



DEPARTEMENT OF PLANT
MOLECULAR BIOLOGY

Unil

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Faculté de biologie
et de médecine

Message du directeur

La biologie végétale est devenue une pierre angulaire dans la quête de réponses aux défis auxquels l'humanité devra faire face au cours du siècle prochain. Malgré le succès de la révolution verte et l'augmentation de la production alimentaire au cours des 50 dernières années, un fait inéluctable est que nous aurons besoin de nourrir une population qui passera de 6,5 à 9 milliards d'êtres humains d'ici à 2050, ceci dans un contexte de réchauffement planétaire et de passage vers de nouveaux modèles climatiques. De plus, la production agricole doit être adaptée afin d'inclure le développement de sources d'énergie renouvelables et de molécules organiques d'une manière durable. Les progrès réalisés dans notre compréhension des aspects fondamentaux de la biologie moléculaire végétale, y compris la croissance et la structure des plantes, l'adaptation à l'environnement et la défense contre les organismes nuisibles et les agents pathogènes, seront essentiels afin d'affronter ces défis.

Au cours de la dernière décennie, la recherche en biologie végétale a été profondément transformée par le développement d'outils très puissants, tels que les séquences complètes des génomes de nombreuses plantes (par exemple : l'*Arabidopsis thaliana*, le riz, le peuplier), les outils encore plus dynamiques de la génomique et de la bioinformatique, la microscopie à deux photons et les nouvelles techniques d'imagerie, la relative facilité de transformation des plantes, et les nouvelles perspectives de recherche issues de la biologie des systèmes. **Au Département de Biologie Moléculaire Végétale, la recherche emploie l'ensemble de ces outils pour l'étude de la plante au niveau moléculaire, en utilisant la biologie cellulaire, la biochimie et la génomique.**

Bien que le premier laboratoire dédié à la biologie végétale expérimentale à l'Université de Lausanne ait été établi en 1919, l'actuel Département de Biologie Moléculaire Végétale (DBMV) a été créé en 2003. Le DBMV s'est fortement développé pour rapidement devenir un département dynamique qui comprend aujourd'hui 8 laboratoires de recherche étudiant les aspects moléculaires des plantes, y compris le pliage des protéines, les composants moléculaires impliqués dans les interactions des cellules végétales avec les agents pathogènes ou les symbiotes, le transport d'ions et les cascades de transduction de signaux impliquées dans la réponse aux stress et aux phytohormones. **Avec un total de plus de 60 collaborateurs, le DBMV est le plus grand département de recherche en biologie moléculaire végétale des universités de Suisse occidentale.** Le DBMV entretient une collaboration fructueuse avec le Centre Intégré de Génomique et participe au programme de Master en Génomique et Biologie Expérimentale.

Des groupes dynamiques et d'excellentes ressources font du DBMV un lieu de travail passionnant et le positionnent comme un acteur majeur dans l'arène internationale de la biologie moléculaire végétale.

Message from the director

Yves Poirier

November 2008

Plant biology has become a cornerstone in the quest to meet the challenges that will face humankind during the next century. Despite the success of the green revolution and the rapid increase in food production over the last 50 years, it is an inescapable fact that we will face new challenges with regard to plant resources. These challenges include the need to secure food production, in a context of global warming and shifts in weather patterns, to feed a population that will increase from 6.5 to 9 billion people by 2050. At the same time, agricultural production needs to be adapted to include the development of renewable sources of energy and organic molecules in a manner that is sustainable. Advances in our understanding of fundamental aspects of the molecular biology of plants, such as growth, adaptation to the environment and defense against pests and pathogens, will be essential in order to meet these challenges.

Research in plant biology has been transformed profoundly over the last decade by the development of very powerful tools. These include the complete genome sequence of numerous plants (e.g. *Arabidopsis thaliana*, rice, poplar); the increasingly dynamic tools of genomics and bioinformatics; two-photon microscopy and new imaging techniques; the relative ease of plant transformation; and novel research perspectives that have arisen as a result of progress in systems biology. **Researchers at the Department of Plant Molecular Biology make use of all these tools to study the plant cell at the molecular level, using cell biology, biochemistry and genomics.**

Although the first laboratory at the University of Lausanne that was devoted to experimental plant biology was established in 1919, the current Department of Plant Molecular Biology (DBMV) was formed in 2003. The DBMV has grown rapidly into a dynamic department, which now includes eight research laboratories that study molecular aspects of plant cells. These aspects include protein folding, ion transport, the molecular components that are involved in the interactions of plant cells with pathogens or symbionts, and the signal transduction cascades that are involved in responses to stress and phytohormones. **With a total of more than 60 researchers, the DBMV is the largest university department in western Switzerland that is devoted to plant molecular biology.** The DBMV has fruitful collaborations with the Center of Integrative Genomics, and participates in the Master Program in Genomics and Experimental Biology.

As a consequence of the dynamism of the groups that comprise the department, and its excellent resources, the DBMV is an exciting place in which to work, and it is positioned as a major player in the international arena of plant molecular biology.

Phosphate homeostasis and carbon metabolism

Yves Poirier
Associate Professor

Presentation

Phosphate is an essential nutrient for all living organisms because it is an integral component of several important biological molecules and plays a key role in the regulation of numerous pathways. In plants, phosphate is also one of the main nutrients that limit plant growth in both natural environments and the agricultural context. The *PHO1* protein was identified in *Arabidopsis* and was shown to mediate the transfer of phosphate from the root to the shoot. Although proteins that are homologous to *PHO1* are found in lower and higher plants, as well as in fungi and animals, the role of *PHO1* has only been described well in *Arabidopsis* so far. In *Arabidopsis*, the *PHO1* gene family contains 11 members, and only two of these are involved in the long-distance transport of phosphate. Our current work has revealed that members of the *PHO1* family are implicated in numerous aspects of plant biology that extend well beyond the transport of phosphate. These functions include an important role in the signal transduction pathway that is involved in the adaptation of plants to phosphate deficiency, the regulation of stomatal opening, responses to different types of stress, and also plant growth.

The second major research theme in my laboratory relates to the study of carbon metabolism in the context of the synthesis of biopolymers. This is a component of the larger goal of using agricultural crops for the effective and sustainable production of organic molecules that are currently produced primarily from fossil carbon sources, such as petroleum. One such biopolymer is polyhydroxyalkanoate (PHA), which is a biodegradable polyester with properties similar to those of plastics. We are using PHA that has been synthesized from intermediates of the peroxisomal β -oxidation pathway as an innovative tool to analyse fundamental aspects of the biochemistry of fatty acid degradation in both fungi and plants. The synthesis of PHA in other subcellular compartments also enables us to study the factors and mechanisms that regulate carbon flux through the isoprenoid pathway in the cytosol, and the fatty acid biosynthetic pathway in plastids. Finally, our interest in biopolymers has extended recently to the synthesis of natural rubber in non-tropical plants, such as the Russian dandelion.

Our research on carbon metabolism and the synthesis of PHA and rubber is at the interface between fundamental biochemistry and biotechnology; our progress in one area can be affected by discoveries in the other.

Selected publications

Goepfert S, Vidoudez C, Tellgren-Roth C, Delessert S, Hiltunen JK, Poirier Y (2008)

Peroxisomal $\Delta 3, \Delta 2$ -enoyl-CoA isomerases and evolution of cytosolic paralogs in embryophyte. *Plant J*: in press

Van Beilen JB, Poirier Y (2008)

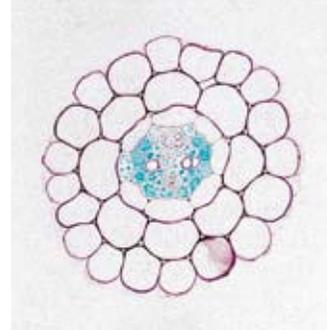
Production of renewable polymers from crop plants. *Plant J* 54: 684-701

Stefanovic A, Ribot C, Rouached H, Wang Y, Chong J, Belbahri L, Poirier Y (2007)

Members of the *PHO1* gene family show limited functional redundancy in phosphate transfer to the shoot, and are regulated by phosphate deficiency via distinct pathways. *Plant J* 50: 982-994

Goepfert S, Hiltunen JK, Poirier Y (2006)

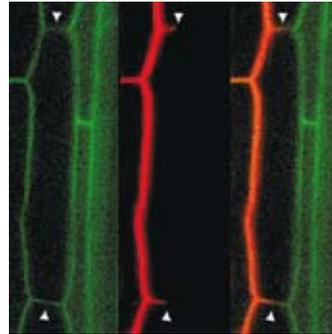
Identification and functional characterization of a monofunctional peroxisomal enoyl-CoA hydratase 2 that participates in the degradation of even-cis unsaturated fatty acids in *Arabidopsis thaliana*. *J Biol Chem* 281: 35894-35903



The PHO1 promoter:GUS construct reveals strong expression of PHO1 in the root vascular cylinder.



Yves Poirier received his PhD in 1989 from McGill University (Canada) for his work on the induction of lymphomas by the Abelson murine retrovirus. He then performed postdoctoral studies with Dr Chris Somerville on *Arabidopsis*, first at Michigan State University, and later at the Carnegie Institution at Stanford University (1990–1994). He then moved to the University of Lausanne, first as Assistant Professor (1995), and later as Associate Professor (2000). He has been the Director of the Department of Plant Molecular Biology since 2003.



Outer and inner plasma membrane of an endodermal cell are separate. Green (left) is a YFP fusion protein highlighting the plasma membrane. Red (middle) shows that a membrane tracer (FM4-64) cannot diffuse past the outer half of the cell. Right shows overlay of both channels.

Niko Geldner studied biology at the Universities of Mainz, Bordeaux and Tuebingen. He carried out the research for his diploma (1998) and PhD (1998-2003) theses in the laboratory of Gerd Juergens, where he worked on plant development. In 2004, he went as a Fellow of the Human Frontier Science Program to the Salk Institute in California, where he joined the laboratory of Joanne Chory for his postdoctoral training. Since the summer of 2007, he has been an Assistant Professor in the Department of Plant Molecular Biology. He recently received a European Starting Grant from the European Research Council in order to work on epithelial polarity in plants.

Presentation

Higher plants are nature's other great experiment with complex multicellular life. However, plants are profoundly different from animals and one has to go down to the level of the individual cell, the common unit of all life, in order to identify the common starting point from which these two branches of life have evolved. At the cellular level, plants and animals show an intriguing mixture of divergence and conservation. The development of multicellular organisms has forced pre-existing cellular structures to undergo enormous increases in complexity, and plants and animals have responded to this challenge in independent ways. It is these specific adaptations of plant cell structures to complex multicellular life that our research group is interested in, because we feel that they are the key to an unbiased, mechanistic understanding of plant life.

Epithelial polarity is a fundamental feature of multicellular life, and model systems in animals have been studied intensely for decades. The establishment of this polarity is a very complex process, and higher plants represent the only other relevant group of organisms that has achieved this independently. However, nothing is known about the mechanisms whereby layers of plant tissue establish and maintain differences between their outer and inner cellular surfaces.

One tissue that has all the hallmarks of an epithelium is the root endodermis. The endodermis is an invariant feature of all vascular plants and fulfils a crucial barrier function; it separates the extracellular space of the outer cell layers, which are in contact with the soil, from the inner space of the vascular bundles that form the transport route to aerial tissues. In order to do so, endodermal cells secrete bands of hydrophobic material in a highly localized and coordinated fashion. These "Casparian bands" are thought to be composed of the cork-like substance suberin, which is a compound polymer that forms extensive supra-cellular networks. The plasma membrane that underlies the Casparian strip appears to be dense and ordered, which suggests the presence of localized proteins that are attached tightly to the extracellular matrix. Our observations suggest that lateral diffusion across this domain is blocked. The endodermal plasma membrane is therefore divided into separate outer and inner subdomains. In accordance with this, we have found transporters that are localized exclusively in one of the two membrane regions, depending on their function in nutrient uptake. We are currently investigating the mechanisms that target proteins to these specific subdomains. In addition, we are working on identifying the proteins that are associated with the Casparian strip by proteomic approaches, and are undertaking forward genetic screens in order to identify the factors that are involved in the localization and establishment of the Casparian strip domain.

Selected publications

Richter S, Geldner N, Schrader J, Wolters H, Stierhof YD, Rios G, Koncz C, Robinson DG, Jürgens G (2007)

Functional diversification of closely related ARF-GEFs in protein secretion and recycling. *Nature* 448: 488-92

Geldner N, Hyman DL, Wang X, Schumacher K, Chory J (2007)

Endosomal signaling of plant steroid receptor kinase BRI1. *Genes Dev.* 21: 1598-602

Jürgens G, Geldner N (2007)

The high and the low road: trafficking choices in plants. *Cell* 130: 12-14

Geldner N, Anders N, Wolters H, Keicher J, Kornberger W, Muller P, Delbarre A, Ueda T, Nakano A, Jürgens G (2003)

The Arabidopsis GNOM ARF-GEF mediates endosomal recycling, auxin transport, and auxin-dependent plant growth. *Cell* 112: 219-30

Natural genetic variation of molecular mechanisms

Christian Hardtke
Associate Professor

Presentation

The focal research interest in our laboratory is to understand how naturally occurring intra-specific genomic variation translates into phenotypic differences, by characterizing the changes that occur in the underlying molecular mechanisms. To investigate this topic, we primarily utilize the model plant *Arabidopsis thaliana*, which suits our purpose particularly well because of its excellent genomic resources, compact genome, and easily monitored phenotypes.

In our analyses, we focus on quantitative aspects of growth and development whose genetic modification is likely to have contributed to the evolution of plant morphology. We are interested in investigating both the systemic impact of natural genetic variation at multiple loci, in order to decipher universal patterns of inheritance, and the impact of individual loci on particular traits. For instance, we have characterized natural strains of *Arabidopsis* by using genome tiling array techniques and whole genome sequencing. These analyses have revealed an unexpectedly high level of gene gain and loss when natural strains are compared with the reference genome. We are investigating the systemic impact of this variation in gene content on traits related to plant performance through a combination of molecular genetic analyses and novel phenotypic assays. Our approaches also enable us to identify discrete novel genes, or new alleles of known genes, that underlie quantitative trait loci of interest. We are investigating these genes and their respective protein products at various levels, from their genetics to their cell biology and biochemistry. We have, for example, identified a novel, plant-specific gene that underlies a quantitative trait locus for root growth. The corresponding protein, which is a putative transcriptional co-activator, mediates responses to plant hormones. Its cell biological and biochemical characterization have identified a novel intracellular signaling pathway between the plasma membrane and the nucleus that regulates cellular growth. Our approach requires expertise in a wide range of techniques. Accordingly, our technical repertoire encompasses state of the art large-scale genomics approaches, histology and confocal microscopy, together with molecular biology, cell biology and biochemistry techniques.

Conceptually, the research in our laboratory contributes to the understanding of rate-limiting factors in plant growth, which is relevant in the context of plant biomass productivity for sustainable development, and to the universal understanding of molecular genetic mechanisms.

Selected publications

Sibout R, Plantegenet S, Hardtke CS (2008)

Flowering as a condition for xylem expansion in *Arabidopsis* hypocotyl and root. *Current Biol* 18: 458-463

Sibout R, Sukuma P, Hettiarachchi C, Holm M, Muday GK, Hardtke CS (2006)

Opposite root growth phenotypes of *hy5* vs. *hy5 hyh* mutants correlate with increased constitutive auxin signaling. *PLoS Genetics* 2: 1898-1912

Mouchel CF, Osmont KS, Hardtke CS (2006)

BRX-mediated feedback between brassinosteroid levels and auxin signaling in root growth. *Nature* 443: 458-461

Mouchel CF, Briggs GC, Hardtke CS (2004)

Natural genetic variation in *Arabidopsis* identifies BREVIS RADIX, a novel regulator of cell proliferation and elongation in the root. *Genes Develop* 18: 700-714



Root meristems, confocal images. The compact root system reflects natural genetic variation in a cellular growth regulator.



Christian Hardtke received his doctoral degree in developmental biology in 1997 from the Ludwig-Maximilians University of Munich, where he worked on plant embryogenesis at the Genetics Institute. After a brief stay at the University of Toronto, he joined the Department of Molecular, Cellular and Developmental Biology at Yale University (New Haven) in 1998 as a Human Frontier Science Program Postdoctoral Fellow to study the perception of light. He started working on the natural genetic variation of molecular mechanisms after becoming an Assistant Professor in the Biology Department of McGill University (Montreal) in 2001. He joined the Department of Plant Molecular Biology as Associate Professor in 2004.



Plants that lack JA (aos mutant) are eaten more quickly than wild-type (WT) plants that produce JA.

Edward Farmer carried out his thesis research in Liverpool, England, working with J. S. Easterby on heart metabolism. Since that time, he has been working on plant defence mechanisms. With the help of a Royal Society fellowship, he started his postdoctoral training in the laboratory of K. Hahlbrock (University of Freiburg, Germany) and then moved to the USA to work with C. A. Ryan (Washington State University). He moved to Switzerland and established a laboratory at the University of Lausanne in 1993. Each few years he does an extended field trip to see plant defense in action.

Presentation

Together, plants and the organisms that eat them (herbivores) dominate the terrestrial biosphere, and there are, on average, at least ten herbivores for every species of plant. Therefore, the immune system of the plant is being challenged constantly at all stages of plant development. Although they cannot escape attack by moving away, plants fight back in remarkably sophisticated ways. The many and varied defence responses of plants are mostly invisible to the human eye and they can only be revealed with sophisticated equipment and powerful methods. These defence strategies include the synthesis of enzymes that interfere with digestive processes within the herbivore, changes in the nutritional value of the leaf itself, and altered growth after attack. Our contribution to the field was to show that they are controlled by a lipid-based signalling pathway centred on the compound jasmonic acid (JA). JA controls a large number, and perhaps most, of all known defence responses to herbivores. Our research, which uses the plant *Arabidopsis*, has also shown that these events happen extremely quickly when the plant is bitten. Within less than one minute, JA starts to accumulate – not only at the site of wounding but also elsewhere throughout the plant. Activated forms of JA are produced, and these promote the expression of at least 1.3% of the protein-coding genes that are needed to reprogramme growth, sequester vital resources (such as nitrogen), and produce defence-related proteins.

Active forms of JA (such as JA–amino acid conjugates) function in signalling within the nucleus, where they bind to massive protein complexes that contain both a ubiquitin ligase complex (the SCF/CO11 complex) and smaller complexes that are thought to consist of JAZ proteins and the transcription factors to which they bind. JA conjugates bring these two protein complexes together, and this results in the destruction of the JAZ proteins. This is thought to liberate the transcription factors so that they can then get to work. We are investigating both the molecular mechanisms that lead to the rapid accumulation of JA in response to wounding and how JA and its derivatives alter gene expression. This involves genetic approaches (mutant identification), analysis of gene cloning and expression (including large-scale sequencing), and quantitative biochemistry (JA-derivative analysis). Our work is aimed at understanding the mechanism of JA signalling and the place of the JA pathway in nature, where it forms a molecular nexus at the interfaces of the primary terrestrial predator/prey relationship.

Selected publications

Glauser G, Grata E, Dubugnon L, Rudaz S, Farmer EE, Wolfender J-L (2008)

Spatial and temporal dynamics of jasmonate synthesis and accumulation in *Arabidopsis* in response to wounding. *J. Biol. Chem.* 283: 16400-16407

Bonaventure G, Gfeller A, Proebsting WM, Hoerstensteiner S, Chételat A, Martinoia E, Farmer EE (2007)

A gain of function allele of TPC1 activates oxylipin biogenesis after leaf wounding in *Arabidopsis*. *Plant J.* 49: 889-898.

Yan Y, Stolz S, Chételat A, Reymond P, Pagni M, Dubugnon L, Farmer EE (2007)

A downstream mediator in the growth repression limb of the jasmonate pathway. *Plant Cell* 19: 2470-2483.

Mène-Saffrané L, Davoine C, Stolz S, Majcherczyk P, Farmer, EE (2007)

Genetic removal of tri-unsaturated fatty acids suppresses developmental and molecular phenotypes of an *Arabidopsis* tocopherol deficient mutant: Whole-body mapping of malondialdehyde pools in a complex eukaryote. *J. Biol. Chem.* 282: 35749-35756.

Plant defence against insects

Philippe Reymond
Group Leader

Presentation

Plants that are under attack from herbivorous insects have developed an array of inducible defences that are aimed at slowing the growth or development of the aggressor and are tailored to protect the plants from further attack. We study the model plant *Arabidopsis thaliana* and use genomic tools to monitor the transcriptional changes that occur after the plants are challenged with leaf-chewing insects. Our research is focused mainly on two projects:

1) The changes in transcriptional patterns that occur in response to attack by insects reveal, at the molecular level, how plants respond to aggressors. We study the interaction between *Arabidopsis* and the larvae of specialist (*Pieris brassicae*) or generalist (*Spodoptera littoralis*) caterpillars using DNA microarrays and bioassays. Our aims are to determine the nature of the insect-derived elicitors that trigger transcriptional reprogramming, and to identify the signals that are involved in the transduction events and the transcription factors that control the induction of defence genes after insect attack. For this purpose, we use *Arabidopsis* signalling mutants and knockout lines to correlate transcriptional changes with insect performance. The aim of this project is to identify sets of genes that contribute to the resistance of plants to herbivores.

2) Insect eggs that have been deposited on a leaf represent a future threat because larvae that hatch from the eggs will ultimately feed on the plant. Using a whole-genome microarray, we have shown that the presence of eggs that have been laid by *Pieris brassicae* results in the modified expression of hundreds of genes. This indicates that plants are capable of detecting the presence of insect eggs and that they respond by activating the expression of defence genes. We want to identify the nature of the egg-derived elicitor(s), to characterize the signalling pathways that control the oviposition-induced responses, and to analyse the benefits and costs of defence against oviposition. The findings could provide the first example of a molecular dialogue between *Arabidopsis* and insect eggs, and would extend the known range of basic innate immunity mechanisms. The long-term application of this project will be the development of strategies to enhance the detection or inhibit the survival of insect eggs.

With an estimated loss of 15% of agricultural crops to insect herbivores, and the banned or restricted usage of many pesticides, knowledge of the innate immunity of plants to insect attack is crucial to improving the possibility of developing a durable and environmentally friendly strategy of crop protection.

Selected publications

Schlaeppli K, Bodenhausen N, Buchala A, Mauch F, Reymond P (2008)

The glutathione-deficient mutant *pad2-1* accumulates lower amounts of glucosinolates and is more susceptible to the insect herbivore *Spodoptera littoralis*. *Plant J* 55: 774-786

Little D, Gouhier-Darimont C, Bruessow F, Reymond P (2007)

Oviposition by pierid butterflies triggers defense gene expression in *Arabidopsis*. *Plant Physiol* 143: 784-800

Bodenhausen N, Reymond P (2007)

Signaling pathways controlling induced resistance to insect herbivores in *Arabidopsis*. *Mol Plant-Microbe Interact* 20: 1406-1420

Reymond P, Bodenhausen N, Van Poecke RMP, Krishnamurthy V, Dicke M, Farmer EE (2004)

A conserved transcript profile in response to a specialist and a generalist herbivore. *Plant Cell* 16: 3132-3147

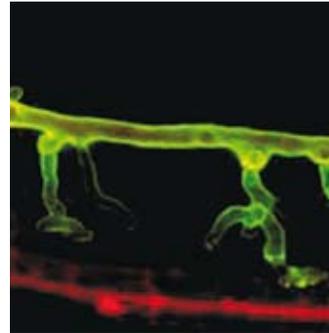


Pieris rapae feeding on an *Arabidopsis* leaf. In the background is a pseudocolour representation of a DNA microarray.



Philippe Reymond carried out his thesis research at the Institute of Plant Biology and Physiology, where he analysed IAA and ABA phytohormones in maize in the laboratory of Dr Paul-Emile Pilet. He received his PhD from the University of Lausanne in 1989. He then studied blue light photoperception during his postdoctoral training with Dr Winslow Briggs at Stanford University. In 1992, he returned to the University of Lausanne and joined the group of Prof. Edward Farmer to investigate signal transduction in plant defence using DNA microarray technology. In 2002, he was appointed as a group leader (Maître d'Enseignement et de Recherche) and started his independent research on plant-insect interactions.

Molecular genetics of the arbuscular mycorrhizal (AM) symbiosis in cereals



Intracellular growth of G. intraradices hyphae within the epidermis of the rice root.

Uta Paszkowski is German, and she graduated in molecular biology from the University of Cologne. In 1993, she received her PhD on "Gene targeting in tobacco" from the ETH-Zurich. As a Marie-Heim-Vogtlin Fellow, she worked at the Botanical Institute in Basel, where she started her work on the genetics of the arbuscular mycorrhizal symbiosis in cereals. In 2000, she became a staff scientist at the Torrey Mesa Research Institute in San Diego and cloned the first mycorrhiza-specific phosphate transporter from rice. She started her own group as a junior group leader at the University of Geneva in the Department of Plant Biology in 2003. Since November 2006, Uta Paszkowski has been a Swiss National Science Foundation Assistant Professor at the DBMV.

Presentation

Plants are involved in symbiotic and parasitic interactions with a wide variety of microorganisms. Of these, the mutually beneficial AM symbiosis represents an exceptionally widespread and evolutionarily old association. The appearance of this interaction coincided with the colonization of land by plants. Extant plant lineages diverged later in evolution, which explains the universal distribution of the AM symbiosis in contemporary terrestrial ecosystems. Most other plant interactions arose later during evolution but appear to partially employ plant programmes already in place for the AM symbiosis.

The lifelong association is mutually beneficial because of a bi-directional exchange of nutrients, in which the fungus receives carbohydrates and in turn enhances the mineral, particularly phosphate, nutrition of its host. The AM fungi associate intimately with the roots of the host, and grow inter- and intracellularly within the root cortex. Intraradical colonization enables the fungi to access the carbohydrates that are required for the formation of the extensive extraradical mycelium, which is involved in acquisition of nutrients from the soil and in the formation of vegetative spores.

The complexity of the processes that are involved in the establishment of the symbiosis indicates a well coordinated orchestration of plant and fungal molecular factors. The research group is interested in the discovery of the molecular mechanisms that underlie AM symbiosis in cereals and the conservation of these mechanisms in symbiosis and parasitism. Amongst the hosts of AM fungi, unique resources are available for rice and maize, and these therefore provide powerful and economically important systems for studying the "genetics and genomics" of AM symbiosis. We have taken two complementary approaches. Using forward genetic screens in maize, we intend to unravel the molecular basis of phenotypic variation. In the second approach, we aim to identify rice compatibility factors that are either involved in interactions with symbiotic fungi or that are involved in interactions with both symbiotic and pathogenic fungi by using a combination of transcriptomics and functional genomics.

Selected publications

Paszkowski U, Jakovleva L, Boller T (2006)

Maize mutants affected at distinct stages of the arbuscular mycorrhizal symbiosis. Plant J 47: 165-173

Paszkowski U (2006)

Mutualism and parasitism: the yin and yang of plant symbioses. Curr Opin Plant Biol 9: 364-370

Güimil S, Chang H, Zhu T, Sesma A, Osbourn A, Roux C, Ioannidis V, Oakeley EJ, Docquier M, Descombes P, Briggs SP, Paszkowski U (2005)

Comparative transcriptomics of rice reveals an ancient pattern of response to microbial colonization. Proc Natl Acad Sci 102: 8066-8070

Paszkowski U, Kroken S, Roux C, Briggs S (2002)

Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. Proc Natl Acad Sci USA 99: 13324-13329

Heat-shock biology and mechanisms of molecular chaperones

Pierre Goloubinoff
Associate Professor

Presentation

1) The biology of the heat-shock response in plants and animals.

We are interested in deciphering the cellular mechanism by which eukaryotes sense mild variations in temperature and react by producing a variety of defence molecules, such as proteases and molecular chaperones, in anticipation of potentially more harmful environmental conditions. We find that specific calcium channels in the plasma membrane act as the initial stress-sensing devices in plant and animal cells. A better understanding of the various steps that are involved in the heat-shock signalling pathway in eukaryotic cells has important agricultural and biomedical applications. For example, the onset and outcome of many diseases in humans that occur as a result of protein misfolding, such as diabetes type 2, Parkinson's, Huntington's, Prion's and Alzheimer's diseases, can be affected dramatically by chaperone-inducing drugs that act on specific targets in the heat-shock signalling pathway. Using plant- and animal-based screens for chaperone induction, we expect to gain control of diseases with impaired chaperone expression and to arrest toxic protein misfolding in aging and in neurological diseases, as well as to block unchecked cell division in some cancers. By using chaperone-inhibiting drugs, we expect to be able to control some cancers that are characterized by the over-expression of chaperones, thereby promoting apoptosis.

2) The molecular mechanisms of aggregate-scavenging chaperones.

We are interested in elucidating the mechanisms by which molecular chaperones, in particular Hsp70 and Hsp100, use the energy of ATP hydrolysis to prevent the formation of cytotoxic protein aggregates and to convert previously formed misfolded protein conformers into unfolded species that can refold to their native structure or be degraded by specific proteases, such as the proteasome. We find that the only disaggregating molecular chaperone known in animal cells, Hsp70, can scavenge toxic protein aggregates actively using a mechanism that we have named "entropic pulling". Together with its co-chaperone Hsp40, Hsp70 can use the energy of ATP hydrolysis to transiently recruit local Brownian movements of the aggregates and the chaperone molecules to generate a local unfolding force. This force causes the global unfolding of aggregates and leads to the refolding into the native structure or the degradation of proteins that have been misfolded as a result of stress or mutation.

Selected publications

De Los Rios P, Ben-Zvi A, Slutsky O, Azem A, Goloubinoff P (2006)

Hsp70 chaperones accelerate protein translocation and the unfolding of stable protein aggregates by entropic pulling. *Proc Natl Acad Sci U S A* 103: 6166-6171

Goloubinoff P, de Los Rios P (2007)

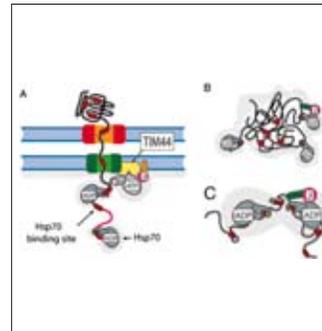
The mechanism of Hsp70 chaperones: (entropic) pulling the models together. *Trends Biochem Sci* 32: 372-80

Hinault M-P, Ben-Zvi A, Goloubinoff P (2006)

Chaperones and proteases: cellular fold-controlling factors of proteins in neurodegenerative diseases and aging. *J Mol Neurosci* 30: 293-310

Saidi Y, Schaefer D, Finka A., Zryd JP, Goloubinoff P (2005)

Controlled expression of recombinant proteins in *Physcomitrella patens* by a conditional heat-shock promoter: a tool for plant research and biotechnology. *Plant Mol Biol* 59: 695-709



Schematic view of the role of Hsp70 in protein translocation and in protein disaggregation and unfolding.



Pierre Goloubinoff obtained a PhD degree from the Weizmann Institute of Science in 1988. He then did postdoctoral research at I.E. DuPont de Nemours and at UC Berkeley. In 1991, he was senior lecturer at the Hebrew University of Jerusalem, and was named Associate Professor from the same university in 1999. He joined the Department of Plant Molecular Biology in 2001 as an Associate Professor.



*Symptoms that develop after inoculation with *Botrytis cinerea* in *Arabidopsis* plants that have a permeable cuticle (below) and in wild type plants (above).*

Christiane Nawrath carried out her thesis research at the Max-Planck-Institut für Züchtungsforschung in the laboratory of Dr Csaba Koncz, and obtained her doctoral degree from the Freie Universität of Berlin in 1990. She received her postdoctoral training with Dr Chris Somerville at Michigan State University in East Lansing and at the Carnegie Institution of Washington in Stanford, USA. She then moved to Switzerland in 1995 to join the laboratory of Dr Jean-Pierre Métraux in Fribourg as an EMBO postdoctoral fellow to work on plant defence mechanisms. She joined the Department of Plant Molecular Biology in Lausanne as an independent group leader in 2004.

Presentation

The aerial portions of plants are covered with a continuous extracellular layer of hydrophobic material, the cuticle, which plays an important role in protecting these organisms from the loss of water and solutes, and from ultraviolet irradiation, as well as pathogen and insect attack. The cuticle consists of two major components: cutin and wax. Cutin, which is a biopolymer that is composed of hydroxy fatty acid derivatives and glycerol, forms the scaffold of the cuticle. Waxes, which consist of a complex mixture of hydrophobic material that comprises very-long-chain fatty acids and their derivatives, are deposited within the cuticle and on the plant surface to seal the cuticle.

A number of genes that are critically involved in the formation of cutin monomers have been identified recently. However, several aspects that relate to the formation of cutin still remain to be discovered. These include the mechanisms by which cutin monomers are transported to the extracellular space and through the cell wall, the polymerization of cutin, the regulation of the well-structured deposition of cutin within the extracellular matrix, and the effects of altered deposition of cutin on the development and physiology of the plant.

Our studies are mainly, but not exclusively, centred on the analysis of *Arabidopsis* mutants that have a permeable cuticle, in order to learn more about the different aspects of the formation of a functional cuticle. Plants with a permeable cuticle exhibit increased water loss and increased sensitivity to herbicides, as well as the rapid permeation of dyes through the cuticle. Two unexpected phenotypes that are also often seen in these plants are the fusion of organs via the epidermal extracellular matrix and an increased resistance to infection by *Botrytis cinerea*. The resistance to *Botrytis* was found to be based largely on the induction of defence responses, which include the production of antifungal substances, by the rapid diffusion of elicitors through the permeable cuticle. The molecular mechanism of the formation of fused organs has not yet been determined.

We combine chemical and ultrastructural studies of mutants that possess an altered cuticle with characterization of the genes and gene products that are responsible for the mutant phenotype. Two mutants that have been characterized recently in our laboratory are botrytis resistant 1 (*bre1*) and permeable cuticle 1 (*pec1*), in which LONG-CHAIN ACYL-COA SYNTHETASE2 and the ATP-binding cassette transporter AtPDR4, respectively, are mutated. Whereas the amount of cutin in the *bre1* mutant is reduced to 20% of the normal level, *pec1* shows no reduction in lipidic cuticular components, and its function in the formation of the cuticle remains to be determined.

Selected publications

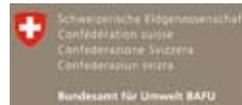
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