Book of Abstracts

4th European Symposium on Plant Lipids

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- R. Franke, Bonn/D
  "Mutations in genes encoding long chain fatty acid modification effect barrier properties of suberized tissues"

- J. Joubes, Bordeaux/F
  "Activation of wax production by CER1 ovexpression in *Arabidopsis* confers drought tolerance but enhances pathogen susceptibility"

Industrial Fatty Acids

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Oxylipins

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- C. Gatz, Göttingen/D
  "Arabidopsis class II TGA transcription factors function as salicylic acid-sensitive positive regulators of jasmonic acid/ethylene-induced PDF1.2 expression"

- C. Wasternack, Halle/Saale/D
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- G. Pohnert, Jena/D
  "Tracing down the Structural diversity of Moss-oxylipins with the help of Chemometric methods"

Sphingolipids

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  Ed Cahoon, Donald Danforth Plant Science Center, St. Louis/USA
  "Probing the Metabolic and Functional Basis for Sphingolipid Structural Diversity in Plants"

- J.D. Faure, Versailles/F
  "Functional analysis of ceramide synthases in *Arabidopsis*"

Isoprenoids

- **Keynote lecture**
  Dirk Warnecke, University of Hamburg, Biocenter Klein Flottbeck, Plant Physiology, Hamburg/D
  "Functions of steryl glucosides in plants, fungi and bacteria"

- P. Doermann, Bonn/D
  "Chlorophyll and phytol catabolism during senescence and abiotic stress in *Arabidopsis*"

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Membrane Transport and Trafficking

- **Keynote lecture**
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  "Exocytotic membrane fusion of synaptic vesicles - what we know - or better do not know ? about the role of membrane lipids"

- M. Gierth, Köln/D
  "Peroxisomal Fatty Acid Import and beta-Oxidation are Vitally Important in Mature *Arabidopsis* Leaves during Extended Darkness"

- N. Linke, Düsseldorf/D
  "Peroxisomal ATP import is essential for seedling development in *Arabidopsis thaliana*"

Poster session

Programme

Sunday, 15 March 2009

18:00	Opening Mixer, at the Paulinerkirche

Monday, 16 March 2009

1. Seed Biology
Chair: J. Napier

08.30 - 09.00	Keynote lecture Ian Graham, University of York, Department of Biology, York/UK
09.00 - 09.15	S. Vandana, New Delhi/IND "Development-associated tissue specific changes in the Lipid composition and Lipid content of dark-grown Sunflower seedlings"
09.15 - 09.30	L. Hernandez. York/GB "Metabolomic and transcriptomic analysis of oil mobilization during seed germination in *Arabidopsis thaliana*"
09.30 - 09.45	C.E. Christensen, Valby/DK "Interactions in peroxisomal -oxidation, a structural point of view"
09.45 -10.00	A.S. Carlson, Alnarp/S "Nutsedge - A novel model system for studying accumulation of oil and starch"
10.00 -10.30	Coffee break

2. Fatty Acid Modifications
Chair: M. Pollard

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11.00 -11.15	F. Domergue, Bordeaux/F "Fatty acyl-CoA reductases from *Arabidopsis thaliana* that generate Fatty Alcohols associated with Suberin deposition"
11.15 -11.30	M. Heilmann, Göttingen/D "A new pathway to produce omega3-fatty acids in plants"
11.30 -11.45	E.S. Averlen, Ulan-Ude/RUS "Polyunsaturated fatty acids from Siberian pine seed oil: A perspective source for medicine"

11.45 -12.00	E. Kombrink, Köln/D "A novel fatty acyl-CoA synthetase (ACOS5) is required for pollen development and sporopollenin biosynthesis in *Arabidopsis*"
12.00 - 13.00	Lunch
13.00 – 15.00	**Poster session**

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Chair: S. Stymne

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Chair: A. Green

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Chair: M. Fulda

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17.30 -17.45	J.D. Faure, Versailles/F "Functional analysis of ceramide synthases in *Arabidopsis*"
17.45	Business meeting: Discussion of the Future Site & Scheduling
19.30	**Conference dinner at the Rathskeller** Markt 9, 37073 Göttingen

Wednesday, 18 March 2009

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Chair: E. Cahoon

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10. Membrane Transport and Trafficking
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"Peroxisomal ATP import is essential for seedling development in *Arabidopsis thaliana*"

11.00 Closing Remarks

11.30 -12.30 Lunch

Sponsors

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Lecture Abstract

In order of Presentation

An essential role for trehalose metabolism in seed biology

Ian A. Graham, Anja Hellwege, Alison D. Gilday and Leonardo D. Gómez,
Centre Novel Agricultural Products, Department Biology, University York, UK

Trehalose-6-phosphate synthase 1 (TPS1), which catalyses the first step in trehalose synthesis, is essential for embryo maturation in Arabidopsis. Patterning in *tps1* embryos appears normal but they do not progress past the torpedo stage to cotyledon stage, which is when storage reserves start to accumulate in the expanding cotyledons. Ultrastructural, biochemical, and transcriptomic analysis shows that, in contract to torpedo stage wild type embryos, torpedo stage *tps1* exhibits many features typically associated with cotyledon stage embryos (Gomez *et al*., 2006, Plant J. 46, 69-84). The cell walls of *tps1* embryos show a remarkable degree of thickening at the ultrastructural level and immunodetection of cell wall components shows that altered deposition of pectins accounts for this change in morphology. The frequency of cell division in *tps1* embryos is half that of the wild type at the heart and torpedo stages. Overall, the flux of carbon is shifted away from oil and into sugars, starch and cell wall components. Despite the dramatic phenotype of the *tps1* embryos there is limited effect on expression of genes associated with primary carbon metabolism. However, there are significant differences in the patterns of a number of enzymes of primary carbon metabolism in the mutant background (Baud *et al*., 2006, Plant J. 46, 155-69). Master regulatory kinases such as AKIN11, whose expression actually correlates with trehalose-6-P levels, could play a key role in regulating carbon metabolism during seed development through the phosphorylation of key enzymes. Recent advances in this area will be discussed in the context of trehalose-6-P acting as an essential regulatory metabolite in embryos and vegetative tissues.

Development-associated, tissue specific changes in the Lipid composition and Lipid content of dark-grown Sunflower seedlings

Shweta Vandana* and S. C. Bhatla @

* Department of Botany, Gargi College, University of Delhi, South Campus, New Delhi, India ;@ Department of Botany, University of Delhi, North Campus, Delhi, 110007, India

* Presenting Author (e mail: shwetavandana@gmail.com)

Abstract

In order to understand the turnover of storage lipids in oilseeds, a qualitative and quantitative analysis of total lipids and fatty acids was undertaken in the roots, hypocotyls and cotyledons of sunflower seedlings at different stages of development, employing biochemical and fluorescence imaging techniques. The total lipid content was highest in the cotyledons, followed by hypocotyls and roots. With seedling development the cotyledons and hypocotyls showed gradual decrease in the total lipid content but the total lipid content of roots was maintained at a level, indicating preferential accumulation/lesser mobilization. Thin layer chromatographic analysis of the lipid profile of tissue homogenates from these tissues revealed the presence of MAGs predominantly in roots and hypocotyls as against cotyledons where they were detectable in minimal quantity. Roots exhibited highest carotene content, followed by hypocotyls and cotyledons in decreasing order. The free fatty acids were most abundant in cotyledon, hypocotyl and root extracts of 4 d old seedlings, suggesting the synthesis of new fatty acids during seedling development. Nile Red treatment of protoplasts from cotyledons, hypocotyls and roots followed by fluorescence and confocal microscopic analysis revealed oil bodies as the major sites of neutral lipids. The cotyledon protoplasts fluoresced uniformly red where as the fluorescence from oil bodies varied from green, golden-yellow to deep red in protoplasts isolated from roots and hypocotyls. The observed differences in the fluorescence from oil bodies are likely to be due to differences in the lipid composition of oil bodies. Gas liquid chromatographic analysis of fatty acids from cotyledons, hypocotyls and roots indeed revealed these compositional variations. FFA profiles showing relative content of a fatty acid (%) in imbibed seeds, 1 d, 4 d and 6 d old seedling cotyledons showed linoleic acid as the major component. Its relative content during seedling development remained more or less unchanged. None of the fatty acids exhibited any significant change in their relative content in cotyledons during seedling development. In the free fatty acid profile of hypocotyls, palmitic and oleic acids were completely depleted with the progress of seedling development while linoleic acid content increased almost three-fold. Linoleic acid was the only detectable fatty acid present in 6 d old seedling hypocotyls. The fatty acid profile of roots also showed highest relative content of linoleic acid and the occurrence of linolenic acid in 6d old root extract was a unique feature of FFA profile of roots. The depletion and synthesis of various fatty acids during seedling development was thus responsible for difference in fluorescence from the protoplasts upon treatment with Nile Red.

Metabolomic and transcriptomic analysis of oil mobilization during seed germination in *Arabidopsis thaliana*

Luisa Hernandez, Zhesi He, Lynne Whitehead, Ekaterina Kozhevnikova,
Tony R. Larson, Ian A. Graham.
CNAP, Deparment of Biology, University of York, PO Box 373
York, United Kingdom

In oilseed plants, initiation of seed germination triggers breakdown of storage lipids to produce energy for seedling establishment. This mobilization of storage oil involves the coordinated induction of a number of biochemical pathways in different subcellular compartments. The first step in oil breakdown occurs in the membrane of the oil bodies and is catalyzed by lipases, which hydrolyze triacylglycerol to produce free fatty acids and glycerol. The fatty acids are activated to acyl-CoAs by the action of acyl-CoA synthetases, and imported into the peroxisome where fatty acid ß-oxidation takes place. The isolation of mutants disrupted in different steps of oil mobilization revealed that peroxisomal ß-oxidation is required for the termination of seed dormancy and germination in *Arabidopsis*. In addition, it is known that the carbon/nitrogen ratio regulates various aspects of seedling growth including oil storage mobilization. The objective of this work is to identify genes and metabolites that are key in regulating triacylglycerol breakdown. To achieve this, metabolite and transcriptomic analyses has been carried out using WT and different oil breakdown *Arabidopsis* mutants tissues during seed germination and seedling establishment. Profiling of the different triacylglycerol species has revealed that substantial recycling of fatty acid into triacylglycerol is occurring in mutants that are blocked in fatty acid breakdown. The extent of recycling depends on when the block occurs and whether it is due to genetic or metabolic factors. Integrated metabolomic and transcriptome data will be presented on different mutants in an effort to identify key regulators in this process.

Interactions in peroxisomal b-oxidation, a structural point of view

Caspar E. Christensen[1,2], Valerie E. Pye[1], Penny von Wettstein-Knowles[2], Birthe B. Kragelund[2],
Anette Henriksen[1]
[1]Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Denmark.
[2]Department of Biology, University of Copenhagen, Ole Maaløes Vej 5, DK-2200 Copenhagen N, Denmark.

Peroxisomal ß-oxidation in plants is essential for a plethora of physiological roles including germinating, senescence and starvation, fatty acid turnover and regulation of plant lipid composition. β-oxidation is also responsible for the synthesis of jasmonic acid and involved in indole-3-acetic acid synthesis. Both serve as crucial plant hormones regulating plant development and responses to biotic and abiotic stress. In barley, ß-oxidation is required for production of hydrolytic enzymes in the aleurone layer during germination and is involved in the regulation of the lipid profile of the malt.

In *Arabidopsis*, the four enzymatic activities required for one cycle of β-oxidation are harboured on three discrete enzymes each represented by 2-5 isozymes. Stable complexes are formed between the 3-oxoacyl-CoA thiolase and the multifunctional enzyme in other β-oxidation systems (e.g. mammalian mitochondria and bacteria). We are seeking to characterize the activity and structure of a range of the isozymes participating in β-oxidation of *Arabidopsis thaliana* peroxisomes by applying UV-vis spectroscopy, fluorescence spectroscopy, surface plasmon resonance spectroscopy, analytical ultracentrifugation, X-ray crystal diffraction, SAXS and ^{15}N NMR spectroscopy to approach the role of protein-protein interactions, multigene families and redox regulation in plant fatty acid degradation.

Nutsedge – A novel model system for studying accumulation of oil and starch

Anders S. Carlsson*, Salla Marttila**, Sten Stymne* and Helle Turesson*.
*Department of Plant Breeding and Biotechnology and **Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, Sweden

Much of our understanding on triacylglycerol (TAG) production and accumulation has come from studying oil seeds of dicotyledonous plants such as Arabidopsis. The specific enzymes involved in the metabolic pathway along which TAGs are synthesised and stored in the oil bodies, as well as the pathway that supplies the precursors generated from imported sucrose are to a large extent known. However, we have a less good understanding of the factors regulating fatty acid synthesis and the level of oil content in storage tissues. How the flux of photosynthates into different storage compounds and organs in the plant is regulated is also still largely unknown. Some progress in the understanding of carbon partitioning has recently been made from mutation studies and detailed metabolic flux analysis. However, this knowledge has mostly been achieved from investigations using the seed as a model.

Mature tubers of cultivated varieties of yellow nutsedge (*Cyperus esculentus*), contains typically 25% oil, 30% starch and 20% sugars (on dry weight basis). This plant species represents therefore a quite different environment to study the processes leading up to TAG. We are studying nutsedge as one of several model systems that are focused at unravelling important mechanisms by which plants regulate the fluxes of carbon into oil and starch, respectively. The specific potential with the novel model system nutsedge is the possibility to get directions for future work towards developing oil accumulating tuber plants, such as potato or cassava.

This presentation will cover the results from the first year of basic physiological, biochemical and ultrastructural studies of developing nutsedge tubers with regard to using this plant as a model system for lipid and starch accumulation.

Functional characterisation of the microsomal fatty acid elongase: Defining the role of very long chain fatty acids in plant development

Johnathan Napier, Harpenden/GB

Abstract not yet available

Fatty acyl-CoA reductases from *Arabidopsis thaliana* that generate Fatty Alcohols associated with Suberin deposition.

Domergue, F. [1], Vishwanath, S.J. [2], Alhattab, R. [2], Joubès, J. [1], Pascal, P. [1],Lowe, C. [2], Lessire, R. [1], and Rowland, O. [2]

[1] Université Bordeaux 2 - Laboratoire de biogenèse membranaire - CNRS UMR 5200, Bordeaux, FRANCE.

[2] Dept. of Biology and Institute of Biochemistry - Carleton University, Ottawa, ON, CANADA.

Fatty acyl-CoA reductases (FAR) catalyze the reduction of fatty acyl-CoAs to primary fatty alcohols. An eight member FAR gene family has been identified in the model plant *Arabidopsis thaliana*. FAR2/MS2 (At3g11980) generates fatty alcohols associated with the pollen coat (Aarts et al., 1997) while FAR3/CER4 (At4g33790) generates fatty alcohols associated with the cuticle (Rowland et al., 2006). The functions of the other six Arabidopsis FAR family members are still unknown.

Using heterologous expression in yeast, we functionally characterized the activities of three Arabidopsis FAR enzymes. In yeast, FAR1 (At1g22500), FAR4 (At3g44540) and FAR5 (At3g44550) expression specifically resulted in the predominant accumulation of C22:0 alcohols, C20:0 alcohols and C18:0 alcohols, respectively. In addition, our analyses showed that fatty alcohols were secreted from yeast when produced in high amounts. We also showed that *FAR1*, *FAR4*, and *FAR5* are highly expressed in roots and specifically in the endodermal cells surrounding the central vasculature of roots. Detailed gene expression studies showed in addition that expression of FAR4 and FAR5 was limited to flowers in aerial tissues, whereas FAR1 was found to be expressed in flowers, leaves and stems. The expression of these three FAR genes was induced by wounding in aerial tissues.

All together, our data support the hypothesis that these three FARs generate C18:0 to C22:0 fatty alcohols associated with suberin, a fatty acid- and glycerol-based cell wall-associated polymer that serves to control water and solute transport as well as to provide a barrier against pathogens.

A new pathway to produce omega3-fatty acids in plants

Mareike Heilmann[1], Martin Wagner[1], Amine Abbadi[2], Martin Fulda[1] and Ivo Feussner[1]

[1]Department of Plant Biochemistry, Albrecht-von-Haller-Institute for Plant Sciences, Georg-August-University Göttingen, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany; [2]Norddeutsche Pflanzenzucht, Hans-Georg Lembke KG, Hohenlieth, Holtsee, Germany

? 3-Very long chain polyunsaturated fatty acids (VLCPUFA) are essential for human development and brain function and thus indispensable components of the human diet; our research is aimed at determining feasible means of producing VLCPUFAs, such as eicosapentaenoic acid (EPA), in transgenic plants and, ultimately, in transgenic oil crops. VLCPUFA biosynthesis requires repeated desaturation and repeated elongation of long-chain fatty acid-substrates. In previous experiments the production of EPA in transgenic plants was found to be limited by an unexpected bottleneck represented by the acyl exchange between the site of desaturation, ER-associated phospholipids, and the site of elongation, the cytosolic acyl-Coenzyme A (CoA)-pool (Abbadi et al., Plant Cell 16, 2734-2748, 2004). In principle, there are several possible ways how this bottleneck can be avoided; either by optimizing fatty acid transfer between desaturases and elongases, or by performing desaturation and elongation within the same fatty acid pool. Here we report on the establishment of a coordinated, exclusively acyl-CoA-dependent pathway, which avoids the rate-limiting transesterification steps between the acyl-lipids and the acyl-CoA-pool during VLCPUFA biosynthesis. The pathway is defined by previously uncharacterized enzymes, encoded by cDNAs isolated from the microalgae *Mantoniella squamata* and *Ostreococcus tauri*. The conceptual enzymatic pathway was established and characterized first in yeast, in order to provide proof-of-concept data for its feasibility, and subsequently in seeds of *Arabidopsis thaliana*. The comparison of the acyl-CoA-dependent pathway with the known lipid-linked pathway for VLCPUFA-biosynthesis showed that the acyl-CoA-dependent pathway circumvents the bottleneck of switching the ?6-desaturated fatty acids between lipids and acyl-CoA in *Arabidopsis* seeds.

Polyunsaturated fatty acids from Siberian pine seed oil: a perspective source for medicine.

Averina E.S., [1] Pintaeva E.Ts.,[1] Radnaeva L.D.,[1,2]

[1]Baikal institute of nature management SB RAS, Ulan-Ude

[2]Buryat state university, Ulan-Ude

Siberian pine (*Pinus Sibirica*), also known as Siberian cedar, can truly be called a treasure of Siberian nature. Widely spread on the immense territories of Baikal region (Russia), these beautiful huge trees have become a symbol of longevity and stamina. Siberian pine seed oil is a concentrate of powerful antioxidants, mono- and polyunsaturated fatty acids.

Stable tendency to employment of medical preparations and biologically active food additives based on natural raw materials attracts an attention to oils, containing biologically active polyunsaturated fatty acids including omega-3 acids. The concentrate of pine seed oil enriched by polyunsaturated fatty acids was obtained by method of complex formation with urea. Investigation by gas chromatography of fatty acid composition of concentrate has revealed the high concentration of biologically active polyunsaturated fatty acids, including essential: oleic – 1,7 %, linoleic - 50,7 %, linolenic –43,4 %, eicosadenoic - 2,4 %, arachidonic – 1,6 % acids.

Liposomal preparations based on concentrate of polyunsaturated fatty acid of pine seed oil, including tibetan phytocollection are possess to increase the immunnobiological and hepatoprotective activities of liposome-encapsulated medicine.

The amphiphilic structure of polyunsaturated fatty acids allows to synthesize polymers with superficially-active properties. The medicinal systems based on esterified hyperbranched polyglycerine by fatty acids from pine seed oil are capable to hydrolise with forming the nontoxic products of the disinteration (polyglycerine, aliphatic fatty acids), and also to liberate the essential fatty acids.

Thus, synthesis of the polymeric and liposomal systems based on concentrate of fatty acids from pine seed oil opens wide prospects for creation of medicinal preparation and their carriers.

A novel fatty acyl-coenzyme A synthetase (ACOS5) is required for pollen development and sporopollenin biosynthesis in *Arabidopsis*

Erich Kombrink[1], Clarice de Azevedo Souza[2], SungSoo Kim[2], Stefanie Koch[1], Lucie Kienow[1], Katja Schneider[1], Sarah M. McKim[2], George Haughn[2], and Carl J. Douglas[2],

[1] Max-Planck-Institute for Plant Breeding Research, Köln, Germany,

[2] Department of Botany, University of British Columbia, Vancouver, Canada

Arabidopsis thaliana contains a large number of genes that encode carboxylic acid-activating enzymes, including nine long-chain fatty acyl-CoA synthetases (LACS), four 4-coumarate:CoA ligases (4CL), and twenty-five 4CL-like proteins of largely unknown biochemical function. We systematically explored the functions of these proteins by applying an extensive substrate screen to recombinant and affinity-purified proteins. This analysis uncovered that activation of fatty acids of variable chain length is the common feature of all active members of this protein family, thereby defining a new group of fatty acyl-CoA synthetase, which is distinct from the known LACS family. Phylogenetic analysis showed that one protein of this family, named ACOS5 (At1g62940), is conserved in several angiosperm lineages as well as in the moss *Physcomitrella patens*. Although closely related to 4CLs, ACOS5 has no significant activity towards hydroxycinnamic acids but rather has a preference for hydroxy-fatty acids of variable chain length. The *ACOS5* gene has a flower-prefered expression pattern in all angiosperms that have been examined. We demonstrate that the *Arabidopsis* mutant *acos5* produced no pollen in mature anthers, no seeds upon self-fertilization, and was severely compromised in pollen wall formation with an apparent lack of sporopollenin or exine. Combination of light and electron microscopy revealed that the phenotype was first evident at stage 8 of anther development, which correlated with maximum *ACOS5* mRNA accumulation in tapetum cells at stages 7-8. Promoter-GUS fusions likewise demonstrate that expression of the *ACOS5* gene is restricted to the tapetum, whereas GFP fusions showed that ACOS5 protein is located in the cytoplasm. We propose that *ACOS5* encodes a novel fatty acyl-CoA synthetase that participates in a conserved and ancient biochemical pathway(s) required for sporopollenin monomer biosynthesis, and may work together in this pathway with other enzymes suggested to participate in sporopollenin biosynthesis in *Arabidopsis*, such as CYP703A2 (fatty acid hydroxylase) and MS2 (fatty acid reductase).

Towards an Understanding of Cutin and Suberin Biosynthesis

Mike Pollard, Fred Beisson, Yonghua Li, Isabel Molina,
Weili Yang, John Ohlrogge
Department of Plant Biology, Michigan State University
East Lansing, MI 48824, USA

The plant lipid polymers cutin and suberin are complex materials with multiple barrier functions, for example in the control of water and ion fluxes, and to restrict pathogen penetration. Additional functions may include maintenance of organ identity, abscission, cell wall signaling, and in certain cases provision of a cell wall structural component. Both forward and reverse genetic screens, particularly with Arabidopsis, have lead to the identification of genes that are required for biosynthesis as well as for the transcriptional regulation of biosynthesis. Analysis of Arabidopsis KO lines and of lines where putative biosynthetic genes are ectopically expressed has helped identify "general" biochemical function, and in many cases pointed to biological function. In particular, genes for P450 oxidases, glycerol-3-phosphate acyltransferases (GPATs) and acyl-CoA synthetases are prominent. Genes for glycerol-methanol-choline oxidases, lipases and ABC transporters may also play important biosynthetic roles. In our lab we have focused on GPAT4/6/8, which are required for cutin synthesis, on GPAT5, which is associated with aliphatic suberin synthesis, and on a clade of BAHD acyltransferases, one of which clearly has a role in the aromatic suberin biosynthesis. These studies will be reviewed. Despite the identification of an increasing number of genes, the exact biochemical functions of the enzymes identified to date remains uncertain, as do the pathways that lead to the assembly of cutin and suberin. Furthermore, a comprehensive description of the polymer structures of cutin and suberin is lacking. To begin to tackle the biochemical issues we have initiated a comparative study of recombinant GPAT and BAHD enzymes. A series of normal, ?-hydroxy and α,ω-dioic acid acyl-CoAs have been synthesized for the GPAT study. Although this study is still at an early stage, the enzyme activities observed are consistent with function. Progress will be described.

Mutations in genes encoding long chain fatty acid modification effect barrier properties of suberized tissues

R. Franke, L. Schreiber, Bonn/D

Land-living higher plants are characterized by lipophilic plant/environment interfaces characterized by specific cell wall modifications such as cutin, waxes and suberin. Suberized cell walls provide physiologically important barriers limiting the apoplastic flow of water and dissolved ions, thereby preventing uncontrolled water and nutrient flow. In addition suberized tissue provides a barrier for pathogen attacks and is also formed upon wounding. Chemically, suberin is a heterogeneous polyester build of aromatic phenylpropanoids and aliphatic, oxygenated fatty acid derivatives, ranging in chain length from C16 to C34. The aliphatics are responsible for the barrier properties of suberized cell walls, therefore fatty acid elongating enzymes, such as ketoacyl-CoA synthases (KCS), and enzymes capable of fatty acid hydroxylation, e.g cytochrome P450 (CYP), have been proposed as essential factors in suberin biosynthesis. Reverse genetics approaches have identified several suberin-involved KCS and CYPs, which effect suberin amount and/or composition when transcriptionally modified. Both, modifications in chain-length distribution and compositional alterations lead to significant changes in the permeability of suberized tissues. Furthermore, Arabidopsis and rice plants with enhanced root suberin amounts show an increased tolerance towards environmental stress condition such as drought and salinity.

Activation of wax production by *CER1* ovexpression in Arabidopsis confers drought tolerance but enhances pathogen susceptibility.

Joubès J[1], Bourdenx B[1], Bernard A[1], Pascal S[1], Domergue F[1], Pervent M[2], Vile D[2], Leger A[3], Roby D[3] and Lessire R[1]

1-Laboratoire de Biogenèse Membranaire, Université Victor Ségalen Bordeaux 2, CNRS, UMR5200, Bordeaux, France. 2-LEPSE, UMR 759 INRA-SUPAGRO, IBIP, Montpellier, France. 3-LIPM, UMR CNRS/INRA 2594/441,Castanet-Tolosan Cedex, France.

The aerial portions of plants are covered with a continuous extracellular layer of hydrophobic material, the cuticle, which plays an important role in protecting plants from water and solute loss, UV irradiation, mechanical damage, as well as pathogen and insect attack. The cuticle is a rather thin membrane consisting of a polymer matrix (cutin) and associated solvent-soluble lipids (cuticular waxes). Cuticular wax is a general term for complex mixtures of homologue series of very long chain aliphatic lipids issued from the VLCFAs with predominant chain lengths from 20 to 32 carbons. The wax compounds are produced in the epidermal cells of plants according to two different pathways: an acyl reduction pathway, which produces primary alcohols and wax esters, and a decarbonylation pathway, leading to the formation of aldehydes, alkanes, secondary alcohols and ketones. In Arabidopsis, the decarbonylation pathway leads mainly to the formation of alkanes which represent more than 70% of total wax components in leaves and more than 50% in stems. To learn more about the regulation of cuticular wax biosynthesis in *Arabidopsis thaliana*, we focused on the *CER1* gene which encodes a putative aldehyde decarbonylase involved in alkane production. The analysis of an Arabidopsis *cer1* mutant and *CER1* overexpressing transgenic lines shows a strong correlation between *CER1* expression, alkane content and cuticle properties. Overexpression of *CER1* induces an increase in the total wax amount and especially in the alkane amount. Overproduction of the leaf wax alkanes increases the resistance to hydric stress. Surprisingly the modification of wax quantity and quality increases the sensitivity of the plants to pathogen infection. Overall data show that correct wax biosynthesis is essential for plant response to biotic and abiotic stresses.

Engineering synthesis and accumulation of novel industrial fatty acids in oilseeds

Allan Green[1], Paul Dribnenki[2], Qing Liu[1], Surinder Singh[1],
Craig Wood[1] and Xue-Rong Zhou[1]
[1] CSIRO Plant Industry, Canberra, ACT, Australia
[2] Viterra, Vegreville, Alberta, Canada

Oilseeds have the potential to provide renewable, cost-competitive and environmentally friendly sources of industrial oils as alternatives to those currently derived mainly from non-renewable and increasingly costly petroleum sources. In contrast to edible oilseeds that typically contain only a small number of fatty acids, there is enormous genetic diversity in fatty acid structure in nature, with many molecules having considerable industrial potential if they could be produced in large volume at low cost. Significant progress has already been made in cloning genes for key enzymes responsible for the introduction of a range of new functionalities (such as acetylenic and conjugated bond systems, hydroxy and epoxy groups) into conventional fatty acids. However, transgenic expression of these enzymes in oilseeds has consistently resulted in disappointingly low accumulation (< 20%) of the functionalised fatty acid compared to the very high levels (> 80%) often found in the species where the genes were sourced from. Biochemical analysis has suggested that a significant impediment to increased accumulation of unusual fatty acids (UFAs) in these transgenic oilseeds may be the inefficient transfer of the UFAs from the metabolic pool in which they are synthesized to the principal oil storage molecule, triacylglycerol (TAG). It is reasonable to expect that oilseeds that do not naturally synthesise these UFAs may not be equipped with phospholipases and acyltransferases capable of efficiently handling their divergent molecule structures. In contrast, organisms that naturally produce oils with extremely high concentrations of UFAs must have the requisite enzymatic machinery to efficiently process these UFAs. This presentation will outline recent progress in reverse engineering strategies to develop oilseeds with high levels of epoxy and hydroxy fatty acids by elucidating the TAG assembly routes used by wild plants that are rich in these UFAs, and then transgenically expressing appropriate TAG assembly pathway enzymes in oilseeds.

Increasing erucic acid content through combination of endogenous low polyunsaturated fatty acids alleles with *Ld-LPAAT+Bn-fae*1 transgenes in rapeseed (*Brassica napus* L.)

Ujjal K. Nath[1], Jeroen A. Wilmer[2,3], Emma J. Wallington[2,4], Heiko C. Becker[1] and Christian Möllers[1]

[1] Department of Crop Sciences, Plant Breeding, Georg–August–Universität Göttingen, Von–Siebold–Str. 8, 37075 Göttingen, Germany. Email: cmoelle2@gwdg.de

[2] BIOGEMMA UK Ltd., 200, Science Park, Cambridge, CB4 0GZ, United Kingdom,

[3] current address: BIOGEMMA, Domaine de Sandreau, Chemin de Panedautes, 31700 Mondonville, France

[4] current address: NIAB, Huntingdon Road, Cambridge CB3 OLE, United Kingdom

High erucic acid rapeseed (HEAR) oil is of interest for industrial purposes because erucic acid (22:1) and its derivatives are important renewable raw materials for the oleochemical industry. Currently available cultivars contain only about 50% erucic acid in the seed oil. A substantial increase in erucic acid content would significantly reduce processing costs and could increase market prospects of HEAR oil. The objective of the present study was to increase erucic content of HEAR winter rapeseed through over expression of the rapeseed fatty acid elongase gene (*fae*1) in combination with expression of the lysophosphatidic acid acyltransferase gene from *Limnanthes douglasii* (*Ld-LPAAT*), which enables insertion of erucic acid into the *sn*-2 glycerol position. Furthermore, mutant alleles for low contents of polyunsaturated fatty acids (18:2+18:3) were combined with the transgenic material. Selected transgenic lines showed up to 63% erucic acid in the seed oil in comparison to a mean of 54% erucic acid of segregating non-transgenic HEAR plants. Among 220 F_2 plants derived from the cross between a transgenic HEAR line and a non-transgenic HEAR line with a low content of polyunsaturated fatty acids, recombinant F_2 plants were identified with an erucic acid content of up to 72% and a polyunsaturated fatty acid content as low as 6%. Regression analysis revealed that a reduction of 10% in polyunsaturated fatty acids content led to a 6.5% increase in erucic acid content. Results from selected F_2 plants were confirmed in the next generation by analysing F_4 seeds harvested from five F_3 plants per selected F_2 plant. F_3 lines contained up to 72% 22:1 and as little as 4% polyunsaturated fatty acids content in the seed oil.

Nath, U.K., J. A. Wilmer, E. J. Wallington, H.C. Becker, and C.Möllers, 2008: Increasing erucic acid content through combination of endogenous low polyunsaturated fatty acids alleles with *Ld-LPAAT* + *Bn-fae* 1 transgenes in rapeseed (*Brassica napus* L.). Theoretical an Applied Genetics: DOI:10.1007/s00122-008-0936-7.

Probing Structure and Function in Diacylglycerol Acyltransferases from Plants and Yeast

Qin Liu[1], Rodrigo M.P. Siloto[1], Martin Truksa[1], Thomas McKeon[2], Xiaohua He[2], Randall J. Weselake[1]

[1] University of Alberta, Edmonton, Canada

[2] United States Department of Agriculture, Albany, USA

Acyl-CoA;diacylglycerol acyltransferase catalyzes the acylation of *sn*-1, 2-diacylglycerol to generate triacyglycerol and CoA. The level of DGAT activity during seed maturation may have a substantial effect on carbon flow into triacylglycerol. DGAT with improved catalytic efficiency and/or modified substrate selectivity could have important applications in crop biotechnology. Such efforts, however, are hampered by our limited knowledge of structure/function relationships in DGAT. We have been using different approaches to gain insights into the function of two non-homologous DGATs, a castor bean DGAT1 (*Ricinus communis*, RcDGAT1) and a budding yeast DGAT2 (*Saccharomyces cerevisiae*, ScDGAT2). When expressed in yeast these truncations confirmed the importance of the C-terminal regions of both enzymes which were critical for maintaining enzyme activity. We have also demonstrated that an internal hydrophilic segment of yeast DGAT2, absent in genes from animals, plants and most fungi, greatly affects ScDGAT2 activity. Currently, we are using cysteine-scanning mutagenesis combined with thiol modification to study the topology of ScDGAT2. Independent conversions of all seven cysteine residues to alanine had little effect on enzyme activity, suggesting that cysteine may not be essential for ScDGAT2 activity. In an alternative approach, currently focused on *Brassica napus* DGAT1 (BnDGAT1), we have developed a high throughput system to select genes encoding active DGAT mutants in randomly mutagenized libraries. Our first application of this technology has focused on increasing the catalytic efficiency of BnDGAT1, but information derived from this study will also shed light on structure-function relationships in this important enzyme.

Engineering healthy plant oils: Sustainable production of marine long-chain fatty acids in land based plants

Joerg Bauer, Thorsten Zank, Ernst Heinz[+], Guohai Wu*, Xiao Qiu*, Anne-Marie Kuijpers, Josef Kuhn, Andreas Mordhorst, Toralf Senger

BASF Plant Science Company GmbH, Germany, [+] University of Hamburg, Germany, * Bioriginal Food&Science Corp., Canada

Very long-chain polyunsaturated fatty acids (VLC-PUFA) are essential for human health and well-being. Numerous scientific studies have shown the importance of VLC-PUFA such as arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Dietary VLC-PUFA not only provide protection against common chronic diseases such as cardiovascular diseases, metabolic syndrome and inflammatory disorders, but can also enhance the performance of the eyes, brain and nervous system.

However, the current sources of these valuable compounds from fish oil or fermentation are limited and may not be sustainable in the long term, limiting the availability for a broad population.

Considerable progress has been made in identifying genes involved in the biosynthesis of VLC-PUFA. Recently, the successful reconstitution of pathways leading to the synthesis of ARA, EPA and finally DHA in oil-seed plants has been shown by different groups. Our and other approaches indicate the feasibility of using transgenic crops as alternative sources of VLC-PUFA (e.g. Wu et al., Nat. Biotech 2005).

Different approaches used to engineer transgenic crops producing VLC-PUFA and their constraints will be discussed. A focus will be put on precise expression of the various pathway genes and the finding of new genetic elements for timely and spatial expression of pathway genes in seeds of *Brassica napus*. The further use of the new genetic elements as valuable tools in modifying oil biosynthesis or other seed-specific processes in *Brassica* species will be addressed.

DGD can be functionally replaced with a bacterial glycolipid during phosphate starvation, but not during photosynthesis

Georg Hölzl[1], Sandra Witt[2], Michael Melzer[3] and Peter Dörmann[1]

[1] Institute for Molecular Physiology and Biotechnology of Plants, Bonn, Germany

[2] Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany

[3] Department of Molecular Cell Biology, Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany

The two galactolipids monogalactosyldiacylglycerol (MGD) and digalactosyldiacylglycerol (DGD) represent the main lipid building blocks for thylakoid assembly. Analysis of the DGD-deficient Arabidopsis mutant *dgd1* and the DGD-free double mutant *dgd1dgd2*, which are characterized by dwarf phenotypes and reduced photosynthetic activity, demonstrated the importance of galactolipids for growth and photosynthesis. Galactolipids were identified at specific sites within crystallized photosynthetic protein complexes implying specific galactolipid functions during electron transport. Under phosphate deficiency, the galactolipid DGD replaces plastidial and extraplastidial phospholipids. To reveal more details on the roles of galactolipids during growth and photosynthesis, DGD was replaced with glucosylgalactosyldiacylglycerol (GGD) by introducing a bacterial glucosyltransferase (GlcT) into *dgd1* or *dgd1dgd2*. Growth and chloroplast ultrastructure of the GGD accumulating mutants (*dgd1*-GlcT, *dgd1dgd2*-GlcT) were similar to wild type. Chlorophyll content and photosynthesis under normal light were only partially restored. Cultivation under high light resulted in a drastic decrease of photosystem II quantum yield in the DGD-free *dgd1dgd2* double mutant and in *dgd1dgd2*-GlcT. The lower quantum yield of *dgd1dgd2*-GlcT compared to *dgd1*, however, did not affect growth. We conclude that the *dgd1* growth phenotype is not primarily caused by a reduced photosynthetic activity, but it can be attributed to a reduced capacity for chloroplast membrane assembly. Under phosphate deficiency, GGD can serve as surrogate lipid for DGD, and it accumulates in plastidial and extraplastidial membranes. GGD accumulation and transport presumably is regulated via the same mechanisms as for DGD.

A novel class of MGDG synthase inhibitors

Eric Maréchal, CNRS
Grenoble, France

Monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) are the most abundant lipids of photosynthetic membranes and are essential for plastid biogenesis and function, particularly for photosynthesis in green tissues. These galactolipids are synthesized by specific galactosyltransferases localized in the envelope membranes of plastids, namely MGD and DGD. In Arabidopsis, the multigenic family of MGDG synthases comprises three members, i.e. atMGD1, atMGD2 and atMGD3. AtMGD1 is the most abundant and active in green tissues, is localized in the envelope inner membrane and is essential for the biogenesis of thylakoids.
In this presentation, we describe a high throughput screening of molecules allowing the identification of two inhibitors of atMGD1. We will present the most active structures based on the study of analogues, and the phenotype analyses of Arabidopsis plants treated at non-lethal doses (chlorophyll content, protein profile, gene expression profile, lipidome profile).

Molecular cloning and expression analysis of a phospholipid: Diacylglycerol acyltransferase (PDAT) gene from olive

José M. Martínez-Rivas and Ana Pérez
Instituto de la Grasa, CSIC, Sevilla, Spain

Unlike oilseeds, little is known on the fatty acid and glicerolipid biosynthesis in oil fruits. Olive fruit represents an interesting system to investigate the regulation of fatty acid and lipid metabolism, because it contains two oil accumulating tissues, the seed and the mesocarp. Moreover, the mesocarp tissue possesses the remarkable characteristic of having high triacylglycerol (TAG) content together with active chloroplasts. Furthermore, because the olive fruit mesocarp is photosynthetic and, indeed, provides directly about half of the total carbon for oil synthesis, then it contains membranes which are highly unsaturated.

Continuing with the molecular characterization of the enzymes involved in TAG biosynthesis and oleate desaturation in olive fruit, we have isolated a full-length cDNA clone encoding a phospholipid:diacylglycerol acyltransferase (*OepPDAT*) from an olive fruit (cv. Picual) cDNA library, using a PCR approach. Alignment of the sequence and phylogenetic analysis revealed a high degree of identity to known plant PDAT sequences. In addition, its expression level has been determined in different olive tissues, particularly in mesocarp and seeds from olive fruits corresponding to Picual and Arbequina cultivars at different developmental stages. The possible contribution of PDAT to olive oil accumulation in olive fruit will be discussed.

CDP-diacylglycerol synthases of *Arabidopsis thaliana*

André Haselier and Margrit Frentzen

RWTH Aachen University, Institute of Biology I, Botany, 52056 Aachen, Germany

Cytidine-diphosphate-diacylglycerol (CDP-DAG) synthases (CDSs) are integral membrane proteins that catalyse the formation of CDP-DAG from cytidine-triphosphate and phosphatidic acid. CDP-DAG serves as a central intermediate in glycerolipid biosynthesis of both prokaryotic and eukaryotic organisms. In plant cells it is the direct precursor of phosphatidylinositol, -serine, -glycerol and cardiolipin and is supposed to be involved in regulating glycerolipid biosynthesis as well as in lipid dependent signal transduction processes. Plant CDSs have been shown to be located in plastids, mitochondria and the endomembrane system, but apart from their subcellular localisation very little is known about these isozymes and the respective genes.

The genome of *Arabidopsis thaliana* appears to possess five CDS genes which encode eight different proteins. The respective cDNAs have been cloned and expressed in suitable *Saccharomyces cerevisiae* mutants to experimentally verify their identity and to analyse the properties of the encoded proteins. In addition, the cDNAs have been expressed as fluorescing fusion proteins in tobacco cells to determine the subcellular localisation of the encoded proteins in plant cells. We have also started to develop and analyse single and double knockout T-DNA mutant lines of *A. thaliana* to investigate the functional role of CDSs *in planta*. The results of these experiments especially with regard to plastidial isozymes will be presented.

Fatty Acid Amide Hydrolase Expression Influences Plant Growth and Susceptibility to Environmental Stresses.

Chapman, K.D.[1], Blancaflor, E.B.[2], Venables, B.J.[1], and Mysore, K.S.[2]

[1]University of North Texas, Center for Plant Lipid Research, Department of Biological Sciences, P.O Box 305220, Denton, TX 76203 USA; [2]Samuel R. Noble Foundation, Plant Biology Division, 2510 Sam Noble Parkway, Ardmore, OK 73401 USA

N-Acylethanolamines (NAEs) are fatty acid derivatives that occur mostly at trace levels in both animal and plant systems. These lipids exhibit diverse biological activities including the modulation of motor, cognitive and immune function in vertebrates and the regulation of growth in plant systems. The machinery for the metabolism of NAEs is highly conserved in multicellular eukaryotes and includes, in part, the termination of lipid mediator function by a fatty acid amide hydrolase (FAAH). A functional homologue of the rat FAAH enzyme was identified in Arabidopsis and it is encoded by the At5g64440 locus. Disruption of this FAAH gene resulted in enhanced sensitivity to growth inhibition by NAE, whereas the ectopic overexpression of this AtFAAH cDNA resulted in tolerance to the negative growth effects of NAEs. AtFAAH overexpressors actually exhibited enhanced growth phenotypes under normal growth conditions (compared with wildtype plants), but they were remarkably susceptible to both abiotic and biotic insults. Consistent with increased susceptibility to abiotic stresses, AtFAAH overexpressing lines also exhibited enhanced sensitivity to the negative growth effects of ABA. Overexpression of an inactive, recombinant FAAH protein (active-site mutation) in the AtFAAH T-DNA insertional mutant background maintained ABA sensitivity, but eliminated the enhanced growth phenotype, suggesting that FAAH may function independent of its catalytic activity in its interaction with ABA. Progress toward identifying FAAH interacting proteins in Arabidopsis and the discovery of additional FAAH proteins in Arabidopsis will be presented. A better understanding of the role(s) for FAAH *in planta* will help to explain its position at the balance between plant growth and responses to stress.

Salt-stress-induced Association of Phosphatidylinositol-4,5-bisphosphate with Clathrin-coated vesicles in plants

Sabine König, Till Ischebeck, Jennifer Lerche, Irene Stenzel, and Ingo Heilmann
Department of Plant Biochemistry, Albrecht-von-Haller-Institute for Plant Sciences, Georg-August-University Göttingen, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany

Plants exposed to hyperosmotic stress undergo changes in membrane dynamics and lipid composition to maintain cellular integrity and avoid membrane leakage. Various plant species respond to hyperosmotic stress with transient increases in phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2), however, the physiological role of such increases is unresolved. The plasma membrane represents the outermost barrier between the symplast of plant cells and its apoplastic surroundings. Here, the spatio-temporal dynamics of stress-induced changes in phosphoinositides (PIs) were analyzed in subcellular fractions of *Arabidopsis* leaves to delineate possible physiological roles. Unlabeled lipids were separated by thin-layer-chromatography and quantified according to gas-chromatographic detection of associated fatty acids. Transient PtdIns(4,5)P_2 increases upon hyperosmotic stress were detected first in enriched plasma membrane fractions, however, at later time points PtdIns(4,5)P_2 increased in endomembrane-fractions of the corresponding two-phase systems. When major endomembranes were enriched from rosette leaves prior to hyperosmotic stress and over 60 min of stimulation, no stress-induced increases in the levels of PtdIns(4,5)P_2 were found in fractions enriched for endoplasmic reticulum, nuclei, or plastidial membranes. Instead, increased PtdIns(4,5)P_2 contents were found in clathrin-coated vesicles (CCVs), which proliferated several-fold in mass within 60 min of hyperosmotic stress according to the abundance of CCV-associated proteins and lipids. Monitoring the subcellular distribution of fluorescence-tagged reporters for clathrin and PtdIns(4,5)P_2 during transient coexpression in onion epidermal cells indicates rapid stress-induced colocalization of clathrin with PtdIns(4,5)P_2 at the plasma membrane. The data indicate that PtdIns(4,5)P_2 may act in stress-induced formation of CCVs in plant cells, highlighting evolutionary conservation of the PI system between organismic kingdoms.

PIPK family in the moss *Physcomitrella patens*. PpPIPKs are required for caulonemal and rhizoid cell elongation.

Laura Saavedra[1,4], Stephen Dove[2], Koji Mikami[3] and Marianne Sommarin[1,4].

[1]Center for Chemistry and Chemical Engineering, Lund University, Lund, Sweden

[2]School of Biosciences, University of Birmingham, Birmingham, UK

[3]Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Japan

[4]Umeå Plant Science Centre, Umeå University, Umeå, Sweden

Phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P_2] plays a major role in many cellular processes such as exocytosis, intracellular vesicular trafficking and cytoskeletal regulation. We are focusing on phosphatidylinositol phosphate kinase (PIPK), which catalyzes the production of PtdIns(4,5)P_2, using the moss *Physcomitrella patens* as a model system due to advantageous features including its high frequency of homologous recombination, a simpler pattern of development compared to flowering plants, and a haploid gametophyte that dominates the moss life cycle. *P. patens* has only two PIPK genes, *PpPIPK1* and *PpPIPK2*, with differently regulated expression and with protein sequences displaying a conserved PIPK catalytic domain and nine MORN (Membrane Occupation Recognition Nexus) domains in accordance with the description of PIPKs class I/IIB in higher plants. In vitro biochemical characterization showed that the two enzymes exhibited different substrate specificities. Interestingly, PpPIPK1 can synthesize PtdIns(3,4,5)P_3, a PI which has not yet been detected in intact plant cells. In order to study the physiological role of these proteins, we have disrupted *PpPIPK1* and *PpPIPK2* by gene targeting and our results show a strong phenotype for *pipk1* but not for *pipk2* single knock outs. *Pipk1* lines are delayed in growth, protonemal filaments show irregular branching patterns, and gametophores are impaired in rhizoid and caulonemal cell elongation. In addition, double *pipk1-2* lines show a dramatic phenotype with defects in cell and gametophore morphology manifested as one short cell type in the protonemal tissue, the lack of the rapidly elongating caulonemal cell type, and leafy gametophores with almost absence of rhizoids. Our data support an essential role for PpPIPKs in cell growth and further analysis are in progress to more in detail understand the role of PtdIns(4,5)P_2 in cell elongation.

Requirement of phosphoinositide-derived signals in the wounding response of *Arabidopsis thaliana*

A. Mosblech, C. Göbel, I. Feussner, and I. Heilmann

Department of Plant Biochemistry, Albrecht-von-Haller-Institute for Plant Sciences, Georg-August-University Göttingen, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany

Various biochemical signals are implicated in *Arabidopsis* wound signaling, including jasmonic acid (JA), salicylic acid, auxin, and Ca^{2+}. We have previously reported on cross-talk of oxylipin signals with phosphoinositide-signals not previously implicated in plant wound-responses (Mosblech et al., Mol Plant 1: 249-261, 2008).

By using *InsP 5-ptase*-plants with attenuated phosphoinositide-signaling, we could show that the induction of wounding-inducible genes was attenuated compared to wild-type-plants, suggesting a role for phosphoinositide-signaling in mediating plant wound-responses. Genes with severely attenuated wound-induction patterns included candidates for defensins, protease inhibitors, or transcription factors of the WRKY-family, which represent major control hubs for wound-induced gene expression. The analysis of multiple gene-expression patterns suggests that phosphoinositides contribute to both JA-dependent and JA-independent aspects of wound-signaling to mediate defense responses in *Arabidopsis*. In support of compromised defensive capabilities of *InsP 5-ptase*-plants, weight gain of *Plutella xylostella* caterpillars feeding on *InsP 5-ptase*-plants was increased compared to that of caterpillars feeding on wild type-plants. While gene expression data and the caterpillar experiments suggest an attenuated defense response in the absence of phosphoinositide-derived signals, detailed biochemical analysis of oxylipin precursors and conjugates of JA indicates that the levels of these compounds are substantially higher in *InsP 5-ptase*-plants than in wild type plants analyzed in parallel.

Because an inositol-polyphosphate might be required for proper functioning of the jasmonate receptor, COI1, *ipk1-1* plants with intact phosphoinositide-signaling but attenuated in the formation of inositol-hexakisphosphate (IP_6) were analyzed. *ipk1-1* plants showed elevated wound-induced levels of jasmonates as did *InsP 5-ptase*-plants, however, contrary to our expectation *ipk1-1* plants were more sensitive to MeJA treatment. Also, *Plutella xylostella* caterpillars performed worse on the IP_6-deficient plants compared to wild type-plants. The data suggest that plants with reduced contents of phosphoinositide-derived signals are partially insensitive to the feed forward regulation of JA biosynthesis. JA perception via COI1 may be dependent not on IP_6 but another inositolpolyphospohate, for instance IP_5.

JAZ Repressor Proteins Control Jasmonate Signaling

John Browse, Bryan Thines and Yajie Niu

Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340, USA

Jasmonate (JA) and related signaling compounds are required for flower development and also regulate many other plant processes, particularly defense responses against both insects and pathogens. However, the molecular details of the signaling mechanism are poorly understood. *Arabidopsis* mutants that are unable to synthesize JA, such as the *fad3-2 fad 7-2 fad8* and *opr3* mutants, are male-sterile and stamen development arrests at stage 12. In these mutants, fertility can be restored by exogenous application of JA. Mutations in the F-box protein COI1 also abolish JA responses. *coi1* mutant plants are male-sterile but the phenotype cannot be rescued by application of JA. To identify JA-responsive genes that are critical to male fertility, we carried out transcriptional profiling over the course of 22 hours following JA treatment of *Arabidopsis opr3* stamens, using Affymetrix ATH1 arrays. From this investigation, we identified members of a gene family without previously ascribed function, where eight of these genes were rapidly induced by JA. We have named the gene products JAsmonate Zim-domain (JAZ) proteins and shown that they are key regulators of jasmonate signaling. JAZ proteins act to repress transcription of jasmonate-responsive genes. Jasmonate treatment causes JAZ degradation and this degradation is dependent on activities of the SCF^{COI1} ubiquitin ligase and the 26S proteasome. Furthermore, the jasmonoyl-isoleucine (JA-Ile) conjugate, but not other jasmonate-derivatives (such as JA, 12-oxo-phytodienoic acid, or methyl-JA), promotes physical interaction between COI1 and JAZ proteins in the absence of other plant proteins. Our results suggest a model in which jasmonate ligands promote the binding of the SCF^{COI1} ubiquitin ligase to and subsequent degradation of the JAZ repressor proteins, and implicate the SCF^{COI1}-JAZ protein complex as a site of perception of the plant hormone JA-Ile. Significantly, the *JAZ* genes are among those that are rapidly and strongly induced by JA. In this way, resynthesis of JAZ proteins can autoregulate the signal to help prevent runaway expression of defense genes and possible tissue damage. Our characterization of the JAZ family provides the basis for understanding the many different roles for JA in the development and environmental responses of plants.

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Arabidopsis class II TGA transcription factors function as salicylic acid-sensitive positive regulators of jasmonic acid/ethylene-induced PDF1.2 expression

Christiane Gatz, Mark Zander, Göttingen, Germany

Abstract

Jasmonic acid/ethylene (JA/ET) and salicylic acid are crucial signaling molecules orchestrating plant defense responses against biotrophic and necrotrophic pathogens, respectively. Whereas the JA- and JA/ET-dependent pathways depend on the the regulatory proteins COI1, AtMYC2, JAZ repressors, and AP2 transcription factors ERF1 and ORA59, the SA pathway relies on NPR1 and TGA and WRKY transcription factors. Here we show that the triple mutant *tga6-1 tga2-1 tga5-1* is also compromised with respect to induction of the JA/ET-responsive genes *PDF1.2* and *bChi* and that it is more susceptible to the necrotrophic fungus *Botrytis cinerea*. JA/ET-induced *PDF1.2* expression is restored to wild-type levels in the *tga6-1 tga2-1 tga5-1 jin1-1* quadruple mutant, which does not express transcription factor AtMYC2. In this mutant background, JA/ET-induced expression of the AP2 transcription factor ORA59, which is a positive regulator of *PDF1.2* expression, is enhanced as compared to wild-type plants. Thus, elevated ORA59 levels can substitute for the lack of class II TGA transcription factors. SA suppressed JA/ET-induced transcription of *PDF1.2* in wild-type and *jin1-1* plants. In contrast, SA had no inhibitory effect in the *tga6-1 tga2-1 tga5-1 jin1-1* quadruple mutant. It is concluded that class II TGA factors act together with ORA59 to activate *PDF1.2* expression and that this activation is compromised by SA. These results describe a novel NPR1-independent function of TGA factors as mediators of the cross-talk between the SA- and the JA/ET pathway.

Different signalling properties of Oxylipins in leaves and flowers of Tomato

C. Wasternack, S. Goetz, A. Hellwege, J. Neumerkel, M. Widder, I. Stenzel[1], R. Kramell, O. Miersch, B. Hause

Leibniz Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle (Saale), Germany

[1]present address: Albrecht-von-Haller-Institute for Plant Sciences, Georg-August University of Goettingen, Juistus-von-Liebig-Weg 11, D-37077 Goettingen, Germany

Oxylipins such as 12-oxophytodienoic acid (OPDA) or jasmonic acid (JA) are signals in plant stress responses and development (1). Beside OPDA and JA, 12-hydroxylated, 12-*O*-sulfated, amino acid- conjugated and *O*-glucosylated JA-derivatives accumulate in different organs and plant species to high levels. In wounded tomato leaves, 12-OH-JA, 12-*O*-Glc-JA and 12-HSO_4-JA accumulate JA-dependently to above 100-fold higher levels than JA but do not induce JA-induced gene expression suggesting partial switch-off in JA-signaling by hydroxylation and subsequent metabolism (2). Using transgenic tomato lines overexpressing a 12-OH-JA - sulfotransferase constitutively, the predominant accumulation of 12-*O*-Glc-JA upon wounding is shifted to the sulfated 12-OH-JA accompanied by an altered flowering phenotype. Such flower-specific signaling properties of oxylipins is also suggested by a distinct “oxylipin signature” in tomato flower organs (3,4) and an OPDA-specific effect on embryo development. The JA- and OPDA-deficient mutant *spr2* but not the OPDA-accumulating and JA-deficient *acx1* mutant is blocked in proper embryo development and can be normalized with OPDA. These data and the strong accumulation of OPDA in the seed coat suggest role of OPDA in embryo development of tomato.

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Crucial regulatory nodes and new physiological scenarios for the Jasmonate Signalling Network in Arabidopsis

Alessandra Devoto, School of Biological Sciences, Royal Holloway - University of London, Egham Hill, Egham, Surrey, TW20 0EX, UK. Email: Alessandra.Devoto@rhul.ac.uk

Plant development and stress responses are regulated by complex signalling networks that mediate specific and dynamic plant responses upon activation by various types of exogenous and endogenous signals. Jasmonates mediate responses to stress and act like growth inhibitors. The latest work on jasmonates (JAs) signalling has identified new regulatory nodes in the transcriptional network that regulates a number of diverse plant responses to developmental and environmental cues. The key elements mediating cross-talk between JAs with other signalling pathways that are activated during stress response will be discussed. Most of the work on JAs has been traditionally done in the context of stress; however, new findings implicating JAs in regulating senescence and plant responses to pathogens suggest a common mechanism of JAs action via distinct groups of transcription factors. In my laboratory, we are interested in discovering the cellular components linking plant stress responses to growth processes with the aim to improve seed production, yield and adaptation of plants to their environment. JAs blocks cell cycle progression by inhibiting G1/S and G2/M transitions in tobacco cells. While the molecular mechanisms and downstream responses have not been clarified yet, we are excited by the likelihood that jasmonate is a distress signal, a physiological role of which is to block cell cycle, slowing vegetative growth during defense responses. A summary of the results obtained so far will be presented.

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Oxylipins contribute to the up-regulation of Auxin Biosynthesis

Stephan Pollmann, Mathias Hentrich, Ruhr-University Bochum, Department of Plant Physiology
Bochum, Germany

In recent years, a number of biotic and abiotic stress conditions have been described to be mediated by signaling molecules of the oxylipin family. In this context, jasmonic acid (JA) as well as its biosynthetic precursor, 12-oxo-phytodienoic acid (OPDA), plays a pivotal role. In a survey for genes that are specifically regulated by either OPDA or JA, we identified a number of intriguing target genes, some of which suggested a direct connection between oxylipin signaling and auxin production. Subsequently conducted genetic and mass spectrometric experiments corroborated our suggestion and provided further evidence for an interconnection of oxylipin signaling and indole-3-acetic acid as well as ethylene production in *Arabidopsis thaliana*. Here we will present first molecular and genetic evidence for an oxylipin mediate induction of two isogenes of the YUCCA family that contribute efficiently to auxin formation when expressed *in planta*. Besides elevated auxin contents and corresponding phenotypic alterations, overexpression lines of these two genes exhibit additional ethylene related phenotypes and an increased resistance towards ethylene biosynthesis inhibitors. In conclusion, our data unveil the existence of a so far undiscovered oxylipin-dependent induction of an auxin-ethylene loop which is seemingly involved in plant wound responses.

Jasmonates in symbiotic interactions of *Medicago truncatula*

Bettina Hause, Cornelia Mrosk, Jan Heumann, Leibniz Institute of Plant Biochemistry, Halle, Germany

The mutualistic interactions between plants and arbuscular mycorrhizal (AM) fungi or nitrogen-fixing rhizobacteria are believed to be regulated from the plant side among other signals by the action of phytohormones. Jasmonates, known as regulators in plant response to biotic or abiotic stresses, are good candidates for such a role.

Therefore, the functional analysis of jasmonates during the interaction between barrel medic (*Medicago truncatula*) and *Shinorhizobium meliloti* or *Glomus intraradices* was performed. The capacity of roots of *M. truncatula* to synthesize JA was changed by transformation with *A. rhizogenes* leading to chimeric plants with wild-type shoots and transformed roots. In that way, we achieved a modulation of the transcript level of the *MtAOC1* gene encoding the allene oxide cyclase (AOC), one of the enzymes involved in JA biosynthesis. A role of JA in the interaction of *M. truncatula* with *S. meliloti* leading to the formation of nodules could not be demonstrated. Here, overexpression and partial suppression of *MtAOC1* did not lead to an altered nodule phenotype: Neither the morphology of nodules nor the number of nodules are different in these plants in comparison to control transformations with the empty vector.

In contrast to nodulation, transgenic roots exhibiting partial suppression of *MtAOC1* followed by lower JA levels showed a significant delay in the process of colonization with *G. intraradices*. Both the mycorrhization degree, quantified by fungal rRNA, and the formation of arbuscules, analyzed by the expression level of the AM-specific phosphate transporter gene *MtPT4*, were affected. In conclusion, it appears that jasmonates affect mycorrhization, possibly in multiple ways. Analyses of global transcript profiles of transgenic AM roots revealed changes in the level of several transcripts. Moreover, analyses of the content of secondary metabolites showed that roots with modulated JA content exhibited altered levels of isoflavonoids pointing to a regulatory role of JA in the biosynthesis of these compounds.

Tracing down the Structural diversity of Moss-oxylipins with the help of Chemometric methods

Georg Pohnert, Martin Rempt, Emanuel Croisier, Institut für Anorganische und Analytische Chemie, Bioorganische Analytik, Friedrich-Schiller-Universität Jena, D-07743 Jena, Germany

Mosses are a nearly unexplored resource for structurally new oxylipins. We investigated the transformation of lipids and free fatty acids by the moss *Dicranum scoparium* by means of stable isotope labeled precursors and mass spectrometry. Chemometric evaluation of the rather complex data revealed multiple new pathways towards unusual and novel oxylipins. A broader survey of fatty acid transformations in mosses supported a rather general trend that these non-vascular plants are a diverse source for oxylipins. We will introduce structural elucidation and exploration of HPL-pathways.

Probing the Metabolic and Functional Basis for Sphingolipid Structural Diversity in Plants

E. Cahoon, Chen, M., Lincoln/Nebraska/USA, Markham, J., Saint Louis Missouri/USA

More than 200 different sphingolipid molecular species occur in plants. The structural differences found in plant sphingolipids are in part due to variations in unsaturation and hydroxylation of the long-chain bases (LCBs) and fatty acids that compose the ceramide backbone of sphingolipids. To date, the functional basis for the structural diversity in plant sphingolipids has received limited study, as has the *in planta* activity and interactions of sphingolipid metabolic enzymes that give rise to this structural diversity. Our recent studies are focused on understanding the impact of alterations in LCB hydroxylation and unsaturation on sphingolipid metabolism and on growth and development of Arabidopsis. These structural features of LCBs arise from the activities of LCB C4-hydroxylases and ?4 and ?8 desaturases. We have found that complete loss of LCB C-4 hydroxylation in Arabidopsis double mutants of the two C-4 hydroxylase genes *SBH1* and *SBH2* have greatly reduced growth and do not progress to reproductive stages of growth. These mutants also have a 2.5- to 3-fold higher content of sphingolipid, due primarily to the aberrant accumulation of sphingolipid species with C16 fatty acids rather than the more typical C22 to C26 fatty acids. Such findings highlight the important role that LCB modification reactions can play in mediating growth and sphingolipid metabolism in plant cells. Data will also be presented for phenotypes associated with loss of LCB ?8 unsaturation and enhanced accumulation of ?4 unsaturated LCBs in sphingolipids of Arabidopsis.

Functional analysis of ceramide synthases in Arabidopsis

Faure JD, Bellec Y., Gissot L, Marion J., Molino D., Palauqui JC.
Laboratoire de Biologie Cellulaire INRA Versailles, France (faure@versailles.inra.fr)

Sphingolipids were found in yeast and mammals to be essentials in several basic cellular functions such as endocytosis, protein transport, apoptosis and stress responses. A key step of the sphingolipid biosynthetic pathway is the acylation of long chain bases (LCBs) catalyzed by the sphingoid base N-acyl transferase or ceramide synthase. Ceramide synthases were found to be involved in cell proliferation and cell death in many organisms including plants. However, their precise cellular functions are still poorly known in plants. Three ceramide synthases homologues (named LOHs) have been identified in Arabidopsis and characterised as ER localized proteins (Marion et al. 2008). We are investigating now the role of LOHs enzymes and their sphingolipid products in plant development using both pharmacological and genetic approaches. The absence of ceramide synthesis was found to be essential for plant development. However, reduced levels of ceramide synthesis led to viable plants that showed altered development. In vivo imaging of several endomembrane and plasma membrane markers showed that sub-cellular trafficking and plasma membrane dynamics were also modified. In particular, sub-cellular distribution of membrane proteins will be discussed. A model describing how ceramide synthesis and ceramide-derived molecules could be involved in membrane dynamics and sub-cellular protein distribution will be presented.

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Functions of steryl glucosides in plants, fungi and bacteria

D. Warnecke, Hamburg/D, Basse, CW, Marburg/D, Churin, Y, Berlin/D, Grille, S, Hamburg/D, Heinz, E, Hamburg/D, Hildebrand, J, Hamburg/D, Kusmakow, O, Hamburg/D, Lebrun, A-H, Hamburg/D,

Plants, most fungi and a few bacteria are able to attach a sugar moiety to sterols. Obviously, this headgroup alters the biophysical properties of the membrane lipid, but in contrast to our detailed knowledge of sterols, we have only little knowledge of the physiological functions of steryl glucosides: They have been assigned to cellulose biosynthesis in plants, peroxisome degradation in a yeast and pathogenicity of a bacterium.

To gain deeper insight into the functions of these glycolipids**,** we identified, cloned and characterized several sterol-glucosyltransferase genes from plants, fungi and bacteria. The sterol-ß-glucosyltransferases from plants and fungi belong to the same family of glucosyltransferases whereas a bacterial sterol-α-glucosyltransferase does not show significant sequence similarities to its eukaryotic counterparts. The organisms contain either only a single sterol glucosyltransferase (yeasts, slime molds and bacteria) or several isoenzymes (2-3 in plants, 2-9 in filamentous fungi). Interestingly, the enzymes do not contain any putative transmembrane domains although one of their substrates, the sterol, is membrane-bound.

The fungal sterol glucosyltransferases contain different non-catalytic domains which attach the enzymes to biomembranes via binding to phosphatidylinositol phosphates. In contrast, it still remains unclear how the sterol glucosyltransferases from plants bind to the membranes.

The availability of the sterol glucosyltransferase genes allowed the generation of gene deletion mutants of fungi and bacteria and additional manipulations concerning the steryl glucoside biosynthesis. Analysis of these mutants will improve our knowledge of the functions of steryl glucosides in the degradation of peroxisomes in *Pichia pastoris* and in immune evasion of the human pathogenic bacterium *Helicobacter pylori*.

Chlorophyll and phytol catabolism during senescence and abiotic stress in *Arabidopsis*

Peter Dörrmann, Felix Lippold and Anna Zbierzak,
Institute for Molecular Physiology and Biotechnology of Plants, University of Bonn, Germany; Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany

Chlorophyll hydrolysis during senescence or abiotic stress results in the release of large amounts of phytol, a C20 alcohol derived from plastidial isoprenoid synthesis. Analysis of phytol metabolism revealed that this isoprenoid alcohol is channeled into different metabolic pathways. After two phosphorylation reactions, phytol-diphosphate can be used for tocopherol de novo synthesis. A large proportion of phytol is esterified to fatty acids resulting in fatty acid phytyl ester accumulation in the chloroplasts. We identified candidate genes presumably involved in the conversion of phytol into phytyl esters during senescence and chlorotic stress conditions. In a second line of experiments, we analyzed chloroplast lipid changes during low temperature stress in Arabidopsis wild type and the chilling sensitive mutant *chs1*. Characterization of the *CHS1* mutation on a molecular level will further our understanding on the relationship between abiotic stress and alterations in lipid composition of extraplastidial and plastidial membranes.

Involvement of the Phospholipid Sterol Acyltransferase 1 in Plant Sterol Homeostasis and Leaf Senescence

Pierrette Bouvier-Navé[1], Anne Berna[1], Alexandre Noiriel[1,a], Vincent Compagnon[1], Anders Carlsson[2], Antoni Banas[2b], Sten Stymne[2] and Hubert Schaller[1]

[1]Institut de Biologie Moléculaire des Plantes, CNRS-Université Louis Pasteur UPR 2357 Département Réseau Métabolique, 28 Rue Goethe, F-67083 Strasbourg Cedex, France. [2]Department of Plant Breeding and Biotechnology, Swedish University of Agricultural Sciences. P.O. Box 101, S-230 53 Alnarp, Sweden.
Present addresses: [a]Institut de Chimie et Biochimie Moléculaires et Supramoléculaires, Université Lyon 1, Bâtiment Chevreul-Porte C227, F-69622 Villeurbanne Cedex; [b]Intercollegiate Faculty of Biotechnology UG-MUG, Kladki 24, 80–822 Gdansk, Poland.

Sterol ester forming enzymes identified in plants belong to the Lecithin Cholesterol Acyl Transferases family or to the superfamily of membrane-bound O-acyltransferases. The physiological function of a Phospholipid Sterol Acyl Transferase 1 (PSAT1) and of an Acyl-CoA Sterol Acyl Transferase 1 (ASAT1) was investigated using Arabidopsis T-DNA insertional lines. Sterol ester content was reduced in leaves of both *asat1* and *psat1* mutants, but in seeds of *psat1* mutants only. Interestingly, the amount of sterol esters found in flowers was very close to that of the wild-type for all lines studied. This indicated further functional redundancy of sterol acylation in Arabidopsis. Feeding experiments in which we supplied sterol precursors to *psat1* and *asat1* mutants were performed. This triggered the accumulation of sterol esters (stored in cytosolic lipid droplets) in the wild-type and the *asat1* lines but not in the *psat1* lines indicating therefore a major contribution of the PSAT1 in maintaining free sterol homeostasis in plant cell membranes. An important biological effect associated with the lack of sterol ester formation in the *psat1* mutants was an early leaf senescence. The results presented here suggest that *PSAT1* plays a role in lipid catabolism as part of the intracellular processes at play in the maintenance of leaf viability during developmental aging.

Exocytotic membrane fusion of synaptic vesicles – what we know - or better do not know – about the role of membrane lipids

Reinhard Jahn, Department of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany

Fusion of biological membranes in the secretory pathway is mediated by SNARE-proteins. It is well established that SNAREs operate as nanomachines that assemble into tight helical complexes bridging the two membranes and pulling them together, which ultimately leads to fusion. While most scientists agree that the intermediate non-bilayer transition states are governed, at least in part, by the biophysical properties of membrane lipids, there is surprisingly little knowledge about the role of specific lipids in this process (with the exception of polyphosphoinositides). Indeed, membrane fusion can be robustly reproduced in artificial membrane using a variety of different lipid mixes. Furthermore, SNARE-mediated fusion events are conserved in all eukaryotes which includes species lacking membrane steroids, suggesting further that the SNARE fusion machine is tolerant to a large variety of lipid mixes. Recently, we have carried out a quantitative analysis of the molecular composition of synaptic vesicles, which also includes a quantitative analysis of membrane lipids. Furthermore, we have obtained new insights into the membrane-anchored parts of the SNARE fusion complex. The implications of these findings for the fusion mechanism and for the role of lipids will be discussed.

Peroxisomal Fatty Acid Import and beta-Oxidation are Vitally Important in Mature *Arabidopsis* Leaves during Extended Darkness

Markus Gierth[a], Hans-Henning Kunz[a], Michael Scharnewski[b], Kirstin Feussner[c], Ivo Feussner[b], Ulf-Ingo Flügge[a], Martin Fulda[b]

[a]Department of Botany II, University of Cologne, Köln

[b]Department for Plant Biochemistry, Albrecht-von-Haller-Institute for Plant Sciences, Georg-August-University, Göttingen

[c]Department of Developmental Biochemistry, Institute for Biochemistry and Molecular Cell Biology, Georg-August-University, Göttingen

Arabidopsis mutants impaired in peroxisomal beta-oxidation display a germination and/or seedling establishment phenotype, however, little is known about the function of beta-oxidation in leaves of mature plants. We report a severe phenotype for loss of function mutants in the peroxisomal ABC-transporter PXA1 and the core beta-oxidation enzyme KAT2 in response to extended darkness. After 36 h of dark treatment mutant plants developed leaf necrosis, bleached rapidly after re-transfer into the light and died subsequently. Before any visible symptoms became obvious, a negative impact on photosystem II efficiency could be detected after as little as 16 h of darkness in mutant leaves. Dark-treated mutants accumulated free fatty acids, especially alpha-linolenic acid, and pheophorbide a, a phototoxic chlorophyll catabolite as revealed by analyses of leaf lipid and pigment composition. Interestingly, exposure to exogenous alpha-linolenic acid showed that *pxa* mutants were more susceptible to its toxic effects than wild-type plants. Microscopical analysis of leaf sections from dark-treated plants indicated severe structural damage of *pxa* leaf tissues in general and chloroplasts in particular. We conclude that the accumulation of free polyunsaturated fatty acids causes severe membrane damage in *pxa* and *kat2* plants and propose a model in which fatty acid respiration via peroxisomal beta-oxidation plays a major role in dark-treated plants.

Peroxisomal ATP import is essential for seedling development in *Arabidopsis thaliana*

Nicole Linka, Heinrich-Heine University, Department of Plant Biochemistry, Düsseldorf, Germany;
Frederica L. Theodoulou, Biological Chemistry Department, Rothamsted Research, Harpenden, UK;
Ekkehard H. Neuhaus, University of Kaiserslautern, Department of Plant Physiology, Kaiserslautern, Germany;
Andreas P.M. Weber, Heinrich-Heine University, Department of Plant Biochemistry, Düsseldorf, Germany

Storage lipid mobilization is critical for seed germination. Until the photosynthetic apparatus is established, the seedling depends on degradation of fatty acids released from lipids to fulfil its carbon and energy requirements. The subsequent break down of fatty acids occurs in the peroxisome by the b-oxidation, which requires activation of free fatty acids to their respective Coenzyme A derivatives by ATP dependent Acyl-CoA sythethases. Consequently, loss-of-function in fatty acid uptake and/or activation within peroxisomes leads to seedlings with defective b-oxidation. The inability to synthesize ATP necessitates ATP import into peroxisomes. The existence of transport proteins that supply plant peroxisomes with energy for fatty acid oxidation and other ATP-dependent processes has not previously been demonstrated. Here, we present two *Arabidopsis thaliana* genes that encode peroxisomal adenine nucleotide carriers, PNC1 and PNC2. Both proteins, when fused to enhanced yellow fluorescent protein (EYFP), are targeted to peroxisomes. Complementation of a yeast mutant deficient in peroxisomal ATP import and *in vitro* transport assays using recombinant transporter proteins revealed that PNC1 and PNC2 catalyze the counter-exchange of ATP with ADP or AMP. Transgenic *A. thaliana* lines repressing both *PNC* genes were generated using ethanol-inducible RNA interference (RNAi). A detailed analysis of these plants showed that an impaired peroxisomal ATP import inhibits fatty acid breakdown during early seedling growth and other β-oxidation reactions, such as auxin biosynthesis. We show conclusively that PNC1 and PNC2 are essential for supplying peroxisomes with ATP, indicating that no other ATP generating systems exist inside plant peroxisomes.

Poster Abstracts

Degradation of lipids in oat seeds during germination

Svetlana Leonova, Åsa Ekman, Anders Carlsson and Sten Stymne, Swedish Agricultural University (SLU)
Alnarp, Sweden.

Oat (*Avena sativa*) a traditional crop, differs in one respect from other cereals in that it can accumulate substantial amount of oil in its seeds. Oat cultivars differ in oil content between 3-15% and the difference is due to differences in oil amount in the endosperm cells, which is negatively correlated to the starch content. With the ultimate goal of understanding the biogenesis and biological functions of these oils, different parts of grain (e.g. endosperm, embryo and scutellum) of medium- and high-lipid oat were studied at several stages during germination. Thing layer chromatography and gas chromatography were the methods of the study. Similarities and differences to other species containing oil in the endosperm are discussed.

Changes in storage nutrients in developing tubers of Nutsedge (Cyperus esculentus)

Helle Turesson*, Salla Marttila**, Leif Bulow***, Sten Stymne* and Anders S. Carlsson*
*Department of Plant Breeding and Biotechnology and **Department of Plant Protection Biology,, Swedish University of Agricultural Sciences, Alnarp, Sweden. ***Department of Pure and Applied Biochemistry, Centre for Chemistry and Chemical Engineering, Lund University, Lund, Sweden

Yellow nutsedge (*Cyperus esculentus*) is a perennial C4 plant belonging to the *Cyperaceae* or sedge family. The genus *Cyperus* consists of several hundreds of species of mostly tropical or warm-temperate annuals and perennial herbs, including the well-known “papyrus” (*Cyperus papyrus*). Wild varieties of yellow nutsedge as well as a related species, the purple nutsedge (*Cyperus rotundus*), produces tubers that contain substantial amounts of starch and only small amounts of sugars and oil. However, a cultivated variety of yellow nutsedge (*Cyperus esculentus* var. *sativa*) has much higher content of oil (20-30%) in its mature tubers along with starch (25-35%) and sugar (about 20%).

Using an aeroponic culture system, we have followed the accumulation of starch, oil, sugar and proteins in developing tubers of the cultivated variety of nutsedge during 42 days of tuber development. The overall results show that a sugar peak that accumulated to about 28% of dry weight during the first week is quickly reduced to a content of less than three percentage of dry weight at 15 days. The reduction in sugar is accompanied by concomitant increase in the content of starch and oil reaching 35% and 12% of dry weight at 15 days. Starch content shows minor changes over the remaining time (to 32% of dry weight at 42 days) while oil increases a few percentage over the next two weeks followed by a sharp increase during the last two weeks, reaching more than 25% of dry weight at 42 days. The content of soluble proteins falls from 14% at three days to two percentage after two weeks and remains at this content.

The biochemical data are supported by structural changes shown from analyses by light and electron microscopy. Early presence of starch granules is visible from 7 days and tiny oil droplets are visible from 15 days. At 35 and 42 days starch granules have grown considerably in size and lipid droplets are filling up the remaining space of the cells.

Production of unusual fatty acids: ^{13}C central metabolic flux analysis

Mohamed KOUBAA[1], Abdelghani IDRISSI TAGHKI[1], Albrecht ROSCHER[2], Brigitte THOMASSET[1],

[1]Génie Enzymatique et Cellulaire UMR CNRS 6022, Université de Technologie de Compiègne
Compiègne, France

[2]Génie Enzymatique et Cellulaire UMR CNRS 6022, Université de Picardie
Amiens, France

A new pathway leading to the synthesis of unusual fatty acids in seed oil was created in rapeseed (*Brassica napus* L.). In order to assess and understand the resulting metabolic changes, embryos at 15 days after flowering, were incubated with different ^{13}C labelled subtract ([1-^{13}C]-glucose, ^{13}C-pyruvate, ^{13}C-alanine and $^{13}CO_2$) and analysis by GC-MSMS and NMR.

The comparison of central metabolic flux for native and transgenic plants (producing branched chain fatty acids), was used to identify global perturbations of the metabolic pathways

In previous studies, significant differences between native and transgenic rapeseed embryos were observed in free amino acid concentrations. *In vivo* NMR analysis of isolated embryos incubated with [1-^{13}C]-glucose show that despite the transformation the embryos maintain their main metabolic fluxes, and that the differences observed for steady-state amino acid concentrations are not accompanied by major changes in fluxes regarding amino acid metabolism [1].

In our studies, metabolic flux analysis using different ^{13}C labelled, serve to (1) identify possible targets for genetic modification and (2) identify biological bottlenecks for the biosynthesis and accumulation of branched fatty acids in rapeseed embryos.

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Modification of the acil-ACP thioesterase type A from sunflower (*Helianthus annuus* L.) by means of site directed mutagenesis

Moreno-Pérez A.J., Salas J.J., Garcés R. and Martínez-Force E.
Instituto de la Grasa (CSIC), Seville, Spain

The substrate specificity of acyl-acyl carrier protein (ACP) thioesterases determines the fatty acids available for the biosynthesis of storage and membrane lipids in seeds. Consequently, there is a big interest in modifying their specificity to produce new oils in oilseed crops. These enzymes are encoded by nuclear genes but they are targeted to the plastid and are usually categorized into two groups, FatA and FatB, according to their sequence and acyl-ACP preference. The FatA genes encode thioesterases with a preference for C18 monounsaturated acyl-ACPs and the FatB genes encode thioesterases with a higher affinity for saturated acyl-ACPs. In the present work we tried to improve *Ha*FatA specificity towards saturated and short monosaturated fatty acids by site directed mutagenesis. Six amino acid residues from the FatA protein, Thr 182, Arg 184, Met 206, Thr 172, Leu 118 and Gln 215, were selected to be modified attending to their position into the active site of the enzyme. To stablish the relative importance of these residues, they were changed to tryptophan, an aromatic amino acid that takes up a big volume within the enzyme hydrophobic pocket. These mutant alleles were heterologously expressed in *Escherichia coli* inducing changes in the lipid composition, then, the mutated proteins were purified and enzymatically characterized. The most efficient mutant was selected to transform *Arabidopsis thaliana fatA* knockouts.

Cloning and molecular characterization of ß-keto-acyl-ACP synthase II from *Helianthus annuus* L.: Implications in high-palmitic mutants

Damián González-Mellado, Rafael Garcés and Enrique Martínez-Force
Instituto de la Grasa. CSIC. Adva. Padre García Tejero, 4. 41012 Seville. SPAIN.

The fatty acid biosynthetic pathway in plants and bacteria is catalyzed by individual polypeptides type II fatty acid synthase (FAS). Using acetyl-CoA and malonyl-acyl carrier protein (ACP) as substrates, ß-ketoacyl-ACP synthase III (KASIII; EC 2.3.1.180) catalyses the first condensing step. The elongation cycle is completed by the sequential actions of the enzymes of the FAS complex. The subsequent two carbon condensation steps are accomplished by KASI and KASII (EC 2.3.1.41), producing as final products, palmitoyl-ACP and stearoyl-ACP.

In order to increase genetic variability, some saturated sunflower mutant lines had been generated. From these mutants, two independent high-palmitic acid lines had been selected on a standart high-linoleic acid background (CAS-5) and on a high-oleic acid background (CAS-12).

To determine the mechanisms involved in the biosynthesis of stearic acid in sunflower developing seeds, we isolated, cloned and sequenced a cDNA coding for ß-ketoacyl-ACP synthase II, *Ha*KASII (EC 2.3.1.41). Its protein sequence is as much as 77% identical to other KASII proteins like ones such as those from *Glycine max*, *Cuphea pulcherrima*, *Jatropha curcas* and *Arabidopsis thaliana.* The analysis by Southern blot reveals one single copy in the genome. The expression levels of this gene in seeds and vegetative tissues were examined by Q-PCR revealing higher levels in developing seeds than in leaves, stems, roots or seedling cotyledons.

Once the wild type allele was characterized, the KASII gene alleles from high-palmitic mutant lines were cloned, revelling truncated proteins due to deletions in the coding DNA sequence. The normal development of the mutant lines suggested that another protein activity ought to replace KASII activity. Assays with crude extract, together with arsenite and cerulenine in wild type and mutants lines suggest that KASII function is replace by KASI.

Identification of a very long-chain polyunsaturated fatty acid ?4-desaturase from the microalga Ostreococcus lucimarinus

Katharina Hoppe, Mareike Heilmann and Ivo Feußner
Department of Plant Biochemistry, Albrecht-von-Haller-Institute for Plant Sciences
Georg-August-University Göttingen, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany

Ostreococcus lucimarinus is a close relative of the microalgal species *Ostreococcus tauri* which has been shown to harbour an acyl-CoA-dependent desaturase and which contains remarkably high amounts of the very long-chain polyunsaturated fatty acid docosahexaenoic acid (DHA, 22:6(n-3)). DHA is synthesized from 18:3(n-3) via consecutive desaturation and elongation cycles in which ?4-desaturation displays the last enzymatic activity required. Here we describe the identification of a cDNA coding for a ?4-fatty acid desaturase which exhibits a cytochrome b_5 domain fused to its N-terminus and three histidine boxes that are typically found in front-end desaturases. Heterologous expression of the codon-optimized version of the cDNA in *Saccharomyces cerevisiae* revealed that the encoded protein catalyzes the conversion of 22:5(n-3) into 22:6(n-3).

Wax ester synthesis and phytol metabolism in Arabidopsis

Felix Lippold, Nicole Gaude, Peter Dörmann

Institute for Molecular Physiology and Biotechnology of Plants, University of Bonn, Germany

Waxes are esters of fatty acids and primary alcohols. Cuticular waxes play an important role in the impregnation of plant surfaces and, due to their hydrophobic properties, are of high industrial interest. In addition to cuticular waxes which are composed of long chain alcohols and very-long-chain-fatty acids (VLCFAs), there are less prominent wax esters in other compartments, e.g. the phytol esters in the chloroplasts. During senescence or stress, chlorophyll is degraded and the phytol moiety is released from chlorophyll. Because phytol destabilizes biological membranes, it is quickly incorporated into tocopherol synthesis, but also into fatty acid phytol esters production (FAPEs). The enzymes synthesizing phytol esters are unknown in plants. In the present project, we study the the enzyme family of bifunctional wax synthases/diacylglycerol acyltransferases (WS/DGAT, 11 members) from Arabidopsis. These enzymes are characterized with a focus on FAPE and cuticular wax ester production. FAPE formation and composition in wild type, mutants and overexpression plants is analyzed in nitrogen starved, senescent and phytol fed plants via GC-MS. Furthermore, enzyme activity is recorded after heterologous expression in E. coli and yeast.

Characterisation of Acyl-CoA Reductases and Wax Ester Synthases from Preen Glands

Eva-Maria Biester, Janine Hellenbrand and Margrit Frentzen

RWTH Aachen University, Institute of Biology I, Botany, 52056 Aachen, Germany

To achieve wax ester production in developing seeds of industrial oil seed crops by genetic engineering and, thus, improve the oleochemical prospects of plant storage lipids, we have started to clone and functionally characterise genes essential for wax ester synthesis from birds' preen glands. These glands effectively produce wax esters of complex chemical composition, which varies significantly from order to order. Galliform birds like chickens or turkeys, for instance, synthesize fatty acid diesters of long chain 2,3-*n*-alkanediols in their preen glands. On the other hand, preen glands of Anseriformes like geese and ducks produce monoester waxes in which multi-branched fatty acids are esterified to *n*-fatty alcohols while wax monoesters of certain bird species contain both branched fatty acids and alcohols.

So far chicken genes encoding putative acyl-CoA reductases and wax ester synthases are available in data bases. To verify their identity and to utilize suitable sequence information for the isolation of respective cDNAs from other birds encoding branched chain specific enzymes, we have cloned cDNA sequences from chicken preen glands. The results of functional expression studies in *Saccharomyces cerevisiae* mutants obtained with two different chicken acyl-CoA reductase and wax ester synthase cDNAs will be presented.

Characterization of an Aldehyde-forming Fatty Acyl-CoA Reductase from Arabidopsis (*Arabidopsis thaliana*)

Thuy Doan[a], Per Hofvander[a], Frederic Domergue[b], Anders S. Carlsson[a], Sten Stymne[a]

[a] Swedish University of Agricultural Science, Alnarp, Sweden.

[b] Victor Segalen University, Bordeaux 2, France.

Very long chain primary alcohols and aldehydes are significant components in the formation of cuticle waxes of plants. Fatty acyl-CoA reductases (FARs) catalyse the formation of fatty aldehydes and/or alcohols from acyl-CoAs. The formation of alcohol is conducted in two steps with aldehyde as an intermediate product. In green algae (*Euglena gracilis*), pea (*Pisum sativum L.*), jojoba (*Simmondsia chinensis*) wheat (*Triticum aestivum L.*) and Arabidiopsis (*Arabidopsis thaliana*), both steps are carried out by a single acyl CoA reductase (alcohol – forming FAR) without releasing a free fatty aldehyde intermediate. In pea, enzymatic activities corresponding to both aldehyde-forming and alcohol-forming FARs have been found. Generally, alcohol forming FARs are NADPH- dependent with the molecular weight in the range of 56-58 kDa whereas aldehyde-forming FARs so far found are much smaller (28-35 kDa) and NADH- dependent. However, the understanding about biochemical and molecular biology of FARs in plants is still limited. Recently, four Arabidopsis genes which are homologous to jojoba FAR have been cloned, expressed and analyzed for their FAR activities in *E.coli* extracts. Enzymatic assays using extracts from *E.coli* expressing At3g56700 (FAR6) revealed the production of fatty aldehyde in addition to the produced fatty alcohol. Expression of other Arabidopsis FARs resulted exclusively in the production of fatty alcohol. Either an inefficient second reduction step, thus releasing fatty aldehyde, or a true fatty aldehyde forming enzyme were both plausible explanations for this observation since *E. coli* has endogenous enzyme activity catalyzing the second step of reduction. Furthermore, FAR6 was found to contain a predicted chloroplast transit peptide sequence which could disturb when expressed in a non-processing organism such as *E. coli*. By applying palmitoyl-CoA agarose as a purification step, the alcohol forming activity could be separated from the aldehyde forming FAR activity in extracts of E. coli expressing FAR6 both with and without predicted signal peptide. FAR6 utilized NADPH as a preferred cofactor
and, in contrast to other plant FARs characterized belonging to the same family, could be shown to only produce fatty aldehydes.

Molecular analysis of a fatty acyl reductaseand a wax synthase from *Euglena gracilis*

Prapapan Teerawanichpan[1], Patricia Vrinten[2], Xiao Qiu[1, 2]

[1] Dept of Food & Bioproduct Sciences, College of Agriculture & Bioresources, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8

[2] Bioriginal Food and Science Corporation, 110 Gymnasium Place, Saskatoon SK S7N 0W9

Euglena gracilis is a unicellular phytoflagellate in the kingdom Protista. It can accumulate a large amount of medium-chain wax esters (C12-C16) under anaerobic growth conditions. Two genes involved in wax biosynthesis were identified from this species. One encodes a fatty acyl reductase (*EgFAR*) involved in the conversion of fatty acyl-CoAs to fatty alcohols, and the other codes for a wax synthase (*EgWS*) involved in the esterification of fatty acyl-CoAs to fatty alcohols. The enzymatic activity and substrate specificity of EgFAR and EgWS were analyzed in yeast (*Saccharomyces cerevisiae*). The results showed that *EgFAR*-expressing yeast produced myristyl alcohol (14:0-OH) and palmityl alcohol (16:0-OH) when fed with the corresponding substrates. However, dodecyl (10:0-OH), lauryl (12:0-OH) or stearyl (18:0-OH) alcohols were not produced when the yeast was fed with the substrates corresponding to these alcohols. *EgWS*-expressing yeast produced myristyl myristate, myristyl palmitoleate, and myristyl palmitate when fed with myristic acid and myristyl alcohol. To reconstitute the wax biosynthesis pathway in yeast, *EgFAR* and *EgWS* were co-expressed and myristic acid was used as a substrate. Myristyl myristate was detected in *EgFAR*-*EgWS* co-expressing yeast, but was not found in *EgFAR*-expressing yeast or in yeast carrying only the vector control.

Selectivity of enzymes involved in the incorporation of unusual fatty acids into triacylglycerol for lipid biosynthesis in oilseed plants

Nlandu Mputu M., Rhazi L., El Kassis E., Vasseur G., Vu T-D., Gontier E. and Thomasset B., UMR CNRS 6022, University of Technology of Compiegne, Compiegne, France

In plants, more than 200 types of fatty acids are produced by a limited number of species. Among these various unusual structures, branched chain fatty acids have interesting physical and chemical properties. Thus they are good candidates to replace petroleum as the raw material for a variety of products such as lubricants, plastics, paints, dyes, and coatings. This industrial potential has led several groups to work on their production in transgenic plants (Duhot et al. 1998). Unfortunately, their research generated disappointing results and lead to very modest accumulation in seeds. A better knowledge of the processes underlying the synthesis of unusual fatty acids and their storage in seeds is needed. Enzymes required for the incorporation of such fatty acids into storage triacylglycerols (TAG) are classically acyltransferases of the Kennedy pathway. In order to identify the bottlenecks responsible for this limited unusual fatty acid production, we studied the substrate selectivity of the Kennedy pathway acyltransferases in developing seeds of agronomic plants (flax (*Linum usitatissimum)* and rape *(Brassica napus*)) and from a plant able to produce high amounts of hydroxy fatty acids (castor bean (*Ricinus communis*)). For this, we compared the incorporation of an equimolar mixture of [^{14}C] oleoyl-CoA and unusual acyl-CoAs into Glycerol-3-Phosphate (G3P), Lysophosphatidic acid (LPA) and Diacylglycerol (DAG), by the Glycerol-3-Phosphate Acyltransferase (G3PAT), Lysophosphatidic acid Acyltransferase (LPAAT) and Diacylglycerol Acyltransferase (DAGAT), respectively. Our assays demonstrated that (1) G3PATs of the three plant species have shown no particular selectivity toward incorporation of unusual acyl-CoAs, (2) LPAATs and DAGATs of the three studied cultivars incorporated preferentially oleoyl-CoA. However, they weakly incorporated branched chain acyl-CoAs when presented with oleoyl-CoA in an equimolar mixture. In conclusion, because of their low affinity for unusual acyl-CoAs, LPAAT and DAGAT constitute bottlenecks for the storage of branched chain acyl-CoA into TAG and limit their uses in the industry.

Duhot P., Gontier E., Thomas D., Thomasset B., Ménard M. (1998)

Patent N° PCT/FR 98 02116 (02/10/98) EU - CA - J – USA.

Expression profiling and functional characterization of genes involved in arachidonic acid biosynthesis in the green microalga *Parietochloris incisa*

Iskandarov U, Khozin-Goldberg I and Cohen Z

The Microalgal Biotechnology Laboratory, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede Boqer Campus, 84990 Midreshet Ben-Gurion, Israel

Under nitrogen starvation the oil content of the green microalga *Parietochloris incisa* (Trebouxiophyceae) can reach up to 35% of dry weight. This microalga is unique in its ability to produce and deposit in high amounts the VLC-PUFA, arachidonic acid (AA) in triacylglycerols (TAG). These features make *P. incisa* an interesting source of PUFA and TAG biosynthesis genes. We have amplified cDNAs for the three sequential desaturases (?12, ?6 and ?5) and the ?6 PUFA elongase from nitrogen starved cells of *P. incisa*. The corresponding ORFs were transformed into *Saccharomyces cerevisiae* for functional characterization. The GC analysis of fatty acid methyl esters of transformed yeasts confirmed the functionality of the cloned cDNAs. Since AA accumulation increases following nitrogen depletion we also performed a quantitative analysis of mRNA levels of these genes by real time PCR at different time periods following N starvation. The results showed that mRNA levels for AA biosynthesis enzymes are induced to the highest levels after 3 days of the onset of starvation in accord with the highest increase in AA share of total fatty acids and preceded the increase in fatty acid content. The Acyl CoA dependency of the ?6 and ?5 desaturases is currently under study.

The Role of Glycolipids at the Interface of Plant Microbe Interactions during Nodulation and Mycorrhiza Formation in *Lotus japonicus*

Vera Wewer, Nicole Gaude, Peter Dörmann,

Institute for Molecular Physiology and Biotechnology of Plants, University of Bonn, Germany

The model legume *Lotus japonicus* undergoes symbiotic interactions with the nitrogen fixing soil bacterium *Mesorhizobium loti* and the endomycorrhizal fungus *Glomus intraradices.* The symbiotic partners contribute to the plant's nitrogen and phosphate supply under nutrient limiting conditions. Membrane components such as glycolipids are of particular interest in plant-microbe interactions. We investigated the functions of the glycolipids digalactosyldiacylglycerol (DGDG), sterol glucoside (SG) and glucosylceramide (GC) in *Lotus* during nodulation and mycorrhiza formation. We detected the galactolipid DGDG as a component of the peribacteroid membrane at the interface between *Lotus* and *Mesorhizobium*. Downregulation of two *DGDG* synthases (*DGD1*, *DGD2*) in *Lotus* RNAi plants lead to a conditional nodulation phenotype where nodulation was almost completely abolished under phosphate deprivation. This phenotype was rescued by phosphate supplementation. We thus conclude that DGDG is essential for nodulation but can be replaced by phospholipids under high phosphate conditions. To study the role of the other two plant glycolipids, SG and GC synthesis was down-regulated by generating transgenic RNAi *Lotus* plants for sterol glucoside transferases (*SGT1*, *SGT2*) and glucosylceramide synthase (*GCS*). Expression analysis by rt-PCR revealed an increase in gene expression of *SGT1*, *SGT2* and *GCS* in nodules as compared to roots, indicating a potential function during nodulation. GC-MS analysis was employed to measure sterol composition in different tissues. Sterol content was increased in nodules as compared to roots, and nodules contained elevated amounts of campesterol. Taken together, these findings indicate a potential function of SG and GC during nodulation.

Lysophospholipid acyltransferases (LPLATs) catalyse the transfer of acyl groups from phosphatidylcholine to CoA (backward reaction)

*A. Banas, *M. Miklaszewska, **W. Banas, ***U. Stahl, ***S. Stymne

* Intercollegiate Faculty of Biotechnology of University of Gdansk and Medical University of Gdansk, ul. Kladki 24, 80-822 Gdansk, Poland

**Institute of Biology, University of Podlasie, ul. Prusa 12, 08-110 Siedlce, Poland

***Department of Plant Breeding, SLU, PO Box 44, S-230 53 Alnarp, Sweden

Lysophospholipid acyltransferases are found in several organisms, including animals, plants and fungi. In forward reaction they utilise usually a broad spectrum of both lysophospholipids and acyl-CoA, producing different type of phospholipids. In addition to their role in the syntheses of phospholipids, it is proposed that LPLATs (especially LPCAT) have a significant role in exchanging fatty acids between phospholipids and acyl-CoA pool (*i.e.* catalysing also the backward reaction). In the presented study, we used *in vitro* assays of microsomal preparations of yeast expressing yeast or Arabidopsis LPLATs to demonstrate that the enzyme could operate reversible.

Yeast (BY4742 strain) with disrupted *YOR175c* gene (encoding one of yeast LPLAT) were transformed with pYES2 (empty plasmid) or one of four different gene encoding enzymes with LPLAT activity: *At1g63050*, *At1g12640* (Arabidopsis LPLATs), *YOR175c* and *SLC1* (yeast LPLATs). Microsomes prepared from transformed yeast cells were used in assays for determining LPCAT (lysophosphatidylcholin acyltransferase) activity of tested enzymes.

Yeast transformed with *SLC1* showed a very low LPCAT activity (but higher than microsomes from yeast transformed only with the empty plasmid), whereas microsomes from other three transformants synthesized PC efficiently from added 18:1-LPC and [14C]18:1-CoA. Without addition of 18:1-LPC very little synthesis of [14C]PC from [14C]18:1-CoA was observed. However, when these incubations were supplemented with BSA and free CoA, efficient synthesis of [14C]PC occurred, suggesting that backward reaction is promoted under these conditions.

After preliminary experiments with microsomes of all yeast transformants, yeast transformed with *At1g63050* were selected for a more detailed study. In optimal BSA (1mg/0,1ml) and CoA (0,2umol/0.1 ml) concentrations, the rate of backward reaction was around 16 pmol PC/1min/1nmol microsomal PC and was around 40 times lower than forward reaction. The addition of DTNB (combine CoA) to the reaction mixture strongly inhibited backward reaction but had no significant effect on forward reaction.

To verify that observed *de novo* syntheses of PC from added [14C]18:1-CoA occur *via* exchange of fatty acids between acyl-CoA pool and PC, microsomal preparations were incubated with sn-2-[14C]18:2-PC, BSA, CoA and 18:1-CoA (non-radioactive) at optimal concentrations. During the incubation time [14C]18:2 in acyl-CoA pool was gradually increasing and in the same time similar amount of [14C]18:2 disappeared from added PC, whereas no re-distribution of radioactivity occurred in control incubations (the empty plasmid). The obtained results confirm that LPLAT can operate in both forward and reversible mode.

Composition of lipids of *Ulva intestinalis* from rivers in arid area of Caspian lowland

O.A. Rozentsvet[1]*, N.N. Suschik[2], V.M.Dembitsky[3]

[1]Institute of Ecology of the Volga River Basin, Russian Academy of Sciences Togliatti, Russia;[2]Institute of Biophysics of the Russian Academy of Sciences, Siberian Department; Krasnoyarsk, Russia; [3] School of Pharmacy, Hebrew University, Jerusalem, Israel

Lipid composition of *Enteromorpha intestinalis* from brackish and saline small rivers in the arid area of the Caspian Lowland (head water and estuary of the river Khara, estuary of the river Solyanka) was studied.

The multicellular green macro alga *U. intestinalis* belongs to ? hlorophyta and is widespread in the ocean littoral shallow waters. It is an eurybiont species characteristic for isolated, polluted and desalinated marine areas successfully developing both on natural substrates and man-made structures. Particularly, the genus *Ulva* is one of the widespread species among the macrophytes found in the saline and brackish rivers flowing into Lake Elton (Volgograd Region, Russia).

Lipid composition of *U. intestinalis* is characterized by a high glycolipid (GL) level (44.4 to 69.0% of total lipids). Neutral lipids, a betaine lipid 1,2-diacylglycerol-3-? -4′-(N,N,N-trimethyl)homoserine (DGTS) and phospholipids (PL) make up 15.9 to 25.8%, 9.3 to 17.4% and 5.7 to 12.9%, respectively. In the presently investigated case it appeared that the corresponding content of DGTS and total PL increase with the salinity rise.

As for fatty acids (FA) of the examined species, it should be stated that its qualitative acid composition corresponds to that of marine species. However, the presently studied river populations were found to contain a number of saturated acids great enough, compared to the marine species. At the same time, tetra and triunsatirated acids ? $_{16\text{-}18}$ are present among the FA in the river populations. As for the impact of habitat conditions on the FA composition, there is a marked trend toward an increase in the content of acid ? $_{16:0}$ as a function of salinity and abundance of biogenic elements.

So, the changes in the lipid composition of the *E. intestinalis* induced by various hydrochemical conditions involve both polar (GL, PL and DGTS) and reserve (NL) lipids. GL fraction appears to be the most sensitive to changes. Various restructuring processes of lipid molecules are obviously of adaptive character which is proved by a widespread distribution of *E. intestinalis* in flat-land rivers having different levels of salinity and concentration of biogenic and organic compounds.

Profiling of galactolipids and Arabidopsides in two lipid biosynthesis mutants of *Arabidopsis thaliana*

Ibrahim A., Göbel C., Fulda M. and Feussner I.

Department of Plant Biochemistry, Albrecht-von-Haller-Institute of Plant Sciences, Georg-August-University Göttingen, D-37077 Göttingen, Germany

(aibrahi@uni-goettingen.de)

Galactolipids are synthesized and localized in the plastidial membranes of plants. Wounding of Arabidopsis leaves leads to the accumulation of the oxygenated derivatives of these galactolipids which are called Arabidopsides. For profiling of the galactolipids and Arabidopsides a sensitive and fast method was established based on reversed-phase LC separation coupled to ion trap mass spectrometry detection. More than 100 different oxidized and non-oxidized galactolipid species were identified in Arabidopsis leaves and were analyzed after wounding in *act1* and *faa1faa2* mutant plants. These two mutants were chosen to investigate the Arabidopside biosynthesis and to address the question, if free oxylipins are esterified to the lipid backbone to form the Arabidopsides or if the Arabidopsides are synthesized directly by the oxidation of lipid-bound fatty acids. In the *act1* mutant, the activity of the acyl-ACP glycerol-3-phosphate acyltransferase is reduced leading to an almost complete loss of the prokaryotic lipid biosynthesis pathway. The *faa1faa2* double mutant lacks the activity of the two plastidial acyl-ACP synthetases in Arabidopsis, FAA1 and FAA2. The comparison of the wild type and mutants profiles of the galactolipid species revealed that the levels of the Arabidopsides containing oxidized hexadecatrienoic acid-derived side chains are reduced in parallel to the reduced levels of the prokaryotic galactolipid species in the *act1*. However, in the *faa1faa2* double mutant the levels of all detected galactolipid species as well as Arabidopsides are almost unaffected by the reduction of the acyl-ACP synthetase activity. The results on the analysis of galactolipid profiles of these two lipid biosynthesis mutants may indicate that Arabidopsides are synthesized via an enzymatic activity involved in oxylipin biosynthesis which might act on lipid-bound fatty acids.

Regulation of MGDG synthesis by phosphatidic acid

Emmanuelle Dubots, Eric Maréchal and Maryse A. Block

Laboratoire de Physiologie Cellulaire Végétale, CNRS / CEA / INRA / Université J. Fourier, CEA-Grenoble, F-38054, Grenoble, France

MGD1 catalyses the galactosylation of diacylglycerol (DAG) to form monogalactosyldiacylglycerol (MGDG) in the chloroplast envelope inner membrane and is essential for the formation of thylakoid membranes. In *Arabidopsis thaliana,* different routes can provide the DAG substrate. On one hand, DAG is synthesized *de novo* inside plastids by a sequence of acylations of glycerol-phosphate, generating phosphatidic acid (PA), and by dephosphorylation of PA into DAG; on the other hand, DAG is produced by phosphatidylcholine (PC) hydrolysis. PC hydrolysis can be performed by phospholipase C (PLC) activities producing DAG directly, or by phospholipase D (PLD) activities, leading to formation of phosphatidic acid (PA) that can then be dephosphorylated into DAG by the mean of a phosphatidate phosphatase. In plants, in addition to its role as a precursor for glycerolipid syntheses, PA is also a signalling molecule. In the past, data indicated that MGDG synthase activity might be stimulated by PA. We will here present results showing the allosteric regulation of MGD1 by PA.

Lipid signalling in *Aspergillus* species

Sorrentino F., Roy I. and Keshavarz T., University of Westminster
London, England

Bacterial populations regulate their behaviour and expression of certain properties through the production of low molecular-mass signalling molecules. When the concentration of these molecules, called quorum sensing signals, reaches a threshold they activate or inhibit gene expression to modify the behaviour of the whole population. While already established in bacteria and yeasts, very little research has been done on fungal quorum sensing (QS). This project is aimed to exploit fungal communication trough quorum sensing for overproduction of fungal bioproducts.

The oxygenated lipid farnesol function as QS molecule in the dimorphic fungus *Candida albicans*, where it regulates morphological transitions associated with virulence. Interestingly, endogenous oxygenated lipids called psi factors, regulate the onset of sporulation, as well as virulence and secondary metabolites production in the filamentous fungus *Aspergillus nidulans*. This mechanism seems to resemble the quorum sensing system of filamentous bacteria.

In silico analysis of *A. terreus* genome revealed the presence of 5 putative genes responsible for lipid oxygenase. Hence, partially purified oxylipins from *A. nidulans* and their precursor linoleic acid were added to *A. terreus* cultures to investigate their effects on sporulation and lovastatin production. The sporulation rate was inhibited by the crude extract and to a lower extent also by linoleic acid. Results also showed that lovastatin production was either enhanced or inhibited, dependeding on the time of addition and levels of linoleic acid added. Both sporulation and lovastatin production are indeed affected by addition of linoleic acid, indicating a regulation of this process by the oxylipins precursor.

Further investigation of the involvement of oxylipins precursor on enhancement on transcription of oxylipins genes should provide important clues regarding the role of oxylipins as autoinducers in *A. terreus*.

The *Arabidopsis* double mutant *pip5k1 pip5k2* is severely impaired in stomatal opening

Till Ischebeck[1], Irene Stenzel[1], Yangju Im[2], Imara Y. Perera[2], Wendy F.Boss[2], Ingo Heilmann[1], [1] Department of Plant Biochemistry, Albrecht-von-Haller-Institute for Plant Sciences, Georg-August-University Göttingen, Göttingen, Germany; [2] Department of Botany, North Carolina State University, Raleigh, NC, USA

The phospholipid phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$) has been found in the plasma membrane of guard cells and shown to be important for stomatal opening. A dwarfed *Arabidopsis thaliana* double mutant impaired in the phosphatidylinositol-4-phosphate 5-kinases PIP5K1 and PIP5K2 was identified. This double mutant showed severe defects in stomatal opening. *In vitro* tests on recombinant enzymes indicate that both PIP5K1 and PIP5K2 predominantly phosphorylated phosphatidylinositol-4-phosphate to form $PI(4,5)P_2$. Based on *in vivo* labeling data, the contribution of PIP5K1 and PIP5K2 to total cellular $PI(4,5)P_2$ was relevant. PIP5K1 and PIP5K2 are expressed in *A. thaliana* guard cells according to promoter-GUS experiments. When fluorescence-tagged PIP5K1 or PIP5K2 were transiently expressed in *Vicia faba* guard cells both kinases localized to plasma membrane areas not immediately adjacent to the stomatal pore. Together the data suggest a mutually redundant function of PIP5K1 and PIP5K2 in the regulation of stomatal opening. Data on the physiological characterization of the *pip5k1 pip5k2* double mutant will be presented with a focus on guard cell functionality.

Protein domains required for plasma membrane association of *Arabidopsis* phosphatidylinositol-4-phosphate 5-kinases

I. Stenzel and I. Heilmann, Department of Plant Biochemistry, Albrecht-von-Haller-Institute for Plant Sciences, Georg-August-University Göttingen
Göttingen, Germany

The regulatory phospholipid, phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2), accumulates in the apical plasma membrane of tip-growing plant cells, such as pollen tubes and root hairs. Recent studies indicate that apical membrane trafficking and cytoskeletal structures involved in the maintenance of apical cell polarity are regulated by PtdIns(4,5)P_2. Importantly, the precise subcellular localization of PtdIns(4,5)P_2 formation is important for the coordination of different regulatory functions of PtdIns(4,5)P_2. PtdIns(4,5)P_2 is formed by phosphatidylinositol-4-phosphate 5-kinases (PI4P 5-kinases), which are represented in the *Arabidopsis* genome by a family of eleven genes. Using a systematic deletion approach and transient expression of fluorescence-tagged conserved and non-conserved domains, it was attempted to define protein domains of *Arabidopsis* PI4P 5-kinases that were necessary and sufficient for plasma membrane targeting. The data indicate that not individual domains but rather combinations of domains are required for plasma membrane targeting. Based on the localization data a model for the plasma membrane orientation of the *Arabidopsis* PI4P 5-kinase, PIP5K3, was conceived.

Oxylipin-induced *YUC8* and *YUC9* are involved in an auxin-ethylene loop in *Arabidopsis thaliana*

Mathias Hentrich, Stephan Pollmann
Ruhr University Bochum, Department of Plant Physiology
Bochum, Germany

Indole-3-acetic acid (IAA), the predominantly occurring auxin in nature, is involved in virtually every aspect of plant growth and development. A precise regulation of the physiologically active IAA level is achieved by transport, conjugation, compartmentalization, as well as by catabolism and *de-novo* biosynthesis. The current knowledge about the physiological functions of auxins increased significantly in the past decades but the understanding of IAA biosynthesis still remains fragmentary.

There is evidence that several pathways for IAA production exist in higher plants. These pathways seemingly operate either in parallel or can be induced under specific environmental circumstances. It has previously been shown that one very prominent proposed pathway proceeds in a YUCCA enzyme-dependent manner. Recently, we identified a subset of *Arabidopsis YUCCA* genes to be efficiently induced by oxylipins, i.e. jasmonic acid and its bioactive precursor 12-oxo-phytodienoic acid. Those compounds are known mediators of plant responses towards a multitude of environmental cues like, for example, herbivore and pathogen challenge, osmotic stress, and mechanotransduction.

Here, we provide first genetic and molecular evidence for an oxylipin-mediated induction of *YUC8* und *YUC9*. Overexpression of YUC8 leads to a strong auxin-overproducing phenotype, an increase in cellular auxin levels as well as in an improved resistance to ethylene biosynthesis inhibitors. In line with the latter finding, we observed increased secondary growth and lignification to take place in this line, which is indicative for elevated ethylene levels. Accordingly, the presented data strongly suggest that signal compounds of the oxylipin-family are involved in the transcriptional regulation of certain *YUC* genes, thereby linking external stimuli with the activation of a so far undiscovered auxin-ethylene loop.

Lipoxygenases from cyanobacteria

Alexandra Andreou, Ivo Feussner, Department of Plant Biochemistry, Georg-August-University Göttingen, Albrecht-von-Haller Instititute for Plant science Göttingen, Germany

Oxylipins are a large family of oxygenated metabolites derived from polyunsaturated fatty acids. Their biosynthesis is initiated by lipoxygenases (LOX), which consist of a class of non-heme iron containing fatty acid dioxygenases. These enzymes catalyze the stereo- and regiospecific insertion of molecular oxygen into polyunsaturated fatty acids that contain one or more 1,4-pentadiene moieties to give the corresponding hydroperoxides. Plant LOX can be classified according to the position of linoleic acid oxygenation to 13- and 9-LOX. This specificity is dependent on the primary sequence of the enzyme, which is predicted to determine the orientation and depth of substrate penetration into the active site. The hydroperoxide products can be further metabolized by other enzymes to yield secondary oxylipin metabolites, such as aldehydes and jasmonate, which in plants have been shown to play a role in development and in responses to wounding and pathogen attack. LOX have been for long considered to be exclusively present in eukaryotic organisms. Recently, however, the first LOX enzymes from cyanobacteria were isolated and characterized. From an evolutionary point of view, plant chloroplasts, which harbor the 13-LOX pathway, have evolved from cyanobacteria. It is, therefore, of particular interest to determine whether the function of oxylipin signaling in cyanobacteria is an ancestor of that present in plant plastids. In our work, using the increasing amount of available sequencing information, we have identified and cloned LOX from cyanobacterial origin and expressed them recombinantly in *E. coli*. The enzymes have been tested for their catalytic activity against various fatty acid substrates and were biochemically characterized. Our studies are aimed in obtaining a better understanding of cyanobacterial LOX and the biological role of LOX signaling in prokaryotes.

Identification of metabolic changes after wounding in *Arabidopsis thaliana* by an unbiased UPLC-MS approach

Kirstin Feussner[1], Cornelia Göbel[2], Alexander Kaever[3], Peter Meinicke[3], Burkhard Morgenstern[3], Petr Karlovsky[4] and Ivo Feussner[2]

[1]Department of Developmental Biochemistry, Institute for Biochemistry and Molecular Cell Biology, Georg-August-University Göttingen/Germany;

[2]Department of Plant Biochemistry, Albrecht-von-Haller-Institute of Plant Sciences, Georg-August-University Göttingen/Germany;

[3]Department of Bioinformatics, Institute of Microbiology and Genetics, Georg-August-University Göttingen/Germany;

[4]Molecular Phytopathology and Mycotoxin Research Unit, Georg-August-University Göttingen/Germany

The wound response of *Arabidopsis thaliana* is a well described model to investigate plant defence in case of herbivore attack. Fatty acid derived signals, like jasmonic acid and its derivatives play a crucial regulatory role in mediating this response. To extend our knowledge on global metabolic changes at early time points after wounding wild type as well as the jasmonate-deficient *dde2-2* mutant plant were analyzed.

Aqueous and non-polar extracts of a wounding time course were used for an unbiased UPLC-TOF-MS analysis and data processing was performed by the software package MarkerLynx™ resulting in an output table of several thousands marker candidates. We present an in-house produced Perl-script application which allows to group samples, delete non-informative marker candidates, identify isotopomers and adducts as well as to search interactive metabolite databases. The resulting data sets contain the intensity pattern representing 8 different experimental conditions for at least several hundred marker candidates. For the interpretation of data sets of such high complexity, we used one-dimensional self-organizing maps (1D-SOM) for metabolite-based clustering and identification of meaningful clusters. A significant number of oxylipins was identified as wounding markers as expected from the current literature. But in particular our approach supports the discovery of so far unknown markers on the basis of their location in the 1D-SOM array with respect to the previously identified markers.

Knocking down the cyclopentenone production results in lack of spore formation in the moss *Physcomitrella patens*

Ellen Hornung[1], Cornelia Göbel[1], Michael Stumpe[1], Bernd Faltin[2], Kiyoshi Himmelsbach[2], Bettina Hause[3], Wolfgang Frank[2], Ralf Reski[2], Ivo Feussner[1]

[1]Department of Plant Biochemistry, Albrecht-von-Haller-Institute for Plant Sciences, Georg-August-University, Göttingen, Germany, [2]Freiburg University, Dept. of Plant Biotechnology, Schänzlestrasse 1, D-79104 Freiburg, Germany; [3]Leibniz Institute of Plant Biochemistry, Dept. of Secondary Metabolism, Weinberg 3, D-06120 Halle (Saale), Germany

We used the model organism *Physcomitrella patens* to gain insight into the biosynthesis of oxylipins and their influence on phytohormones in a non-flowering plant. In contrast to higher plants, *P. patens* contains in addition to C18-derived polyunsaturated fatty acids (PUFAs) also C20-derived PUFAs like arachidonic acid. Therefore we wanted to investigate the biosynthesis and function of cyclopentenones derived from these PUFAs. Two cDNAs encoding allene oxide synthases (PpAOS1 und PpAOS2) were isolated and the recombinant PpAOS1 was characterized in more details, demonstrating that the enzyme has a broad substrate range and accepts all hydroperoxides derived from endogenous fatty acids as substrates. Two cDNAs encoding for allene oxide cyclases were also isolated (PpAOC1 and PpAOC2) and both recombinant proteins were demonstrated to catalyze the formation of the cyclopentenone 12-oxo phytodienoic acid (12-OPDA) which is derived from 13-hydroperoxy linolenic acid. PpAOC2 catalyzed in addition the formation of 11-oxo-phytotrienoic acid (11-OPTA) from 12-hydroperoxy arachidonic acid. Analysis of phytohormones in AOC knock out lines and wild type of *P. patens* was performed to elucidate the role of cyclopentenones in the moss.

A novel P450 fusion protein from *A. nidulans*

Florian Brodhun, Cornelia Göbel, Ellen Hornung, and Ivo Feussner
Department of Plant Biochemistry, Albrecht-von-Haller-Institute for Plant Sciences,
Georg-August-University, Göttingen, Germany

The homothallic ascomycete *Aspergillus nidulans* serves as model organism for filamentous fungi due to its ability to propagate with both an asexual as well as a sexual life cycle. Analyses showed that fatty acid-derived substances regulate the balance between both cycles. These so-called psi factors (precocious sexual inducer) are produced by psi-factor producing oxygenases (Ppo). The genome of *A. nidulans* harbors three different genes possibly encoding for Ppo: *ppoA*, *ppoB* and *ppoC*. Bioinformatic analysis predicted the presence of two different heme domains within Ppo: in the N-terminal region a mammalian heme peroxidase domain is predicted while a cytochrome P450 domain is predicted for the C-terminal region of the proteins.

In order to analyze the properties of Ppo and the reaction catalyzed by these enzymes, PpoA was expressed in *E. coli* as active enzyme. The purified protein showed a 62-fold increase in activity and was identified to be a homo tetrameric ferric heme protein that metabolizes mono- as well as polyunsaturated C-16 and C-18 fatty acids around pH 7.25. The presence of thiolate ligated heme as it is known for all P450 enzymes was confirmed by the formation of a reduced carbon-monoxide complex that exhibited a characteristic absorption maximum at 450 nm.

Studies on its reaction mechanism revealed that PpoA uses different heme domains to catalyze two different reactions: Within the peroxidase domain (8*R*)-hydroperoxy octadecadienoic acid ((8*R*)-HPODE) is formed by abstracting an H-atom from the C-8 of linoleic acid in a first reaction step yielding a carbon centered radical that reacts with molecular dioxygen. In a second reaction step this intermediate product is isomerased within the P450 domain to 5,8-dihydroxy octadecadienoic acid (5,8-DiHODE).

This present study identified PpoA as a novel bifunctional P450 fusion protein that uses a previously undescribed reaction mechanism for the catalysis of forming psi-factors.

Pichia pastoris Bar1p Produces Ceramides Destined for Glucosylceramide Biosynthesis

Philipp Ternes[1], Tobias Wobbe[2], Marnie Schwarz[1], Kirstin Feussner[1], Isabelle Riezman[3], Dirk Warnecke[2], Howard Riezman[3], and Ivo Feussner[1] [1] Georg-August-Universität Göttingen, Germany
[2] Universität Hamburg, Germany
[3] Université de Genève, Switzerland
pternes@uni-goettingen.de

Baker's yeast, *Saccharomyces cerevisiae*, has a single class of complex sphingolipids, glycosyl inositol phosphoryl ceramides (GIPCs , including $M(IP)_2C$ and its precursors MIPC and IPC). In contrast, the methylotrophic yeast *Pichia pastoris* and many other fungi contain glucosylceramide (GlcCer) in addition to GIPCs. Besides the differences in their headgroup, both lipid classes are distinguished by their ceramide backbone: While GIPCs have a hydroxylated sphingoid base and a C_{24}–C_{26} fatty acid, GlcCer has a desaturated, methyl-branched sphingoid base and a C_{16}–C_{20} fatty acid. The aim of this study is to investigate how these specific structures are generated.

Phylogenetic analysis shows that Pichia possesses two ceramide synthase homologs, Lag1p and Bar1p, with Pichia Lag1p being very similar to Saccharomyces Lag1p and Lag1p, and Pichia Bar1p being more distantly related. An intriguing possibility is that Pichia Bar1p is responsible for the biosynthesis of the ceramide backbone of GlcCer. By an in vitro enzyme assay, we now show that Pichia Bar1p indeed has a preference for C_{16}–C_{20} acyl-CoA, matching the fatty acyl chain lengths found in GlcCer. LC/MS analysis of the free ceramides in Pichia shows the presence of two ceramide pools matching the structures of the complex sphingolipids described above, a desaturated one with an acyl chain length of C_{16}–C_{20}, and a hydroxylated one with an acyl chain length of C_{24}–C_{26}. In a Pichia *bar1* KO strain, the desaturated pool is entirely missing while the hydroxylated pool is unaffected. Strikingly, the *bar1* KO strain is lacking GlcCer altogether, confirming that the desaturated ceramide pool is the precursor for GlcCer biosynthesis. These data show that there is a division of labor between the two Pichia ceramide synthases, with Bar1p playing a key role in the biosynthesis of GlcCer.

Identification of precursors for carotenoid biosynthesis during tomato fruit ripening

Djedoux Maxime Angaman, Irene Pateraki, Montserrat Busquets and Albert Boronat
Departament de Bioquímica i Biologia Molecular, Facultat de Biologia, Universitat de Barcelona, 08028-Barcelona, Spain

The characteristic pigmentation of red ripe tomato fruit is due to the deposition of high amounts of lycopene, the predominant carotenoid found in tomato fruit. Lycopene intake is associated with decreased incidence of prostate cancer and cardiovascular disease. Carotenoid biosynthesis is a very active process during tomato fruit ripening and requires the supply of pyruvate and glyceraldehyde 3-phosphate (the metabolic precursors of the plastidic methylerytritol 4-phosphate pathway). The origin of these precursors in the chromoplast is currently unknown. In developing tomato fruits malic and citric acid accumulate in nearly equal amounts. However, as fruit begins to ripen, malic acid level declines whereas citric acid level still increases. Since malic acid has been reported to be an efficient precursor for anabolic processes in some nongreen plastids, we have explored the role or this compound as a precursor for carotenoid biosynthesis during tomato fruit ripening. Malic acid can be converted into pyruvate by the action of malic enzyme, the activity of which has been demonstrated in tomato fruit chromoplasts. Using isolated tomato fruit chromoplasts we have found that ^{14}C-malic acid served as a precursor for lycopene biosynthesis. Unexpectedly, most of the incorporated radioactivity was found in membrane lipids. Similar incorporation rates were found when ^{14}C-pyruvate and ^{14}C-glucose were used. Incorporation of ^{14}C-pyruvate in pericarp tissue samples gave results similar to those found in isolated chromoplasts. All together, these results indicate that lipid biosynthesis is a very active process during tomato fruit ripening. ^{14}C-acetate was also an efficient precursor for lipid and sterol biosynthesis in pericarp tissue samples. Interestingly, we also detected incorporation of radioactivity from ^{14}C-acetate into lycopene, suggesting that the cytosolic mevalonate (MVA) pathway may be contributing to carotenoid biosynthesis in the chromoplast. This observation has been confirmed using ^{14}C-MVA, which was used as a precursor for both sterol and carotenoid biosynthesis. The use of inhibitors specifically blocking carotenoid, sterol and fatty acid biosynthesis suggests that the operation of these pathways may be regulated in a coordinated way during tomato fruit ripening.

Arabidopsis mutant plants lacking the peroxisomal ABC-Transporter PXA1 are sensitive to extended darkness

Michael Scharnewski[a], Hans-Henning Kunz[b], Kirstin Feussner[c], Ivo Feussner[a], Ulf-Ingo Flügge[b], Markus Gierth[b] and Martin Fulda[a], [a]Department for Plant Biochemistry, Albrecht-von-Haller-Institute for Plant Sciences, Georg-August-University, D-37077 Goettingen, Germany, [b]Department of Botany II, University of Cologne, [c]Department of Developmental Biochemistry, Institute for Biochemistry and Molecular Cell Biology, Georg-August-University, Göttingen, Germany

The full-size peroxisomal ATP binding cassette (ABC) transporter PXA1, homologue of human adrenoleukodystrophy protein (ALDP), is required for fatty acid degradation and plays a major role in the regulation of seed germination and seedling development in *Arabidopsis thaliana*. PXA1 was also reported to be involved in the biosynthesis of jasmonic acid and auxin. Whereas quite some information on the role of PXA1 on germination and on postgerminative growth was accumulated recently our knowledge about its role in mature tissue is rather poor. However, when challenging *pxa1* mutant plants by periods of extended darkness a dramatic phenotype was observed. The *pxa1* mutant plants exhibited severe leaf necrosis, bleached rapidly and died after extended dark treatment of 36 h whereas Arabidopsis wild-type plants remained unaffected. Further analyses of leaf lipids from dark-treated plants showed a significant accumulation of free fatty acids and of acyl-CoAs. The composition of these elevated pools indicated plastidial lipids as major source for the release of the fatty acids. This data was supported by microscopical analysis of leaf sections from dark-treated plants showing severe structural damage of *pxa1* leaf tissue in general and of chloroplasts in particular. Probably as consequence of the changes in fatty acid metabolism,dark-treated *pxa1* plants started to accumulate triacylglycerol in leaf tissue. In addition the *pxa1* mutant exhibited a strong increase in the amount of jasmonic acid upon extended darkness. In conclusion, our findings demonstrate an important role for both PXA1 and ß-oxidation in mature plants exposed to extended darkness.

New polymers from Bio-sourced Polyols

Carine Alfos*, Eric Cloutet2, Henri Cramail*2,

1. ITERG, Institut des Corps Gras, Avenue Gaspard Monge – 33600 Pessac (France)
2. Université de Bordeaux, Laboratoire de Chimie des Polymères Organiques (LCPO, UMR 5629), ENSCPB, 16, Avenue Pey-Berland 33607 Pessac Cedex (France)

The anticipated exhaustion of petroleum resources, the accompanying geopolitical concerns and the high degree of fluctuation of crude oil prices, justify the focus on renewable materials.To this purpose, natural vegetable oils (triglycerides and fatty acid methyl esters) have recently attracted renewed attention as raw materials for the preparation of bio-sourced polymers, appropriate to a multitude of applications. The main vegetable oils currently exploited as sources of polyols are soybean and palm with a world production of 35.9 millions tons and 36.8 millions tons in 2006/07, while the main European vegetable oils are rapeseed and sunflower with a world production of 17.8 and 10.8 millions tons, respectively. The synthesis of polyols -from fatty oleic and linoleic acid methyl esters- with controlled structure and functionality is currently investigated at ITERG and LCPO (Pessac, France). These polyols are used as building-blocks of various polymers such as polyesters and polyurethanes. The synthesis of these bio-sourced materials will be discussed in the presentation.

Antioxidant efficiency of the phenolic compounds originated from vegetable water of olive oil industry in increasing the stability of Biodiesel

Zohreh Rabiei[1], Sattar Tahmasebi Enferadi[2,3], Gian Paolo Vannozzi[2]

[1] Faculty of New Technologies and Energy Engineering, Shahid Beheshti University, G. C., Tehran, Iran

[2] Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi di Udine, Udine, Italy

[3] National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

During olive oil extraction, large quantities of olive oil waste, vegetable water / wet olive oil cake, are produced which contain a high level of phenolic compounds of strong antioxidant potential.

The phenolic compounds of vegetable water of the classical three-phase centrifugation and the two-phase centrifugation systems were isolated by methanol extraction and were added to B100 (biodiesel made entirely from Soya) and B80 (a mixture of 80 percent biodiesel and 20 percent diesel) to investigate the antioxidant capacity of natural resources in ensuring the storage stability of biodiesel. 28.8% and 42.2% of total phenols present in vegetable water originating from three-phase and two-phase systems, respectively were recovered. It was observed the highest concentration in hydroxytyrosol (1.16% (w/w) dry residue) and the strongest antioxidant activity obtained from the phenolic extract from the two-phase system. The stability of biodiesel samples against oxidation process was determined using either Schaal oven storage test (at 50 ºC) or Rancimat method (at 70 ºC). The induction times obtained by Rancimat method were consistent with the values determined by oven test. The highest protective effect in both types of samples containing 3% (w/w) of added antioxidants was observed.

Minor components of pistachio oil: A potent natural antioxidant for industry

Sattar Tahmasebi Enferadi[1,3], Zohreh Rabiei[2], Gian Paolo Vannozzi[3]

[1] National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

[2] Faculty of New Technologies and Energy Engineering, Shahid Beheshti University, G. C., Tehran, Iran

[3] Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi di Udine, Udine, Italy

A comprehensive study was carried out on the study of the effects of naturally occurring antioxidants of the oil extracted from the discarded aflatoxic pistachio on the stability of refined soybean oil. Commercially available antioxidants such as, TBHQ, vitamin E, and citric acid are usually added to edible oils with the aim of increasing the stability. Instead, natural antioxidants derived from industrial / agricultural wastes can be more advantageous from both economic and ambient point of view. Pistachio oils were extracted using supercritical CO_2 and Soxhlet extraction method. The unsaponifable matters (USM) of the oils were isolated by alcoholic potassium hydroxide saponification and extraction with diethyl ether. The USMs were then added either separately or when accompanied with citric acid to identify its synergistic effect on augmenting the stability of refined soybean oil. It was concluded that the oil extraction method has significant effect on the quality of recovered USM. The use of USM of pistachio oil increased the stability of refined soybean oil; moreover the presence of citric acid exhibited the higher antioxidant efficiency.

Fatty acid metabolism in cyanobacteria

Danuta Kaczmarzyk, Martin Fulda

Department of Plant Biochemistry, University of Göttingen, 37077 Göttingen, Germany

We identified the acyl-acyl carrier protein synthetase (AAS) from cyanobacteria and generated knockout mutants devoid of this activity on the background of two different strains. Further experiments revealed that the deletion of *aas* resulted in two phenotypes: First, the mutant cells were unable to utilize exogenous fatty acids and second, the mutant cells secreted endogenous fatty acids into the culture medium. Based on profiles of fatty acids secreted to the medium and esterified to membrane lipids, and on results of radio labeling experiments the aspects of lipid remodeling will be discussed.

Identification of metabolical markers in *Arabidopsis thaliana* after infection with *Verticillium longisporum*

Stefanie Götze, Kirstin Feussner, Cornelia Göbel, Ivo Feussner
Deptartment of Plant Biochemistry, Albrecht-von-Haller-Institute for Plant Sciences, Georg-August-University Göttingen, D-37077 Göttingen, Germany

Verticillium longisporum is a soil borne pathogen which infects plants of the Brassicaceae family. The fungus enters through the roots and spreads within the plant through the xylem. Infection of oilseed rape causes wilting symptoms and early fruit ripening which leads to economical damage by yield depression.

Using the model plant *Arabidopsis thaliana* our work focuses on the metabolical mechanisms underlying this plant-fungus interaction. A non-targeted profiling approach by UPLC-MS was established to find metabolical changes in leaves of Arabidopsis upon infection. With this method different markers were identified that derive from the phenylpropanoid pathway and from the biosynthesis of long chain hydroxy and a,?-dicarboxy fatty acids. Both metabolite classes are reported to be involved in suberin and cutin biosynthesis.

Quantification of the phenylpropanoids by RP-HPLC-DAD measurements could confirm the data of the non-targeted approach showing an accumulation of sinapoylglucose already at 10 days post infection. Therefore we tested the susceptibility of a ferulate-5-hydroxylase mutant (*fah1-2*) to the fungus. This mutant does not have sinapic acid and its esters.

With this direct method we could additionally identify some indolic compounds like camalexin and the glucosinolate glucobrassicin. Based on this finding we also tested the *cyp79b2/cyp79b3* double mutant that is defective in an early step of the tryptophan metabolism.