2nd European Symposium on Plant Lipids
17-20 August 2005, Copenhagen, Denmark

Programme

Wednesday 17 August 2005
17:00-20:00 Welcome reception

Thursday 18 August 2005
09:15-09:30 Opening remarks

Session A

09:30-10:00 **Identification and functional analysis of a new triacylglycerol-lipase-family in *Arabidopsis thaliana***
I. Feussner, Goettingen, Germany
10:00-10:15 **Characterisation of sterol carrier protein-2**
J. Edqvist, Uppsala, Sweden
10:15-10:30 **The structure of peroxisomal short length specific acyl-CoA oxidase sheds light on the re-oxidation mechanism**
J. Berglund, Copenhagen, Denmark
10:30-10:45 **Lipid storage in soybean seeds occurs gradiently and is related to differentiation of plastids**
L. Borisjuk, Gatersleben, Germany
10:45-11:45 Coffee, tea and posters

Session B

11:45-12:30 **Plenary Lecture: Biosynthesis and secretion of plant cuticular wax**
L. Kunst, Vancouver, Canada
12:30-12:45 **Extracellular BODYGUARD involved in the formation of cuticle**
A. Yephremov, Cologne, Germany
12:45-14:00 Lunch

Session C

14:00-14:15 **The roles of Comatose, a peroxisomal ABC transporter, in lipid metabolism and signalling**
F. L. Theodoulou, Harpenden, Great Britain
14:15-14:30 **Fatty acid metabolism in *Arabidopsis***
M. Fulda, Goettingen, Germany
14:30-14:45 **Phosphate deprivation induces transfer of DGDG galactolipid from chloroplast to mitochondria**
J. Jouhet, Grenoble, France
14:45-15:00 **Biochemical and structural analyses of glycosyltransferases involved in plant glycolipid biosynthesis**
A.A. Kelly, Stockholm, Sweden
15:00-15:15 **PLAMs (plastid associated membranes): Biophysical evidence for their endoplasmic reticulum origin and for non-covalent PLAM-chloroplast zones of attachment**
A.S. Sandelius, Göteborg, Sweden
15:15-16:00 Coffee, tea and posters

Session D

16:00-16:30 **Biosynthesis and function of galactolipids**
P. Doermann, Golm, Germany
16:30-16:45 **Cycloartenol synthase deficiency in *Arabidopsis***
H. Schaller, Strasbourg, France
16:45-17:00 **Functional role of phosphatidylglycerol and cardiolipin in plants**
M. Frentzen, Aachen, Germany
18:50 Canal trip and dinner, meeting point is Nyhavn 71 (Tour company "Netto-Bådene")

Friday 19 August 2005

Session E

09:00-09:15 ***Arabidopsis* phospholipid: Sterol acyltransferase (PSAT) regulates the composition of free sterols in the membranes**
A. Banas, Alnarp, Sweden
09:15-09:30 **Linoleate biosynthesis and aroma biogenesis in olive fruit: a molecular approach**
J.M. Martinez-Rivas, Sevilla, Spain
09:30-09:45 **Biochemical analysis of a fatty acid isomerase from *Propionobacterium acnes***
A. Liavonchanka, Goettingen, Germany
09:45-10:00 **Castor oil biosynthesis and expression profiles of lipid genes in developing seed of *Ricinus communis* L. (castor bean)**
G. Chen, Albany, USA
10:00-11:00 Coffee, tea and posters

Session F

11:00-11:45 **Plenary Lecture: Oxylipins in plant-herbivore interactions : Tales from *Nicotiana attenuate* in the real world**
I. T. Baldwin, Jena, Germany
11:45-12:00 **Functions of sterol glycosides and glycosylceramides in host/pathogen interactions**
D. Warnecke, Hamburg, Germany
12:00-12:15 **Phospholipid and oxylipin signaling of the plant innate immune system**
M. Ellerström , Göteborg, Sweden
12:15-12:45 **Molecular basis for the elicitation of plant innate immunity by lipo-oligosaccharide of *Xanthomonas campestris***
M.A. Newman, Frederiksberg, Denmark
12:45-14:00 Lunch

Session G

14:00-14:30 **The role of sphingolipids in plants and yeast: from signalling to lipid rafts**
J.A. Napier, Harpenden, Great Britain
14:30-14:45 **Fungal sphingolipids as targets of plant defense reactions**
P. Sperling, Hamburg, Germany
14:45-15:00 ***In vitro* effects of ursolic acid on purified rat intestinal alkaline sphingomyelinase**
D. Andersson, Lund, Sweden
15:00-16:00 Coffee, tea and posters

Session H

16:00-16:30 **The use of plant lipids as functional designer-made ingredients in foods**
J. Holstborg, Braband, Denmark
16:30-17:00 **Applications of Plant Lipids in Food Products**
K. Nielsen, Aarhus, Denmark
17:00-17:30 **Production of enzymes and industrial usage - special focus on improving oil processing**
H.C. Holm, Denmark
17:45-18:15 The Future

Saturday August 20, 2005

Session J

09:00-09:30 **Engineering the vitamin E content in transgenic *Brassica napus* seeds**
D. Weier, Aachen, Germany
09:30-09:45 **Transgenic oilseeds as a new renewable source for very long chain polyunsaturated fatty acids**
A. Abbadi, Goettingen, Germany
09:45-10:00 **Characterisation of primula □6-desaturases with distinct substrate specificities in transgenic yeast and plants**
O.V. Sayanova, Harpenden, Great Britain
10:00-10:15 **Accumulation of medium-chain fatty acids in oilseed rape (*Brassica napus* L.) due to heterologous gene expression**
C. Stoll, Giessen, Germany
10:15-10:30 **High variabilty of eicosanoid-transformation in lower plants**
T. Wichard, Jena, Germany
10:30-11:00 Coffee, tea and posters
11:00 **Closing Remarks**

Identification and functional analysis of a new triacylglycerol-lipase-family in *Arabidopsis thaliana*

Martina Körner, Maike Rudolph, Xuan Wang, Karin Athenstaedt[1], Günther Daum[1] and Ivo Feussner

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Many organisms accumulate carbon skeletons in the form of triacylglycerol (TAG) that is stored in lipid bodies. In plants lipid bodies are found in many tissues and are particularly abundant in seeds, where upon germination the TAG is broken down to fuel post-germinative growth. The initial step is catalysed by a lipase, which hydrolyses TAG at the lipid body surface. Little is known about the molecular identity of these TAG-hydrolysing lipases (TGLs) in plants. In order to identify this enzyme, a reverse genomic analysis was performed on the *Arabidopsis* genome. A protein of about 45-kDa was identified (AtTGL1), which shares homology with lipases from the GDSL-acylhydrolase family and is preferentially expressed in seedlings. In *Arabidopsis* it belongs to small gene family with 9 members (AtTGL1-9). AtTGL1 was expressed heterologously in *E. coli* and was characterized in detail after expression in insect cells. It was shown to hydrolyze triolein at a neutral to acid pH, but is neither active on phospholipids (PC, PI) nor on sterol esters and has no acyltransferase activity. In addition it complements a TGL mutant from yeast (*tgl3*). Transient expression studies in soybean embryos of AtTGL1-YFP were used to show that the protein is associated with the surface of lipid bodies. Moreover homozygous knock out lines of AtTGL1 failed to germinate in the absence of sucrose, and it is likely that AtTGL1 play an important role in regulating lipolysis during post-germinative growth.

Characterisation of sterol carrier protein-2.

Johan Edqvist[1], Bing Song Zheng[1], Kristina Blomqvist[1], Matts Nylund[2], Lenita Viitanen[2], Jessica Tuuf[2], Tiina A. Salminen[2], Peter Mattjus[2]

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We have recently shown that plants encode and express sterol carrier protein-2 (SCP-2). The biological function of SCP-2 is unclear, although there are indications that at least the mammalian SCP-2 may be involved in the catabolism of branched-chain and very long chain fatty acids. Bacterial and archaean SCP-2 are expressed from separate, independent genes, while in eukaryotes SCP-2 is often expressed in the C-terminal of multi-domain proteins. However, according to current knowledge plant SCP-2 is also expressed from independent, unfused genes. The *A. thaliana* locus At5g42890 encodes the *A. thaliana* SCP-2 (AtSCP-2), which is a 13.6 kDa protein with pI of 9.2. AtSCP-2 is predominantly localized to the peroxisomes. Structural models of apo-AtSCP-2 and the ligand-bound conformation of AtSCP-2 revealed high similarity with mammalian SCP-2-like domains. The AtSCP-2 models in both forms have a similar hydrophobic ligand-binding tunnel, which is extremely suitable for lipid binding. AtSCP-2 protein has lipid transfer activity *in vitro*, showing a preference for phosphatidylcholine, ergosterol and phosphatidic acid. We have also cloned and characterised SCP-2 from *Euphorbia lagascae*. The amino acid sequence of ElSCP-2 shares 67% identity and 84% similarity with AtSCP-2. Although ElSCP-2 and AtSCP-2 have a high sequence identity some of their cavity amino acids are different, which might explain the divergent ligand-binding preference of the two proteins. Proteomic analysis revealed that ElSCP-2 protein is accumulating in the endosperm during seed germination. We are interested in understanding the biological function of SCP-2 and will present our recent results on function, expression, evolution, specificity and structure of SCP-2 from plants.

The structure of peroxisomal short length specific acyl-CoA oxidase sheds light on the re-oxidation mechanism

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Peroxisomes are essential structures in all eukaryotic organisms. The organelles are characterized by the presence of catalase, but also by the enzymatic activities needed for β-oxidation of fatty acids. In β-oxidation cycles, which are the centers of fatty acid breakdown, the lipid chain is shortened by a C_2 unit in each round. Where mammals need both mitochondrial and peroxisomal β-oxidation cycles in order to oxidize all lipids, plants are able to conduct the full process in the peroxisome. The acyl-CoA oxidase family catalyzes the first and rate limiting step in the perixosomal β-oxidation cycle, converting acyl-CoA to *trans*-2-enoyl-CoA. *Arabidopsis thaliana* express at least 4 different acyl-CoA oxidases, each with different chain length specificities.

The structure of the short length specific *Arabidopsis* acyl-CoA oxidase, ACX4, from *Arabidopsis thaliana* has been determined in complex with the substrate analogue acetoacetyl-CoA. It is the second structure of a plant acyl-CoA oxidase available, long chain specific ACX1 was previously determined in our group. Where ACX1 is active as a dimer, ACX4 instead forms an active tetramer in solution, thereby being structurally much more similar to the related family of acyl-CoA dehydrogenases (ACAD). ACAD's catalyze the equivalent oxidative reaction in mitochondria, but transfers the reducing equivalent from FADH- to another flavoprotein, whereas ACX's are reoxidized by molecular oxygen. The structure of ACX4 makes is possible to better understand the mechanism of the oxygen reactivity in the oxidative half-reaction, substrate selectivity as well as the catalysis of ACX.

Lipid storage in soybean seeds occurs gradiently and is related to differentiation of plastids

Ljudmilla Borisjuk, Ulrich Wobus, Hardy Rolletschek, Institut fuer Pflanzengenetik und Kulturpflanzenforschung (IPK), 06466 Gatersleben, Germany

This study establishes a topographical framework for functional investigation of lipid biosynthesis and its interaction with photosynthesis [1] and introduces oxygen production/delivery as a key factor for the regulation of lipid storage in seeds in vivo [2]. In soybean seeds, the gradual transformation of chloroplasts into storage organelles starts from inner regions going outwards. This is evidenced by EM, CLSM, in situ hybridisation of plastid related genes and histo-/biochemical data. As a consequence, photosynthesis becomes gradiently distributed within the developing embryo. Electron transport rate and oxygen production rate is maximal in the embryo periphery as documented by imaging PAM-fluorescence and oxygen release via microsensors. Non-invasive NMR-spectroscopy of mature seeds revealed steep gradients in lipid deposition with highest concentrations in inner regions. The gradual loss of photosynthetic capacity was accompanied by a similarly gradual accumulation of lipids. The inverse relationship between photosynthesis and lipid biosynthesis argues against a direct metabolic involvement of photosynthesis in lipid biosynthesis during late storage stage, but points to a role for photosynthetic oxygen release. Photosynthesis supplies significant amounts of oxygen to the hypoxic seed tissue. This is followed by an increase in local ATP levels, most prominently within the lipid-synthesizing (inner) regions of the embryo and higher rates of lipid biosynthesis (14C flux analysis). We empazise a central role of chloroplasts in lipid storage [3, 4] and conclude that both respiratory and biosynthetic fluxes are dynamically adjusted to photosynthetic activity of plastids supplying oxygen to the lipid-synthesising regions.

[1] Borisjuk L, Nguyen TH, Neuberger T, Rutten T, Tschiersch H, Claus B, Feussner I, Webb AG, Jacob P, Weber H, Wobus U, Rolletschek H. (2005) The New Phytologist (in press).

[2] Rolletschek H, Radchuk R, Klukas C, Schreiber F, Wobus U, Borisjuk L. (2005). The New Phytologist (in press).

[3] Weber, H., Borisjuk, L., Wobus, U. (2005). Annual Review of Plant Biology 56: 253-279.

[4] Rolletschek H. & Borisjuk L. (2005). Recent Res. Plant Devel. 3: 25-45.

Biosynthesis and secretion of plant cuticular wax

Ljerka Kunst
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The cuticle is a continuous lipid structure that covers the surfaces of all land plants. It serves as a waterproof barrier protecting plants against non-stomatal water loss, UV-light, pathogens, and insects. The cuticle consists of intracuticular wax embedded in a cutin matrix and epicuticular wax that coats its outer surface. Major wax components are derived from very-long-chain fatty acid (VLCFA) precursors with chain lengths between 20 and 34 carbons. In most plants, there are two major pathways producing aliphatic wax components from VLCFAs: the acyl-reduction pathway generating primary alcohols and wax esters and the decarbonylation pathway generating aldehydes, alkanes, secondary alcohols and ketones. We are taking advantage of a large number of wax-deficient (*cer*) mutants in *Arabidopsis thaliana* to identify and functionally characterize gene products involved in wax biosynthesis and deposition.

Mutations in the *CER4* gene specifically affect synthesis of wax products via the acyl reduction pathway as evidenced by major decreases in the levels of primary alcohols and wax esters in *cer4* mutants. This phenotype is consistent with a lesion in a gene encoding an alcohol-forming fatty acyl-CoA reductase (FAR). There are eight FAR-like genes in *A. thaliana*. Molecular characterization of *CER4* alleles and genomic complementation confirmed that one of these genes encodes the CER4. The CER4 FAR activity was verified by expression in yeast, which resulted in the accumulation of C24 and C26 primary alcohols. RNA blot analysis demonstrated that *CER4* was expressed in leaves, stems, flowers, siliques and roots. Its transcription is limited to the epidermal cell layer consistent with its role in cuticular wax biosynthesis.

Cuticle synthesis requires extensive export of wax out of the epidermis to the plant surface. The mechanism of wax export is currently unknown. TEM studies of the *A. thaliana* wax-deficient *cer5* mutants revealed striking sheet-like inclusions in the cytoplasm of the *cer5* epidermal cells. Gas chromatographic analysis showed that these inclusions represent abnormal deposits of cuticular wax. The wax load on the surface of *cer5* mutants was reduced to 47% of wild-type levels. In contrast, total wax content of *cer5* epidermal cells manually separated from the stem was qualitatively and quantitatively comparable to the wild-type. These results indicated that biosynthesis of wax constituents in *cer5* mutants was not impaired, but that they were trapped inside the epidermal cells. The isolation of the *CER5* gene established that it encodes an ABC transporter of the WBC subfamily exclusively localized in the plasma membrane of epidermal cells. The CER5 ABC transporter is the first component of the cuticular wax export pathway identified in plants.

Extracellular BODYGUARD involved in the formation of cuticle

Alexander Yephremov[1], Andrea Faust[1], Derry Voisin[1], Sergey Kurdyukov[1], Christiane Nawrath[2], Nadia Efremova[1], Rochus Franke[3], Lukas Schreiber[3], Heinz Saedler[1]

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The extracellular matrix produced by epidermal cells is specialized to protect plants against adverse environmental conditions and pathogens and includes the cuticle barrier the outermost cell wall of the epidermis made up of lipid molecules. Some extracellular lipids are soluble and can be extracted with organic solvents into the wax fraction, while others are connected to cell wall carbohydrates and interconnected by ester bonds. A number of Arabidopsis mutants, which show apparent defects in the cuticular barrier, represent a valuable genomic tool not only to identify genes involved in the formation of the cuticle but also to elucidate how cuticular lipids modulate development of plants. Here we report characterization of an Arabidopsis cuticular mutant, called *bodyguard* (*bdg*), which displays pleiotropical effects of the mutation on growth, morphology, cell viability and differentiation. The *bdg* mutant accumulates significantly more cell wall-bound lipids and epicuticular waxes than wild-type plants. The RT-PCR analysis shows in *bdg* transcriptional activation of genes involved in the decarbonylation pathway, which catalyzes the formation of alkanes with odd-numbered chain lengths in epicuticular wax. However, with regard to cuticular appearance and structure, *bdg* is reminiscent to transgenic Arabidopsis plants expressing extracellular fungal cutinase, which exhibit defects characteristic of loss of cuticle structure. Strikingly, molecular cloning of *BDG* shows that it encodes a member of the α/β-hydrolase fold superfamily of proteins, to which extracellular fungal cutinases belong. Expression of *BDG* is restricted to epidermal cells. Furthermore, subcellular immunolocalization shows that BDG is a polarly localized protein that accumulates in the outermost cell wall in the epidermis. Thus, BDG defines a new family of plant-specific α/β hydrolases. We propose that BDG is required for polymerization processes in the cuticular layer of the cell wall or the cuticle proper.

The roles of Comatose, a peroxisomal ABC transporter, in lipid metabolism and signalling.

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The *Arabidopsis* COMATOSE (CTS) locus was identified in a genetic screen for primary activators of germination. CTS encodes a homologue of human Adrenoleukodystrophy protein (ALDP), a peroxisomal ABC transporter which has been implicated in lipid transport. Accordingly, a role in regulating fatty acid or fatty acyl-CoA import into the peroxisome has been demonstrated for CTS: *cts* mutant seed retain oil bodies, accumulate very long chain fatty acyl CoAs and do not germinate in the absence of classical seed dormancy-breaking treatments (1). Moreover, *cts* mutant seedlings cannot establish in the absence of sucrose, suggesting an inability to mobilise storage lipid. Alleles of *cts* were identified independently in screens for resistance to indole butyric acid (IBA) and the synthetic auxin precursor, 2,4-dichlorophenoxy butyric acid (2,4-DB) (2,3). Mutation of CTS blocks beta-oxidation of IBA and 2,4-DB, suggesting that CTS is also able to transport (or regulate the transport of) these compounds. This prompted us to test whether CTS might mediate the transport the jasmonic acid (JA) precursor (9*S*, 13*S*)-12-oxo-phytodienoic acid (OPDA). In *cts* mutants, basal JA is reduced and wound-induced JA accumulation is impaired, suggesting a role for CTS in JA biosynthesis (4). A model can be proposed whereby substrates for beta-oxidation enter the peroxisome by two routes: a pathway requiring CTS, and, dependent on the physicochemical properties of the substrate, a parallel “leak” pathway involving anion-trapping. However this remains to be demonstrated experimentally and approaches to address CTS-mediated transport of lipid substrates will be discussed.

Real-time PCR and analysis of array data demonstrate that CTS is expressed throughout the plant, suggesting roles beyond germination. Characterisation of *cts* alleles revealed a subtle fertility phenotype in which the defect in lipid catabolism is separable from that in JA biosynthesis. Current research is focussed on defining more clearly the role(s) of CTS in lipid homeostasis and dissecting how specific aspects of the *cts* mutant phenotype relate to the roles of this transporter in lipid metabolism and signalling.

(1) Footitt et al., 2002 EMBO J. 21: 2912 (2) Zolman et al., 2001 Plant Physiol. 127: 1266 (3) Hayashi et al., 2002 Plant Cell Physiol. 43: 1 (4) Theodoulou et al., 2005 Plant Physiol. 137 : 835.

Fatty acid metabolism in Arabidopsis

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Our research aims on tracking the pathways of fatty acids in plant cells. A keyrole in fatty acid metabolism play the enzymatic activities of acyl-CoA synthetases which can be found in various subcellular compartements and which are responsible for the metabolic availability of fatty acids. One tool to elucidate the specific role of these enymatic acitivities are the analysis of knock-out mutants from Arabidopsis. By this means mutants with severe phenotypes were identified. Some of these mutants will be discussed. One mutant lacks the activity of two peroxisomal long-chain acyl CoA synthetases and proofed these proteins to be essential for early seedling growth. The phenotype of the mutant challenges the current model of fatty acid import into peroxisomes involving an ABC transporter. We will report on a newly created triple mutant which provided some clues on how fatty acids might get metabolized during seedling establishment.

Phosphate deprivation induces transfer of DGDG galactolipid from chloroplast to mitochondria

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In plant cell galactolipids are main lipids of photosynthetic membranes and are absent from non plastidic membranes. During phosphate deprivation, a condition encountered by plants in many soils, there is an important change in membrane lipid organization. Total phospholipid content decreases and is balanced by a major increase in DGDG galactolipid content. DGDG is then not only present in plastids but also in non plastidic membranes. We show that in Arabidopsis cell cultures grown in absence of phosphate a high proportion of DGDG is present in mitochondria (Jouhet et al., 2004, J Cell Biol. 167:863-874). DGDG is expected to be synthesized in chloroplast outer envelope membrane mainly from diacylglycerol provided by phospholipids. Indeed we observed that synthesis of mitochondria DGDG is dependent on chloroplast envelope and that its structure is characteristics of DGD enzyme activity. The transfer of galactolipid is investigated and detected between mitochondria-closely associated envelope membranes and mitochondria. This transfer apparently does not involve the endomembrane system but is likely dependent upon contacts between plastids and mitochondria. These contacts are favoured at early stages of phosphate deprivation when DGDG cell content is just starting to respond to phosphate deprivation.

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Biochemical and structural analyses of glycosyltransferases involved in plant glycolipid biosynthesis

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The galactolipids mono- (MGDG) and digalactosyldiacylglycerol (DGDG) are the most abundant plant lipids predominantly found in chloroplasts, where they constitute around 78 mol% of total membrane lipids in the thylakoids. Three MGDG synthases and two DGDG synthases are known to be involved in galactolipid biosynthesis in *Arabidopsis thaliana*. Mutant analyses (of *mgd1*, *dgd1*, *dgd2* and of the double mutant *dgd1dgd2*) revealed, that these lipids are critical to sustain photosynthesis. Furthermore, the amounts of galactolipids increase during phosphate deficiency to substitute for certain phospholipids, not only in the chloroplast, but also in extraplastidial membranes, suggesting extensive lipid-trafficking between the different compartments. The ratio of MGDG (a non-bilayer forming lipid) to DGDG (a bilayer forming lipid) is crucial for the physical phase of thylakoid membranes and therefore tightly regulated. It has been shown, that the corresponding membrane-associated glucolipid-synthesizing enzymes from the gram-positive bacterium *Acholeplasma laidlawii* are regulated by lipid bilayer-properties and key phosphate-containing metabolites, indicating a close relationship between membrane lipid homeostasis and cellular metabolism. We therefore expect, that regulation of the plant enzyme activities occurs not only on the transcriptional level, but also on the protein level as shown in the bacterial system. We are currently investigating how the lipid environment regulates activity of the various galactolipid enzymes from *Arabidopsis*. These results are then correlated to 3D-models obtained by bioinformatics tools (including sequence-based comparisons with available structures from other glycolsyltransferases, fold recognition and threading methods) to develop working models for the 3D- structures for this important plant enzyme class.

PLAMs (PLastid Associated Membranes): Biophysical evidence for their endoplasmic reticulum origin and for non-covalent PLAM-chloroplast zones of attachment.

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To synthesize their galactolipids, all higher plant chloroplasts are partially or completely dependent on import of lipid precursors from the endoplasmic reticulum (ER). We have previously reported that lipid metabolising activities generally assumed to be localized in the ER co-isolated with chloroplasts isolated from young leaf tissue (J. M. Kjellberg *et al.* 2000, Biochim. Biophys. Acta 1485:100). We have partially characterized a light membrane fraction that could be separated from isolated chloroplasts and suggested an ER origin (M.X. Andersson and A.S. Sandelius, 2002, Aachen lipid symposium, poster). We have now utilized ER-specific fluorescent probes as well as *Arabidopsis* transformed with a GFP-tagged lumenal ER protein (Matsushima *et al.* 2002, Plant Physiol 130:1807; the seeds kindly provided by the authors) to demonstrate chloroplast-ER connections by confocal laser beam microscopy. In leaf cells of transformed *Arabidopsis*, the network appearance of the ER was evident, as was its close association with chloroplasts. In isolated chloroplasts, GFP-fluorescing spots were visible on the chloroplast surface. The latter result not only suggests the occurrence of specific areas of association between the ER and the chloroplast strong enough to survive the chloroplast isolation procedure, but also demonstrates that the attached ER had been part of the ER continuum of the cell. Manipulating the ER-chloroplast using optical tweezers and laser scalpels (M. Goksör *et al.* 2003, Proceeding of SPIE, 4966) demonstrated the tight association of ER to chloroplasts. This was demonstrated in both isolated chloroplasts as well as in laser dissected protoplasts. In conclusion, we demonstrate that a membrane clearly of ER origin is at discrete areas associated with the chloroplast surface. Supported by The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (A.S.S). Part of this study was conducted at the centre for Biophysical Imaging (financed by SWEGENE) at Chalmers University of Technology and Göteborg University.

Biosynthesis and function of galactolipids

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Chloroplasts are the site of photosynthesis and harbor a unique set of lipids, e.g. the galactolipids (monogalactosyldiacylglycerol, MGDG; digalactosyldiacylglycerol, DGDG). MGDG and DGDG are crucial for photosynthetic light reactions. Therefore, DGDG deficiency caused by mutations in the two DGDG synthases DGD1 and DGD2 result in reduction in chlorophyll and photosynthetic efficiency. Introduction of a bacterial glucolipid synthase from Chloroflexus into the dgd1 mutant leads to the accumulation of a novel lipid, glucosylgalactosyldiacylglycerol (GlcGalDG) in the chloroplasts, and to complementation of growth and photosynthetic efficiency. Therefore, galactose in the second position of the DGDG head group can to some extent be functionally replaced by glucose.

DGDG accumulates in extraplastidial membranes after phosphate deprivation, but also in the peribacteroid membranes surrounding nitrogen fixing Rhizobia in root nodules of legumes. Similar to *Arabidopsis*, the model legume *Lotus japonicus* contains two DGDG synthase genes, DGD1 and DGD2. cDNAs were obtained for the two DGDG synthases, and used to generate transgenic *Lotus* plants with reduced expression of DGD1 or DGD2 by RNAi technique. Data will be presented on the nodulation efficiency of transgenic *Lotus* plants after inoculation with *Sinorhizobium loti*.

Cycloartenol synthase deficiency in Arabidopsis

Hubert Schaller *, Elena Babiychuk £, Pierrette Bouvier-Navé *, Sergei Kushnir £

- * Isoprenoid department, IBMP/CNRS, Strasbourg, France *
- £ Department of Plant Systems Biology, Ghent University/VIB, Belgium

Plant isoprenoids are involved in all fundamental biological processes. Higher plants such as *Arabidopsis thaliana* produce campesterol and sitosterol from cycloartenol. This metabolite, which is the plant sterol precursor, comes from the cyclisation of 2,3-oxidosqualene. A leaky mutant of *CAS1* (*CYCLOARTENOL SYNTHASE 1*) has been shown to produce high amounts of 2,3-oxidosqualene. Biochemical and physiological implications of this mutation in *Arabidopsis* will be presented.

Functional role of phosphatidylglycerol and cardiolipin in plants

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Phosphatidylglycerol (PG) and cardiolipin (CL) are anionic phospholipids, which constitute the biomembranes of prokaryotes and eukaryotes. In plant cells PG represents a minor component of the different membrane systems usually amongst other phospholipids, but it is the only phospholipid in the thylakoid and inner envelope membranes, whereas CL is exclusively located in the mitochondrial membranes. Apart from their structural role of forming a lipid bilayer, these anionic phospholipids appear to have additional important functions.

PG is synthesised by the concerted action of a phosphatidylglycerophosphate (PGP) synthase, which transfers a phosphatidyl group from CDP-diacylglycerol to glycerol-3-phosphate, and a PGP phosphatase, which rapidly converts PGP to PG. These enzymic activities are located in the inner mitochondrial membrane of all eukaryotic cells where they provide PG for the formation of CL catalysed by the CDP-diacylglycerol dependent CL synthase. In addition to the mitochondrial pathway conserved in all eukaryotes, plants have a plastidial as well as a microsomal pathway for PG synthesis.

In order to investigate the functional role of PG and CL in plant cells, we have functionally characterised the PGP and CL synthase genes of *Arabidopsis thaliana* and started to analyse *A. thaliana* loss-of-function mutants having defects in one of these genes. The results of these experiments will be reported.

Arabidopsis Phospholipid: Sterol Acyltransferase (PSAT) regulates the composition of free sterols in the membranes

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Recently, we identified an Arabidopsis gene (At1g04010) encoding an enzyme (Phospholipid: Sterol Acyltransferase, PSAT) responsible for most of the sterol ester formation in this plant. The enzyme belongs to the LCAT/PDAT family and is the only intracellular enzyme so far found which catalyses an acyl-CoA independent sterol ester formation. The enzyme utilises phospholipids (preferentially PE) as acyl donors and acylate various sterols and sterol biosynthesis intermediates.

The enzyme had poor activity for sterol intermediates compared to end-product sterols when the acyl acceptors were presented as single substrates. However, it had been shown previously that sterol intermediates are preferentially accumulated as esters in plants overproducing sterols. An explanation for these apparent conflicting *in-vivo* and *in-vitro* results was given by sterol selectivity experiments. Using RPHPTLC, we were able to separate some of the acylated sterol intermediates from acylated end-product sterols. Competition experiments were performed using sitosterol as an end-product sterol and 24-ethylidene lophenol, cycloartenol or obtusifoliol as intermediate. When the sterol intermediates were used as single substrate, the PSAT activity was less than half that of sitosterol. However, when they were presented to the enzyme together with sitosterol, the acylation of sitosterol was drastically decreased and the acylation rates of the intermediates increased up to 3-fold.

Based on these results we conclude that PSAT is activated by the end-product sterols and that the activated PSAT has selectivity towards sterol intermediates. In this way, PSAT can both regulate the pool size of free sterols as well as limit the amount of free sterol intermediates in the membranes.

Linoleate biosynthesis and aroma biogenesis in olive fruit: a molecular approach

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Olive oil is a vegetable oil with outstanding nutritional properties due to its well balanced fatty acid composition, as well as the presence of minor components such as natural antioxidants. In addition, olive oil is characterized by its unique organoleptic characteristics including aroma, color and flavor. However, very little information is available in olive fruit on the pathways involved in the biosynthesis of the compounds responsible for these exceptional properties. We have initiated a program with the aim of improving olive oil quality using a molecular approach.

The first goal is the reduction of linoleic acid in some olive cultivars which posses a relatively high content of this fatty acid, yielding olive oils with low oxidative stability. To achieve this three different cDNA sequences, encoding two microsomal (*OepFAD2-1*, *OepFAD2-2*) and one plastidial (*OepFAD6*) oleate desaturases have been isolated. Genomic Southern blot data are consistent with the presence of at least two copies of each *FAD2* gene and only one copy of the *FAD6* gene in the olive genome. Expression studies show that they are tissue and developmentally regulated. Possible differential physiological roles related to their contribution to the linoleic acid content in different tissues will be discussed.

On the other hand, we have started the molecular characterization of the lipoxygenase (LOX) pathway in olive fruit, in relation to the biosynthesis of olive oil aroma. Two different cDNA clones with homology to 9-LOX and 13-LOX respectively, have been isolated. In addition, a sequence that shows homology to 13-hydroperoxide lyases has also been cloned. The physiological function of these genes regarding their contribution to the olive oil aroma will be discussed.

Biochemical analysis of a fatty acid isomerase from *Propionobacterium acnes*

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Several positional and geometric isomers of linoleic acid (LA) are collectively known as conjugated linoleic acid (CLA) and were reported to have anti-carcinogenic, anti-atherogenic, anti-adipogenic, anti-inflammatory and anti-diabetic effects. So far, only two isomers, namely 9-*cis*,11-*trans*-CLA and 10-*trans*,12-*cis*-CLA, were shown to elicit these effects in experiments on the cell cultures, animals and humans volunteers. This broad range of CLA activities is partly due to inhibition of cyclooxygenase (COX) by CLA and activation of PPARγ transcription factor; possibly other biochemical pathways are also involved.

The industrial method of CLA preparation is the catalytic isomerization of plant oils rich in LA, yielding of mixtures with varying amounts of 9-*cis*,11-*trans*-CLA and 10-*trans*,12-*cis*-CLA and with minor content of other isomers. A biotechnological approach based on use of selective isomerases would provide the advantages of having pure isomers and enable to produce CLA-containing nutrients with precise composition.

In the present work we optimized the heterologous expression of a gene encoding for a fatty acid double bond isomerase from *P. acnes* (PAI) in *E. coli* and purified the correspondent protein. In contrast to the majority of similar isomerases, PAI is soluble; it catalyzes conversion of 9-*cis*,12-*cis*-LA into 10-*trans*,12-*cis*-CLA by a yet unknown mechanism. To learn more on the underlying mechanism we determined its substrate specificity and characterized the products formed. Furthermore we were able to crystallize the protein and determine the structure of the enzyme by X-ray diffraction. The structure is mainly α-helical, however two β-domains are present, on of them is a variation of typical FAD-binding domain. The mode of substrate binding remains to be determined and currently we are attempting to co-crystallize PAI with its substrate/product. As no structural information for related enzymes is published until now, the structure of PAI is not only of applied but also of general biochemical interest and it will provide additional insights in the mechanism of cis-trans fatty acid double bond isomerases.

Castor Oil Biosynthesis and Expression Profiles of Lipid Genes in Developing Seed of Ricinus communis L. (Castor bean).

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Castor oil contains 90% ricinoleate, an unusual fatty acid with numerous industrial applications. Although the biochemical pathways of ricinoleate biosynthesis have been well studied, the mechanism of such high accumulation of ricinoleate in castor seed remains unknown. In order to better understand the key steps of this biosynthetic process, we have conducted a series of seed development studies including morphogenesis, fatty acids accumulation and expression profiles of genes involved. We have identified two visual markers, seed coat color and endosperm volume, and defined three phases that encompass the course of castor seed development. Castor seed matured at about 60 days after pollination (DAP), and the endosperm tissue underwent rapid expansion during the mid-phase (26-40 DAP). Ricinoleate was not detectable before the endosperm emerged but rose quickly to 77% of its final content when seeds developed into 26 DAP. The ricinoleate reached to a dominant level up to 86% at 33 DAP, and increased to top 90% at 40 DAP and later.

In theory, the efficient accumulation of the ricinoleate should involve transcriptional activation of genes associated with the ricinoleate and oil synthesis. We have designed quantitative RT-PCR analysis and investigated the transcript levels of 14 lipid genes at each stage of seed development. All of the transcripts were induced to higher levels when seed developed into 26 DAP, coinciding with the onset of endosperm expansion and ricinoleate/oil synthesis. The transcripts reached to their peak levels at later different stages, displaying various temporal patterns. Furthermore, the difference of maximum induction varies dramatically among genes, ranging from 5 folds to >200,000 folds. By comparing these genes in the fatty acid biosynthesis pathway in plastid and the Kennedy pathway in the endoplasmic reticulum, we propose several possible limiting steps or control points in castor ricinoleate/oil synthesis. The genes involved the Kennedy pathway seem to have stronger control over this process.

Oxylipins in plant-herbivore interactions : Tales from Nicotiana attenuata in the real world

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It has become abundantly clear that oxylipins could play important roles in a plant's interactions with its environment. Demonstrating that this is in fact the case has required developing genetic tools in plant systems with a rich and well understood ecology. This talk will describe the ongoing research with *Nicotiana attenuata*, a native tobacco species that colonizes the post-fire environment of the Great Basin desert in North America. In this species, attack from adapted lepidoptern herbivores is "recognized" by the plant when fatty acid amino acid conjugates (FACs), present in the oral secretions from the larvae, are introduced into plant wounds during feeding. Since a JA burst appears to be one of the first and organizing hormonal responses in the plant to FAC elicitation, our group has cloned a number of genes for enzymes involved in oxylipin biosynthesis (NaLOX3, NaAOS, NaHPL) oxylipin decoration (NaTD) and perception (NaCOI, NaWRKY3). For many of these genes, plants have been transformed to silence their expression and the transformed plants have been returned to their native habitat for an evaluation of their ecological performance. For many of these transformants, the field work has confirmed laboratory results, but for others, the transformants have suggested entirely unexpected functions of oxylipins.

Functions of Sterol Glycosides and Glycosylceramides in Host/Pathogen Interactions

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Sterol glycosides and glycosylceramides occur in eukaryotes, and in some bacteria. We will report on the cloning and characterization of novel sterol glycosyltransferases and ceramide glycosyltransferases from plants, fungi, and bacteria. Sterol glycosyltransferase isozymes are localized to different intracellular membranes due to varying targeting signals. Both sterol glycosyltransferase and ceramide glycosyltransferase knock-out mutants of phytopathogenic fungi show reduced pathogenicity.

Phospholipid and oxylipin signaling of the plant innate immune system

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Bacterial pathogens deliver type III effector proteins into the plant cell during the course of infection. In susceptible (*r*) hosts, type III effectors contribute to virulence, but in the case of resistant host-plant (*R*), they betray the pathogen to the plants surveillance system. Recognition induces a complex suite of cellular and molecular events comprising the plants inducible defense. As plant recognition of bacterial Avr proteins occurs in the cytosol, the response can be mimicked using a transgenic system. The gene encoding the bacterial type III effector AvrRpm1 of *Pseudomonas syringae* was introduced as a chemically inducible construct in wild type (*R*) Arabidopsis and the *rpm1* (*r*) mutant. Recognition of AvrRpm1 causes the sequential activation of phosphoinositide specific phospholipase C (PLC) and phospholipase D (PLD). PLC acts in concert with diacylglycerol kinase (DAGK) to produce phosphatidic acid (PA). The early PLC-DAGK dependent PA production is followed by activation of PLD that produces large amounts of PA directly from structural phospholipids. That inhibition of either PLD or PLC inhibited response and direct feeding of PA or PLD caused response, strongly argues for that PA is an integral part of the signaling pathway and not just a consequence of response. We have also found that, as a consequence of AvrRpm1 recognition, a large amount of the jasmonate 12-oxo-phytodienoic acid (oPDA) accumulates. However, the vast majority of the oPDA is esterified to a previously uncharacterized galactolipid. Structural determination of this novel galactolipid (lipid X) is in progress. The accumulation of both free and esterfied oPDA could be inhibited by inhibition of PLC or PLD, thereby establishing a link between phospholipid signaling and oxylipins. Moreover, a mutant unable to synthesize oPDA was clearly hampered in its defense against *P. syringae*. In summary, our data supports that PA as well as oxylipins are integral and necessary components of the signal transduction pathways of the plant innate immune system.

Molecular basis for the elicitation of plant innate immunity by lipo-oligosaccharide of *Xanthomonas campestris*

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Lipopolysaccharides (LPSs) and lipooligosaccharides (LOSs) are major components of the cell surface of Gram-negative bacteria with diverse roles in bacterial pathogenesis of animals and plants that include elicitation of host defences. Little is known about the mechanisms of perception of these molecules by plants and about the associated signal transduction pathways that trigger plant immunity. We have addressed the issue of the molecular basis of elicitation of plant defences through the structural determination of the LOS of the plant pathogen *Xanthomonas campestris* pv. *campestris* strain 8004 and examination of the effects of LOS and fragments obtained by chemical treatments on the immune response in A*rabidopsis thaliana*. The structure shows a strong accumulation of negatively charged groups in the lipid A-inner core region and has a number of novel features, including a galacturonyl phosphate attached at Kdo residue and a unique phosphoramide group in the inner core region. Intact LOS and the lipid A and core oligosaccharides derived from it were all able to induce the defence-related genes *PR1* and *PR2* in *Arabidopsis* and to prevent the hypersensitive response (HR) caused by avirulent bacteria. Although LOS induced defence-related gene transcription in two temporal phases, the core oligosaccharide induced only the earlier phase and lipid A only the later phase. These findings indicate that plant cells can recognize lipid A and core oligosaccharide structures within LPS to trigger defensive cellular responses and that this occurs via two distinct recognition events.

The role of sphingolipids in plants and yeast: from signalling to lipid rafts

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Sphingolipids are ubiquitous and essential components of most eukaryotic cells, playing roles in a diverse range of processes such as apoptosis, cell cycle progression and vesicular trafficking. Sphingolipid functionality is believed to be derived from their bulk membrane properties and also their phosphorylated metabolites (such as sphingosine-1-phosphate; S-1-P) which acts as a potent signalling compound in animal systems. In addition, there is also good evidence for the importance of sphingolipids in so called lipid rafts (discrete microdomains usually found in the plasmamembrane). Whilst there is a large volume of research to demonstrate the importance of sphingolipids and their metabolites in animal systems, the situation is less clear in higher plants. Moreover, whilst the yeast *S. cerevisiae* has served as an excellent model for the genetic dissection of sphingolipid biosynthesis, this particular yeast is atypical in its sphingolipid complement, lacking sphingosine. We have used two post-genomic model systems to investigate the role of sphingolipid functionality with particular reference to variation in long chain base (LCB) modifications. Firstly, the fission yeast *S. pombe* serves as simple genetically-tractable system with which to test the role of sphingosine (and S-1-P) as a signalling system in lower eukaryotes. Secondly, arabidopsis provides an excellent model with which to examine aspects of plant-specific sphingolipid biosynthesis. We have generated *S. pombe* and arabidopsis mutants disrupted in the biosynthesis of sphingosine, allowing us to critically evaluate the role of this LCB in these organisms. These mutants, in combination with quantitative measurement of endogenous LCBs by LC-MS, provide insights into the suitability of applying animal sphingolipid signalling paradigms to lower eukaryotes. We have also examined the evidence for sphingolipids in lipid rafts in plants, using diagnostic LCB composition to quantitate levels in detergent-resistant membrane fractions. This has indicated the enrichment of sphingolipids in DRMs, in particular the presence of specific LCB isomers diagnostic for inositol-containing sphingolipids. Thus, a combination of genetic and biochemical analyses provide new insights into the functionality of LCBs in lower eukaryotes.

Fungal sphingolipids as targets of plant defense reactions

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In eukaryotic cells, sphingolipids have become a focus of interest, because they are involved in signal transduction pathways and other cellular processes. Glucosylceramide (GlcCer) represents the unique glycosphingolipid which plants, fungi and animals have in common. On the other hand, organism-specific modifications occur at the ceramide backbone of GlcCer. While plants possess a large variety of different, mainly delta 8-(*Z*)-unsaturated GlcCers, most fungi share a consensus GlcCer structure which is characterized by (4*E*,8*E*)-9-methylsphinga-4,8-dienine linked to a saturated or monounsaturated alpha-hydroxy fatty acid.

Recently, we have shown that fungal GlcCers act as targets for plant defensins, which induce fungal growth arrest by plasma membrane permeabilization through specific interaction with high affinity binding sites on fungal cells (Thevissen et al., 2004, JBC 279: 3900-3905). The structural features of fungal GlcCer which contribute to the recognition by antifungal peptides, are presently unknown. To study this question, we cloned several fungal genes enabling specific manipulations of the GlcCer structure from *Pichia pastoris*. *In situ* engineering of the GlcCer structure by generating *P. pastoris* mutants lacking either glucosylation, desaturation, hydroxylation or methylation shows that the absence of some of these structural modifications affects the recognition by an antifungal peptide isolated from radish seeds.

In vitro effects of ursolic acid on purified rat intestinal alkaline sphingomyelinase

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Purpose: Alkaline sphingomyelinase (alk-SMase) is an enzyme that hydrolyses sphingomyelin in a bile salt dependent manner in the gastrointestinal tract, and has been proposed as an inhibitor of colon carcinogenesis. Ursolic acid (UA) is a plant component that has been shown to have anti-proliferative and apoptotic effects on HT29 human colon adenocarcinoma cells, with activation of alk-SMase as an early event. The aim of this study was to study the in vitro effects of UA on the activity of purified rat intestinal alk-SMase. **Methods**: Rat intestinal alk-SMase activity was determined after incubation with UA in the presence and absence of taurocholate (TC). The effect was compared with boswellic acid, another plant component with similar structures. **Results**: UA and boswellic acid enhanced the activity of rat intestinal alk-SMase. The maximal effect of UA was comparable to that induced by acetyl-keto-□-boswellic acid, but UA exhibited the effect at a much lower concentration. UA did not similarly enhance the activity of neutral sphingomyelinase. Activation of alk-SMase by TC at low concentration was enhanced by UA but that at high concentration was suppressed. **Conclusions**: UA and its homologue boswellic acids have stimulatory effect on the activity of intestinal alk-SMase.

The Use of Plant Lipids as Functional Designer-made Ingredients in Foods

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Plant lipids are a major global food source. In 2002-03, the total world production of vegetable fats and oils was estimated at approx. 100 million tons (Mt), of which more than 80% is used for human consumption.

Whereas the main focus of the crop scientists and vegetable oil producers today are to provide fats and oils for food uses, industry is focusing increasingly on the specific properties of the various lipids and their derivatives. The presentation will provide examples of how plant lipids and their derivatives can be utilised in the design of functional ingredients in industrial scale, with specific focus on two areas: food emulsifiers and low calorie fats (structured lipids).

Food emulsifiers are amphiphilic lipids manufactured from edible fats and oils or fatty acids esterified with polyvalent alcohols together with organic acids such as lactic acid, citric acid, acetic acid, or tartaric acid. They provide a vast range of functionalities in foods such as:

- Binding oil and water in emulsions (e.g. dressings, margarines, and low fat spreads)
- Improving the quality of baked goods with respect to volume, texture, and shelf life.
- Destabilising emulsions in whipped emulsions such as ice cream, toppings, and mousse.

Several food products on the market, for example reduced-fat or low-calorie products only exist because of the emulsifiers they contain.

In the area of *Low calorie fats* derived from oleochemicals, various types have been developed over the last 20 years e.g. OLESTRA, CAPRENIN, and SALATRIM.

SALATRIM is a family of triglyceride blends made from short and long-chain fatty acids. The short-chain acids (C2-C4) may be acetic, propionic, butyric, or a combination of all three. The long-chain fatty acid (C16-C22) is predominantly stearic and derived from fully hardened vegetable oil.

Due to the unique fatty acid composition SALATRIM contains only 5-6 calories/g compared to the 9 calories/g of naturally occurring triglycerides, at the same time giving foods a similar creamy taste, texture, and mouth feel as full-fat products.

Finally a new alternative PVC plasticiser based on castor oil will be mentioned briefly as an example from the non-food area.

Applications of Plant Lipids in Food Products.

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Plant lipids are widely used in the food industry as commodity and specialised vegetable oils to provide texture, richness in flavour and flavour release or to interact with other food ingredients.

In products like chocolate confectionery or margarine the fat is the continuous phase in a blend or an emulsion and as the fat is “the outer phase” the functionality of the fat controls the sensory functionality as hardness, structure and how the product melts away in the mouth and releases the flavour.

Most dairy products on the other hand are oil-in-water emulsions and in these products the sensory properties of the product are more dependent on the interaction between the fat and the continuous water-phase. In all cases the flavour stability of the oil is important

Native oils such as soybean oil, rapeseed oil or palm oil may provide the required functionality, but over the years more specialised products have been developed based upon hybrids of the plants or upon different modification techniques. Hydrogenation of oils, saturation or trans isomerisation of unsaturated fatty acids has, for many years, been the preferred technology used to increase the hardness, melting point and oxidative stability of vegetable oils, but the increased focus on the possible negative health effects of trans fatty acids has increased the focus on other ways to achieve the right functionality.

Fractionation of natural fats with a high content of saturated fatty acids, primarily palm oil, is one way to achieve fats which can provide structure to products like chocolate confectionery, margarine or other applications which require a solid fat system. But the potential of fractionation is limited by the fatty acids and triglycerides, which are available in the native oils, so new sources of fatty acids and triglycerides as well as new technologies to modify the oils will emerge in the future.

Production of enzymes and industrial usage - special focus on improving oil processing

Hans Christian Holm

The developments within biotechnology has enabled cost effective production of a variety of enzymes that improve the processes and final products for a number of industrial producers. The production of enzymes from screening in nature to final product will be shown. As well as case stories showing how enzymes have replaced harsh chemicals and improved final products. We believe the time has now at last come for bringing the enzymatic processes into the oils and fats industry.

The presentation will show:

- how lipases are today used in production of margarine and shortenings enabling production free of trans fatty acids formation,
- how phospholipases remove phospholipids from oils ensuring high processing yields and high quality of refined oils
- how a growing number of "nutraceutical" specialty oils are prepared from vegetable and fish oils using enzyme technology
- and finally how vegetable oils through enzymatic conversion are used to make new and improved ingredients for cosmetic usage.

- Join the industrial evolution.

Engineering the vitamin E content in transgenic *Brassica napus* seeds

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Tocochromanols are essential components of animal diets and are therefore collectively known as vitamin E. They consist of a polar chromanol ring with a lipophilic prenyl side chain and the different homologous forms vary only in the number and position of methyl substituents on the chromanol head group. Apart from their varying antioxidative abilities tocochromanols play presumably a role in the preservation of membrane integrity as well as in the regulation of transcription and post-translational processes.

Tocochromanols are synthesised in photosynthetic organisms and in higher plants their biosynthesis proceeds at the inner envelope membrane of chloroplasts utilising homogentisate derived from the cytosolic shikimate pathway and prenyl diphosphates, isoprene intermediates from the plastidial 1-deoxyxylulose-5-phosphate pathway. The first committed step is catalysed by homogentisate prenyltransferases producing 2-methyl-6-prenyl-1,4-benzoquinols. A cyclase forms the typical chromanol ring structure by generating an additional oxygen heterocycle next to the aromatic ring originating from homogentisate, while the methyl substituents on the chromanol ring are inserted by methyltransferases.

In this presentation we will report the characterisation of key enzymes involved in tocochromanol biosynthesis via overexpression as recombinant proteins in microorganisms. In addition, we will discuss the importance of these key enzymes in tocochromanol biosynthesis by showing that the overexpression of the respective chimeric genes in developing seeds of transgenic rapeseed plants increased the total content and changed the composition of tocochromanols in the seed oil.

Transgenic Oilseeds as a New Renewable Source for Very Long Chain Polyunsaturated Fatty acids

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A constant supply of very long-chain polyunsaturated fatty acids (VLCPUFAs) such as arachidonic (ARA), eicosapentaenoic (EPA), and docosahexaenoic acid (DHA) as part of the human diet is considered beneficial. These ω6- and ω3-fatty acids function as constituents of membrane phospholipids and as precursors of different classes of eicosanoids. VLCPUFA biosynthesis starts from linoleic and α-linolenic acids. They are not present in reserve triacylglycerols of angiosperm plants and, therefore, enter the human diet mainly in the form of marine and freshwater fish. In view of the increasing world population and the problem of over fishing marine resources, transgenic oilseeds might constitute a sustainable source of VLCPUFAs. To establish VLCPUFA biosynthesis by genetic engineering of annual oilseed crops additional fatty acyl-desaturases, elongases and acyltransferases are required that have regiospecificities not present in agronomically important plants. As a result of parallel efforts in several laboratories, cDNAs for all the desaturases and elongases required for the conversion of linoleic and α-linolenic acid to ARA, EPA and DHA are available from different organisms. Heterologous expression of appropriate ensembles in different plants has resulted in the production of dietary significant amounts of ARA and EPA in transgenic seed oils. The results of these strategies will be discussed.

Characterisation of Primula Δ6-desaturases with distinct substrate specificities in transgenic yeast and plants

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The biosynthesis of 20-carbon polyunsaturated fatty acids (PUFAs) has been the subject of intensive research in recent years. PUFAs perform many important functions in human metabolism and are especially known for their key role in the biosynthesis of eicosanoids. PUFAs can be classified into the n-6 and n-3 families, derived from the two essential fatty acids, linoleic acid (LA , 18:2, n-6) and α -linolenic acid (ALA, 18:3, n-3) respectively. Eicosanoids derived from n-6 PUFAs are functionally distinct to those derived from n-3 substrates. Eicosanoids derived from n-6 substrates are generally pro-inflammatory, whereas the eicosanoids derived from n-3 fatty acids have little or no inflammatory activity. There is also mounting evidence of the therapeutic role of n-3 PUFAs in human disorders such as Metabolic Syndrome and Cardiovascular disease. Taking into account the importance of PUFAs in human health, the successful production of PUFAs (especially the n-3 class) in transgenic oilseeds can provide a sustainable source of these essential fatty acids.

The “conventional” aerobic pathway which operates in most PUFA-synthesising eukaryotic organisms, starts with Δ^6 desaturation of both LA and ALA to yield γ-linolenic (GLA, 18:3, n-6) and stearidonic acid (STA, 18:4, n-3). The first example of the microsomal Δ^6-fatty acid desaturase was cloned from Borago officinalis and orthologs of the Δ^6 -desaturase have been identified from many different species, including mammals, fungi, mosses and plants. All these Δ^6 -desaturases showed no major preference for n-6 or n-3 substrates. Recently we have cloned and characterized several examples of Δ^6 -fatty acid desaturases from Primula which show strong specificity towards n-3 substrates when expressed in yeast and transgenic plants. We also have identified Δ^6-desaturase from Primula which displayed a distinct preference for the n-6 substrate. The identification of fatty acid Δ^6 -desaturases with n-3/n-6 selectivity has great potential in the heterologous production of PUFAs.

Accumulation of medium-chain fatty acids in oilseed rape (*Brassica napus* L.) due to heterologous gene expression

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The fatty acid composition is an important objective of molecular genetics and plant breeding to make different seed oils more competitive in various segments of the vegetable oil market. With the purpose to improve the industrial usefulness of oilseed rape we aim at developing genetically engineered *Brassica napus* that accumulates medium-chain fatty acids (MCFA) in its seed oil (Stoll *et al*., Eur. J. Lipid Sci. Technol., 107, 2005, 244-248). Therefore relevant genes from the genus *Cuphea* encoding the synthesis of MCFA have been selected for *Agrobacterium*-mediated transformation. Gene constructs harbouring the β-ketoacyl-acyl carrier protein synthase III gene (wild- and mutant type) either from *C. lanceolata* (*Cl*KASIII) or *C. wrightii* (*Cw*KASIII) were used alone or in combination with different *Cuphea* thioesterase genes (*Ch*FatB2, *Cl*FatB3). Compared to the KASIII wild type the mutated enzyme (KASIIIbmut) is not sensitive for regulatory acyl-ACPs (decanoyl-ACP), and therefore the latter was predestined to be used in double constructs with thioesterases relevant for the biosynthesis of caprylic (C8:0) and capric acid (C10:0). The best transformants were analysed further by half-seed analyses and the number of integrated transgene copies were determined by Southern blotting. Both single *Cl*KASIII constructs (wild- and mutant type) showed no effect on the production of MCFA. Concerning the double constructs an increase of MCFA could be shown in the T2 seeds of which the best phenotypes were selected to produce individual T2 plants. The resulting T3 seeds were analysed as pooled seed samples and revealed stable fatty acid contents for both double constructs: 3.9% C10:0 and 12.6% C16:0 for *Cl*KASIIIbmut/*Cl*FatB3 and 2.0% C8:0 and 8.8% C10:0 for *Cl*KASIIIbmut/*Ch*FatB2, respectively. In order to derive increased levels of MCFA and to get information about the stability of the gene expression, further progenies of selected plants showing the best phenotype in the T3 seeds are developed.

High variabilty of eicosanoid-transformation in lower plants

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Recently, polyunsaturated aldehydes (PUA) and oxo-acids produced by diatoms came into the focus of ecologists since they interfere with the reproductive success of their predators [1]. We set out to investigate the formation of these PUA in lower plants, such as diatoms and mosses. Interestingly, the production of these oxylipins follows generally different routes from C_{20}- fatty acids in these organisms. Whereas lipoxygenase / lyase (?)-mediated transformations that result in volatile PUA are widely distributed (one third of 51 screened diatom species) in diatoms [2], *Asterionella formosa* and *Gomphonema parvulum* exhibit different fatty acid metabolising activities resulting in hydrocarbons and unsaturated oxo-acids [3]. As found for diatoms, the wounded moss *P. patens* transforms C_{20}-fatty acids into similar or same oxo-acids starting from C_{20} precursors. But here again different pathways are responsible for the release of unsaturated C_8-alcohols and C_9-aldehydes as second cleavage product derived from arachidonic acid [4]. In order to understand the high variability of eicosanoid-transformations, we cloned two enzymes involved in fatty acid conversion from *P. patens*. One exhibits multifunctional activities and combines the action of a 12-lipoxygenase and lyase activity introducing a hydroperoxy-functionality in arachidonic acid and cleaving the hydroperoxide into C_8-alcohols and the 12-oxo-acid [5]. The second cloned enzyme bears a lyase activity and catalyses the production of (*Z*)-non-3-enal and the (5*Z*,8*Z*)-11-oxoundeca-5,8-dienoic acid from 12-HPETE. Since the production of volatile PUA is activated by wounding, trapping of PUA required an *in situ* derivatisation protocol based on the reaction of the crude cell-preparations with *O*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA•HCl) under near physiological conditions [6]. Interestingly, (*Z*)-non-3-enal was only trapped by this *in situ* derivatisation during the cell disruption. Otherwise it is directly transformed into (*E*)-2-nonenal that is detected by headspace solid phase microextraction by a proposed 3*Z*:2*E* -enal isomerase.

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Posters

Off-flavors during storage of potato flakes

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Off-flavors frequently appear during storage of potato flakes. We postulated that green note compounds formed by the lipoxygenase pathway [1] or by autooxidation are responsible for this undesirable odour. It is well known that the process of fatty acid degradation can reduce shelf-live of various food stuffs by generation of volatile or non volatile oxylipins [2].

In order to confirm our hypothesis, volatile compounds emitted by potato flakes stored for 6 months at 25°C were identified and quantified by SPME-GC-MS. Analysis revealed that the hexanal is the main compound formed and that its release gradually increases during the storage period. HPLC analysis confirmed the hexanal presence in potato flakes.

To elucidate the mechanism leading to hexanal synthesis, further experiments were undertaken. Lipoxygenase activity and oxylipin profiles (free and esterified forms) were determined [3]. Considering the predominant 9-lipoxygenase activity in potato tubers, the presence of large amounts of 13-fatty acid hydroperoxides and the very low lipoxygenase activity strongly suggest a non enzymatic origin of hexanal. This statement was confirmed by the chiral analysis of fatty acid hydroperoxides that are almost racemic.

Further investigations are necessary to determine formula or process modifications that can avoid volatile formation.

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Application of pulsed electric fields at oil yield and phytosterols content at the production of edible oils

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Vegetable oils and fats containing phytosterols are essential nutrients in the human diet. They are beneficial especially due to their cholesterol lowering effect. The aim of the current work was to study the impact of pulsed electric fields on the oil content, oil quality and phytosterols content of wet milled corn germs in comparison to the different separation methods solvent extraction, pressing as well as supercritical CO_2 extraction.
The basic principle of the effect of pulsed electric fields is the permeabilisation of biological membranes. In dependency of the impulse characteristic and the treatment intensity, the permeabilisation can be reversible or irreversible. At a reversible treatment of plant membranes at electric field strengths below 1.0 kV/cm stress is induced and the production of secondary metabolites is initiated, whereas at field strengths higher than 1.0 kV/cm the cells are irreversibly stressed and pores are built. This leads to enhanced mass transfer processes which is beneficial for oil extraction or pressing. Main parameters for the pulsed electric field treatment are process parameters as electric field strength, number of pulses, treatment time, pulse characteristics as well as a homogeneity, sufficient moisture content and conductance of the probe, respectively.
In this work wet milled corn, steeped for 48 hr at 30°C, 40°C and 50°C in steeping water, was irreversibly permeabilised at a field strength of 3.0 kV/cm and 120 pulses. Subsequently, oil was separated by hexane extraction, pressing and supercritical CO_2 extraction, respectively. At an electrical field strength of 3.0 kV/cm and a steeping water temperature of 50°C the oil yield could be increased by 27.8 % at hexane extraction, by 25.2 % at pressing and by 14.9 % at supercritical CO_2 extraction. Further increase of oil yields could be reached by the combination of pressing and hexane extraction as well as CO_2 - and hexane extraction. Most phytosterols could be gained by the supercritical CO_2 extraction. By the application of pulsed electric fields no quantitative variances of the content of stigmasterol, sitosterol and campesterol were obtained, whereas, at the CO_2 extraction more sitosterol was determined.
From the established results a concept for the wet milling process of corn to enhance the oil yield and to produce an oil with an additional nutritional benefit by the application of pulsed electric fields was developed.
In current investigations the impact of pulsed electric fields on the oil yield and content of different functional food ingredients at the production of rapeseed is studied.

Evening primrose *seed* (*Oenothera biennis)* oil extraction, by an enzymatic aided cold pressing process

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Evening Primrose (*Oenothera biennis*) seeds have 18 % of oil which is composed by more than 90 % of PUFA which contains 12 % w.w of γ-linolenic acid (GLA) In fatty acid human metabolic pathway, GLA is transformed to di-homo-γ-linolenic acid (DHGLA), being prostaglandin 1 (PGE1) precursor which generates anti-inflammatory and anti-thrombotic properties. Otherwise significant DHGLA amounts don't exist in nature, and then a dietary supplementation with GLA is necessary to recover PGE1 deficiency diseases.

Evening primrose oil extraction is mainly done by cold pressing to preserve end-products quality. However, its yield is lower than the obtained by traditional oil extraction process by solvent. Oil extraction yield by pressing could be increased with enzyme treatment incorporation previous to pressing stage. Enzymes like pectinases, cellulases and hemicellulases have been used on vegetable oil extraction, like coconut, chilean hazelnut, soybean, rice brain, borage, reporting a better oil recovery than solvent extraction and good quality end – products (oil and meal). The type of enzymes and hydrolysis conditions are important factors to raise oil yield extraction.

The aim of this work is develop an enzymatic process to produce GLA concentrated oil from evening primrose seeds. In the evening primrose oil extraction process by pressing is studied the effect of enzymatic treatment conditions, like as period of treatment, commercial enzyme preparation, enzyme-substrate ratio (E/S), temperature and moisture on evening primrose oil extraction yield by pressing, using. Pressing stage was carried out at standardized conditions, 49 MPa, 45 minutes and ±10% of sample moisture.

Crushed evening primrose seed samples were conditioned with heat in a bath of boiling waters by 15-20 minutes. The enzymes and water were then incorporated. Best results were observed when Ultrazym commercial enzyme was applied by 15 h using an E/S ratio over than 2 % w.w., 45 ºC and moisture of 40% obtaining 69% of extraction yield. This result represents 15% of enhancement of oil extraction yield by a single stage cold pressing. Extracted oil increased up to 76.4 % when cold pressing were carried out by two stages with a preheated matter.

Residual meal quality improves by enzymic treatment. Residual meal crude fibre content diminishes and its phenolic compounds content and antioxidant activity increases, when the enzymes were used.

Carbohydrate metabolism in developing linseed embryos

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Lipid accumulation in oilseeds depends on the efficient conversion of photosynthetic sugars into fatty acids. However, offer and demand to not always coincide, due to day-night cycles on the one hand and to developmental shifts on the other hand. Discrepancies between offer and demand can be buffered by temporary carbohydrate stores inside the embryo. For example, it has been shown that developing rapeseed (*Brassica napus* L.) embryos accumulate starch in the days prior to the onset of maximal lipid synthesis, and that this starch subsequently disappears in favour of storage lipids [1].

We first analysed carbohydrate dynamics in developing linseed (*Linum usitatissimum* L.) embryos on a developmental timescale by measuring the storage product contents over time. Contrary to rapeseed, linseed accumulates very little starch, and the rate of net starch degradation is about a hundredfold lower than the rate of lipid synthesis [2].

In a second approach, the capacity of isolated linseed plastids to synthesise starch was tested. When fed with [1-^{14}C]-glucose 6-phosphate, the isolated plastids surprisingly synthesise starch at a rate which is nearly a hundred times higher than the net rate of synthesis *in planta* [2].

Thirdly, the metabolism of [1-^{13}C]-glucose by excised linseed embryos was directly followed by *in vivo* NMR. The label was incorporated into sucrose allowing to assess the rate of sucrose synthesis and the sucrose pool turnover. Label incorporation into fatty acids however occurred much faster suggesting that most of the incoming sugar bypasses the sucrose pool, and the latter might rather serve as a buffer on a timescale of several hours. Label analysis in the starch pool after the incubation period showed that the starch pool, too, is renewed in less than a day.

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In vivo-regulation of lipid storage in seeds occurs via local energy state and oxygen supply

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Oilseed crops accumulate large amounts of lipids accounting for a significant portion of the global food and feed supply.The metabolic pathways and the genes involved are well known now. However, progress in understanding the regulation of oil biosynthesis is hampered by very little information on the spatio-temporal arrangements of major substances and pathways. To overcome this limitation, we applied topographical methods to different types of seed (soybean, maize, sunflower, barley) and demonstrated the distribution of lipids (NMRI), oxygen (microsensors), energy (ATP-bioluminescence) and photosynthesis (PAM-fluorescence). We combined these data with metabolite profiles (LC-MS), flux estimations (stable isotopes) and mRNA expression pattern (cDNA arrays) to achieve a more comprehensive view on oil storage. According to our investigations (1) in oil-storing seed organs accumulation of lipids coincides with high local energy (ATP-) levels (Borisjuk et. al., 2005). (2) At the onset of storage, oxygen inside seeds drops to very low levels, restricting seed metabolism (hypoxia). This affects energy supply towards lipid-synthezising regions, pool sizes of metabolic intermediates (e.g. acetyl-CoA) and finally lipid biosynthetic rates (Rolletschek et al., 2005a). (3) Non-green seeds rely entirely on diffusive oxygen uptake whereas green seeds are able to generate significant amounts of oxygen via photosynthesis. In such a way hypoxic constraints are released, allowing the seed to increase its metabolic (biosynthetic) activity (Rolletschek et al., 2005b). These data emphazise the regulatory role of local energy and oxygen availability for storage lipid biosynthesis.

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Physical and structural properties of caleosin, a protein probably involved in the mechanisms of lipid bodies maturation

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Plants store energy as polysaccharides or lipids. Lipids are stored in intracellular oil particles called oil or lipid bodies, which can be found in all types of cells, but mainly in seeds. Lipid bodies are made up of a core containing mainly neutral lipids. The core is surrounded by a single monolayer of phospholipids embedded with structural proteins (oleosins). The function of oleosins is to maintain oil bodies in small stable droplets. Next to the oleosins the phospholipids monolayer contains also small amount of caleosin. Caleosin structure is oleosin-like but on the other hand unique thanks to the presence of calcium binding site.

The mechanisms of lipid biosynthesis in plants is well known. However not so much is known about the process of formation and maturation of lipid bodies. Lipid bodies are believed to arise from specific microdomains of the endoplasmic reticulum membrane that contain the full complement of TAG-biosynthesis enzymes. The small nascent lipid bodies undergo a series of fusions which led to a formation of mature lipid body. As achieving of correct size of lipid bodies is important for the life of cell, this process is highly regulated. The way of this regulation in plants is not known. The presence of calcium binding protein (caleosin) in mature lipid bodies and the similarity with the process of the formation of milk lipid globules in mammalian cells, could indicate that a cellular second messenger, calcium, and caleosin itself play an important role in the maturation process.

For better understanding of caleosin role, structural and physical properties of caleosin were examined using different methods (Langmuir balance, pendant hanging drops and circular dichroism). Special emphasis were paid to the effect of calcium on these properties. Measurement were held with recombinant proteins. These proteins were prepared by cloning of one of *A. thaliana* caleosin genes (At4g26740) in two different bacterial expression systems and their purification to electrophoretic homogeneity.

Functional analyses of different proteins of the Arabidopsis oil bodies

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Following the proteomic analysis of the Arabidopsis oil bodies (Jolivet et al., Plant Physiol. Biochem, 42 (2004), 501-509) mutants for the different constitutive proteins have been obtained. The effects of the mutations on the biogenesis and the properties of the oil body are under study. Additional functional analyses are under progresses including the regulation of gene expression and localisation of the encoded proteins.

Phosphoinositide molecular species and their subcellular distribution in cultured tobacco (NT1) cells

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Cellular membranes contain polyphosphorylated inositol-containing phospholipids (phosphoinositides). The localized presence of phosphoinositides imparts specificity to signaling pathways by recruiting and activating signaling proteins that mediate cellular processes during development or stress conditions. The phosphoinositide isomer, phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2], for instance can interact with different proteins, thereby regulating ion transport, cytoskeletal dynamics, vesicle flow, or phospholipase D activity. PtdIns(4,5)P_2 is also the precursor of the second messenger, inositol 1,4,5-trisphosphate ($InsP_3$). We have previously demonstrated the presence of distinct subcellular phosphoinositide pools in plant cells. These pools can be localized in the plasma membrane or in endomembranes. It is our hypothesis that compartmentalization into phosphoinositide pools allows individual lipid-protein interactions and facilitates the orchestration of regulatory phosphoinositide functions. It is our goal to elucidate mechanisms by which subcellular phosphoinositide pools may be distinguished and maintained in plant cells. Tobacco (NT1) cultured cells were subjected to subcellular fractionation. From subcellular fractions, phosphatidylinositol (PtdIns), PtdIns 4-phosphate (PtdIns 4P), or PtdIns(4,5)P_2 were isolated by thin layer chromatography, and lipids analyzed by gas chromatography/mass spectrometry (GC/MS) for the associated fatty acids. The data presented indicate that acyl-moieties differ between phosphoinositides from individual subcellular pools. The observed acyl distribution may be related to the origin of individual phosphoinositide pools. Supported by an Emmy Noether Fellowship from the German Research Foundation (DFG, to I.H.).

Characterization of *Arabidopsis* mutants involved in cuticle formation

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The aerial portions of plants are covered with a continuous extracellular layer of hydrophobic material, the cuticle, that plays an important role in protecting these organisms from water and solute loss, UV irradiation, frost damage, as well as pathogen and insect attack. The cuticle consists of two major components, cutin and wax. Cutin, a structural polymer, is in many species composed of interesterified hydroxy and epoxy-hydroxy fatty acids. In *Arabidopsis*, however, cutin consists of a high proportion of C16 and C18 dicarboxylic acids, with octadeca-cis-6, cis-9-diene-1,18-dioate being the main component.

In order to study the molecular aspects of the biosynthesis and the functions of cutin in *Arabidopsis* a mutant screen has been developed to detect *Arabidopsis* plants with an increased permeability of the cuticle. 6 *permeable cuticle* (*pec*) mutants have been isolated that have a characteristic pattern of organ- and development-specific alterations in cuticle permeability as well as an altered ultrastructure of the cuticular membrane. Similar to cutinase-expressing transgenic *Arabidopsis* plants, *pec* mutants exhibit increases in the resistance to *Botrytis cinerea*. Some of the *pec* mutants show also occasional fusions between rosette leaves. The features identify *pec* mutants as a new group of cuticle mutants in *Arabidopsis*. All 6 *pec* mutations are monogenic and recessive. Mutations in *PEC1* and *PEC2*, leading both to organ fusions and a very strong resistance to *Botrytis* were chosen for a map-based cloning procedure. *PEC1* is located in the middle of chromosome 2 in a distance of 0.5 cM from marker nga1126 and *PEC2* is located in the middle of chromosome 1 close to marker ciw1.

Lipid metabolism in arbuscular mycorrhizal roots of *Medicago truncatula*

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The peroxidation of polyunsaturated fatty acids, common to all eukaryotes, is mostly catalysed by members of the lipoxygenase enzyme family of non-heme iron containing dioxygenases. Lipoxygenase products can be metabolised further in the oxylipin pathway by several groups of *CYP74* enzymes. One prominent oxylipin is jasmonic acid (JA), a product of the 13-allene oxide synthase branch of the pathway and known as signalling substance that plays a role in vegetative and propagative plant development as well as in plant responses to wounding and pathogen attack. In barley roots, JA level increases upon colonization by arbuscular mycorrhizal fungi. Apart from this first result regarding JA, no information is available on the relevance of lipidperoxide metabolism in arbuscular mycorrhizal symbiosis. Thus we analysed fatty acid and lipidperoxide patterns in roots of *Medicago truncatula* during mycorrhizal colonization. Levels of fungus-specific fatty acids as well as palmitic acid (16:0) and oleic acid (18:1 n-9) were increased in mycorrhizal roots. Thus the degree of arbuscular mycorrhizal colonization of roots can be estimated *via* analysis of fungal specific esterified fatty acids. Otherwise, no significant changes were found in the profiles of esterified and free fatty acids. The 9- and 13-LOX-products of linoleic and α-linolenic acid were present in all root samples, but did not show significant differences between mycorrhizal and non-mycorrhizal roots, except JA which showed elevated levels in mycorrhizal roots. In both types of roots levels of 13-LOX products were higher than those of 9-LOX products. In addition, three cDNAs encoding *CYP74* enzymes, two 9/13-hydroperoxide lyases and a 13-allene oxide synthase, were isolated and characterized. The transcript accumulation of these three genes, however, was not increased in mycorrhizal roots of *M. truncatula*.

Identification of an allene oxide synthase (*CYP74C*) that leads to formation of α-ketols from 9-hydroperoxides of linoleic and linolenic acid in below ground organs of potato

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Allene oxide synthase enzymes are members of the cytochrome P-450 enzyme family, subfamily *CYP74*. Here we describe the isolation of three cDNAs encoding AOS from potato (StAOS1-3). Based on sequence comparisons they represent members of either the *CYP74A* (StAOS1 + 2) or the *CYP74C* (StAOS3) subfamily. StAOS3 is distinguished from the other two AOS isoforms from potato by its substrate specificity for 9-hydroperoxides of linoleic and linolenic acid whereas 13-hydroperoxides are only poor substrates. The highest activity was shown with (9*S*,10*E*,12*Z*)-9-hydroperoxy-10,12-octadecadienoic acid (9-HPODE) as a substrate. This hydroperoxide was metabolized *in vitro* to α- and γ-ketols as well as to the cyclopentenone compound 10-oxo-11-phytoenoic acid. They represent hydrolysis products of the initial StAOS3 product 9,10-epoxyoctadecadienoic acid, an unstable allene oxide. By RNA gel hybridization blot analysis, *StAOS3* was shown to be expressed in sprouting eyes, stolons, tubers and roots, but not in leaves. StAOS3 protein was found in all organs tested, but mainly in stems, stolons, sprouting eyes and tubers. As (*in vivo*) reaction products, the α-ketols derived from 9-hydroperoxides of linoleic and linolenic acid were only found in roots, tubers and sprouting eyes. Immunolocalization showed that StAOS3 was found exclusively in the envelope of proplastids and young amyloplasts. From these data one may assume a role of StAOS3-derived α-ketols in regulation of plastid development or sugar metabolism.

Very Long Chain Fatty Acids in sunflower oil: Occurrence and Biosynthesis.

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Very long chain fatty acids (VLCFAs, those with more than 18 carbon atoms) occur in small amounts in most seed oils. The main VLCFAs present in sunflower seeds are arachidic, behenic and lignoceric, accounting for 1 to 3 % of the total fatty acids in the triacylglycerols from common lines. Although these percentages are minor, the low melting points of these fatty acids affected the behavior at low temperature of the resulting oils.

VLCFA species are synthesized in the endoplasmic reticulum of by the action of the dissociated enzyme complex called fatty acid elongase (FAE) from cytoplasmic malonyl-CoA and acyl-CoAs exported from plastids. This reaction implies the condensation of these substrates by the action a ketoacyl-CoA synthase followed by the reduction and dehydration of the ketoacyl-CoA to yield a 2-C elongated acyl-CoA that is ready for further elongation or incorporation into glycerolipids. The FAE complex was characterized in sunflower seed kernels, where it was demonstrated to be mainly dependent on NADPH. In this regard, two condensing enzymes with different substrate specificities were solubilized and partially purified. These isoforms displayed strong substrate inhibition kinetics and different optimum pH values. The content of VLCFA of the oils from different sunflower mutants and the perspectives of the production of improved sunflower oils will be discussed in function of the properties of these enzymes.

Spatial distribution of three microsomal oleate desaturase (*FAD2*) transcripts in sunflower developing seeds

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The proportion of linoleic acid in oilseeds is strongly modified during embryo development by environmental conditions, mainly temperature, producing oils with a final composition that depends on the year and geographical area. The enzyme responsible for the synthesis of linoleic acid from oleic acid is the microsomal oleate desaturase (FAD2). In sunflower, three different *FAD2* genes have been isolated and characterized. The *FAD2-1* gene encodes the major and seed-specific isoform, whereas *FAD2-2* and *FAD2-3* are weakly expressed in seeds. We have reported two mechanisms by which temperature regulates the FAD2 activity in sunflower seeds: i) a direct, slow and non-reversible effect, due to the low thermal stability of the FAD2-1 isoform and the repression of the *FAD2-1* gene at high temperature and ii) an indirect, fast and reversible effect by which temperature acts on the availability of oxygen, that, in turn, regulates the FAD2 activity.

In order to elucidate the mechanism of this second effect, we have investigated this phenomenon at the tissue level by *in situ* hybridization using *FAD2* gene-specific probes labeled with a fluorescent nucleotide. These experiments showed localization of *FAD2-1* transcripts in all cell types of the sunflower seed. However, in longitudinal sections of the seed a higher hybridization signal was detected in the procambial ring and the radicle, whereas in transversal sections a preferential accumulation was observed in the palisade parenchima compared to the spongy parenchima. In contrast, *FAD2-2* and *FAD2-3* transcripts were evenly distributed throughout the seed. This pattern of expression will be discussed in relation to the oxygen concentration inside the sunflower seed.

Phospholipid turnover in the plasma membrane of phosphate limited oat (*Avena sativa*)

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As much as one third of all organically bound phosphate in the plant cell is associated with phosphate-containing membrane lipids. We have shown that oat grown under phosphate-limited conditions replaced up to 70% of the plasma membrane and the tonoplast phospholipids with non-phosphorous lipids, especially the galactolipid digalactosyl diacylglycerol (DGDG). The phospholipid-to-DGDG exchange appears to be a general phosphate-saving feature of angiosperms. In parallel to the phospholipid replacement, phosphate-limited growth condition also led to enhanced phospholipase activities in the oat plasma membranes: a Ca^{2+}-independent phospholipase D (PLD) as well as a phosphatidic acid phosphatase (PAP) were induced. Native gel electrophoresis of oat plasma membranes revealed that both the PLD and the PAP activities co-migrated with a native protein size probably exceeding 100 kD. Three candidate polypeptides for the lipases were identified by mass spectrometry. However, one of the three candidates was previously described as a phosphate-deficiency induced phospholipase C (PLC) in *Arabidopsis*. A phylogenetic analysis did not reveal any preferential relation to a PLC or a PLD for any of the polypeptides. The *in vivo* significance of the phospholipid turnover in phosphate-limited oat is presently investigated.

Influence of irradiance on fatty acid composition of symbiotic dinoflagellates of hermatypic corals

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Lipid production and accumulation by photoautotrophic symbionts is a principal source of energy for the animal-host. The effect of variation in light intensity on the fatty acid profiles of symbiotic dinoflagellates (SD) isolated from hermatypic corals *Millepora intricata*, *Pocillopora damicornis*, *Seriatopora caliendrum*, *S. hystrix*, and *Stylophora pistillata* preferentially subjected to 95%, 30%, 8% and 2% of the incident photosynthetic active radiation (PARo) was investigated. Irradiance had a significant effect on fatty acid composition of polar lipids, associated with photosynthetic membranes and on triacylglycerols, the storage substances of the cells. SD showed substantial changes in the proportion of 16:0 as a response to variation in PARo. During adaptation to high light percentage of 16:0 increased in polar lipids and triacylglycerols. Additionally, percentage of 14:0, 16:1(n-7) increased in triacylglycerols. A substantial portion of these fatty acids is located in triacylglycerols. Thus, high light conditions imposed increase in storage products providing utilization of the excess energy for energy-consuming processes. Polar lipids of SD adapted to low level of PARo had greater concentrations of 18:4(n-3), 20:5(n-3), 18:5(n-3) and 22:5(n-6) commonly involved in the formation of thylakoid membranes. Conversely, the amounts of 20:4(n-6) and 22:6(n-3) increased with increasing irradiance, it may indicate on their association with photosynthesis. The elevation in the percentage of 18:4(n-3), 20:5(n-3) and 18:5(n-3) during exposure to low light was accompanied by increase in chlorophyll *a* content in the SD cells. It supports the suggestion that chlorophyll molecules are associated with some specific polyunsaturated fatty acids. In spite of the proportions of individual fatty acids varied significantly in response to light conditions, in general, the ratio of saturated and polyunsaturated fatty acids changed slightly in both lipid classes. Hence, variation in PARo levels did not affect in considerable changes of unsaturation of the membrane fatty acids. Thereafter the adaptive response of algae to the variation of light intensity in physiological ranges does not result in the changes in the membrane fluidity. Light-dependent changes in the fatty acid composition arise, apparently, from correlation of activity of photosystems with processes of production and desaturation of fatty acids in algae.

Composition of the surface waxes of some edible Solanaceae fruits: tomatoes, bell pepper and eggplant

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From numerous cultivars of tomatoes, bell pepper and egg plant, the surface wax of the ripe fruits was rinsed and the wax components were separated into three fractions by elution from a 1 g silica gel SPE cartridge. Fraction 1 (hexane/toluene 1 + 2) yielded hydrocarbons and aldehydes. Fraction 2 (hexane/tert-butylmethyl ether 3 + 1) naringenin-chalcone (only for tomatoes). All compounds present were identified by GC/MS analysis in comparison with appropriate references, for fractions 2 and 3 also in silylated form. Wherever possible, the identity was confirmed by Ag^+-TLC, Ag^+-HPLC, specific derivatization, hydrogenation, etc. For quantitative analyses, GC with an FID was used.

The overall results are presented in an extensive table which gives an overview on the broad range of components and their average contents down to levels of 0.1 g/100 g. The following compound classes were found in varying amounts: n-alkanes, iso- and anteiso-alkanes, n-alkadienes and n-alkatrienes, n-alkanols, aldehydes, n-alkanoic acids and 2-hydroxy-alkanoic acids, triterpenols and sterols as well as naringenin-chalcone (for tomatoes only). Evidently it is the first time that n-alkadienols and n-alkatrienols present in tomato wax were identified in plant material.

The role of sphingolipid long chain base heterogeneity

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We have identified the gene responsible for the C4-hydroxylation of the sphingolipid long chain base (LCB) in the model organism *Schizosaccharomyces pombe*. This gene when heterologously expressed in the *Saccharomyces cerevisiae* mutant *sur2D* is able to restore LCB C4 hydroxylation and also displays a low level of activity as a sphingolipid D^4desaturase, mirroring previous data showing that the sphingolipid D^4 desaturase can show some bifunctional hydroxylase activity.

We disrupted the endogenous *SUR2* gene alone, and in combination with the other LCB modification gene in *S. pombe* (the previously characterised dihydroceramide/ dihydrosphingosine D^4 desaturase, *dsd1*$^+$). This latter enzyme activity is responsible for the synthesis of sphingosine in *S. pombe*. The *DSpsur2* mutant and the *Dsur2/Ddsd1* double mutant were viable at 30ºC indicating that the presence of the modified LCBs sphingosine and phytosphinganine (resulting from *dsd1*$^+$ and *sur2*$^+$, respectively) is not essential for *S. pombe* viability at 30ºC.

However, this double mutant shows a cold-sensitive phenotype by not growing at temperatures below 20^{o}C. Although LCB have been implicated in heat stress responses in *S. cerevisiae*, and several *S. cerevisiae* sphingolipid biosynthetic mutants display a high temperature sensitivity, our *S. pombe Ddsd1/Dsur2* double mutant is to our knowledge the first example of conditional-sensitivity at low temperatures. We were able to chemically rescue this cold sensitivity by the addition of C2 ceramides. These data have implications for our understanding of the role of LCBs in lower eukaryotes, and may indicate the importance of the ratio of free to *N*-acylated LCBs in cellular processes. This work may have implications for the roles of long chain base heterogeneity in plants.

Characterization of oxylipin producing enzymes from *Aspergillus nidulans*

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A sexual life cycle is unknown for most filamentous fungi of the *Aspergillus* group. *A. nidulans* has been established as one of the major scientific model organisms for filamentous fungi due to its ability to produce both asexual as well as sexual spores in combination with an extensive secondary metabolism. It has been shown that the formation of sexual fruitbodies containing the sexual ascospores is largely regulated by oxylipins, which are oxygenated derivatives of different unsaturated fatty acids found in the fungus. They function as so-called *Psi* factors (precocious sexual inducer). The synthesis of these oxylipins is believed to be catalysed by the sequential action of at least three groups of enzymes whereby the first reaction step is the best characterized step till now. A first hydroxylation at position 8 adjacent to the Δ9-double bond of unsaturated fatty acids is observed leading to formation of group of compounds named psiB. It is catalyzed by a group of enzymes designated as “Ppo” enzymes (psi producing oxygenase). The group contains three members namely Ppo A, B & C. The second step is another hydroxylation at C-5 of the mono hydroxylated fatty acid leading to 5,8-dihydroxy fatty acids, so-called psiC compounds. The last reaction step is a lactonase reaction leading to cyclic modification of the carboxy group. These substances are called psiA. Enzymes catalyzing the later two reaction steps have not been identified till now. This study aims at investigating the formation of psiBs by PpoA and PpoC. Thus the enzymes were heterologously expressed in the bakers yeast. The enzymes were characterized by *in vivo* and *in vitro* studies using different fatty acids substrates, namely linoleic, oleic, α-linolenic and palmitoleic acids. Products formed were analyzed by HPLC/DAD and GC/MS.

Oxylipin Database - A tool for browsing the plant oxylipin pathway and downloading profiling results

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Plant oxylipins are products of polyunsaturated fatty acid oxidation and comprise a wide array of molecules (e.g. fatty acid hydroperoxides, divinyl ethers, jasmonic acid). The formation of these metabolites may occur either by autoxidation or by the action of enzymes. The enzymatic formation of hydroperoxy fatty acids represents the first step in the synthesis of oxylipins. The hydroperoxides can be converted by enzymes within the so-called oxylipin pathway which seems to be the most prominent pathway of enzymatic lipid peroxidation in plants. The oxylipin pathway leads to the synthesis of a multitude of oxylipins. In general these metabolites can be classified into α-DOX-, 9-LOX-derived and 13-LOX-derived oxylipins with respect to the C18 fatty acids, linoleic acid and linolenic acid, as well as to the C16 fatty acid, roughanic acid. *In vivo* oxylipins are involved in abiotic and biotic stress responses. Some of them have direct antimicrobial properties; others may act as regulators of plant defence gene expression.

To investigate the involvement of distinct oxylipins in plant stress responses in various plant species, analytical methods have been developed to record oxylipin profiles. This profiling consists of a set of HPLC, GC and GC/MS steps and allows the measurement of more than 150 metabolites of the oxylipin pathway in parallel.

Due to the complexity of the oxylipin biosynthesis, a web-based interface has been set up for browsing the plant oxylipin pathway. According to the involved enzymes and converted substrates the pathway has been sub grouped into distinct schemes. In these images, the formation of around 200 oxylipins can be displayed in detail interactively together with spectral information. In addition, the interface is connected to a SQL database and is capable of graphically representing data derived from oxylipin profiling experiments. This database has been programmed for the calculation and management of the profiling data. Moreover, detailed practical protocols are provided to follow the underlying analytical procedures.

Profiling of labile plant oxylipins by *in situ* derivatization with pentafluorobenzyl-hydroxylamine

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Signaling compounds derived from the family of unsaturated C_{18}-fatty acids play an important role during plant development and stress response. Although the oxylipins comprise a broad range of chemically diverse compounds, most previous studies focus on the analysis of classical phytohormones such as jasmonic acid and 12-oxophytodienoic acid. We developed a rapid and reliable GC-MS-based method for the identification and quantification of oxylipins which includes labile and highly reactive compounds. A key feature of the method is the *in situ* derivatization of leaf extracts with pentafluorobenzyl hydroxylamine (PFBHA) yielding stable PFB-oximes that prevent degradation and rearrangements during sample preparation. The raw extract is partitioned with hexane and CH_2Cl_2 to separate less polar from polar compounds followed by aminopropyl solid phase extraction and derivatization with diazomethane and *N*-methyltrimethylsilyltrifluoroacetamide (MSTFA).

Moreover, PFB-oximes exhibit characteristic mass spectra and can be analyzed with high sensitivity by negative ionization mass spectrometry (NCI-MS). This approach greatly facilitates the identification of unknown oxylipins in complex plant matrices and allows monitoring of temporal and spatial changes of the oxylipin pattern in response to stress factors. High levels of oxylipins were observed locally in caterpillar damaged leaf tissue. Some of the oxylipins were already produced after mere mechanical wounding; whereas others required an additional action of herbivore-characteristic elicitors.

Additionally, the *in situ* derivatization completely suppresses the isomerization of *cis*-JA to the less active *trans*-JA. After wounding, *cis*-JA is produced by *de novo* biosynthesis via the octadecanoid pathway. Then, *cis*-JA is slowly converted into its less active *trans*-isomer until a resting level is reached. Although substantial differences in physiological activity of various JA isomers have been reported, it is not entirely clear to which extend the epimerization is under enzymatic control or occurs spontaneously.

The Production and Accumulation of Very Long Chain Polyunsaturated Fatty acids (VLC-PUFAs) in *Arabidopsis thaliana*.

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Humans are not capable of *de novo* synthesis of VLC-PUFA such as, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Therefore the human diet must contain these essential fatty acids. In particular EPA and DHA derived from fish have been shown to improve human health. In particular it is suggested that *n-3* VLC-PUFAs may reduce the risk of metabolic syndrome, a term used to describe a collection of pathologies indicative of a progression towards heart disease, diabetes, stroke and obesity, which constitutes an increasing public health problem in industrialised societies. The ability to produce such fatty acids in plants, as an alternative to marine sources, is therefore of considerable interest.

The efficient reconstitution of VLC-PUFA biosynthesis in transgenic plants has been demonstrated. However, a “substrate dichotomy” between the two key enzyme activities (desaturases and elongases) of PUFA biosynthesis currently prevents high level accumulation of these fatty acids in transgenic plants. In order to investigate this bottleneck, *Arabidopsis* transgenic lines of different backgrounds are being characterised expressing conventional and alternative desaturation pathways for arachidonic acid (AA) and EPA. In particular, we are examining the contribution of acyl-channelling enzymes such as PDAT and LPAAT to the distribution of substrates for VLC-PUFA biosynthesis. This is being achieved by using *Arabidopsis* mutants with T-DNA disruptions in target genes that encode such activities. This approach will provide an insight into lipid biosynthesis and hopefully deliver transgenic plants accumulating high levels of VLC-PUFAs.

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Characterisation of a novel microsomal Lysophosphatidic Acid Acyltransferase isoform of *Brassica napus*

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In higher plants, isozymes of Lysophosphatidic acid acyltransferase (LPAAT) located in plastid and cytoplasmic compartments play a central role in determining the acyl composition of phosphatidic acid, a key intermediate in the biosynthesis of membrane and storage lipids. We have identified a multigene family in *Arabidopsis thaliana* encoding proteins that contain motifs present in glycerolipid acyltransferases and that possess sequence homologies to LPAATs but not to other acyltransferases. With the objective to determine whether each member of the family encodes an authentic LPAAT, we have cloned cDNA sequences coding for each isozyme from *Arabidopsis thaliana* and we have begun the characterisation of certain homologous sequences from *Brassica napus*. The strong sequence divergence, the distinct compartmentalisation and the varying patterns of expression argue for a specific role for each LPAAT-like protein in the production of phosphatidic acid required for diverse cellular functions.

Since we are particularly interested in the role played by acyltransferases in the synthesis of storage lipids we have assessed the contribution of seed expressed LPAAT isoforms to triacylglycerol biosynthesis, (Maisonneuve, 2003). We have isolated cDNAs from a *Brassica napus* immature embryo library that were predicted to code for two distinct membrane proteins sharing approximately 60% amino acid identity and possessing the glycerolipid acyltransferase molecular signature. Complementation of an acyltransferase-deficient mutant of *E. coli* together with activity assays suggested that each of the cDNAs encoded a functional LPAAT with the properties of a eukaryotic pathway enzyme. Studies using RT-PCR revealed differences in the expression of the genes encoding the two isoforms. One of the cDNAs was detected in all rapeseed tissues and at each stage of embryo development, whereas the expression of the second cDNA was restricted to roots, flowers and immature embryos. LPAAT promoter::reporter fusions in Arabidopsis revealed a strong expression of this second isoform in pollen.

Taken together, these results suggest that the ubiquitously expressed LPAAT isoform may perform an essential housekeeping role in glycerolipid biosynthesis in all tissues.

The function of the phosphatidic acid produced by the second microsomal LPAAT remains obscure. However, the characterisation of a cytoplasmic glycerol-3-phosphate acyltransferase (Zheng et al 2003) whose disruption is associated with an impaired male fertility, a consequence of a loss of pollen competence, suggests the possibility of the existence of a distinct or additional eukaryotic pathway in floral organs employing distinct acyltransferase isoforms. The characterisation of loss of function mutants of LPAAT together with a detailed tissue localisation of glycerol-3-phosphate acyltransferase and LPAAT isoforms will be necessary to confirm this hypothesis.

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Euro Fed Lipid	Short Course on Food Emulsions and Emulsifiers	Prague, Czech Republic	24-25 September 2005
Eurolipids	Eurolipids – International Trade Fair for Fats & Oils and related Technologies	Frankfurt, Germany	2-4 November 2005
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Euro Fed Lipid	Workshop Olive Oil - Legal Regulations and Quality Assessment	Frankfurt, Germany	3 November 2005
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DGF	Kaufmann Tage 2005	Heidelberg, Germany	24-25 November 2005
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