H.C.J. Hoefsloot.

Academic publications (refereed)

Aerts, J.M., Breemen, M.J. van, Bussink, A.P., Ghauharali, K., Sprenger, R., Boot, R.G., Groener, J.E., Hollak, C.E., Maas, M., Smit, S., Hoefsloot, H.C., Smilde, A.K., Vissers, J.P.C., Jong, S. de, Speijer, D. & Koster, C.G. de (2008). Biomarkers for lysosomal storage disorders: Identification and application as exemplified by chitotriosidase in Gaucher disease. *Acta Paediatrica, 97*(s457), 7-14.

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Membership editorial board

Kampen, A.H.C. van (Ed.). (2008). *Advances in Bioinformatics*.
Smilde, A.K. (Ed.). (2008). *J. Chemometr.*
Westerhuis, J.A. (Ed.). (2008). *J. Chemometr.*

Invited lectures

Smilde, A.K. (2008, January 31). *Analysing complex metabolomics data.* Rennes, France, Congress Statistical methods for post-genomics data.

Smilde, A.K. (2008, March 18). *From metabolomics data to biological networks.* Leuven, Belgium, Catholic University Leuven.

Smilde, A.K. (2008, November 12). *LC-MS and multivariate analysis: a happy marriage?* Montreux, Switzerland, 25th LC/MS Symposium.

Smilde, A.K. (2008, January 15). *Statistical analysis of metabolomics data.* Kaiseraugst, Switzerland, Invited lecture DSM.

Westerhuis, J.A. (2008, October 10). *Metabolic network discovery.* Amsterdam, NISB workshop Top down or bottom up.

Westerhuis, J.A. (2008, November 11). *Metabolomics data analysis.* Somerset, New Jersey, USA, Eastern Analytical Symposium.

Micro Array Department and Integrative Bioinformatics Unit

Group leader: Dr. T.M. Breit

Dr.Ir.R.A.Wittink	Project management "wet-lab"
Dr.M.J. Jonker	Project management "dry-lab"
J.S.Batson	Project Administration
Drs.J.Rauwerda	Senior Researcher IBU

Introduction

MicroArray Department (MAD) & Integrative Bioinformatics Unit (IBU):
Microarray technology is a well-established tool in the analysis of genome-wide gene expression studies. The ultimate goal of a microarray experiment is simultaneous examination of the expression of all genes of a specific organism, in a cell type, during specific growth or stress conditions, to unravel complex cellular mechanisms or identify and use biomarkers. Transcriptomics biomarkers are genes whose expression profile can be used for diagnostic purposes or to monitor and predict cellular processes. Because microarray experiments produce a vast amount of data, extensive bioinformatics infrastructure, methods and expertise are needed to cope with these data effectively. Microarray bioinformatics comprise data-handling (storage and exchange), data-preprocessing (normalization and validation), and data-analysis (clustering, biomarker selection, etc.).
The MAD-IBU consist of i) a microarray technology section (Wet-lab) with ~5 specialists that provide transcriptomics service & support and perform microarray technology R&D; ii) a microarray data-analysis section (Dry-lab) with ~11 bioinformaticians that provide transcriptomics data analysis service & support and performs bioinformatics R&D; and iii) a management part with 2 staff members. Together, the MAD operates as a transcriptomics technology and bioinformatics expertise centre and core facility for UvA scientists, as well as external academic and industrial customers. The MAD is an official Affymetrix Service Provider and is in the process of becoming a certified Agilent Service Provider.
The focus of the Wet-lab R&D is to improve the microarray technology for transcriptomics with a strong focus on sample size reduction. We aim to eventually analyze all kinds of single cells by microarray technology. The focus of the Dry-lab is focussed on the methods, tools and infrastructure necessary to perform advanced transcriptomics data-analysis starting from array design until publication. Another important focal point for the whole group is design-for-experimentation. Performing well designed range finding experiments should elucidate the role of time and space in microarray transcriptomics experiments. To this end, MAD-IBU participates in three nationwide projects: "BioRange", a nationwide bioinformatics research project; "BioAssist", a national bioinformatics support programme and "Virtual Lab for e-Science (VL-e)", the Dutch e-science project in the field of ICT infrastructure and methods.

Research Highlights

The Wet-lab:
Developed a microarray protocol for single-embryo and single-egg transcriptomics analysis on Zebrafish.
Developed a sample protocol for high-resolution analysis of Zebrafish embryogenesis.
The Dry-lab:
Developed advanced methods to identify transcriptomics biomarkers for toxicogenomics.
Performed an extensive study on RNA damage in aging brain and neurodegenerative diseases.
Developed an organism-centric approach for prokaryotic transcripomics (PROGENIUS)
Developed an advanced zebrafish microarray.

Other Highlights

The whole group further strengthened the strategic collaborations with several external research organisations: Laboratory for Health Protection Research, RIVM, (Bilthoven); Medical Microbiology, UMC (Utrecht); ACTA, VU-AMC (Amsterdam); Molecular Cell biology, UL, (Leiden).

Future Prospects

- Analyze maternal RNAs in unfertilized Zebrafish eggs.
- Analyze the transcriptomics of the earliest stages of Zebrafish embryogenesis.
- Perform a hallmark time-axis microarray experiment on Zebrafish.
- Perform several range-finding experiments in the context of design-for-experimentation.
- Extend the MicroArray Problem Solving Environment for microarray data analysis and interpretation
- Set-up the infrastructure for prokaryote transcriptomics data handling for 5 model organisms.
- Develop methods, tools, and infrastructure to translate prokaryote high-throughput de-novo sequence information into microarray designs.
- Write > 15 (collaborative) research articles.

Key Publications

Inda, M.A., Batenburg, M.F.van, Roos, M., Belloum, A.S., Vasunin, D., Wibisono, A., Kampen, A.H. van, Breit, T.M. (2008). SigWin-detector: a Grid-enabled workflow for discovering enriched windows of genomic features related to DNA sequences. *BMC Res Notes.* 8;1:63.

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Academic publications (refereed)

Aronica, E., Boer, K., Becker, A., Redeker, S., Rijen, P.C. van, Wittink, F., Breit, T., Spliet, W.G.M., Wadman, W.J., Lopes da Silva, F.H., Troost, D. & Gorter, J.A. (2008). Gene expression profile analysis of epilepsy-associated gangliogliomas. *Neuroscience, 151*(1), 272-292.

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Bošnački, D., Pronk, T.E. & Vink, E.P. de (2008). In silico modelling and analysis of ribosome kinetics and aa-tRNA competition. In R.-J. Back & I. Petre (Eds.), *Proceedings of COMPMOD 2008: Workshop on Computational Models for Cell Processes Vol. 47. TUCS general publications* (pp. 23-38). Turku: Turku Centre for Computer Science (TUCS).

Bruning, O., Bruins, W., Jonker, M.J., Zwart, E., Hoeven, T.V. van der, Pennings, J.L.A., Rauwerda, H., Vries, A. de & Breit, T.M. (2008). The absence of Ser389 phosphorylation in p53 affects the basal gene expression level of many p53-dependent genes and alters the biphasic response to UV exposure in mouse embryonic fibroblasts. *Mol. Cell. Biol., 28*(6), 1974-1987.

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Luppens, S.B.I., Kara, D., Bandounas, L., Jonker, M.J., Wittink, F.R.A., Bruning, O., Breit, T.M., Cate, J.M. ten & Crielaard, W. (2008). Effect of Veillonella parvula on the antimicrobial resistance and gene expression of Streptococcus mutans grown in a dual-species biofilm. *ORAL MICROBIOL IMMUN, 23*(3), 183-189.

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Book Charpter

Wassink, I., Rauwerda, H., Vet, P. van der, Breit, T.M. & Nijholt, A. (2008). E-BioFlow: Different Perspectives on ScientificWorkflows. In *Bioinformatics Research and Development* (Communications in Computer and Information Science , Vol. 13, XXI) (pp. 243-257). Springer Verlag.

Invited lecture

Wassink, I., Rauwerda, H., Vet, P. van der, Breit, T.M. & Nijholt, A. (2008, July 09). *E-BioFlow: Different Perspectives on ScientificWorkflows.* Vienna, Austria, Bioinformatics Research and Development, BIRD 2008.

Management

Finance

The integrated results for 2008 show an operating shortage of 351 k€ where a negative result of 122 k€ was budgeted.

Revenues and costs over 2008, compared with previous years were:

	2001	2002	2003	2004	2005	2006	2007	2008
university funding *	5838	6131	7364	8987	7577	13234	12795	13848
external funding	3852	3883	4474	6167	4515	4701	4952	5489
total revenues	9690	10014	11838	15154	12092	17935	17747	19337
personnel costs	7096	7465	8919	9626	9122	12816	13918	14448
bench fees	2236	2450	3310	4989	2729	5362	4138	5240
total costs	9332	9915	12229	14614	11851	18178	18056	19688
result	358	99	-391	540	241	-243	-309	-351

* All amounts are given in K Euro.
Figure: Graphic representation of revenues and costs of the Swammerdam Institute for Life Sciences, in k€, for the years 2001-2008. In this table –starting from 2004-external funding is considered to be 2nd and 3rd funding source only.

Funding

The funding system of Dutch universities distinguishes three different kinds of funding resources. These are referred to as so called “funding sources” and are numbered one to three. Resources originating from the university itself are referred to as the first funding source. External funding is divided into funding from the Netherlands Organization for Scientific Research (second funding source) and money originating from all other resources such as EU and contract research (third funding source).

	2003	**2004**	**2005**	**2006**	**2007**	**2008**
Revenues	7793	8987	7577	13234	12795	13848
Costs	8291	8902	7357	13580	13259	14115
Result	-498	85	220	-346	-464	-306

Figure 1: representation of income and costs in the 1st funding source, in k€, for the years 2003-2008.

	2003	**2004**	**2005**	**2006**	**2007**	**2008**
Revenues	2279	2303	2160	2032	2299	2434
Costs	2279	2303	2160	2048	2226	2436
Result	0	0	0	-16	73	-2

Figure 2: representation of income and costs in the 2nd funding source, in k€, for the years 2003-2008.

	2003	**2004**	**2005**	**2006**	**2007**	**2008**
Revenues	1766	3864	2355	2669	2653	3055
Costs	1659	3409	2334	2550	2571	3097
Result	107	455	21	119	82	-42

Figure 3: representation of income and costs in the 3rd funding source, in k€, for the years 2003-2008.

Figure 1 shows that in 2006, first funding source income and costs increased by almost 6000 k€ compared to 2005. This increase is the result of the introduction of a new university wide full-cost financial system. Essentially, overhead costs that were not calculated in previous years such as housing and computer infrastructure were calculated to the institute as of 01-01-2006. Simultaneously budgets were increased.

Funding from the Dutch Organization for Scientific Research (2nd funding source) remains stable in the past years. Third funding source income slightly increased in the past years. The numbers of 2004 profited from two major incidental incomes and explain the increase numbers of 2004. The first incidental revenue in 2004 existed of 1045 k€ that SILS received for its role as coordinator of an EU program. This was transferred directly to other partners in this program. The second was a 404 k€ result of the successful sales of a spin-off company.

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