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General information

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Vice-presidents: Prof. Isabelle DONNAY
Prof. François CHAUMONT

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Administrative Assistant: Mrs. Véronique LEBRUN
Finance Manager: Mrs. Michèle ROCHAT
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BGMB Group of Biochemistry and Molecular Genetics of Bacteria
Prof. Bernard HALLET
Prof. Pascal HOLS
Prof. Patrice SOUMILLION (Spokesperson)

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Prof. Michel GHISLAIN
Prof. Pierre MORSOMME
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Prof. Jacques MAHILLON (ELI)
Prof. Jacqueline MARCHAND (IMCN)

Acknowledgments

Here, I would like to thank our Colleagues of the Institute who contributed to this first annual scientific report.

I also thank warmly our Emeritus Colleagues Claude REMACLE, Jacques FASTREZ and Philippe VAN DEN BOSCH DE AGUILAR who accepted to chair PhD thesis juries. Their excellent work has been recognized by the juries as well as by PhD candidates. I would like to thank them again for the quality of their work, the time they spent to chair the juries and to examine the manuscripts.

Patrice SOUMILLON, Françoise GOFFLOT, Bernard HALLET, Patrick DUMONT, Xavier DE BOLLE are acknowledged for the organization of ISV seminars, the scientific day of the Institute and the PhD students’ day. Of course, I have also to thank our collaborators who play a major role in the organization of these events: Véronique LEBRUN, Philippe BOMBAERTS, Daniel JAL and all the people among technicians, administrative staff and PhD students.

Thanks to the generous gift from a private donor combined to the support of the Fondation Louvain that made possible the ISV post-doctoral fellowship. This is indeed a very nice opportunity to attract talented post-doctoral fellows. I thank Marc BOUTRY for his commitment and his role as president of the selecting committee.

Last but not least, my thanks and gratitude go to Mrs. Isabelle MAGNOLI who collected data and reports (this was probably the most difficult task) to finalize this first annual scientific report of our Institute.
Introduction

Founded in 1998, the Institute of Life Sciences - Institut des Sciences de la Vie (ISV) - is an interfaculty research Institute of the Université catholique de Louvain. At the end of the 90’s, it appeared that at the UCL in Louvain-la-Neuve, research activities in the fields of biochemistry, molecular biology, cell biology, cell physiology and microbiology were dispersed in too many different departments. This fragmentation was clearly not appropriate to create efficient and competitive research groups. Moreover, it was also quite clear that regrouping these groups in an Institute of Life Sciences would be beneficial for promoting interactions between scientists, would favor collaborations between groups, would improve the training of young scientists, would allow sharing equipment and would permit the setup of technological platforms.

In 2011, the ISV brings together six research groups among which twenty principal investigators (academics or FNRS researchers) and about 130 people including post-docs, PhD students, technicians and administrative staff. The main objective of our Institute is still the development of an environment favorable to high profile research activities. Our aims are to promote high quality basic research in life sciences, to improve the scientific training of young researchers, to attract young talented researchers and help them develop a successful team, to favor the setup of interdisciplinary projects, to favor the transfer of know-how, and finally to promote applied projects that can emerge from basic research.

President’s foreword

This first annual scientific report provides an overview of scientific activities of the Institute for the period of January 2011 to December 2011 but additional information can be also found on the website of the ISV (http://www.uclouvain.be/en-isv.html).

Overall, this first report must serve to document the activities of the Institute and the achievements for the year 2011. This report reveals that our research groups are dynamic, providing a stimulating research environment in our Institute. Moreover, I must also point out that this environment is open and friendly. I am convinced this positive atmosphere is beneficial for our research activities and should continue to be so.

Enjoy reading,

Bernard Knoops

President of the Institute of Life Sciences
Louvain-la-Neuve, October 2012
**Group of Cellular, Nutritional & Toxicological Biochemistry (BCNT)**

**Yves-Jacques Schneider**

**Postdoctoral fellows:** Dr. Alexandra Bazes, Dr. Alina Martirosya, Dr. Géraldine Nollevaux

**PhD students:** Pauline Beguin (Prof. Y. Larondelle), Sophie Bourez (Prof. C. Debier), Anne Brasseur (Dr. L. Puussemier), Régis Coco (Prof. V. Préat), Sylvie Hollebeeck (Prof. Y. Larondelle), Anouk Kaulman (Dr. T. Bohn), Jean-François Michiels (Prof. S. Agathos), Laurence Ribonnet (Prof. Y. Larondelle), Laurence Plapied (Prof. V. Préat), Anne-Catherine Schneider (Prof. Y. Larondelle), Sébastien Sart (Prof. S. Agathos), Céline Thiry, Julie Winand

**Graduate students:** Claes Séverine, Marechal Vincent, Polet Madeleine, Serikova Youlia, Hespel Theana, Nguyen Thi Le Thuy, Bormans Mathieu, Dubois Anne-Catherine, Meyer Amandine, Iweins Michael

**Technician:** Nicole Devilez (part time)

**Research activities 2011:**

Set up and validation of cell culture systems for biotechnological applications

- *In vitro* models of the human intestinal barrier, based on mono-, bi- and tri-cultures of Caco-2 cells with either Raji (B lymphocytes), clonal HT29 (goblet cells) or THP-1 (macrophages) cells or adipocytes to mimic physio-pathological and toxicological situations.
- New “smart” biomaterials based on self-associated peptidic hydrogels to optimize the adhesion, proliferation and differentiation of animal cells, including stem cells.

Interactions of food components with the human intestinal barrier.

- Current research aims at investigating interactions of phenolic compounds and prebiotics (upon colonic fermentation) with the human intestinal barrier: cytotoxicity, effect on biotransformation systems and inflammation markers with or without inflammatory stimulation.
- Interactions of nanoparticles (nanoAg and nano SiO₂) present in human food with the human intestinal barrier with special focus on possible inflammation and protection by polyphenols.

**Research papers:**

- Insect cells as factories for biomanufacturing. Drugmand JC, Schneider YJ, Agathos SN. *Biotechnol*


Theses:

-Jean-François Michiels: Investigation of the Role of Peptones in Animal Cell Culture: Case Study of a Soy Peptone in a CHO Cell Serum-Free Medium

-Laurence Plapied: Strategy of oral vaccination by polymeric nanoparticles: M cell targeting or bioadhesion


-Sébastien Sart: Controlled expansion and differentiation of mesenchymal stem cells in microcarrier-based stirred bioreactors.

Master theses:

-Nguyen Thi Le Thuy: Bioaccessibilité et biodisponibilité de la curcumine native dans un modèle in vitro de la barrière intestinale humaine.

-Madeleine Polet: Evaluation de la toxicité de nanoparticules d’argent sur un modèle in vitro de barrière intestinale

The bacterial transposon **Tn4430** as a paradigm for the mechanisms of replicative DNA transposition (E. Nicolas, end of PhD thesis; C. Oger, PhD thesis, B. Hallet, research stay at the CNRS, Toulouse -lab. Of M. Chandler- April-May 2011; A. Draime, Master thesis)

Tn4430 is a transposon of the Tn3 family, a widespread family of replicative transposons in bacteria. Transposons of this family were among the very first transposable elements to be identified and are often presented as paradigms of replicative transposition in textbooks. Paradoxically, very little is known regarding the exact molecular mechanism of transposition of these elements. In particular, the transposase TnpA of Tn3-family transposons is an unusually big protein (~1,000 aa) showing little sequence similarity with other proteins. This protein not only catalyse the DNA cut and re-joining reactions required for transposition, it is also responsible for an intriguing process termed ‘target immunity’ whereby transposons avoid multiple insertions in the same target molecule.

During the past months, we have achieved several decisive steps toward the understanding of these mechanisms by developing biochemical assays for the TnpA protein of **Tn4430 in vitro**. We have also identified TnpA mutants that are selectively affected in target immunity (published in Lambin et al., 2012, Mol Microbiol. 84: 805). These mutants exhibit promiscuous activities compared to wild type TnpA. They form higher-order nucleoprotein complexes that do not normally form with the wild type protein and show higher cleavage activity. We conclude that TnpA activation is tightly regulated at the level of transpososome assembly and that mutations affecting immunity have ‘unlocked’ the transposase making it less demanding with respect to the required activation signal.

More recent observations have revealed an unexpected link between the mechanism of DNA targeting by Tn4430 and DNA replication. **In vivo**, the insertion pattern of Tn4430 into a specific target was found to be influenced by factors affecting the efficiency and direction of DNA replication. **In vitro**, TnpA was found to bind with a high affinity to replication fork-like DNA structures containing a 3’OH at the branch point. When compared to linear DNA fragments, these structures are efficient substrates for TnpA-catalyzed end transfer. We thus propose a model in which the Tn3-family elements target DNA replication or repair intermediates as a mechanism to recruit the host replication machinery during replicative transposition.

**Distribution of functions among Penicillin Binding Proteins (PBPs) in shaping Lactococcus lactis cells** (B. David, end of PhD thesis –in prep.-, co-sup. P. Hols)

Penicillin Binding Proteins (PBPs) are the key enzymes that are responsible for the biosynthesis of peptidoglycan, the major constituent of the cell wall of Gram-positive bacteria. Spatial and temporal coordination of these enzymes is crucial for conferring a specific shape to the cells while preserving their integrity during growth. How is this coordination achieved during morphogenesis of ovoid bacterial cells remains poorly documented.

Analysis of ovococcus *Lactococcus lactis* genome revealed the presence of 7 PBPs: 5 high molecular weight- (HMW-) PBPs (PBP1A, PBP1B, PBP2A, PBP2B and PBPX) and 2 low molecular weight- (LMW-) carboxypeptidases (DacA and DacB). The contribution of these different PBPs in conferring the specific shape of *L. lactis* was investigated by differentially inhibiting their activity using specific beta-lactams, by analyzing the phenotype of knock-out PBPs-mutants, and by localizing the PBP enzymes using generic fluorescent probes (Bocillin-FL) or specific PBP-YFP*Venus* fusions.
We show that the ovoid shape of *L. lactis* results from the activity of functionally distinct PG synthesis machineries involved in cell elongation and cell division (See also Pérez-Núñez et al., 2011, Mol. Microbiol. 79:759). The transpeptidase PBPX is the central component of the cell division machinery, while the cell elongation machinery articulates around the activity of the other class B enzyme, PB2B. The other PBPs appear to play redundant functions, preferentially acting in one or the other biosynthetic pathways. Spatial and temporal coordination of both machineries is currently being studied by time-lapse fluorescence microscopy.

**The replication mechanism of pGIL01 prophage from Bacillus thuringiensis: Toward the development of new tools for the molecular evolution of proteins** (P. Nauny, PhD thesis, co-sup. P. Soumillion)

The bacteriophage GIL01 from Bacillus thuringiensis propagates as a linear DNA molecule using a terminal protein-primed mechanism. The aim of the project is to characterize this mechanism at the molecular level in order to develop new tools for the engineering of proteins by accelerated molecular evolution *in vivo* and *in vitro*. Since the onset of the project (2010) progress has been made to identify the minimal set of phage-encoded functions required for replication and to express the protein in view of their purification.

**Research papers:**


**Theses:**

- **Emilien Nicolas** (April 2011): Biochemical activities of the transposase TnpA in transposition and target immunity mediated by the replicative transposon Tn4430

**Master theses:**

- **Cédric Oger**: Influence de la réplication de l’ADN sur le mécanisme de ciblage du transposon bactérien Tn4430
- **Debout Hélène** (B. Hallet/ P. Hols): Caractérisation biochimique du système de régulation comRS responsable de l’activation de la compétence chez *Streptococcus thermophilus*
- **Aurélie Diman** (B. Hallet/ P. Hols): Caractérisation biochimique du régulateur LarR de l’activité lactate racémase chez *L. Plantarum*
The research activities of the team are focused on a specific group of Gram-positive bacteria, generically referred to as “lactic acid bacteria” (LAB), which are of major importance for food fermentation and for their ability to colonize the gastro-intestinal tract of mammals. Three model species (Lactobacillus plantarum, Lactococcus lactis, and Streptococcus thermophilus) are studied at the genetic and physiological levels by a multidisciplinary range of genetic, (post-)genomics, biochemical, and biophysical approaches. More specifically, research areas are: cell-wall biosynthesis, carbon metabolism, metabolic adaptation to environmental parameters and gene regulation. DNA recombinant technologies are also used to engineer LAB strains as delivery systems for the production of specific compounds for agro-food and pharmaceutical industry.

(i) Biosynthesis and degradation of the cell envelope

The cell wall of Gram-positive bacteria (including LAB) contains three major components: peptidoglycan (PG), teichoic acids (LTA and WTA), and polysaccharides (WPS). The PG is the major constituent of the cell envelope of Gram-positive bacteria and is a polymer of the disaccharide N-acetylmuramic acid (MurNAc)-(1→4)-N-acetylglucosamine (GlcNAc) associated with a peptidic stem linked to MurNAc. The PG is highly post-modified (e.g. O-acetylation, amidation) or decorated (e.g. WTA, WPS). The functional role of these modifications remains poorly understood. In L. plantarum (rod-shape), we showed that PG glycan strands are highly post-modified by O-acetylation and that this modification is present on both MurNAc (OatA-dependent) and GlcNAc (OatB-dependent), the latest being never described before. Furthermore, we demonstrated that these two modifications have antagonistic roles in controlling hydrolases of PG (Bernard et al. a). The PG of L. plantarum contains amidated meso-diaminopimelate (mDAP) in its stem peptide. We identified the first amidotransferase responsible of this amidation. We showed its importance in the control of PG maturation enzymes and a critical role in the control of the septation process (Bernard et al. b). The PG of L. plantarum is decorated by WTA and WPS. Mutants deficient for WTA biosynthesis revealed interesting phenotype characteristics such as an increased autolytic behaviour and a strongly altered morphology. We also initiated a first investigation of the spatial distribution of WTAs by a combined approach of fluorescence microscopy and high resolution AFM imaging (Andre et al.). In addition, we participate to the study of the role of small heat shock proteins regarding to cell morphology and membrane fluidity (Capozzi et al.). In L. lactis (ovoid shape), we studied the functional role of enzymes responsible of PG assembly (PBPs) and their importance in morphogenesis. We contribute to the understanding of the role of PBP2B and PBP2X in cell elongation and septation, respectively (Perez-Nunez et al.).
(ii) Metabolic adaptation to environmental parameters and gene regulation

Recently, we showed that *S. thermophilus* is naturally competent for DNA transformation under specific conditions. We demonstrated that competence development in this species is controlled by a peptide-mediated quorum-sensing system of the PlcR/Rgg/PrgX family, named ComRS. In this context, we contributed to the study of other members of this family in *S. thermophilus* (Fleuchot *et al.*). In addition, we exploited natural transformation to identify the protease PrtS as a key determinant of nitrogen metabolism for growth in milk (Dandoy *et al.*). Finally, we contribute to the understanding of the commensalism between *L. plantarum* and drosophila during larval development by the analysis of mutants deficient in fermentation end-products (Storelli *et al.*).

**Research papers:**


**Master theses:**

- Aurélie Diman (Co-prom, P. Hols): Caractérisation biochimique du régulateur LarR de l’activité lactate racémase chez *Lactobacillus Plantarum*

- Debout Hélène (B. Hallet/ P. Hols): Caractérisation biochimique du système de régulation comRS responsable de l’activation de la compétence chez *Streptococcus thermophilus*
### PhD students: 
Gilles Joachim, Pierre Galka, Mary Doumit, Anastassia Vorobieva, Stéphanie Garcia, Heykel Trabelsi, Bruno Baudoux, Gabrielle Woronoff, Philippe Nauny (coprom B. Hallet), Benoit Desguin (coprom P. Hols)

### Graduate students: 
Olivier Box, Pierre-Yves Laruelle, Antoine Deschamps (coprom P. Morsonne), Lucrezia Rinaldi (coprom R. Rezohazy).

### Technician:  
Laurence Bausiers; **secretary:** Véronique Lebrun (50%)

#### Research activities:

1. **Directed evolution of enzymes.**

   With the general aim of a better understanding of structure-function relationships in enzymes, several projects are dedicated to the engineering of new functions such as specificity, regulation or catalysis.

   We developed new high throughput screening assays for evolving the specificity of penicillin acylase in microfluidic-based microdroplets reactors. The enzyme and a reporter GFP are produced by *in vitro* transcription and translation and a “translatogenic” substrate of the enzyme is autoamplifying the translation reaction, resulting in fluorescence increase (Woronoff *et al.*, in preparation). This work is performed in collaboration with A.D. Griffiths (ISIS, Strasbourg).

   Our efforts in trying to evolve a DD-peptidase (PBP-A) into a beta-lactamase have been pursued. Using a so-called neutralization drift followed by a selection on a sub-lethal concentration of penicillin, we have been able to select PBP-A mutants that confer resistance to the antibiotic. Biochemical characterization is underway.

   Using the phage display technology, beta-lactamase insertants libraries constructed from a stabilized starting enzyme have been used for the selection of allosterically regulated enzymes. Unlike the previous selections performed with libraries constructed from a non stabilized enzyme, the selections failed to provide regulated enzymes. We are currently trying to identify the origin of this problem.

   The isocitrte dehydrogenase (IDH), an enzyme from the Krebs cycle, has been developed as a new dimeric platform for enzyme evolution. This homodimeric enzyme has its active site at the interface between the subunits. By mutating residues on each side of the active site, inactive mutants were capable of restoring activity when coexpressed in the same bacteria, thanks to heterodimerization. Interestingly, with this strategy, we have shown that heterodimerization can occur between subunits of homologous enzymes coming from different organisms and featuring low percentage of sequence identity (< 20%). This has consequences for evolutionary effects that can happen upon horizontal gene transfer.

   In a new project, we are highlighting an ancestral tandem duplication of seven residues that has most probably occurred in the essential omega loop of class A beta-lactamases and may be the preponderant evolutive event that lead to the emergence of the beta-lactamase activity. Sequence alignments indicate that the tandem duplication is highly probable (> 99.7%). The cloning of a
synthetic gene of the beta-lactamase from \textit{S. lavendulae}, an enzyme featuring a quasi perfect duplication, was started with the aim of performing a retro-evolution experiment.

2. Selection of bioactive cyclic peptides

We have recently demonstrated the biosynthesis of combinatorial libraries of backbone cyclic peptides in the cytoplasm and periplasm of \textit{E. coli}. Several projects aim at identifying new bioactive compounds within these libraries.

Using an anti-darwinian selection strategy based on the plasmid recovery after lysis of the interesting clones, we are searching for peptides inhibitors of the AcrAB-ToIC efflux pump in \textit{E. coli}. The addition of oxacillin, a bacteriolytic compound which is a substrate of the pump, is capable of inducing the lysis only if the pump is not functional. Hence, the recovered plasmids are coming from clones that are potentially producing peptide inhibitors of the pump. Model experiments have validated the selection strategy and interesting clones have been selected. A details characterization is underway.

Using a Darwinian selection strategy, we are searching for cyclic peptide inhibitors of aquaglyceroporins (AGPs). Besides their functional water and glycerol transport, these porins are capable of transporting toxic metalloids such as arsenite or antimonite. In an \textit{E. coli} strain which is deleted from its own AGP and from its metalloid detoxification system, it is therefore possible to select inhibitors of a recombinant AGP since it will allow the bacteria to grow in the presence of these metalloids. AGP-dependant toxicity to metalloid was shown for the \textit{E.coli} GlpF porin. Expression clones for human porins are being constructed.

A new thesis with the aim of searching cyclic peptide disruptors of the HoxA1-Pbx interaction has also started in October 2011. The objective is to transfer the cyclic peptide libraries into the yeast and to use a two-hybrid-based assay for identifying interaction disruptors.

3. Other research activities

We are studying the replication system of linear prophage (pGIL01) that has been discovered by the team of J. Mahillon. The short term objective is the cloning and the biochemical characterization of the proteins involved in the replication, mainly the DNA polymerase and the terminal protein. The long term objective is the development of a new method for the directed evolution of proteins. The idea is to use this replication system for the \textit{in vivo} error prone replication of a specific gene of interest without replicating other genes of the organism.

A last project concerns the biochemical characterization of the lactate racemase of \textit{Lactobacillus plantarum}, a new metallo-enzyme containing one or more nickel ions in its active site.

\textbf{Research papers:}


\textbf{Master theses :}

\textbf{René Mbaduko}: Recherche des peptides inhibiteurs d’ApoL1.

\textbf{Gilles Joachim}: Sécrétion de peptides cycliques par \textit{E. coli} au travers de porines plV mutées.

\textbf{Michael Abraham}: Liposome display.
Group of Nutrition Biology and Environmental Toxicology (BNTE)

Cathy Debier

PhD students: Sophie Bourez, Marie Vanden Berghe, Caroline Louis

Graduate students: Stéphanie Suciu (UCL-Agro), Marie Stas (UCL-SC, ends in 2012), Denis Lesoinne (ULg-SC)

Staff shared within the BNTE research group: Technicians: Eric Mignonet (and Christine Turu/Catherine Romanowska, Secretary: Franck Moreau. Account manager: Maria Ruiz

Research activities 2011

My research activities focus on the toxicokinetics of persistent organic pollutants (POPs) in terrestrial and marine animals. More precisely, during 2011, we focused on the biochemical mechanisms involved in the uptake and storage of POPs in the adipose tissue of mammals. Among others, we examined the intracellular distribution of PCBs in mouse cultured adipocytes and tested the potential involvement of caveolin-1, an abundant adipocyte membrane protein, in the uptake of these compounds by fat cells. We showed that PCB-28, PCB-118 and PCB-153 congeners rapidly and extensively accumulate in cultured adipocytes. The dynamics of accumulation differed between the 3 congeners tested. By subcellular fractionation of primary adipocytes, we demonstrated that these pollutants were almost exclusively recovered within the lipid droplet fraction. Our data also indicated that caveolin-1 per se is not required for selective PCB accumulation, but rather point out a primary dependence on adipocyte triglyceride content. In another part of the project, we developed an efficient method of in vitro lipolysis to study the behavior of POPs at the adipose tissue level during a stage of energetic deprivation. The next step will be to study the dynamics of mobilization of different POP congeners (alone or in mixtures) and the relationships with the release of fatty acids.

In addition to in vitro studies, we also focused on the toxicokinetics of various POPs (PCBs, DDTs and PBDEs) during key-periods of the life cycle of free-ranging phocid seals. Among others, we studied the transfer of PCBs, PBDEs and their metabolites (HO-PCBs and HO-PBDEs) as well as DDTs from lactating mothers to their pups in grey seals. Generally, concentrations of total PCBs, PBDEs and DDTs tended to be higher in all tissues at late lactation. The transfer from inner blubber to maternal serum was selective and strongly depended on the Log Kow value of the compounds, with less lipophilic compounds being more efficiently released. Several HO-PCB metabolites were found in maternal and pup serum, indicating a most-likely transplacental transfer of HO-PCBs from mothers to pups. The increasing HO-PCB concentrations found in pup serum at late lactation suggested either endogenous biotransformation in suckling pups or accumulation of undetectable low amounts from milk. The potential impact that such a contamination by POPs may exert on the health and physiology of marine mammals was investigated through the use of biomarkers such as vitamin A. Vitamin A levels in inner blubber and serum of lactating grey seal females appeared to be positively related to ΣPCBs, ΣPBDEs and several individual PCB and PBDE congeners in inner blubber and serum. These findings suggest enhanced mobilisation of hepatic retinoid stores and redistribution in the blubber, a storage site for vitamin A in marine mammals, before the onset of lactation. We have also reported that serum concentrations of ΣHO-PCBs and 4-OH-CB107 tended to increase with circulating vitamin A levels. Our results are in agreement with previous findings highlighting a disruption of
vitamin A homeostasis in the blubber and bloodstream following exposure to environmental pollutants.

In a collaborative work with the University of California at Santa Cruz (UCSC), we also studied the relationships between the foraging ecology (foraging location and depth, trophic level) of northern elephant seals and their POP contamination burden. In collaboration with the University of Liège, we participated to studies investigating the dynamics of trace metals in seals during key periods of their life cycle. In collaboration with JF Rees, we also participated to a study investigating the effects of environmental pollutants on the biology of fish through the use of in vivo and in vitro models (development of precision-cut liver and brain slices)

Research papers:


Short papers (3 pages – Organohalogen compounds database – Dioxin 2011):


Master theses:

Denis Lesoinne (ULg): Mise au point d’une méthode d’analyse des PBDEs et des PCBs: évaluation du niveau de contamination du lait du phoque gris (Halichoerus grypus) dans le cadre d’un test interlaboratoires – co-supervised with JP Thomé (ULg)

Stéphanie Suciu (AGRO UCL): Etude du métabolisme lipidique chez Mirounga angustirostris en cours de jeûne post-sevrage par real-time PCR semi-quantitative
Postdoctoral fellows: Tarik Abboudi; Christelle André; Quynh Chau Dang Van; Laurence Ribonnet

PhD students: Pauline Beguin (50%); Quynh Chau Dang Van; Lorraine Guillame; Sylvie Hollebeek (50%); Lai Thi Ngoc Ha; Claudine Passo Tsamo; Darly Pompeu; Laurence Ribonnet; Anne-Catherine Schneider

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Graduate students: Louis-Pasteur Bamenga; Cédric De Taeye; Céline Debouche; Lova Ralanbomahay; Julie Mellery; Nicolas Nyssen; Eric Radelet; Rina Razanakolona

Staff: Technicians: Marc Michotte; Eric Mignolet; Mustapha Ozalp; Catherine Romanowska; Carole Soulas; Christine Turu; Secretary: Franck Moreau, Account officer: Maria Ruiz

Research activities 2011 (only the research areas are mentioned):

Improvement and evaluation of the quality of cow’s milk fatty acid composition

Modulation of fermentation processes in the rumen in an environmental perspective

Determination of the dietary requirements of different fish species and impact of feeding strategies on the nutritional quality of their flesh

Upgrading of under-exploited foods (Amazonian or Vietnamese fruits, Andean roots and tubers, native European fruits, agricultural by-products, ...), with a specific richness in bioactive compounds (polyphenols, triterpenes, carotenoids, glucosinolates, ...) for functional food development

Understanding of the mechanisms whereby nutrients, food components, xenobiotics and other food contaminants interact with the human intestinal barrier

Development of biological means to lower the toxicity of dietary products contaminated with mycotoxins

Research papers:


-Nang Thu, T.T., Bodin, N., De Saeger, S., Larondelle, Y., Rollin, X. (2011) Substitution of fish meal by sesame oil cake (Sesamum indicum L.) in the diet of rainbow trout (Oncorhynchus mykiss W.), Aquaculture Nutrition, 17, 80-89


- Rogez, H., Pompeu, D.R., Akwie, S.N.T., Larondelle, Y. (2011) Sigmoidal kinetics of anthocyanin accumulation during fruit ripening: a comparison between açai fruits (Euterpe oleracea) and other anthocyanin-rich fruits, Journal of Food Composition and Analysis, 24, 796-800


- Hollebeeck, S., Raas, T., Piront, N., Schneider, Y-J., Toussaint, O., Larondelle, Y., During, A. (2011) Dimethyl sulfoxide (DMSO) attenuates the inflammatory response in the in vitro intestinal Caco-2 cell model, Toxicology Letters, 206, 268-275

**Theses:**

- *Laurence Ribonnet*: Evaluation of the interactions of herbal dietary supplements with molecular targets at the intestinal level: a first step toward risk assessment

- *Quynh Chau Dang Van*: Influence of the diet structure and lipid supplementation on ruminal biohydrogenation and on the bovine milk fatty acid composition

**Master theses:**

- *Louis-Pasteur Bamenga*: Caractérisation des glucides contenus dans le résidu liquide d’extraction des fibres végétales par l’eau super-chauffée

- *Cédric De Taeye*: Vers une huile de lin (*Linum usitatissimum* L.) protégée naturellement et enrichie en lignanes fonctionnelles

- *Céline Debouche*: La disponibilité des lignanes de la graine de lin (Master Biologie)

- *Lova Ralanbomahay*: The influence of protein growth rate on the optimum dietary amino acid pattern for a growing wild strain Loire-Allier of Atlantic salmon (*Salmo salar*) fry


- *Nicolas Nyssen*: Substitution of kieselguhr filter aids and adsorption of undesirable substances (mycotoxins) by vegetable material in the filtration of beer and wine

Postdoctoral fellow: Dr Tie Jun LING (Anhui University - China)

PhD student: Elie Tchuessa KADJI

Research activities 2011:
Research about the tea-beer production
Research about the food quality control by spectrophotometric techniques

Research paper:

Master theses:
-Emilie Flora Lepka Sahaidio : Isolement de l’acide éléostéarique à partir de l’huile de *Ricinodendron Heudelotii*
-Jeremy Farvaque : Propriétés pro- et anti-oxydantes de la chlorophylle
Postdoctoral fellow: Delphine Scaion

Graduate students: Ophélie Colmant, Catherine Rossillon

Research activities:

We have developed a new in vitro approach based on cultured brain slices of European eels *Anguilla anguilla*. The slice can be maintained alive for 24 hours in culture medium. The uptake and the response of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) to Methyl-Mercury (Me-Hg) have been studied. The contamination of wild eels with metals and organochlorines has also been studied in Belgian eels populations. Results indicate that eel brains contain high amounts of heavy metals and organochlorines. The contamination profile in mercury and Me-Hg markedly differ from those of muscle and liver. In another study, we investigated the impact of Me-Hg and Cd2+ on erythrocytes antioxidant resistance. Results indicate that preexposure of erythrocytes to Me-Hg increases the resistance of erythrocytes suggesting that it may increase resistance mechanisms. This resistance is not associated to increased activities of the three tested antioxidant enzymes.

Research papers:

No articles were published in 2011.

Master theses:

- **Ophélie Colmant**: Etude comparée des effets du méthylmercure sur le système nerveux central d’anguilles européennes: études de tranches (in vitro) /milieu naturel

- **Catherine Rossillon**: Impact of environmental contaminants on the resistance of *Anguilla anguilla* erythrocytes exposed to oxidative stress in vitro
The tumor suppressor p53 has the distinction of being the most frequently mutated gene in human cancer. As a transcription factor, p53 induces the expression of a variety of genes which products are involved in the inhibition of cell growth or the induction of apoptosis. Moreover, p53 can repress the expression of pro-survival genes, such as those coding for MDR1 or BCL-2. In addition to this transcriptional activity, different teams including ours have described that in the presence of an apoptosis-inducing stimulus, a fraction of p53 translocates to the mitochondria and triggers mitochondrial outer membrane permeabilization (MOMP), leading to the release of the mitochondrial effectors of apoptosis into the cytoplasm. Notably, p53 is able to interact with BAK, a pro-apoptotic BCL-2 family member, and to induce its oligomerization at the MOM.

We have developed different projects to study the mechanisms of p53-dependent apoptosis, to investigate some aspects of p53’s activity alteration in cancer and to study the mechanisms of action of natural molecules presenting chemopreventive or chemotherapeutic activities in cancer:

1) **Influence of MAGE-A genes on p53-dependent apoptosis.** Although silent in the vast majority of adult tissues, MAGE-A (Melanoma Antigen-A) genes are frequently expressed in different cancer types, including melanoma as well as breast, colon and lung cancer. Our project aims to characterize whether an expression of MAGE-A1, -A2, -A3, -A4, -A6 and -A12 can alter the sensitivity of cancer cells to apoptosis-inducing stimuli, including drugs commonly used in cancer chemotherapy. Our data indicate that overexpression of several of these proteins in MCF-7 breast cancer cells enhances their resistance to the DNA damaging agent doxorubicin. We are currently investigating the mechanisms involved. One hypothesis is that these proteins could interact with p53 and inhibit its activity.

2) **Mitochondrial activity of common p53 mutants.** The tumor suppressor p53 has a direct pro-apoptotic activity at the mitochondria: p53 interacts with BAK and triggers its oligomerization, leading to MOMP and release of the mitochondrial effectors of apoptosis. This project aims to study common p53 mutants (R175H, G245S, R248W, R273H, R282W) with regard to this activity. First, our data show that p53 R175H and R282W display a constitutive cytosolic as well as mitochondrial localization. Surprisingly, these mutants retain a wt ability to trigger BAK oligomerization in the presence of purified mitochondria. Thus, although intrinsically able to trigger MOMP, such mutants remain inactive in cancer cells suggesting that they must be the target of an inhibition. This is currently under investigation. Secondly, our data show that p53 R175H and R273H interact with mitochondrial procaspase-3. Indeed, procaspase-3 is present in the cytosol but also at the MOM in a complex with HSP60 and HSP10. We found that p53 R175H and R273H inhibit the activation of procaspase-3 by caspase-9. These data shed a light on the “gain of function” properties of common p53 mutants. This part of the project has been published in 2011 in *Cancer Biology & Therapy.*
3) Influence of anti-apoptotic BCL-2 family members on the mitochondrial activity of wt p53. Anti-apoptotic BCL2 family members are often overexpressed in cancer cells. This is the case of BCL-2 itself, BCL-XL and MCL1. They participate to the different mechanisms that render cancer cells less sensitive to apoptosis-inducing stimuli. This project aims to analyze whether this has an impact on the mitochondrial activity of p53. Specifically, we aim to study the interactions between these proteins and the tumor suppressor, to analyze whether their expression inhibits the mitochondrial translocation of p53 during apoptosis and to study whether the mitochondrial activity of p53 is itself compromised (BAK oligomerization, release of the mitochondrial effectors of apoptosis).

4) Study of the mechanism of action of resveratrol, a natural polyphenol. Resveratrol (RSV, trans-3,4’,5-trihydroxystilbène) is widely studied because of its chemopreventive activity in different cancer models in mouse. Moreover, possibility of using derivatives of this molecule for chemotherapeutic use is investigated. Our data show that, at high concentration, RSV triggers formation of DNA double-strand breaks. This is due to the ability of the molecule to interfere with topoisomerase catalytic cycle, similarly to the topo II poison etoposide. Moreover, RSV induces p53 in colon cancer cells, resulting in apoptosis. This work is currently submitted for publication.

Research papers:

Master theses:
- Maryse Hermant : Rôle du suppresseur de tumeur p53 dans l’apoptose induite par le resvératrol.
- Cédric Castrogiovanni : Etude de l’activité pro-apoptotique mitochondriale de mutants de p53.
Postdoctoral fellow: Nicolas Theys;  
PhD student: Benoit Lizen (from Sept 2011)  
Graduate students: Diane Bissen (2011-2012), Cécile Coste (2011-2012)  
Technicians: Konstantin Doshishti-Agolli; Marie-Thérèse Ahn (75% CELL);  
Secretary: Véronique Guns (CELL)  

Research activities 2011:

The Hox genes code for a family of transcription factors that determine the identity of cells and tissues in the developing embryo. Although their expression and functions within the embryo are well established, very little is known about their distribution at foetal and post-natal stages. Several studies have reported Hox gene expression in normal adult tissues, opening new questions about their regulation and biological function at adulthood besides their role in development. In addition, inappropriate expression of particular Hox genes is associated with tumour development and malignant progression in adult tissues. Our research project is in keeping with these important aspects of Hox genes, their biological functions at adulthood and their potential involvement in diseases and oncogenesis, with emphasis on the central nervous system (CNS).

To explore Hox genes late functions in the CNS, we have developed several lines of action.

A first action is to establish a comprehensive, quantitative and qualitative, expression atlas of the all Hox genes and their functional partners in the foetal and adult CNS in mouse. This systematic approach should allow bringing to light new individual gene function(s) but also gene networks involved in particular cerebral functions. Indeed, our data show for the first time the presence of transcripts for 24 Hox genes in the adult brain. More importantly, our data, revealing expression of Hox genes in territories where they are not present during embryonic development, suggest that these genes could be recruited to serve new function(s) in postmitotic neurons. These results were presented as poster and oral communication at the COST/ISF Workshop on the Function of Hox and Tale Homeoproteins in Development and Disease (France, October 2011), and they are currently being prepared for publication.

A second action is to investigate in more details the spatio-temporal regulation of a subset of Hox genes, selected on the basis of their particular distribution as revealed in the first action. We have selected two subsets of genes for this analysis: Hoxb1, b3 and b4, for which specific expression has been detected within the cerebral cortex and the thalamus, two regions derived from the embryonic forebrain; and Hoxa5 and Hoxb5, for which specific expression has been detected within the cerebellum. For both groups of genes, we are currently (1) tracing the expression patterns from embryonic to adult stages and (2) identifying expressing cells at the molecular level. To provide an anatomical and quantitative view of the time-course of Hox expression, ISH and RT-qPCR are applied on foetuses and on isolated brains from newborns and adult mice. To investigate in which cells Hox
genes could be active, we are developing double cell labelling procedures to simultaneously detect Hox transcripts/proteins and specific neuronal or glial molecular markers.

To elucidate Hox genes specific function in the nervous system at later stages, our third action is the generation and phenotyping of conditional loss-of-function mutant mice for Hoxa5. A transgenic mouse line with a floxed allele of Hoxa5 has been established by the group of Dr L. Jeannotte (Université de Laval, Canada), and is available through collaboration with this group. This transgenic line will be crossed with an inducible CreER<sup>T2</sup> mouse line, which will allow temporal inactivation of Hoxa5 in all tissues, the CMV-CreER<sup>T2</sup>. This line is available through collaboration with the group of Filippo Rijli (FMI, Basel). Both lines are in the process of being derived in our animal facility.

Finally, to bring this project one step further in the analysis of Hox functions and dysfunctions in the nervous system, the last action focus on Hox gene expression in nervous system tumours, mainly medulloblastoma tumours, using RT-qPCR profiling.

Research papers:


Master theses:

-Hutlet Bertrand (F. Gofflot/R. Rezsohazy): Caractérisation de souris mutantes pour l’homéodomaine de Hoxa1
-Benoît Lizen (F. Gofflot/R. Rezsohazy) : Caractérisation du phénotype de souris portant une mutation pour les résidus 2 et 3 de l’homéodomaine Hoxa1
Antioxidant enzymes of animal cells

Bernard Knoops

Postdoctoral fellow: Julie Goemaere (from June 2011)

PhD students: Julie Goemaere, Valérie Van der Eecken, Oksana Kuznetsova, Geoffroy Walbrecq, Marc Pirson


Technicians: André Clippe, Ing. (50%), Daniel Jal (CELL), Coralie Piget (CELL); secretary: Véronique Guns (CELL), Michèle Rochat (CELL-FYMO-ISV), Liliane Demuylder (CELL-BGMB).

Research activities 2011:

Peroxiredoxins (PRDXs) have been identified as a large family of peroxidases. These enzymes compose a superfamily of peroxide reductases ubiquitously found throughout evolution in bacteria, archaea and eukaryotes. Notably, PRDXs are part of the enzymatic antioxidant system of living organisms, collaborating in animal cells with well-characterized catalase, superoxide dismutases and glutathione peroxidases. Peroxidase activity of PRDXs is depending on cysteines. All PRDXs exhibit a conserved peroxidatic Cys (Cp) in the N-terminal domain of the protein. During the peroxidase reaction, the Cp attacks the O-O bond of the peroxide (hydrogen peroxide, alkyl hydroperoxides or peroxynitrite) and is oxidized to a cysteine sulfenic acid, which is then reduced back during the resolution step. Subsequently, oxidized cysteines are recycled by reducing equivalents derived from thiol-containing donor molecules such as thioredoxins or glutathione. Based on the resolution mechanism and the existence or the lack of a resolving Cys (Cr) localized to the C-terminal region of the enzyme, PRDXs were classified into three subfamilies referred as 1-Cys, typical 2-Cys and atypical 2-Cys. In mammals, there are six PRDXs encoded by six distinct genes.

During the year 2011, our research work has been mainly focused on: (i) the expression and the function of PRDXs and related antioxidant enzymes in the central nervous system of rodents either during development or in the adult (healthy or in a rat model of amyotrophic lateral sclerosis) and (ii) the subcellular localization of PRDX5 in mitochondria (inter-specific variability in mammals) and in peroxisomes (function).

Immunohistochemistry was used to map basal expression of PRDXs throughout mouse brain. We first confirmed the neuronal localization of PRDX2-5 and the glial expression of PRDX1, PRDX4 and PRDX6. Then, we performed an in-depth analysis of neuronal PRDX distribution in the brain. Our results show that PRDX2-5 are widely detected in the different neuronal populations, and are especially well expressed in the olfactory mitral layer, in the cerebral cortex, in pons nuclei, in the red nucleus, in all cranial nerve nuclei, in the cerebellum and in motor neurons of the spinal cord. In the mouse developing spinal cord, our results show that all PRDXs are expressed in ventral or ventro-lateral area of the spinal cord along the rostro-caudal axis. Interestingly, PRDX3, PRDX5 and thioredoxin 2 are highly expressed early during spinal cord development particularly in motoneurons suggesting that these antioxidant enzymes could play a functional role in spinal cord differentiation. We also use
transgenic SOD1G93A rats (model of amyotrophic lateral sclerosis) to analyze the expression and the role of PRDXs in neurodegenerative processes. Our results show that the expression of different isoforms of PRDXs is upregulated in spinal cord of transgenic rats at the end-stage of the disease. Particularly, the expression of the six isoforms (PRDX1 to 6) is observed in activated astrocytes in transgenic tissues, while only PRDX6 is highly expressed in astrocytes in wild-type rats. These results suggest that an important activation of antioxidant defenses is triggered in proliferating astrocytes in amyotrophic lateral sclerosis.

In our analysis of the subcellular localizations of PRDX5, we found that PRDX5 mitochondrial targeting sequence is present and functional in the annelid lugworm Arenicola marina. Surprisingly, although mitochondrial targeting is well conserved among animals, PRDX5 is missing in mitochondria of domestic pig and dog. Thus, it appears that mitochondrial targeting of PRDX5 may have been lost throughout evolution in animal species, including pig and dog, with unknown functional consequences. Also, in order to study the role of PRDX5 in peroxisomes, murine endogenous expression of PRDX5 in 158N oligodendrocytes was knocked-down by RNA interference. PRDX5 expression in peroxisomes was re-established using a vector carrying the human PRDX5 whose weak peroxisomal targeting sequence 1 (PTS1; SQL) was mutated to a strong PTS1 (SKL). Stable 158N clones were obtained. The redox status within peroxisomes was monitored by using redox-sensitive variants of the green fluorescent protein. Our results show that peroxisomal PRDX5 protects 158N oligodendrocytes against peroxisomal oxidative stress. However, the relative importance of catalase and PRDX5 as protective antioxidant enzymes inside peroxisomes still remains to be investigated.

Research papers:

-Van der Eecken V., Clippe A., Van Veldhoven P.P. and Knoops B. (2011) Mitochondrial targeting of peroxiredoxin 5 is preserved from annelids to mammals but is absent in pig Sus scrofa domesticus, Mitochondrion, 11, 973-981

PhD thesis:

-Julie Goemaere: Peroxiredoxin expression in the central nervous system and oxidative modifications: potential role in neurodegeneration

Master theses:

-Amandine HANNOTIAU: Construction de sondes roGFP2 et mesure du statut redox des peroxysomes de la lignée gliale 158N de souris
-Wassila ILIAS : Identification des partenaires de la HSPC152/TRMT112
Group of « Embryologie moléculaire et cellulaire animale » (EMCA)

Isabelle Donnay

**PhD students:** Delphine Paul (assistant), Wendy Sonnet (FRIA), Emmanuelle Ghys (assistant), Caroline Sauvegarde (FRIA)

**Research assistant:** Jean-François Dumasy (RW)

**Graduate student:** Matthew Dallemagne (BBMC)

**Technicians:** Philippe Bombaerts, Raphaël Chiarelli, Nathan N’guyen

**Secretary:** Marie-Anne Mauclet

**Research activities 2011:**

Three projects were carried on in 2011

Roles of Hox genes in the early mammalian embryo development (co-promotor: R. Rezsohazy, ISV)

The general objective is to investigate the potential roles of Hox proteins during oocyte maturation and early embryo development (from the zygote to the blastocyst stage). This work started 6 years ago in the framework of a FNRS-FRFC grant. During 2011, the study of the spatio-temporal expression of several Hox and TALE (known co-factors of Hox proteins) genes was carried on. Expression was studied in two mammalian models, the mouse and the bovine, using complementary approaches: in situ hybridization, quantitative or semi-quantitative RT-PCR, immunofluorescence. One paper has been published (Paul et al., 2011, see infra), and another was written and submitted for publication (Sonnet et al., Characterization of TALE genes expression during the first lineage segregation in mammalian embryos). Posters and short communication were presented to 3 international congresses. W. Sonnet and D. Paul defences are scheduled in 2012. C. Sauvegarde joined the group in October to pursue the study.

Impact of embryonic sex on early embryo development depending on environmental conditions

The general objective is to evaluate the impact of the embryonic sex on embryo development, apoptosis and kinetics of development during the first week of development of the bovine embryo (up to the blastocyst stage). Techniques previously developed by E. Ghys were applied to embryos cultured in two different conditions in order to evaluate the interaction between the sex of the embryo and environmental factors. The results are under analysis and complementary studies are scheduled in 2012. A poster was presented at an international congress.

Setting up of a cryobank for the preservation of local livestock breeds in Belgium (co-promotor: Ph. Baret, ELI)

The main objective is to preserve genetic resources in livestock for future use and research. In 2011, the collection of semen from rams of three local Belgian breeds (Entre-Sambre-et-Meuse, Ardennais Roux et Tacheté and Mouton Laitier Belge) was carried on, a database was built and a scientific and
technical committee regrouping scientists and field actors was set up. A scientific paper was written and submitted for publication (Dumasy et al. Genetic diversity and exchange networks: a combined approach for the conservation of endangered sheep breeds). The project is part of a European network.

Research papers:


Master thesis:

**Bridoux Laure** (I. Donnay/R. Rezsohazy) : Rôle des gènes Hox dans la maturation ovocytaire et la transition embryo-maternelle

**Sauvegarde Caroline**: Développement précoce des embryons bovins mâles et femelles issus de sperme sexé in vitro : cinétique de développement et apoptose
Postdoctoral fellow: Sophie Remacle

PhD students: Isabelle Bergiers, Laure Bridoux, Stéphanie Delval, Miloud Nichane, Arnaud Taminiau, Pierre Deprez (co-supervision with Prof. B. Lengelé, IREC, UCL), Delphine Paul (co-supervision with Prof. I. Donnay, ISV, UCL), Delphine Sauvegarde (co-supervision with Prof. I. Donnay, ISV, UCL), Wendy Sonnet (co-supervision with Prof. I. Donnay, ISV, UCL), Pierre Galka (co-supervision with Prof. P. Soumilion, ISV, UCL).

Graduate students: Olivier Mitri, Lucrezia Rinaldi

Technicians: Nathan Nguyen, Julie Vandeputte; Secretary: Marie-Anne Mauclet. Staff (shared with Prof. I. Donnay and A. Moens): Philippe Bombaerts, Raphaël Chiarelli

Research activities:

*Hox* genes define a subset of the homebox gene family coding for homeodomain transcription factors critically involved in vertebrate embryogenesis. Aside of their roles in normal development, misregulation of *Hox* genes has been associated to oncogenesis.

While the functions of Hox proteins have been intensively investigated since their discovery about 30 years ago, their mode of action has only very recently begun to be investigated in detail. For a few years, the mode of action of two Hox proteins, Hoxa1 and Hoxa2, are under detailed investigation in our group. A functional analysis to identify the structural determinants for the activities of Hoxa1 has revealed several features. Hoxa1 shares a conserved DNA-binding homeodomain with all the Hox family members. However two N-terminal amino acid residues of the Hoxa1 homeodomain are atypical. *In vitro* and *in vivo* studies have shown that these residues importantly contribute to the functional specificity of Hoxa1 [Remacle et al, 2002 and; in preparation].

Most Hox proteins can interact with the Pbx transcription factors. This interaction relies on a conserved hexapeptide motif. For Hoxa1, we previously demonstrated that the activity of the protein critically relies on the integrity of its hexapeptide, suggesting an obligatory partnership between Hoxa1 and Pbx [Remacle et al., 2002, 2004]. Now, we have provided evidence that the partnership between Hoxa1 and Pbx also seems critical for the oncogenic activity of Hoxa1 [Delval et al., 2011].

A genome-wide high-throughput screening for proteins able to interact with Hoxa1 was successfully performed in collaboration with M. Vidal (Harvard, USA). Fifty-nine Hoxa1 interactors were revealed by a yeast two-hybrid assay, among which 45 were confirmed by co-precipitation from live mammalian cells. The intracellular distribution of 41 interactions was further determined by Bimolecular Fluorescence Complementation assay. Most interestingly, in addition to interactors known to be active in transcriptional regulation, several Hoxa1 interacting proteins are involved in cell signalling pathways [Lambert et al., submitted]. The functional consequences and biological relevance of the newly identified Hoxa1-mediated interactions are currently being addressed.
The mode of action of Hoxa2 was approached by searching and characterizing target genes and target enhancers. New direct Hoxa2 target genes have been identified [Miloud Nichane, in preparation]. These genes code for transcription factors or signalling molecules involved in patterning the hindbrain [Matis et al., 2007] or in the control of chondrogenic differentiation [Massip et al., 2007; Deprez et al., 2011; and in preparation]. A handful of responsive cis-regulatory elements were further identified in the vicinity of 4 targets and the regulatory interactions between Hoxa2 and partner transcription factors are under investigation in the context of these enhancers.

Recently, a genome-wide screen for Hoxa2 interactors has also been performed in collaboration with J.C. Twizere (agrobiopole of Gembloux, ULg). Like for Hoxa1, this screen revealed interactors involved in transcription and cell signalling. A first functional group of interactors has been confirmed by co-precipitation [I. Bergiers, submitted]. These interactors could be linked to cell-cycle progression, cell shape, cell migration and DNA damage response. The involvement of Hoxa2 in modulating proteins ubiquitination and cell behavior is currently under investigation.

Research papers:


Theses:


Master theses:

-Bridoux Laure (I. Donnay/R. Rezsohazy) : Rôle des gènes Hox dans la maturation ovocytaire et la transition embryo-maternelle

-Goossens Gérôme: Caractérisation de l’activité transcriptionnelle de sites cibles pour la fixation d’Hoxa2

-Hutlet Bertrand (F. Gofflot/R. Rezsohazy): Caractérisation de souris mutantes pour l’homéodomaine de Hoxa1

-Lamy Juliette: Etude de l’implication de l’interaction Hoxa1-PBX dans la carcinogenèse mammaire

-Benoît Lizen (F. Gofflot/R. Rezsohazy): Caractérisation du phénotype de souris portant une mutation pour les résidus 2 et 3 de l’homéodomaine Hoxa1
Arguably as important as inducing responses to a stress is stopping them when they are no longer required. This aspect of stress responses in plant, and signaling in general, has tended to be overlooked. Cellular homeostasis requires that every signaling process involving up- or down-regulation of a given pathway should only be transient, and returning to steady state after a signaling process is as vital to living cells as being able to perceive and transduce changes of their environment. One of the best studied responses of plant cells subjected to water-related stress is the transient increase of the phytohormone abscisic acid (ABA). The Arabidopsis TSPO (Translocator protein)-related (AtTSPO) is a membrane protein induced transiently by water-related stresses and ABA treatment. AtTSPO belongs to the tryptophan-rich sensory protein/peripheral-type benzodiazepine receptor (TspO/MBR) protein family, which are membrane-anchored proteins, found, with few exceptions, in organisms ranging from Archaea to metazoa. Since their identification in the late 70’s, TSPOs have been the subject of intensive research, almost exclusively in animal cells, to pinpoint their function.

We established an expression profile of AtTSPO using *in silico* analyses of transcriptome data and challenged this information with experimental data at the protein level, the rationale being that physiological conditions requiring expression of AtTSPO may be indicative of its biological role.

AtTSPO is a 196 aminoacids stress-induced, post-translationally regulated, early secretory pathway-localized plant cell membrane protein, and belongs to the TspO/MBR family of regulatory proteins, which can bind porphyrins, although the biological significance of this interaction is not yet clear. We showed that boosting tetrapyrrole biosynthesis enhanced AtTSPO degradation. We used hemin-agarose affinity chromatography, a pulldown assay, spectrophotometric analyses of copurified AtTSPO-ligand, and *in vivo* labeling with the fluorescent heme analog palladium mesoporphyrin IX and showed that AtTSPO could bind heme *in vitro* and *in vivo*. This binding required the histidine residue at position 91 (H91), but not that at position 115 (H115), both residues being relatively well conserved in plant TSPOs. In contrast to the H115A substitution, the H91A and double H91A/H115A substitutions stabilized AtTSPO and rendered the protein insensitive to heme-regulated degradation, suggesting that heme binding regulates AtTSPO degradation. AtTSPO degradation was inhibited in the autophagy-defective *atg5* mutant, and sensitive to inhibitors of type III phosphatidylinositol 3-kinases, which regulate autophagy in eukaryotic cells. The primary sequence of AtTSPO contains a
putative ubiquitin-like ATG8 interacting motif $^{121}$LYLYL$^{125}$ (consensus W/YxxL/V/I), and mutation of the two tyrosines in this motif did not affect heme binding \textit{in vitro}, but stabilized the protein \textit{in vivo}, suggesting that downregulation of AtTSPO requires an active autophagy pathway, in addition to heme. Consistent with this interpretation, fluorescent microscopy revealed that the autophagosome marker GFP-AtATG8e was partially colocalized with YFP-AtTSPO in plant cell. We also showed that overexpression of AtTSPO attenuated aminolevulinic acid-induced porphyria in plant cells. Taken together, these data support a role for AtTSPO in porphyrin binding and scavenging during stress in plants. Work is in progress to understand the structure-function relationship of this membrane protein in the plant cell under stress.

Research papers:


PhD theses:

- Damien Guillaumot: Functional characterization of the Arabidopsis thaliana Translocator-related protein AtTSPO
- Celine Vanhee: The ABA-regulated Arabidopsis TSPO is an ER/Golgi-localized heme binding membrane protein and a potential scavenger of porphyrins via an autophagy-dependent degradation mechanism

Master thesis:

- Jonathan Blondeau: Solubilization and purification At-TSPO heterologously expressed in \textit{Saccharomyces cerevisiae}
Postdoctoral fellows: Catherine Navarre, Antoine Champagne, Désirée Bienert

PhD students: Amandine Baijot, Aurélie Delimoy, Nicolas Jacquet, Raphaëlle Laterre, Bertrand Magy, Marta Niczyj, Dominik Piotrowiack, Julien Roland, Adrienne Sallets, Frédéric Toussaint.

Graduate student: Jérémie Tollet

Technicians: Sébastien Lievyns, Joseph Nader

Research activities 2011:

The research projects of this team are focused on two types of plant membrane primary transporters: H⁺-ATPases and Pleiotropic Drug Resistance transporters. The objective is to understand their physiological and biochemical properties. In this report, two examples of recent achievement will be summarized. As a side project, this team is involved in developing plant culture cells as an expression system for heterologous proteins.

1. Regulation of the plasma membrane H⁺-ATPase

The plant plasma membrane H⁺-ATPase builds a transmembrane pH and potential difference. This in turn activates a whole range of secondary transporters that move nutrients in and out of the cell, sometimes against huge concentration gradients. H⁺-ATPases are highly regulated at the protein level. In particular, this enzyme possesses a C-terminal auto-inhibitory domain which can be deactivated by phosphorylation of the penultimate residue, a Thr, and the subsequent binding of regulatory 14-3-3 proteins. By mass spectrometric analysis of plasma membrane H⁺-ATPase isoform 2 (PMA2) isolated from Nicotiana tabacum plants and suspension cells, we identified a new phosphorylation site, Thr-889, in a region of the C-terminal domain upstream of the 14-3-3 protein binding site. This residue was mutated into aspartate or alanine, and the mutated H⁺-ATPases expressed in the yeast Saccharomyces cerevisiae. Unlike wild-type PMA2, which could replace the yeast H⁺-ATPases, the PMA2-Thr889Ala mutant did not allow yeast growth, whereas the PMA2-Thr889Asp mutant resulted in improved growth and increased H⁺-ATPase activity despite reduced phosphorylation of the PMA2 penultimate residue and reduced 14-3-3 protein binding. To determine whether the regulation taking place at Thr-889 was independent of phosphorylation of the penultimate residue and 14-3-3 protein binding, we examined the effect of combining the PMA2-Thr889Asp mutation with mutations of other residues that impair phosphorylation of the penultimate residue and/or binding of 14-3-3 proteins. The results showed that in yeast, PMA2Thr889 phosphorylation could activate H⁺-ATPase if PMA2 was also phosphorylated at its penultimate residue. However, binding of 14-3-3 proteins was not required, although 14-3-3 binding resulted in further activation. These results were confirmed in N. tabacum suspension cells. These data define a new H⁺-ATPase activation mechanism that can take place without 14-3-3 proteins.

2. Role of NtPDR3 in the plant response to iron deficiency

Although iron is present in large amounts in the soil, its poor solubility requires the plant to use various strategies to facilitate iron uptake. We have found that expression of NtPDR3 (Pleiotropic drug resistance), a Nicotiana tabacum plasma membrane ABC transporter, is strongly induced in the root epidermis under iron deficiency conditions. Prevention of NtPDR3 expression resulted in N.
tabacum plants that were less tolerant to iron-deficient conditions, displaying stronger chlorosis and slower growth than wild-type when not supplied with iron. Addition to the medium of caffeic acid, a phenolic known to be secreted upon iron deficiency, restored growth of the NtPDR3-silenced plants to the same level as that of the wild-type. The homolog p-coumaric acid was not effective, indicating that the catechol function was essential. Expression of NtPDR3 in N. tabacum cells resulted in higher resistance to toxic concentrations of caffeic acid and lower internal concentration of caffeic acid. These results demonstrate that NtPDR3 plays an essential role in the plant response to iron deficiency by exporting a phenolic compound to the rhizosphere.

3. Improving expression of heterologous proteins in Nicotiana tabacum culture cells
The N. tabacum BY2 cell line is used by this team to express plasma membrane transporters and pharmacological proteins. Two transcription promoters have been engineered. The first one, En2NpPMA4, combines the promoter of NpPMA4, a constitutively expressed H⁺-ATPase gene, and two copies of the 3SD from the cauliflower mosaic virus. It allowed strong constitutive expression of the β-glucuronidase reporter, several PDR transporters as well as several antibodies. The second one, NtHSP3, is the promoter of a gene shown to be highly induced upon heath shock of N. tabacum BY2 cells. It allowed strong expression of the β-glucuronidase reporter as well as PDR transporters whose constitutive expression is toxic.

Research papers:


- Van Cutsem E., Simonart G., Degand H., Faber A.M., Morsomme P. and Boutry M. (2011) Gel-based and gel-free proteomic analysis of Nicotiana tabacum trichomes identifies proteins involved in secondary metabolism and in the (a)biotic stress response. Proteomics 11, 440-454


Thesis:

- Emmanuel Van Cutsem: Towards the identification of secondary metabolite transporters in glandular trichomes of Mentha x piperita and Nicotiana tabacum

Master thesis:

- Raphaëlle Laterre: Expression d’un anticorps de type IgG dans des cultures cellulaires d’Arabidopsis thaliana
Aquaporins

**Group of Molecular Physiology (FYMO)**

**Aquaporins**

**François Chaumont**

**Postdoctoral fellows:** Patrick Bienert, Arnaud Besserer, Charles Hachez, Linda Jeanguenin, Marie-Christine Flamand

**PhD students:** Robert Heinen, Hagen Reinhardt, Marie Berny, Adrien Chevalier

**Graduate students:** Emeline Burnotte, Delphine Crappe

**Technician:** Marie-Christine Eloy

**Staff shared within the FYMO research group:** Hervé Degand, Belkacem El Amraoui, Abdelmounain Errachid, Anne-Marie Faber, Monique Leloup, Danièle Masquelier, Régeane Mathieu, Michèle Rochat.

**Research activities 2011:**

Plant growth and development are dependent upon the tight regulation of water uptake and transport across cell membranes and tissues. Water can pass through cell membranes via aquaporins (AQP)

During 2011, we pursued our characterization of PIP trafficking to the plasma membrane. To determine the domains responsible for the ZmPIP1;2 ER retention and ZmPIP2;5 plasma membrane localization, chimeric proteins created by swapping the terminal regions, loops and transmembrane domains of ZmPIP2;5 and ZmPIP1;2 were generated. These mutated proteins were fused to the mYFP and/or mCFP, expressed in maize cells, and then localized by confocal microscopy. Interestingly, swapping the transmembrane 3 between ZmPIP1;2 and ZmPIP2;5 prevented the latter to reach the plasma membrane (Chevalier et al., in preparation).

SNARE proteins regulate membrane fusion and contribute to protein targeting and delivery in all eukaryotic cells. We investigated whether the regulation of the traffic and water channel activity of ZmPIP2;5 is controlled by SYP121, a syntaxin also involved in the regulation of K⁺ channels. Co-expression of ZmPIP2;5 and the dominant negative syntaxin ZmSYP121-Sp2 in maize protoplasts resulted in a marked decrease in the amount of ZmPIP2;5 in the plasma membrane. A combination of *in vitro* and *in vivo* assays showed that ZmSYP121 and ZmPIP2;5 directly interact. These data show that SYP121 regulates the trafficking and possibly the gating of PIP, and suggest that SYP121 could be a main regulator of the maintenance of overall cell osmotic homeostasis (Besserer and Chaumont, in preparation). Other PIP interactors have been identified by affinity chromatography and mass spectrometry, and the interactions are currently tested and characterized in Arabidopsis.

We identified a highly conserved cysteine residue in loop A of ZmPIP1 or ZmPIP2 proteins and demonstrated by mutagenesis that this cysteine is involved in the formation of a disulfide bond between two monomers (Bienert et al., submitted). In addition, modeling analysis allowed us to identify residues that might be important for ZmPIP2;5 at ZmPIP1;2 heterotetramer formation.
Finally, in the frame of the European DROPS project, we started to analyze the expression of PIP genes in leaf of maize lines diverging in drought tolerance.

Using microsatellites approaches, Marie-Christine Flamand studied the genetic phylogeny of trout (Salmo trutta) populations in the Walloon rivers, the genetic diversity of red deer and wild boar populations as well as the reproductive success of male red deer (funded by the Walloon Region).

Research papers:

- Dellicour, S., Frantz, A.C., Colyn, M., Bertouille, S., Chaumont, F. and Flamand, M.-C. (2011) Population structure and genetic diversity of red deer (Cervus elaphus) in forest fragments in north-western France. Conservation Genetics, 12, 1287-1297

Reviews:


Theses:

- Damien Cavez : Towards the elucidation of maize plasma membrane aquaporins interactions
- Urszula Miecielica : Phosphorylation of maize plasma membrane aquaporins modulates their channel activity and trafficking
- Robert Heinen : Expression and role of plasma membrane aquaporins in maize leaves

Master thesis:

- Fraiture Marie-Alice : Rôle des aquaporines dans l’élongation cellulaire et le développement d’Arabidopsis thaliana
Technicians: Hervé Degand, Belkacem El Amraoui, Abdelmounain Errachid, Anne-Marie Faber, Monique Le Loup, Danièle Masquelier, Régeane Mathieu, Michèle Rochat are shared with the other members of the FYMO group.

Research activities 2011:

Mutations in mitochondrial DNA are a frequent cause of genetic diseases in human. It has also been proposed that the accumulation of mitochondrial DNA mutations could play a role in ageing. The unique mitochondrial DNA polymerase, polymerase gamma (pol g), plays a key role in the fidelity of mtDNA replication through its selectivity towards the correct nucleotide and its 3’-5’ exonuclease proofreading activity. Recently, the three dimensional structure of human pol g has been determined.

Based on the good conservation between the catalytic subunits of yeast and human pol g, we have used Saccharomyces cerevisiae as a model organism to isolate and characterize pol g alleles that decrease the frequency of point mtDNA mutations (antimutators). We have designed a large scale in vivo screen involving the analysis of 3000 yeast transformants and finally identified 8 pol g amino acid substitutions scattered in the different domains of the polymerase. We have quantified the frequency of point mtDNA mutations and determined the propensity of these antimutators to lose their mitochondrial genome. We have purified wild-type and mutant pol g and characterized their biochemical properties. Based on the measurements of the polymerization processivity, exonuclease to polymerase activity ratios, and DNA binding affinity, we have found that the antimutators display a shift towards the excision of nucleotides compared to DNA synthesis, suggesting that the primer strand is preferentially localized at the exonuclease site, facilitating removal of the replicative errors. Based on the three dimensional structure of human pol g the antimutator mutations define a new area of the enzyme defined by residues in close proximity to the DNA binding channel and pointing their lateral chain at less than 5Å one from each other i) in the 3’-5’ exonuclease domain, near the Exoll site and in a module composed of two α-helices connected by a short loop; ii) in a long loop in the polymerase domain. Several of these residues are conserved in yeast, human and mouse, and thus our work could be used by others as a basis to generate mice harbouring the same substitutions as those obtained in yeast pol g. It would be interesting to determine the phenotypes of the genetically modified mice, in particular whether the mtDNA mutation rate is decreased and whether the ageing process is modified.

Research paper:


Theses:

-Karolina Szczepanowska, A cluster of pathogenic mutations in the 3’-5’ exonuclease domain of DNA polymerase gamma defines a novel module coupling DNA synthesis and degradation
PhD student: Nathalie Campagnolo

Staff shared within the FYMO research group: Hervé Degand, Belkacem El Amraoui, Abdelmounain Errachid, Anne-Marie Faber, Monique Leloup, Danièle Masquelier, Régeane Mathieu, Michèle Rochat.

Research activities 2011:

Functional characterization of a novel yeast metallopeptidase involved in sumoylation

Functional analysis of the aminocholesterol drug resistance protein Rta1 in yeast

Research papers:


Theses:

-Nathalie Campagnolo: Endoplasmic reticulum-associated degradation of mutated forms of yeast ABC transporter Pdr5
Intracellular protein and membrane transport

Postdoctoral fellow: Louis Gremillon

PhD students: Joanna Dodzian, Bérengère Guerriat, Aleksandra Szopinska, Didier Demaegd, Julien De Block

Graduate students: Jennifer Villers, Anne-Sophie Colinet, Bingyu Wang, Emilie Deffontaines, Antoine Deschamps

Staff shared within the FYMO research group: Hervé Degand, Belkacem El Amraoui, Abdelmounain Errachid, Anne-Marie Faber, Monique Leloup, Danièle Masquier, Régeane Mathieu, Michèle Rochat.

Research activities 2011:

Our research is focused on ion transporters and membrane protein trafficking in yeast.

In a first project, we are studying a yeast membrane protein the human ortholog of which is implicated in a genetic disease. The Congenital Disorders of Glycosylation (CDG) are a heterogeneous group of inborn errors affecting directly or indirectly the glycosylation pathway. Recently, mutations in a new human gene have been identified as a novel cause of CDG. This gene is highly conserved throughout evolution and orthologs of the protein can be found in many bacteria and virtually all eucaryotes, including the yeast Saccharomyces cerevisiae. The proteins encoded by these genes are predicted to possess 6 transmembranes spans, surrounding a central hydrophilic loop, and a signal peptide. Preliminary results show that the yeast protein is localized in the cis- and medial-Golgi apparatus and is involved in calcium tolerance. The calcium-sensitivity phenotype is highly enhanced when known calcium transporters are deleted together with our target gene. This genetic interaction may suggest a shared function in the transport and homeostasis of calcium in the Golgi apparatus. Therefore, we postulate that these proteins could be new Golgi-localized Ca\textsuperscript{2+} transporters. A defect in transport activity would perturb glycosylation and causes CDG in human.

In a second project, we developed a quantitative gel-free proteomic approach to monitor membrane proteins changes in biological membranes. We decided to focus on the yeast plasma membrane and to follow the rapid changes that could happen when yeast cells are exposed to a sudden change of the external medium. We used an optimized plasma membrane purification procedure and a quantitative gel-free proteomic approach based on iTRAQ (isobaric Tags for Relative and Absolute Quantitation) labeling to monitor changes in the plasma membrane proteome in cells exposed to salt stress or to a sudden change of nitrogen source in the medium. For instance, yeast cells have evolved the ability to grow on a wide variety of nitrogen sources that can be classified as rich (represented by ammonium) or poor (represented by proline). Rich nitrogen sources are easier to convert into glutamine and glutamate, which are the precursors of all the cellular nitrogen. Quantitative analysis revealed that 18 plasma membrane proteins were significantly less abundant after growth on ammonium than on proline, and 2 were more abundant on ammonium. In order to follow the
evolution over time of the plasma membrane proteome after ammonium induction, a ‘kinetic experiment’ was designed. Cells were subjected to a sudden change in nitrogen source (from proline to ammonium) and changes in protein abundance were measured after 15, 45 and 90 minutes. Our study confirmed the nitrogen-sensitive feature of a great number of plasma membrane transporters like Gap1p, Put4p, Dal5p, Mep2p, and allowed the identification of new targets of the nitrogen regulation. Interestingly, the kinetic experiment indicated that the internalization rate after ammonium induction varies among the proteins. Some proteins are internalized very rapidly while others much more slowly. This demonstrates that plasma membrane proteome is a highly dynamic system that rapidly reacts in response to different external stimuli.

Research papers:

-Van Cutsem E., Simonart G., Degand H., Faber A.M., Morsomme P. and Boutry M. (2011) Gel-based and gel-free proteomic analysis of Nicotiana tabacum trichomes identifies proteins involved in secondary metabolism and in the (a)biotic stress response, Proteomics, 11, 440-454

Theses:

-Thomas Deplanque: Molecular tools to study plant ER to Golgi transport

Master theses:

-Jennifer Villers: Study of yeast plasma membrane proteins dynamics by quantitative proteomic analysis
-Anne-Sophie Colinet: Identification d’acides aminés essentiels de la protéine de levure Gdt1p par mutagenèse aléatoire
-Bingyu Wang: Improvement of the purification strategy for a yeast membrane protein
-Emilie Deffontaines: Caractérisation de la protéine Sna4p de la levure Saccharomyces cerevisiae
Scientific platforms

The Proteomic/Mass Spectrometry facility platform was founded in 2009 as part of the Institut des Sciences de la Vie (ISV) and the de Duve Institute. The platform provides proteomics services principally through 2D-gel electrophoresis and mass spectrometry. Our tools are ESI-IT and MALDI-TOF-TOF mass spectrometers equipped for online and offline LC experiments. We specialize in the identification and quantification of soluble and membrane proteins from complex samples. We can also provide data on the location of post-translational modifications.

The experimental farm of the UCL (center A. de Marbaix) situated at Corroy-le-Grand is used for both research and teaching purposes. ISV users of the platform are Yvan Larondelle (feeding strategies of dairy cows aiming at lowering methane production and for a sustainable production of CLA-rich milk) and Isabelle Donnay (ram semen collection for the constitution of the Walloon cryobank; bovine embryo transient transfers in recipient cows in order to evaluate the quality of in vitro embryos produced in different conditions).

The "Marcel Huet" platform is concentrating its efforts on aquaculture research and training activities. A major part of the research conducted by ISV members deals with fish physiology and nutrition, as well as with the impact of toxic compounds on these animals. Members of the ELI institute are also using the facilities of the platform to study the genetic influence of reared Fario trouts introduced in the nature on the behaviour of the wild trouts in Belgium.

The “Greenhouses and Phytotrons” technology platform (SEFI) provides a logistic support for research and teaching to the scientists and academics belonging to the ISV and ELI institutes. This platform has been approved by the University authorities in 2012.

IMABIOL is an imaging platform dedicated mainly to biology and bringing together expertise and equipment from 3 institutes, namely ELI, IMCN and ISV. The platform offers state-of-the-art equipments and applications in confocal imaging and atomic force microscopy. Detailed and practical informations about this facility can be found at http://www.uclouvain.be/en-279076.html.
ISV postdoctoral fellowships

Dr Christelle André from October 2011, Promotor: Prof. Yvan Larondelle
PhD Students’ Day Program

ORAL PRESENTATIONS

Beguin Pauline, UCL (Y. Larondelle & Y.-J. Schneider, promoters)
Influence of inflammation on tight junctions and permeability of intestinal epithelium

Kuznetsova Oksana, UCL (B. Knoops, promoter)
Antioxidant status of astroglia and neurotoxicity in a rat model of amyotrophic lateral sclerosis: role of peroxiredoxins

Riquier Hélène, FUNDP
Comparison of X-ray and alpha particle irradiations on survival fraction and gene expression pattern in human lung adenocarcinoma A549 cells

Leclère Lionel, FUNDP
Hydrolysed citrus pectin induces an apoptotic-like cell death in HepG2 and A549 cells

Desguin Benoît, UCL (P. Hols & P. Soumillion, promoters)
Biochemical characterization of the lactate racemase of Lactobacillus plantarum: a new member of the nickel-enzyme family

Trabelsi Heykel, UCL (P. Soumillion, promoter)
Toward directed evolution of a DD-peptidase into a Beta-lactamase

Szopinska Aleksandra, UCL (P. Morsomme, promoter)
Quantitative analysis of the plasma membrane proteome of yeast cells exposed to salt stress

Thiry Céline, UCL (L. Pussemier & Y.-J. Schneider, promoters)
Selenium speciation and bioavailability in food

Lobet Guillaume, UCL (X. Draye, promoter)
New insights on the role of root hydraulic conductivities in the overall water uptake dynamics

Orman Beata, UCL (X. Draye, promoter)
Characterization of auxin-related genes involved in root development in barley (Hordeum vulgare)

Passo Claudine, UCL (Y. Larondelle & B. Delvaux, promoters)
Differentiation of banana varieties by some physicochemical parameters of their fruits

Baijot Amandine, UCL (M. Boutry, promoter)
Functional characterization and purification of plant PDR transporters expressed in culture cells
Piotrowiak Dominique, UCL (M. Boutry, promoter)

*Nicotiana plumbaginifolia* plasma membrane H+-ATPase is differentially regulated and increases plant size when expressed in *Arabidopsis*

Roland Julien, UCL (M. Boutry, promoter)

Identification of the substrates of pleiotropic drug resistance transporters expressed in plant

POSTERS

DEGHELT Michaël (FUNDP) The asymmetric distribution of the tubulin homologue FtsZ in the pathogen *Brucella abortus*

DEMAEGD Didier (UCL) Characterization of Gdt1p, a yeast protein involved in calcium homeostasis

DOUMIT Mary (UCL) Selection and characterisation of cyclic peptide inhibitors of the AcrAB-ToIC multidrug efflux pump

GARCIA Stéphanie (UCL) Selection of human aquaporins inhibitors within combinatorial libraries of backbone cyclic peptides biosynthesized in *Escherichia coli*.

GHYS Emmanuelle (UCL) Does the sex of the embryo affect apoptotic rates at the blastocyst stage?

LOUIS Caroline (UCL) An attempt to differentiate elephant seals progenitors into adipocytes

MAGY Bertrand (UCL) Production of biopharmaceuticals in plants

MALAISSE Jeremy (FUNDP) Involvement of hyaluronan in skin physiology

MICHEL Sébastien (FUNDP) Comparaison de la population mitochondriale entre des cellules cybrides sauvages et portant la mutation A8344G, associé au syndrome MERRF

NOTTE Annick (FUNDP) Role of autophagy in the hypoxia-induced resistance against taxol-induced apoptosis in MDA-MB-231 breast cancer cells

SALLETS Adrienne (UCL) Separation of long and short glandular trichomes from *Nicotiana tabacum* to unravel their respective secondary metabolism

VOROBIEVA Anastassia (UCL) Toxicity of TEM-1 beta-lactamase variants: possible mechanisms and consequences on bacterial fitness and protein evolution

WALBRECQ Geoffroy (UCL) Cell models and new probe to study the redox status in peroxisomes

WINAND Julie (UCL) Inflammation and obesity: coculture model
ISV Scientific Day

Jeudi 22 décembre 2011

Marc Boutry (FYMO) Plant drug smugglers

Michel Ghislain (FYMO) Hypoxia-induced resistance to antifungal drugs

Cathy Debier (BNTE) Adipose tissue and persistent organic pollutants: the "dangerous liaisons"

Patrick Dumont (CELL) Resveratrol and p53-induced apoptosis

Cell-cell signalling and mobility

Sébastien Rigali, Centre d'ingénierie des protéines, University of Liège Camel trekking for non motile bacteria

Claire Périlleux, Plant Physiology, University of Liège Signals that make plants flower

André M. GOFFINET, IONS/DENE, University of Louvain, Planar cell polarity signaling in neural development.

Séance d'éméritats et pensions

Allocution du Professeur Bernard Knoops, Président de l’ISV

Professeur Jean-Paul Declercq "La cristallographie des protéines, comment ça marche ?"

Professeur Claude Remacle "Du passé et du futur"
Seminars

21/01/2011 Iron uptake and homeostasis in *Pseudomonas* Dr Pierre CORNELIS (VUB)

28/01/2011 Regulation of symbiotic infection in actinorhizal symbioses Dr Laurent LAPLAZE (IRD, Dakar)

04/02/2011 Divide and Conquer? Hijacking cell cycle regulators for stomatal differentiation Dr Charles HACHEZ (UCL/FYMO)

18/02/2011 p27kip1 controls cortical interneuron migration by coordinating the actin and microtubule cytoskeletons Dr Laurent NGUYEN (GIGA - ULg)

25/02/2011 Unusual thiamine derivatives : clues to non-cofactor roles of thiamine in prokaryote and eukaryote cell function Dr Lucien BETTENDORFF (GIGA - ULg)

11/03/2011 DNA damage in adult stem cells : implications for cancer and ageing Dr Peggy SOTIROPOULOU (ULB)

18/03/2011 Molecular mechanisms underlying rapid evolution of living organisms Dr Kevin VERSTREPEN (KUL)

01/04/2011 Understanding DNA replication inside living cells Dr Rodrigo REYES-LAMOTHE (Oxford, UK)

08/04/2011 Transcriptional regulation of adipogenesis Dr Isabelle GERIN (UCL/ICP)

29/04/2011 Dr Guillaume PAVLOVIC (Strasbourg)

06/05/2011 Analysis of pancreatic cell differentiation in zebrafish Dr Bernard PEERS (GIGA - ULg)

27/05/2011 Role of endoplasmic reticulum in hepatic steatosis Dr Pascal FERRE (Paris)

14/09/2011 On the origins of maize: a story written in its genome Dr Jean-Philippe VIELLE CALAZDA (Mexique)

16/09/2011 Transcriptional regulation of human papillomavirus type 16 early proteins expression in cervical and trophoblastic cells Dr Véronique FONTAINE (ULB)

23/09/2011 "One health" research in Africa Dr Tanguy MARCOTTY (IMT, Anvers)

30/09/2011 Mechanisms of action of intrinsically disordered proteins: toxin-antitoxin modules as model systems Dr Remy LORIS (VIB)

07/10/2011 Single-molecule enzymology with biological nanopores Dr Giovanni MAGLIA (KUL)

14/10/2011 An apple a day? Evaluation of apple cultivars as a source of inflammation-modulating compounds in Inflammatory Bowel Diseases Dr Christelle ANDRE (ISV/BCNT)

21/10/2011 Transport of vitamins, potassium and water in plants: molecular bases and agronomic perspectives Dr Linda JEANGUENIN (Florida, USA, postdoc ISV)
04/11/2011 Peroxisomes and oxidative stress: facts and hypotheses Dr Marc FRANSEN (KUL)

18/11/2011 Interference of Theiler’s virus proteins with host defenses Dr Thomas MICHELS

25/11/2011 Cell cycle and adult neurogenesis: Where are we today Dr Brigitte MALGRANGE (GIGA, ULg)

02/12/2011 Genome hypomethylation and activation of germline-specific genes in cancer Dr Charles DE SMET (UCL/de Duve Institute)

09/12/2011 Aquaporin-5 and salivary glands Dr Christine DELPORTE (ULB)

16/12/2011 Investigations on allergic immune responses during asthma using mouse models and mould antigens Dr Olivier DENIS (ISP - Bruxelles)
PhD dissertations

14/12/2011 Nathalie DELVAL Etude du mode d'action du facteur de transcription Hoxa1 dans la carcinogenèse mammaire

02/12/2011 Celine VANHEE The ABA-regulated Arabidopsis TSPO is an ER/Golgi localized heme binding membrane protein and a potential scavenger of porphyrins via an autophagy-dependent degradation mechanism

14/11/2011 Robert HEINEN Expression and role of plasma membrane aquaporins in maize leaves

09/11/2011 Damien GUILLAUMOT Functional characterization of the Arabidopsis thaliana translocator-related protein (AtTSPO)

24/08/2011 Sébastien SART Controlled expansion and differentiation of mesenchymal stem cells in microcarrier-based stirred bioreactors

12/08/2011 Quynh Chau DANG VAN Influence of the diet structure and lipid supplementation on the bovine milk fatty acid composition

20/07/2011 Nathalie CAMPAGNOLO Endoplasmic reticulum-associated degradation of mutated forms of yeast ABC-transporter Pdr5

05/07/2011 Benjamin LEMAIRE Etude de la toxicité des micropolluants organochlorés chez le poisson de profondeur Coryphaenoides rupestris : utilisation des tranches de précision de foie

22/06/2011 Julie GOEMAERE Peroxiredoxin expression in the central nervous system and oxidative modifications: potential role in neurodegeneration

20/05/2011 Laurence RIBONNET Evaluation of the interactions of herbal dietary supplements with molecular targets at the intestinal level: a first step toward risk assessment

11/05/2011 Jean-François MICHIELS Investigation of the role of peptones in animal cell culture: case study of a commercial soy peptone in a CHO cell serum-free medium

27/04/2011 Urszula MIECIELICA Phosphorylation of maize plasma membrane aquaporins modulates their channel activity and trafficking

20/04/2011 Thomas DEPLANQUE The plant early secretory pathway: molecular tools to study endoplasmic reticulum to Golgi apparatus transport

01/04/2011 Emilien NICOLAS Biochemical activities of the transposon TnpA in transposition and target immunity mediated by the replicative transposon Tn4430

31/03/2011 Karolina SCSZEPANOWSKA A cluster of pathogenic mutations in the 3'-5' exonuclease domain of DNA polymerase defines a novel module coupling DNA synthesis and degradation

24/03/2011 Emmanuel VAN CUTSEM Towards the identification of secondary metabolite transporters in glandular trichomes of Mentha x piperita and Nicotina tabacum

15/03/2011 Damien CAVEZ Towards the elucidation of maize plasma membrane aquaporin interactions