JOURNÉES CAMPUS D'ILLKIRCH 2008

24 ET 25 AVRIL

RÉSUMÉS DES AFFICHES

Poster number : **1**

Micro-RNA-346 regulates IL-18 release by LPS-activated fibroblast-like synoviocytes in rheumatoid arthritis.

[1]Alsaleh Ghada,, [1]Tom Juncker, Wachsmann, [1]Dominique, Laboratoire Physiopathologie des Arthrites, EA3432, Université Louis Pasteur, Strasbourg, [2]Sebastien Pfeffer, IBMP, CNRS -2357, Strasbourg. [3]Jacques-Eric Gottenberg, Rhumatologie INSERM U802, Hôpital Bicêtre, Université Paris-Sud 11, Le Kremlin Bicêtre, [4]Jean Sibilia, Département de Rhumatologie, Hôpitaux Universitaires de Strasbourg, Strasbourg.

Background: We reported previously that LPS induced IL-18 mRNA expression in synoviocytes fibroblast-like (FLS) isolated from rheumatoid arthritis (RA) patients, but mature IL-18 was not released by activated cells. Furthermore, our studies revealed that the lack of IL-18 release by FLS was due to a defect of translation of mRNA into pro IL-18 because of rapid degradation of IL-18 mRNA. MiRNAs have emerged as key players in the regulation of translation and degradation of target mRNAs. They have been associated with diverse biological processes such as cell differentiation, cancer, viral infection. Recent studies have demonstrated that miRNAs play a role in innate immunity

Objectives: Based on these observations, our group has carried out a study to identify miRNAs that could play a role in the "anti-inflammatory" response of LPS-activated RA FLS

Methods: To analyze the expression profile of miRNAs after activation of RA FLS by LPS, a miRNA microarray analysis was performed using the mirAnalyser microarray which contains human miRNA oligonucleotides from the Sanger miRbase. Results were validated by Real-time quantitative PCR and Northern Blot. FLS were transfected with the Clear-miR^{TM} miRNA inhibitors which consist of sequences of 21 nucleotides, using the Human Dermal Fibroblast Nucleofactor^{TM} kit from Amaxa. IL-18 was evaluated in cell supernatants by ELISA.

Results: We used a DNA microarray containing 409 oligonucleotides probes complementary to mature forms of miRNAs of human origin. After LPS challenge, this initial screening identified 63 miRNAs, the expression levels of which were increased or attenuated in response to LPS. When a cut-off of a 2-fold change in miRNA expression was used, 14 miRNAs were up-regulated. Among them, two miRNAs targeting IL-18 mRNA showed a strong expression level : miR-346 (>7times) and miR-32 (>5times) whereas the expression of 2 miRNAs targeting IL-18 mRNA was attenuated (miR-299 and miR-222). These results were validated by qPCR and Northern blot analysis. To investigate the hypothesis that miR-346 and miR-32 may play a role in regulating the release of IL-18 in LPS-activated RA FLS, we performed transfection experiments of inhibitors targeting miR-346 and miR-32 in RA FLS. Supernatants were tested for level of IL-18 using ELISA. Treatment with LPS significantly induced IL-18 release by activated RA FLS, transfected with antisense molecules targeting miR-346 as compared to activated FLS transfected with non targeting antisense molecules. Transfection with inhibitors of miR-32 did not induce IL-18 release by transfected and activated FLS. These results indicate that tmiR-346 is implicated in the negative regulation of IL-18 secretion by LPS-activated FLS.

Poster number : **2**

THE TRANSCRIPTION FACTOR KLF6 is REGULATED by a COMPLEX NETWORK OF BIOCHEMICAL PATHWAYS INVOLVING JNKs, p38s and GSK-3 ACTIVITIES

Andreoli V., Chatton B. and Bocco J.L.

Departamento de Bioquímica Clínica. CIBICI – CONICET. Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina. Université Strasbourg I, Institut Gilbert Laustriat, ESBS, UMR7175 CNRS-ULP, Bd S. Brandt, 67412 Illkirch, France. E-mail: vandreoli@fcq.unc.edu.ar.

The transcription factor KLF6 is a potential tumor suppressor gene product whose expression level responds to external cell stimulation mediated by growth factors, tumor promoters and DNA-damage agents. KLF6 interacts with the c-Jun proto-oncoprotein and induces its degradation leading to inhibition of cell proliferation. The JNK family of MAP kinases is the main regulator of c-Jun, contributing to its enhanced transcriptional activity and protein stability. In addition, p38 kinases are also involved in the regulation of c-Jun activity through phosphorylation of AP-1 members, including c-Jun itself. The cytoplasmic levels of KLF6 in *jnk*+/+ cells are reduced upon ectopic expression of JNK1 or JNK2. Accordingly, the KLF6 protein levels are markedly enhanced in *jnk* -/- cells though expression of JNK1 and in a lesser extent JNK2 severely diminish the level of KLF6. Moreover, JNK2 activity is associated to the translocation of KLF6 from the cytoplasm to the nucleus and activated p38 isoforms (α, β_2, γ and δ) also decrease KLF6 protein levels in the absence of JNK and ERK activities. KLF6 is constitutively phosphorylated *in vivo* in *jnk*-/- serum starved cells treated with p38 inhibitor, correlating with high protein stability. A phosphorylation consensus sequence for GSK-3 kinase is found next to an ERK/JNK target site within the amino acid sequence of KLF6. Therefore KLF6 stability could be subjected to a phosphorylation mediated degradation mechanism similar to the one operating for the regulation of c-Jun and c-Myc stability by ERK and GSK-3 kinases. Thus, treatment of cells with a GSK-3 inhibitor (LiCl) leads to an increased stability for KLF6 after UV-radiation or specific activation of JNK and p38. Our results clearly show that JNK and p38 pathways are mainly involved in the regulation of KLF6 protein stability and KLF6 subcellular localization. The GSK-3 kinase plays a role in the regulation of KLF6 degradation, probably through a priming phosphorylation mechanism. Increased activity of JNK and p38 diminished KLF6 protein levels allowing high stability and activation of c-Jun. JNK2 substantially increases KLF6 targeting to the nucleus indicating that activated c-Jun and KLF6 activities are mutually exclusive within the nucleus, correlating with enhanced or reduced cell proliferation rate, respectively.

Red wine polyphenols and green tea polyphenols induce NO- and EDHF-mediated endothelium-dependent relaxations through the activation of AMP-activated protein kinase

Modou Oumy Kane, Eric Anselm, Stéphanie Dal, Christian Bronner and Valérie B. Schini-Kerth
UMR CNRS 7175, Département de Pharmacologie et Physiopathologie Cardiovasculaires, Faculté de Pharmacie, Illkirch, France

Several epidemiological studies have shown that regular consumption of moderate amounts of red wine is associated with a decreased total mortality due, in part, to a reduced risk of cardiovascular diseases. The protective effect has been attributable to polyphenols. Polyphenols have been shown to induce pronounced endothelium-dependent relaxations of arteries by causing the formation of nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF). The aim of the present study was to determine the role of the stress-activated AMP-activated protein kinase (AMPK) in the red wine polyphenols (RWPs) and in green tea polyphenols-induced endothelial formation of NO and EDHF.

Vascular reactivity was assessed in organ chambers. Cultured porcine coronary artery endothelial cells were used to study the phosphorylation level of endothelial NO synthase (eNOS) at serine 1177, and AMPK at the threonine 172 by Western blot analysis. RWPs contained 2.9 g/L polyphenols expressed as gallic acid equivalents.

RWPs caused endothelium-dependent relaxations in rings from rat aorta and mesenteric artery, and in those from porcine coronary artery. NO-mediated relaxations to RWPs in aortic rings, and in coronary artery rings, as assessed in the presence of indomethacin and charybdotoxin plus apamin, were inhibited by compound C (an inhibitor of AMPK). Compound C also reduced EDHF-mediated relaxations, as assessed in the presence of indomethacin and N^{ω}-nitro L-arginine, in mesenteric artery and coronary artery rings. In contrast, compound C did not affect endothelium-dependent relaxations to acetylcholine and those to sodium nitroprusside neither in aortic rings nor in mesenteric artery rings. Compound C also inhibited the NO-mediated endothelium-dependent relaxations induced by green tea polyphenols in coronary artery rings. In addition, green tea polyphenols and to a lesser extent RWPs induced the phosphorylation of AMPK at threonine 172 in endothelial cells within 10 minutes.

The present findings indicate that RWPs and green tea polyphenols cause both NO and EDHF-mediated relaxations in several types of isolated arteries. They further indicate that activation of the AMP-activated protein kinase pathway is involved in the signaling pathway leading to the endothelial formation of both NO and EDHF in response to polyphenols.

Poster number : **4**

Development and optimization of alphaviruses for high-level expression of functional membrane proteins.

Benmaamar R.[1, 2], Wagner R.[1]

[1] Dpt Récepteurs et Protéines Membranaires, CNRS-UMR7175, Illkirch - France
[2] Bio-Xtal, Chemin des Croisettes 22, CH-1006, Epalinges - Switzerland

The Semliki Forest Virus, SFV, system has been gaining interest over the past few decades due to its efficiency in heterologous protein expression, and notably the high-level expression of integral functional membrane proteins and receptors (1). The genes encoding the viral capsid and spike (C-p62-k-E1) polyprotein are replaced by the foreign gene, that is placed under the control of the strong subgenomic 26S viral promotor, whereas structural proteins are expressed in *trans* from a helper RNA. This double system allows only recombinant RNAs to be packaged and the generated virus is non-replicative (2). Although these features greatly enhance biosafety handling of the recombinant virus, one of the major side effects is that precise viral titration is not possible, making standardization of viral preparations for large-scale protein expression difficult to achieve.

Based on this, SFV-specific primers were designed and optimized such that viral titration could be accomplished by quantitative real-time RT-PCR (qRT-PCR). To quantify the number of copies of the viral genome, qPCR curves of recombinant SFV particles were compared to a standard RNA curve. Flow cytometry was used for standard titration of infectious particles on BHK-21 cells using a specific FITC-conjugated antibody as a marker. Preliminary data show that there is approximately a 100-fold factor between qRT-PCR and conventional immunofluorescence quantification. This may be due to non-infectious particles generated during viral preparations, or to multiple infection of a single cell.

An alternative recombinant protein expression tool would be to generate replicative, yet non-infectious to humans, alphaviral particles. A number of closely related alphaviruses were carefully studied and although one highly potential candidate has been successfully engineered, protein expression in this alternative system has yet to be optimized.

(1) Hassaine G, Wagner R, Kempf J, Cherouati N, Hassaine N, Prual C, André N, Reinhart C, Pattus F, Lundstrom K., Semliki Forest virus vectors for overexpression of 101 G protein-coupled receptors in mammalian host cells. *Protein Expr Purif*. 2006 Feb; 45(2):343-51.

(2) Berglund P, Sjöberg M, Garoff H, Atkins GJ, Sheahan BJ, Liljeström P. Semliki Forest virus expression system: production of conditionally infectious recombinant particles. *Biotechnology (NY)*. 1993 Aug; 11(8):916-20

Poster number : **5**

Normal and pathological positioning of nuclei in muscle fibres: an *in vivo* study

Johann Böhm[1], Marc Koch[2], Yves Lutz[2], Lama Al-Qusairi[1], Jean-Louis Mandel[1], Jocelyn Laporte[1]

Centronuclear myopathies (CNM) are a group of congenital myopathies where cell nuclei are abnormally located in skeletal muscle. In CNM nuclei are located in the center of the fiber instead of their normal position at the periphery. Generally, the position of the nucleus determines various cellular activities, but the mechanisms and the molecular pathways that regulate the nuclear movement and position events are not well understood. We will therefore analyze the general cellular organization of muscle in WT and CNM mice in order to identify proteins implicated in the aberrant nuclear positioning, and other organelles that might be abnormally located. The recently developed MacroFluo is a confocal macroscope that allows the structural analysis of muscle fibers in the living mouse. The results obtained by this new technology will help to understand how mutations in different genes that are associated with centronuclear myopathies are involved in nuclear positioning.

[1]Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC)
[2]Institut de la Clinique de la Souris (ICS)

Poster number : **6**

Calmodulin's interactors : tools for studying calcium signal

Rania DAGHER, Dominique BONNET, Pascal VILLA, Didier ROGNAN, Marcel HIBERT, Marie-Claude KILHOFFER, Claire PIGAULT and Jacques HAIECH
Faculté de Pharmacie, UMR 7175-LC1, Équipe Chimiogénomique et Pharmacogénomique
74, route du Rhin, 67401 ILLKIRCH, FRANCE

Calmodulin (CaM) is the primary Ca^{2+}-sensor in all eukaryotic cells that interprets the calcium signal, modulates the activities of many target proteins and affects cellular metabolism in response to extracellular signals. It's a prototypic protein of induced fit compared to membrane receptor that exhibits multiple conformational states for its target recognition.
There is, however, limited understanding of how ligand-multisite protein interactions selectivity regulate the activities of multiple protein targets.

Our aim is to provide CaM's small interacting well characterized molecules that can be used as research tools for dissecting CaM-protein interactions and studying calcium signal.
Along this way, we have developed a screening assay for soluble proteins by using fluorescence polarization (FP), a simple and direct tool to study association equilibrium when free- and bound-implicated species have different rotational diffusions.

The FP-screening of a fluorescent chemical library (1328 compounds) with CaM permits us to select four fluorescent non-selective probes. Their binding to CaM, as function of Ca^{2+} concentration, has been characterized by FP-titration with electrostatic mutants. Our experiments demonstrate that full probe binding occurs at molar ratio of two Ca^{2+} per CaM.

These probes were then used in FP-competitive screening assay to detect small specific molecules from the Strasbourg Academic Library (5920 compounds). Binding to CaM of these small molecules was confirmed by FP-displacement titration curves that show a substantially different displacement curve profiles and suggest a differential binding classes of molecules with a given CaM conformations.

This differential binding of hits to CaM correlates with hits docking to known crystallographic structures of CaM interactors complexes. This illustrates a conformational CaM plasticity in its target binding. Our results may lead to differential disruption of the interaction between CaM and target enzymes using different small molecules, therefore providing research tools in deciphering Ca^{2+}/CaM signaling.

Portal hypertension in rats is associated with blunted EDHF-mediated relaxations in the mesenteric artery: role of ROS and activation of AT1 receptor

S. Ros-Dal[1], M. Oswald[2], T. Pestrikova[2], C. Schott[1], C. Bronner[1], T.Chataigneau[1], B. Geny[2] and V.B. Schini-Kerth[1]

[1]Institut Gilbert-Laustriat, UMR7175 CNRS/ Université Louis Pasteur (Strasbourg I), Dépt de Pharmacologie et Physicochimie, Faculté de Pharmacie, Illkirch, France. [2]Service de Physiologie et d'Explorations Fonctionnelles et EA 3072, Hôpitaux Universitaires de Strasbourg, France.

Introduction: Portal hypertension, a common clinical syndrome associated with chronic liver diseases such as cirrhosis, is characterized by an impaired endothelium-dependent relaxation in the liver microcirculation leading to increased hepatic vascular resistance. This study examined whether the endothelial formation of nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF) is altered in an experimental model of biliary cirrhosis with portal hypertension obtained by the ligation of the common bile duct (CBDL) in rats, and if so to determine the underlying mechanism.

Methods: Reactivity of isolated mesenteric arteries from male Wistar rats was measured in organ chambers. The expression level of connexins (Cx37, Cx40, Cx43), IK_{Ca}, SK_{Ca}, eNOS, HIF-2α, NADPH oxidase subunits and nitrotyrosines level were assessed by immunohistochemistry. The vascular formation of reactive oxygen species (ROS) was evaluated with dihydroethidine. In addition, CBDL-rats and sham-rats were treated one week before and 4 weeks after surgery with apocynin (100 mg/kg/d) or losartan (10 mg/kg/d).

Results: Decreased EDHF-mediated relaxations to acetylcholine and red wine polyphenols were observed in CBDL-rats whereas the global and the NO-mediated relaxations were not affected. Impaired EDHF-mediated responses were associated with a reduced vascular expression of Cx37, Cx40, Cx43 and IK_{Ca}, SK_{Ca}, an increased expression of eNOS, HIF-2α and NADPH oxidase subunits, vascular formation of ROS and peroxynitrites. No such effects were observed in CBDL-rats treated with either apocynin (an inhibitor of NADPH oxidase) or losartan (an inhibitor of AT1 receptor).

Conclusion: These findings indicate that CBDL in rats is associated with blunted EDHF-mediated relaxations in mesenteric arteries possibly due to a reduced expression of several connexins and potassium channels. This effect involves an excessive vascular oxidative stress most likely following activation of the AT1 receptor.

Poster number : **8**

Post-translational modifications and alternative splicing modulate ATF7 transcriptional activity

Jessica Diring, Barbara Camuzeaux, Mariel Donzeau, Claude Kedinger, Bruno Chatton

Université Strasbourg I, Institut Gilbert Laustriat CNRS-UMR 7175, Ecole Supérieure de Biotechnologie de Strasbourg, Bd Sebastien Brandt, 67412 Illkirch, France

ATF7 (formerly ATFa) is a ubiquitously expressed leucine-zipper containing transcription factor, initially characterized by its ability to bind ATF/CRE sequences (TGACGTCA) of different adenoviral early promoters. While ATF7 homodimers exhibit weak transcriptional activity, it modulates the activity and DNA-binding specificity of Jun, Fos or related transcription factors, through heterodimerization with these proteins. The transcriptional activity of ATF7 is mediated via interactions with TAF12, the heterodimerization partner of TAF4 in the general transcription factor TFIID. In contrast, TAF4 associates with ATF7 and inhibits its activity. To gain further insight into the molecular mechanisms of ATF7-mediated activation, we have investigated the contribution of post-translational modifications in this process. We found that human ATF7 is SUMOylated, both *in vitro* and *in vivo*. This modification involves covalent attachment of SUMO-1 (Small Ubiquitin-like Protein Modifier) protein to a lysine residue within a (V,I,L)KxE consensus site in ATF7 activation domain. This event has been shown to delay ATF7 nuclear translocation and to inhibit its transcriptional activity by impairing its interaction with TAF12. We have also shown that ATF7 is phosphorylated *in vivo*, under stimulation of p38 MAP kinase pathway. Ongoing experiments are aimed at elucidating the functional role of these two post-translational modifications in ATF7 transcriptional activity.

A novel alternatively spliced ATF7 isoform has recently been characterized. This protein (ATF7-4) shares with full length ATF7 the N-terminal activation domain but lacks the C-terminal basic-region/leucine-zipper domain and therefore does not bind to DNA and is localized within the cytoplasm. Our observations suggest that ATF7-4 may act as a dominant negative form, as it inhibits ATF7 transcriptional activity on reporter promoters. A functional characterization of ATF7-4 will allow us to elucidate the molecular mechanisms involved in ATF7 and other potential transcription factors regulation.

Molecular Motors inactivation using FALI

Emmanuelle Briant, Jan De Mey, Denis Dujardin
Institut Gilbert Laustriat, ESBS, UMR 7175, Parc d'Innovation, Boulevard Sébastien Brandt, BP 10413, 67412 ILLKIRCH Cedex, France

Cytoplasmic dynein is a motor complex implicated in a huge variety of transport along microtubules, including spindle orientation, chromosome segregation, cell migration, and also organelle transport and positioning during interphase. Dynein has been involved in the lissencephaly syndrome, which can be caused by mutations in the Lis1 cofactor of dynein. This syndrome is characterized by a defective development of the cerebral cortex leading to severe mental retardation, and early lethality. Dynein also interacts with the protein EB1 and the tumour suppressor APC which gene is often mutated in colon cancers. Since dynein, EB1, and APC are proposed to be key players of spindle orientation mechanisms, our lab is currently studying how they mediate mitotic spindle orientation, and how failures in this mechanism could contribute to pathologies. However, the multiplicity of functions of spindle orientation factors are complicating greatly the interpretation of the phenotypes obtained after their inactivation by standard methods (SiRNA, antibody injection …), and so far, it prevented us to progress in the dissection of spindle orientation mechanisms. In order to solve these specificity issues and progress in the understanding of the molecular functioning of dynein at different sub-cellular structures, we are implementing the micro-FALI technology (Fluorophore Assisted Light Inactivation) in our lab. It should allow specifically and locally inactivating a subunit of the complex at a specific site. It consists in using proteins tagged with specific fluorophores. Their illumination leads to free radical production denaturizing specifically the grafted protein only at the level of the illuminated structure.

In the present work, we tried two different approaches for FALI, using either proteins of interest tagged with a tetracystein motif that recognizes FALI compatible cell permeable ligands (FlAsH/ReAsH), or intrabodies tagged with the KillerRed fluorescent protein optimized for FALI. Non specific toxic effects could be observed using the FlAsH/ReAsH reagents; however we managed to find conditions to block Golgi vesicles transport along microtubule using FALI targeting cofactors of dynein tagged wit ReAsH. Moreover, we managed to block cytokinesis using an intrabody coupled to KillerRed recognizing Myosin II, an actin based molecular motor also involved in spindle orientation. Our results provide promising evidence that we can block molecular motor functions during mitosis using FALI.

Poster number :**10**

Zinc dependence of the HIV-1 Tat protein interaction with tubulin

Caroline Egelé[1;2], Pascale Barbier[2], Sylviane Muller[3], Vincent Peyrot[2] & Yves Mély[1]
[1]UMR 7175 - LC1, ULP, Faculté de Pharmacie, Illkirch, France
[2]CRO2, Inserm U911, Faculté de Pharmacie, Marseille, France
[3]UPR 9021 CNRS, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France

Depletion of CD4+ T-cells is the hallmark of HIV infection and AIDS progression. In addition to the direct killing of the viral-infected cells, HIV infection also leads to increased apoptosis of uninfected bystander cells. This is mediated in part by the HIV-1 Tat protein, which is secreted by the infected cells and taken up by uninfected cells. Tat is a 86-amino acids protein that binds two Zn^{2+} through its cysteine-rich region. Zinc binding folds the cysteine-rich region [1, 2], and is thought to play a role in Tat-mediated apoptosis [3]. Since one mechanism of Tat-mediated apoptosis involves microtubules [4], the aim of this study was to compare the interaction of apo-Tat and Tat-Zinc with tubulin [5]. To this end, we first characterized the effect of zinc on Tat conformation by fluorescence correlation spectroscopy (FCS) and time-resolved anisotropy. Both apo- and holo-forms were found to be monomeric and poorly folded. However, the two forms differed by local conformational changes in the vicinity of the cysteine-rich region that likely affect the tubulin binding sequence in the Tat core. Then, we characterized the interaction of the two Tat forms with tubulin dimers and microtubules by analytical ultracentrifugation, turbidity measurements and electron microscopy. Both Tat forms bound to tubulin dimers at 20°C, but only Tat-Zn formed discrete complexes with the dimers, while apo-Tat induced a large amount of tubulin aggregates. At 37°C, both forms of Tat promoted the nucleation and increased the elongation rates of tubulin assembly. However, only Tat-Zn increased the amount of microtubules, decreased the tubulin critical concentration, and stabilized the microtubules. Our data suggest that Tat-Zn does correspond to the active Tat form, responsible in the cytoplasm for the Tat-mediated apoptosis pathway.

Bibliography

1. Frankel, A.D., D.S. Bredt, and C.O. Pabo, *Tat protein from human immunodeficiency virus forms a metal-linked dimer*. Science, 1988. **240**(4848): p. 70-3.
2. Huang, H.W. and K.T. Wang, *Structural characterization of the metal binding site in the cysteine-rich region of HIV-1 Tat protein*. Biochem Biophys Res Commun, 1996. **227**(2): p. 615-21.
3. Misumi, S., N. Takamune, Y. Ohtsubo, K. Waniguchi, and S. Shoji, *Zn2+ binding to cysteine-rich domain of extracellular human immunodeficiency virus type 1 Tat protein is associated with Tat protein-induced apoptosis*. AIDS Res Hum Retroviruses, 2004. **20**(3): p. 297-304.
4. Chen, D., M. Wang, S. Zhou, and Q. Zhou, *HIV-1 Tat targets microtubules to induce apoptosis, a process promoted by the pro-apoptotic Bcl-2 relative Bim*. Embo J, 2002. **21**(24): p. 6801-10.
5. Egele, C., P. Barbier, P. Didier, E. Piemont, D. Allegro, O. Chaloin, S. Muller, V. Peyrot, and Y. Mely, *Modulation of microtubule assembly by the HIV-1 Tat protein is strongly dependent on zinc binding to Tat*. Submitted.

Poster number :**11**

Gliomas and EGFR: Search for New Pharmacological / Therapeutical Tools Interfering with EGFR Ligand Induced Signalling Pathways (poster biology)

Fève M, Zeniou-Meyer M, Bernhard S, Junier* M-P, Haiech J, Kilhoffer M-C.
UMR 7175 LC1 Equipe Chimiogénomique et Pharmacogénomique, Faculté de Pharmacie, 74 route du Rhin 67401 ILLKIRCH
*INSERM U752, Département de Pathologie, Hôpital Sainte-Anne, Paris.

Malignant high-grade astrocytomas (glioblastomas) represent the third most common malignant tumor type in children. These tumors are histologically heterogeneous and have a poor outcome. Among the signalling pathways deregulated in glioblastomas, the EGFR (Epidermal Growth Factor Receptor) signalling module seems to have a major contribution. Indeed, aberrant EGFR activation promotes adult malignant glioma proliferation, survival and migration. In addition, EGFR overexpression is observed in 80% of childhood gliomas. Therefore, our hypothesis is that molecules which inhibit the binding of EGFR ligands (EGF, TNF-α) to their receptor, thus preventing EGFR signalling, are good candidates for treatment of brain tumors. So far, antibodies directed against the EGFR ligands or the EGFR ligand binding extracellular domain are the only tools available to block ligand binding to EGFR. However, these antibodies present major drawbacks since they diffuse very poorly through the blood-brain barrier. Our goal is to overcome this limitation by identifying small chemical compounds more likely to diffuse through the blood-brain barrier and able to prevent ligand binding to EGFR, EGFR dimerization, or receptor tyrosine kinase activity. To do so, we will screen small compounds chemical libraries using two major technologies: fluorescence anisotropy and FRET.

Bredel, M., Pollack, I.F., Hamilton, R.L. & James, C.D. Epidermal growth factor receptor expression and gene amplification in high grade non brain-stem gliomas of childhood. *Clin Cancer Res* 5, 1786-92 (1999).

Riese II, D.J., Gallo, R.M., Settleman, J. Mutational activation of ErbB family receptor tyrosine kinases: insights into mechanisms of signal transduction and tumorigenesis. *BioEssays* 29, 558–565 (2007).

Poster number :**12**

Interaction between E6 oncoprotein of human papillomavirus (HPV) and the PDZ 1 domain of MAGI-1 by Biacore approach

Sadek Fournane[1], Sebastian Charbonnier[1], Anne Chapelle[1], Danièle Altschuh[2], Georges Orfanoudakis[1], Gilles Travé[1], Murielle Masson[1], Yves Nominé[1]

1, UMR 7175, Equipe Oncoprotéines, ESBS, Bld. Sebastien Brant, BP 10413, F-67412 Illkirch

2, UMR 7175, Equipe Biocapteurs, ESBS, Bld. Sebastien Brant, BP 10413, F-67412 Illkirch

Human papillomaviruses (HPVs) are small DNA viruses. Infection of mucosal epithelium with high-risk HPVs (HPV16 is the most prevalent) is associated with the development of cervical cancer. The oncogenic potential of high-risk HPVs is mediated by two viral oncoproteins, E6 and E7. HPV E6 is a multifunctional protein that targets for degradation several cellular proteins, including the tumor suppressor protein p53 and PDZ-containing proteins. PDZ (PSD-95, Dlg, ZO-1) domains, comprising around 90 amino-acids, are a large family of protein modular domains and occur more than 450 times in human genome. PDZ domains mediate protein-protein interaction by binding to C-terminus extremity of target proteins.

E6 oncoprotein from high-risk mucosal HPVs presents at its C-terminus extremity a PDZ-binding motif allowing the interaction with some PDZ-containing proteins involved in cell proliferation, polarity or adhesion as hDlg, hScribble, MAGI-1, MUPP1. This interaction is required for HPV-induced carcinogenesis.

The aim of this study is to determine the structural requirements for the E6/PDZ interaction. For this purpose, we expressed MAGI-1 PDZ 1 domain as His-MBP-PDZ fusion protein in E.coli and purified it. The E6Cter peptides were expressed as His-GST-PDZ and purified. We used Biacore approach to determine the affinity of E6/PDZ interaction. We found that the affinity is 2.8 μM for HPV16 E6Cter peptide. Then, a site-directed mutagenesis of PDZ 1 domain and the C-terminus peptide of HPV16 E6 (SRSSRTRRETQL-COOH) has been performed in order to identify residues conferring the specificity of interaction. We shed light on the residues of the E6 peptide which are important for PDZ recognition. In addition, we found potential residues within the PDZ domain required for E6 binding specificity. This study give us new structural insights for the specificity of E6/MAGI-1 PDZ 1 interaction. All these informations will helpful to design an inhibitor of E6/PDZ interaction that may has therapeutic benefit.

Characterization of Vpr oligomers in living cells.

Joëlle V. Fritz[1], Pascal Didier[1], Serge Bouaziz[2], Jean-Luc Darlix[3], Yves Mély[1] and Hugues de Rocquigny[1]

[1] Département de Pharmacologie et Physico-Chimie, UMR 7175 CNRS, Faculté de Pharmacie, ULP, Strasbourg [2] Inserm U640 CNRS UMR8151 UFR des Sciences Pharmaceutiques et Biologiques Paris, France[3] LaboRétro INSERM U 758, IFR 128 ENS de Lyon, LYON, France

Vpr is a human immunodeficiency virus type 1 small and multifunctional auxiliary protein, which influences the survival of infected cells by inducing a G2M arrest and apoptosis. It is located mostly at the nuclear rim and in the nucleus. Vpr is thought to form homo-oligomers, but no direct imaging of Vpr-Vpr interaction in living cells has yet been reported and no role has been attributed to this oligomerization. To address this issue, HeLa cells were transfected with plasmids expressing eGFP- and mCherry-tagged Vpr and investigated with two photon fluorescence lifetime imaging microscopy (FLIM) and fluorescence correlation spectroscopy (FCS). Results show that Vpr-Vpr oligomerization takes place mostly at or close to the nuclear envelope. A significant amount of Vpr dimers and trimers has also been found in the cytoplasm and the nucleus. Point mutations in the three α helices of Vpr, namely L23F, ΔQ44, I60A and L67A, strongly impair Vpr oligomerization. Surprisingly, the same mutants showed a partial or total loss of Vpr at the nuclear rim, suggesting a direct correlation between Vpr oligomerization and its cellular localization. In contrast, point mutations outside the helical regions (Q3R, W54G, R77Q and R90K) show no effect on its oligomerization and cellular localization. The most drastic effect on Vpr oligomer formation and cellular localization has been observed with the ΔQ44 mutant that is thought to disrupt the second helix. In contrast, no significant reduction of Vpr-mediated apoptosis was observed with Vpr mutants, indicating that the Vpr pro-apoptotic properties are not related to its oligomerization and nuclear envelope localization.

Poster number :**14**

Two-photon two-focus Fluorescence Correlation Spectroscopy

Julien Godet, Pascal Didier and Yves Mély
Photophysique des interactions Biomoléculaire Faculté de Pharmacie
UMR 7175 Université Louis Pasteur 74 route du Rhin F-67401 Illkirch

Fluorescence Correlation Spectroscopy (FCS) has emerged as a powerful tool to investigate biological problems [1, 2]. This technique allows characterizing the translational dynamics of fluorescent molecules (or molecular complexes) in any liquid environment. By analysing the intensity fluctuations of fluorescent species within a femtoliter volume (defined by the laser excitation), several physical parameters - diffusion time, local concentration, molecular brightness, related to the hydrodynamic and photophysical properties of these species - can be monitored by means of the autocorrelation function. FCS has been successfully applied in solutions as well as in cells [1, 2]. Among the different derivatives of FCS, two-focus FCS (2fFCS) has been recently introduced by Enderlein et al. in an elegant manner to measure absolute diffusion constants [3, 4]. With this method, fluorescence fluctuations from two overlapping excitation volumes are monitored and the resulting cross-correlation is used to determine absolute diffusion constants.

FCS measurements were performed on a home-built two-photon laser scanning system set-up [5]. To generate two overlapping excitation volumes in the sample, a Michelson interferometer was introduced in the optical path of the microscope. The approach has been validated with an aqueous solution of TetraMethylRhodamine (TMR) with increasing amounts of sucrose. The retrieved diffusion constants scale linearly with the inverse of the viscosity as expected from the Stokes-Einstein equation. Finally, the method was applied to investigate the binding properties of the HIV-1 NCp7 protein to a small oligonucleotide (AATGCC). Through the changes of the diffusion constants as a function of the protein/ODN ratio, we successfully determined the binding parameters.

1. Haustein, E. and P. Schwille, Annu Rev Biophys Biomol Struct, 2007. **36**: p. 151-69.
2. Orden, A.V. and J. Jung, Biopolymers, 2008. **89**(1): p. 1-16.
3. Dertinger, T., V. Pacheco, I. von der Hocht, R. Hartmann, I. Gregor, and J. Enderlein, Chemphyschem, 2007. **8**(3): p. 433-43.
4. Muller, C., K. Weiss, W. Richtering, A. Loman, and J. Enderlein, OPTICS EXPRESS, 2008. **16**(6): p. 4322-4329.
5. Azoulay, J., J.P. Clamme, J.L. Darlix, B.P. Roques, and Y. Mely, J Mol Biol, 2003. **326**(3): p. 691-700.

Poster number :**15**

Prokineticin receptor-1 induces neovascularization and epicardial derived progenitor cell differentiation.

Célia Guilini 1, Kyoji Urayama 1, Gulen Turkeri 1, Selcuk Takir 1, Hitoshi Kurose 2, Nadia Messaddeq 3, and Canan G. Nebigil 1
1. UMR-7175/CNRS/Université Louis Pasteur-Strasbourg I, Ecole Supérieure de Biotechnologie de Strasbourg, Bld. Sébastien Brandt BP. 10413, F-67412 Illkirch, France.
2. Department of Pharmacology and Toxicology, Kyushu University, Fukuoka, Japan.
3. IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire), Inserm, U 596, CNRS, UMR 7104, BP 10142, Illkirch, F-67400 France.

Prokineticins are potent angiogenic factors that bind to two G protein-coupled receptors to initiate their biological effects. Here, our studies on genetically manipulated animal models for prokineticin receptor-1 (PKR1) mouse cardiac explants cultured human epicardial derived progenitor cells (EPDCs) EPDCs and H9c2 cardiomyoblasts co-culture model show a novel mechanism that cardiac-PKR1 signaling upregulates its own ligand, prokineticin-2 as a paracrine factor to induce proliferation and differentiation of the EPDCs, thereby regulating postnatal coronary angiogenesis and vasculogenesis. We found that transgenic (TG) mice overexpressing PKR1 in cardiomyocytes displayed no spontaneous abnormalities in cardiomyocytes, but increased capillary density and number of coronary arterioles. Since epicardin (EPDC marker) + cell numbers were significantly increased in the TG hearts, we hypothesized that excessive PKR1 signaling may induce EPDCs activation to form neovascularization. Prokineticin-2 induces significant outgrowth from mouse epicardial explants and quiescent human EPDCs, restoring epicardial pluripotency and triggering differentiation of endothelial and smooth muscle cells (SMC). Co-culturing H9c2 overexpressing PKR1 with EPDCs promotes differentiation of endothelial and SMC, supporting the TG phenotype. The prokineticin-2 effects were abolished in PKR1-knockout (KO) heart explants or by inhibition of PKR1 in EPDCs, indicating PKR1-mediated effects. Overexpressing PKR1 in H9c2 cells or TG hearts upregulates prokineticin-2 levels. Ablation of PKR1 in KO hearts down regulates prokineticin-2 and decreases capillary network in subepicardial area, suggesting an autocrine/paracrine loop between PKR1 and its ligand that might underline its effects on neovascularization. This study provides insights for possible therapeutic strategies aiming at restoring pluripotency of EPDCs by induction of cardiomyocytes PKR1 signaling.

Communication affichée (poster)
Thématique : biologie

Poster number :**16**

Identification of Amino Acid Residues contributing to The ATP Binding Sites at P2X Receptors

Ruotian JIANG, Adeline MARTZ, Lia PRADO de CARVAHLO, Guillaume GUERLET*, Alexendre SPECHT*, and Thomas GRUTTER
Laboratoire de Biophysicochimie des Récepteurs Canaux
*Laboratoire de Chimie Bioorganique
UMR 7175-LC1 du CNRS
Université Louis Pasteur, Faculté de Pharmacie

ATP-gated P2X receptors represent, beside the nicotinic acetylcholine (nAChR) and ionotropic glutamate (iGluR) receptor families, a third family of ligand-gated ion channels receptors. They are presumably comprised of three identical or homologous subunits (seven subunits identified so far (P2X1-7)) arranged symmetrically around the ion channel.Each subunit has two transmembrane segments joined by a large extracellular ectodomain in which positively charged and aromatic residues were previously proposed to take part in ATP binding. However, these works do not provide evidence that these residues participate directly to the binding of ATP. Consequently, the actual location and the amino acid residues contributing to the ATP-binding site remain to be unambiguously determined.

Our project consists in the identification of extracellular amino acid residues contributing to the ATP binding sites. To provide directed evidence that these residues contribute to the ATP-binding site, we propose to use photoaffinity labelling strategy.

Photoaffinity labelling consists in creating a covalent bond induced by irradiation between a photoactivatable ATP-derived ligand and the wild-type receptor.

This techniques was successfully applied for other ligand-gated ion channels receptors such as nAChR.

As so far, we have found that :

1. 8-Azido-ATP and 2-Azido-ATP are both commercially available photoaffinity probes.
2. They are agonists on $P2X_2$ and $P2X_3$ tested by Patch-Clamp electrophysiology of HEK cells transiently transfected by cDNAs encoding P2X receptors.
3. UV irritations of these two probes without receptors have been performed, some photochemical characters of the photolysis products have been studied.

The irradiation with the receptors will be setted up and the radioactive probes will be used for tracking the labelled amino acid residues.

Poster number :**17**

Study of the immunomodulatory properties of tick saliva on innate immunity in a murine model of Lyme disease

Aurélie Kern[1], Claire Marchal[1], Benoit Jaulhac[2], Jean Sibilia[2], Yves Piémont[2], Nathalie Boulanger[1]
EA 3432: Physiopathologie des Interactions hôte-bactérie, (1) Faculté de Pharmacie, 74 route du Rhin BP 60024, 67401 Illkirch Cedex, (2) Faculté de Médecine, 3 rue Koberlé, 67000 Strasbourg.

Lyme disease is a bacterial infection due to *Borrelia burgdorferi* sensu lato and transmitted by the tick, *Ixodes*. This infection is first characterized by a cutaneous inflammation, the erythema migrans. Dissemination of spirochetes from the site of inoculation can lead to clinical manifestations typically involving the skin, joints or central nervous system.

Several studies have demonstrated that *Borrelia* transmitted by the tick bite are more pathogenic than *Borrelia* cultured *in vitro* and inoculated with a syringue. This indicates a major role of the tick saliva on the pathogenicity of the microorganism. While the immonusuppressive effect of tick saliva on the adaptive immunity is well-documented, its effect on the innate immunity of the vertebrate host has been more recently investigated. To this end, we analyzed whether tick saliva could modulate the skin innate immunity, more specifically, whether the tick saliva might immunosuppress the secretion of anti-microbial peptides (AMPs). These molecules have been described as key elements in the control of cutaneous infections. Two families of AMPs are well-represented in the skin: the cathelicidin (CRAMP) and the defensin (mBD3) families. So far the potential role of AMPs has never been investigated in the context of arthropod borne diseases.

Experiments were carried out with C3H/HeN mice to assess the role of these molecules in the transmission of *B. burgdorferi* in different experimental protocols of infections: *Borrelia* inoculated by syringue injection and *Borrelia* inoculated by infected ticks. These two protocols were compared to the inoculation of *Streptococcus,* well-known to induce cathelicidin. The AMP induction was analyzed by quantitative RT PCR. Our results showed that tick saliva down-regulates the induction of AMPs and the proinflammatory molecule TNF-α. This inhibition of inflammatory genes could facilitate the local multiplication of bacteria before their dissemination to different organs.

Poster number :**18**

A novel Chameleon Epidrug

KHANWALKAR Harshal, GRONEMEYER Hinrich

Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Strasbourg, 67404 Illkirch Cedex (France). In collaboration with the teams of A.de Lera (Vigo, Spain), L.Altucci (Naples, Italy) and H. Stunnenberg (Nijmegen, The Netherlands)

Epigenetic deregulation of transcription that contributes to diseases including cancer is reversible and the corresponding enzymes are promising drug targets. HDAC inhibitors are validated as anti cancer drugs in advanced clinical trials. Novel Psammaplin A (Marine sponge) related compounds display a variety of activities, including the inhibition of histone deacetylases (HDAC), DNA methyltransferases (DNMT) and Sirtuins. These compounds have been extensively characterized in enzymatic HDAC inhibitory assay as well as with cell lines in *in vitro* assays. The results obtained in the *in vitro* experiments show that this compounds inhibit HDAC, DNMT and Sirtuin activities. The most efficient compound (UVI5008) was selected for further testing in *in vitro* enzymatic assays, which revealed that UVI5008 blocks HDAC1, 4 and 6 as well as increases the global and site-specific histone acetylation (H3-K9, H3-K14, H3-K18). In addition, UVI5008 induces the expression of the tumor cell-selective death ligand TRAIL (tumor necrosis factor-related apoptosis-inducing ligand). UVI5008 induces G1-M arrest and apoptosis in established acute myeloid leukemia (AML) cells and AML patient's blasts in ex vivo culture. Apart from their HDAC inhibitory activity, the novel inhibitor blocks CpG island methylation of the p16/INK4 and retinoic acid receptors (RAR)-beta tumor suppressors. Finally we have translated these *in vitro* studies in *in vivo* xenograft model in Nude mice [Crl: Nu (Ico) Foxn1Nu mice (Swiss)]. UVI5008 shows better efficacy than the respective standard compounds like SAHA and MS-275 when all these compounds are tested at their maximum tolerated doses (MTD) given every alternate day.

All these data demonstrate that the novel, Psammaplin A related compound, UVI5008 targets simultaneously three established epigenetic enzymatic systems whose suitability for cancer therapy is promising.

Poster number :**19**

Synthesis of new locally perfluorinated phospholipids for liposome formulation

Jérôme Klein,[a] Emmanuel Klein,[a] Miahela Ciobanu,[b] Benoît Frisch,[b] Luc Lebeau,[a,*]
[a]*Chimie Organique Appliqué, UMR CNRS 7175-LC1, Faculté de Pharmacie, Illkirch*
[b]*Chimie Enzymatique et Vectorisation,* ibid.

The achievement of a drug carrier and delivery system, such as liposomes, has become a major objective in biomedical research. Indeed such carrier can significantly reduce the amount of drug needed for therapeutic effect while lowering unwanted side effects. One of the requirements is that liposomes must be stable in the bloodstream for extensive period of time.

To build liposomes with higher stability, we used the unusual behavior of perfluoro alkyl chains. They are neither hydrophophilic nor lipophophilic; they are fluorophilic.

Long-lived liposomes were obtained by the use of highly fluorinated phopholipids. Dr Vierling's team investigated that field with phospholipids containing a perfluoroalkyl segment at the end of the hydrophobic tail. Liposomes of such fluorinated phospholipids proved to be much more stable than their hydrogenated conterparts, most probably because of the greater cohesion energy between perfluorinated alkyl chains.

In order to further improve the stability of liposomes, we proposed the synthesis of new phospholipids containing a fluorinated segment **near the polar head**. That phospholipids self assembly should form a fluorophilic shield all around the liposome, thus preventing the insertion of hydrophobic tails of amphiphilic compounds and precluding degradation of the liposomes. We first synthesized several fatty acids containing fluoroalkyl and alkyl segments of different lengths and integrated them into various structures with different polar heads.

To evaluate the stability of the resulting liposomes in different media, we encapsulated carboxyfluorescein at a concentration provoking fluorescence quenching. We then monitored leakage of carboxyfluorescein as a function of time, by fluorescence spectroscopy and correlated it with the stability of the lipid bilayer. The results obtained in the presence of FBS (Foetal Bovine Serum) which composition is close to that of extra cellular medium showed that the fluorinated vesicles were significantly more stable than those made of classical EggPC. Surprisingly, opposite results were obtained in HEPES buffer. The determination of the optimal chain lengths and hydrogen/fluorine ratio is actually under investigation.

Poster number :**20**

Inhibitory effect of Salp15, a tick saliva protein, on the inflammation of resident skin cells induced by Borrelia burgdorferi, the causative agent of Lyme disease

Claire Marchal[1], Benoit Jaulhac[2], Aurélie Kern[1], Jean Sibilia[2], Luft Benjamin[3], Xiaohua Yang[3] Yves Piémont[2], Nathalie Boulanger[1]

EA 3432: Physiopathologie des Interactions hôte-bactérie, (1) Faculté de Pharmacie, 74 route du Rhin BP 60024, 67401 Illkirch Cedex, (2) Faculté de Médecine, 3 rue Koberlé, 67000 Strasbourg, (3) Université de Stonybrook, NY, USA.

Lyme disease is an arthropod borne disease transmitted by a hard tick, *Ixodes* spp. and caused by a spirochete *Borrelia burgdorferi*. It is a zoonotic organism that occurs between an arthropod, the tick and a vertebrate host. The disease prevails mainly in the Northern hemisphere. Infection by this organism induces multiple and varied symptoms: articular, neurologic, cutaneous and cardiatis attacks.

The tick –*Ixodes*- is a hematophagous arthropod that requires a 4- to 8- day attachment period on the vertebrate host in order to feed to repletion. In order to secure attachment of the vector and to secure susceptibility of reservoir hosts for future tick infestations, tick saliva contains modulators of host immune responses. Salp15, a 15-kDa salivary gland protein, is a major immunomodulatory protein in *I. scapularis* saliva. It inhibits T lymphocyte activation by binding to the CD4 coreceptor. In addition, Salp15 binds to *B. burgdorferi* outer surface protein (Osp) C, a major antigen expressed in tick salivary glands during the early stages of mammalian infection. Finally, Salp15 binds to dendritic cells and inhibits production of pro-inflammatory cytokines.

As the cutaneous inflammation, the erythema migrans, is the first step of Lyme disease, we analysed the role of resident skin cells such as keratinocytes in this inflammation. Human primary keratinocytes were incubated *in vitro* with *B. burgdorferi* and the inflammatory response (IL-8, IL-6 and antimicrobial peptides) was measured by ELISA and Q-RT-PCR. We then studied a potential immunosuppressive effect of the tick saliva protein, Salp15 and we found that Salp15 inhibited the inflammatory response. This immunosuppressive effect on innate immunity occurs in the very early phase during Lyme disease and it could facilitate the development of the disease.

Poster number :**21**

Purification, cristallisation et études structurales préliminaires de ShuA et de Xcc3358, récepteurs membranaires TonB-dépendants.

A. Meksem, K. Brillet, E. Lauber et D. Cobessi

Département récepteurs et protéines membranaires,
Ecole Supérieure de Biotechnologie de Strasbourg
Email: ahmed.meksem@esbs.u-strasbg.fr

Les récepteurs TonB dépendants (TBDR) sont insérés dans la membrane externe des bactéries à Gram-négatif. Ils sont impliqués dans le transport actif de molécules peu abondantes comme les sidérophores, le saccharose[1] et l'hème.
En dépit d'une faible identité de séquences, les TBDR possèdent un repliement commun en deux domaines : **un domaine C-terminal**, organisé en un tonneau de 22 brins β antiparallèles enchâssés dans la membrane externe et reliés entre eux par des coudes périplasmiques et de longues boucles extracellulaires et **un domaine N-terminal** appelé domaine bouchon qui contient un feuillet mixte de 4 brins β qui scelle le tonneau[2]. Une séquence essentielle au transport des ligands est conservée en N-terminal : la boîte TonB. Elle est caractérisée par la séquence consensus TXXVS/T (X étant des résidus hydrophobes). L'énergie nécessaire au transport actif est fournie par le complexe TonB-ExbB-ExbD de la membrane interne suite à une interaction entre la partie N-terminale du TBDR et TonB.
Les TBDR sont impliqués dans la pathogénicité de *Shigella dysenteriae* et de *Xanthomonas campestris pv*. *campestris* (Xcc). Les résolution des structures tridimensionnelles à résolution atomique du récepteur de saccharose (Xcc3358) et de l'hème (ShuA) avec et sans ligand pourraient permettre de mieux comprendre le fonctionnement et le mécanisme de transport par ces récepteurs ainsi que celui de toute la famille des TBDR et de caractériser les bases structurales des reconnaissance des ligands transporter ou des protéines fixant ces ligands. Les résultats concernant la purification, la cristallogenèse et l'étude structurale préliminaire de ces récepteurs seront présentés dans ce poster.

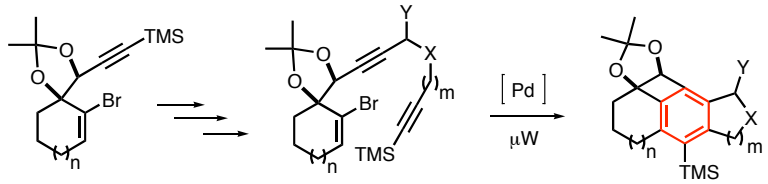
1- Postle K. & Kadner R.J. (2003). Touch and go: tying TonB to transport. *Mol. Microbiol.*, **49**, 869-882.
2- Cadieux N. &, Kadner R.J. (1999). Site-directed disulfide bonding reveals an interaction site between energy-coupling protein TonB and BtuB, the outer membrane cobalamin transporter. *Proc. Natl. Acad. Sci. USA*, **96**, 10673-10678.

A New Pd-Catalyzed Cascade Reaction to the Synthesis of Exotic Polycycles

J. Peluso, Gaëlle Blond, C.D. Muller and Jean Suffert
Université Louis Pasteur de Strasbourg (LC 01 UMR 7175 CNRS/ULP),
Faculté de Pharmacie, 74, route du Rhin 67401 Illkirch-Cedex, France.

The design and elaboration of complex molecules from simple starting material in the minimum of operations is today one of the most challenging goal in organic synthesis. The need of the discovery of new methodologies is necessary. Towards this end, the use of transition metal-catalyzed processes has become a powerful tool for the construction of sensitive and functionalized polycyclic molecules. In particular, cyclocarbopalladation has emerged as a potentially general and versatile synthetic method for the preparation of complex polycyclic systems in a one-pot operation. Palladium catalysed multistep cascades are especially noteworthy in terms of atom economy, stereocontrol and overall efficiency. In the last few years, our chemistry group has developed some methodologies around the 4-*exo*-dig cyclocarbopalladation starting from α,β-insaturated cyclic ketones and stannylated compounds leading to original and complex molecules.[1]

We turn our attention on some different methodologies without using stannylated reactants. Thus, we are developing a new original cascade reaction through a 4-*exo*-dig and (m+4)-*exo*-dig cyclocarbopalladation followed by a 6π electrocyclization reaction starting from **1**. This cascade leads to new strained aromatic systems with good yields.[2]



1 50-92%

To evaluate this new kind of chemical library, we used a new test recently developed by our biology group. It is a fast and accurate high content screening essay for monitoring apoptosis, investigating the biological activity of molecules on living adherent cell lines.[3] Apoptosis plays important roles in development and homeostasis of the human organism. Inappropriate apoptosis can lead to a wide range of acute and chronic diseases including inflammation and cancer. When compared to a classical drug design approach (based on a single target protein involved in a given biological event), the use of a cell-based essay is dramatically efficient for drug discovery and development.

---

[1] a) Suffert, J. ; Salem, B. ; Klotz, P. *J. Am. Chem. Soc.* **2001**, *123*, 12107 ; b) Salem, B. ; Klotz, P. ; Suffert, J. *Org. Lett.* **2003**, *5*, 845 ; c) Salem, B. ; Klotz, P. ; Suffert, J. *Synthesis* **2003**, *1*, 1 ; d) Salem, B. ; Suffert, J. *Angew. Chem. Int. Ed.* **2004**, *43*, 2826; e) Bour, C.; Blond, G.; Salem, B.; Suffert J. *Tetrahedron* **2006**, *62*, 10567

[2] Blond, G. Bour, C.; Salem, B.; Suffert J. *Org. Letters*, **ASAP**, 15 février 2008.

[3] Peluso, J. , H. Tabaka-Moreira, N. Taquet, S. Dumont, J.M. Reimund, and C.D. Muller., *Cytometry A*, 2007. **71**(11): p. 901-4.

Poster number :**23**

Structural Biology and Genomics Platform : techniques for the crystallization of eukaryotic proteins

Pierre Poussin-Courmontagne, Loubna Salim, Matthieu Stierlé, Edouard Troesch, Jean-Claude Thierry, Dino Moras, Patrick Shultz and Didier Busso.

The Structural Biology and Genomics Department is involved in a structural genomics program whose goal is solving crystal structure of protein families members, related to human health, alone or in complex with ligands and protein partners. With this aim, the Department has implemented a Platform (Structural Biology and Genomics Platform), located in the CEBGS (Centre Européen de Biologie et Génomique Structurales) and opened to academic and industrial users. We have developed flexible methods from cloning to crystallization, using automation when appropriate, in order to adapt each step to projects requirements.

At the crystallization level, we especially focused on developping techniques to otpimize crystal growth, including low-cost additives screening, screen refinement, large range of drop volumes (from nanolitres to microlitres), crystallization in gels or under oil.

Here we present a global view of procedures we have established to tackle crystallization of protein samples.

Didier Busso, Jean-Claude Thierry and Dino Moras (2008) *The Structural Biology and Genomics Platform in Strasbourg: an Overview*, Methods in Molecular Biology, Vol. 246: Structural Proteomics: High-Throughput Methods, Chapter 35. Edited by B. Kobe, M. Guss and T.Huber © Humana Press, Totowa, NJ.

Didier Busso, Pierre Poussin-Courmontagne, David Rosé, Raymond Ripp, Alain Litt, Jean-Claude Thierry and Dino Moras (2005) *Structural genomics of eukaryotic targets at a laboratory scale*, J Struct Funct Genomics. 6 (2-3): 81-8.

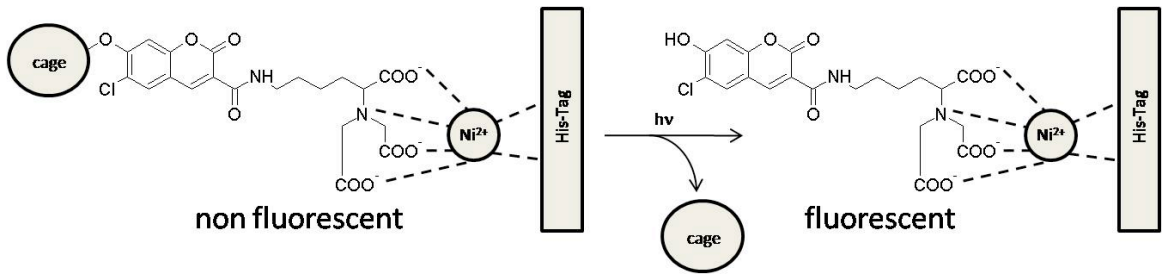
Synthesis of light-activated fluorophores for site-selective protein labelling

David Puliti, David Warther, Sylvestre Gug, Alexandre Specht, Maurice Goeldner

The studies of protein-biodynamics in living cells are most commonly put into practice by expressing the studied protein as a fusion protein with a fluorescent protein.[i] This genetic targeting allows high sensitivity of detection with little background. However, the relatively big size of these fusion proteins may severely perturb folding, trafficking and functioning of the studied proteins.[ii] To overcome these limitations we are developing new labelling techniques using small genetically encoded tags (20 amino acids max.) which recognize the protein with a high enough affinity and which are linked to small organic fluorescent probes.[iii]

Furthermore, we are developing a new generation of photo-activated fluorophores (caged fluorophores), which can be activated instantaneously by photolytic cleavage of a protecting group, with good spatial resolution (two photon uncaging). The selected fluorophores were 6-chloro-7-hydroxycoumarin derivatives, since it has an excellent fluorescence quantum yield (0,95) which is totally quenched by the caging group. In order to limit cellular autofluorescnce we are looking for fluorophores which emit at wavelengths above 600nm.

As genetically encoded Tag we have chosen the Histidine-Tag which will be recognized by a nitrilotriacetate (NTA) moiety[iv,v] coupled to the fluorophore. The search for cell permeable and high affinity NTA-derived tags are in progress.



Prospects: A fully specific labeling method for a protein requires a covalent bond formation using orthogonal chemistry. This can be achieved only by incorporation of a non natural amino acid into a protein using the non-sense suppressor tRNA methodology (see communication by C. Garino). Therefore, the incorporation into the sequence of an amino acid carrying an azido group in the side chain, will allow the covalent bond formation through a "Staudinger ligation" reaction with a cell permeable tag comprising the caged fluorophore. The development of this methodology will be applied first to membrane proteins before tagging intracellular proteins.

[1] Giepmans B. N. *et al.*, Science. **2006**;312(5771):217-24.
[1] Agbulut O. *et al.*, J Biol Chem. **2007**;282(14):10465-71.
[1] Orange C. *et al.*, Chem Commun (Camb). **2008**;(10):1217-9.
[1] Lata S. *et al.*, J Am Chem Soc. **2005**;127(29):10205-15.
[1] Guignet E. G. *et al.*, Chemphyschem. **2007**;8(8):1221-7.

Poster number :**25**

TIF1β s'associe avec les protéines HP1 pour établir et maintenir la répression du gène *MEST*

Raphaël Riclet, Mariam Chendeb, Régine Losson & Florence Cammas

TIF1β (Transcriptional Intermediary Factor 1 β), initialement identifié en tant que corépresseur des facteurs de transcription à domaine KRAB (*Krüppel-Associated Box*), a par la suite été montré capable d'interagir avec diverses machineries de remodelage de la chromatine ainsi qu'avec les protéines HP1 (*Heterochromatin Protein 1*). Nous avons démontré au laboratoire que l'interaction entre TIF1β et les HP1 est essentielle (1) à la relocalisation de TIF1β de l'eu- vers l'hétérochromatine au cours de la différenciation des cellules F9 de carcinome embryonnaire de souris en endoderme primitif (PrE) et (2) pour la différenciation terminale de ces cellules PrE en endoderme pariétal (PE). Dans le but de progresser dans la compréhension du rôle fonctionnel de l'interaction entre TIF1β et les HP1, au niveau moléculaire, nous avons identifié les gènes dont l'expression est directement régulée par les complexes cellulaires contenant TIF1β et les HP1. Pour cela, nous avons combiné une analyse transcriptomique comparative entre des cellules F9 $TIF1\beta^{+/-}$ et des cellules $TIF1\beta^{HP1box/-}$ (exprimant une protéine TIF1β mutée, incapable d'interagir avec les protéines HP1) avec des expériences d'immuno-précipitation de la chromatine (ChIP). Nous avons identifié *MEST* (Mesoderm specific transcript) comme gène cible primaire de TIF1β. Ce gène est réprimé dans les cellules $TIF1\beta^{+/-}$ alors qu'il est très fortement exprimé dans les cellules $TIF1\beta^{HP1box/-}$. Nous avons mis en évidence que TIF1β, via son interaction avec les protéines HP1, maintient une structure de type hétérochromatine dans la région promotrice de *MEST* dans les cellules $TIF1\beta^{+/-}$ (enrichissement en triméthylation de H3K9 et H4K20, présence de HP1, ADN méthylé et niveau réduit d'acétylation de H3K9 et de triméthylation de H3K4). Des expériences de FISH (Fluorescence in situ hybridation) indiquent que cette interaction facilite aussi le recrutement de *MEST* au niveau des régions d'hétérochromatine péricentromérique. Enfin, en exprimant flag-TIF1β dans les cellules $TIF1\beta^{HP1box/-}$, nous avons observé que flag-TIF1β est capable de restaurer partiellement la répression de MEST et de rétablir la structure de type hétérochromatine au niveau du promoteur de ce gène, démontrant ainsi que l'interaction entre TIF1β et les HP1 est impliquée à la fois dans la maintenance, mais aussi dans l'établissement de la répression de ses gènes cibles.

Poster number :**26**

Selection and characterisation of synthetic human single chain antibody fragments binding to the gankyrin oncoprotein.

Anne-Sophie Rinaldi[1], Dominique Desplancq[1], Audrey Stoessel[1], Mireille Baltzinger[2], Annie-Paule Sibler[1] & Etienne Weiss[1].

[1]CNRS, UMR 7175, Equipe Immunobiotechnologie, ESBS, Bld. Sébastien Brant, BP 10413, F-67412 Illkirch
[2]CNRS, UMR 7175, Equipe Biocapteurs, ESBS, Bld. Sébastien Brant, BP 10413, F-67412 Illkirch

Gankyrin is a seven ankyrin-repeat protein overexpressed in hepatocellular carcinomas and was initially identified as a component of the 19S regulatory complex of the 26S proteasome. This liver oncoprotein has been reported to be an important player in the regulation of the tumor suppressors Rb and p53. Several strategies as RNA interference have been explored to knock down the gankyrin oncogenic activity, but it is not clear which domain of gankyrin is involved. On the other hand, the single chain variable antibody fragments (scFvs) have been shown to be valuable tools for blocking specific protein interactions. For this reason, we decided to isolate anti-gankyrin scFvs from a large synthetic human scFv library based on a unique framework and optimized for cytoplasmic expression [1]. The selection of several scFvs among the 10^9 independent clones was performed by phage display against the human gankyrin protein expressed in the cyanobacterium *Anabaena* sp. *PCC7120* [2]. Once they were selected, the anti-gankyrin scFvs were expressed in the bacterial cytoplasm, purified and characterised *in vitro* in terms of solubility, activity and affinity for their target. These results suggest that the isolated anti-gankyrin scFvs represent promising candidates for testing the reduction of the degradation of p53 and Rb *in cellulo*.

References:

1. Philibert P., Stoessel A., Wang W., Sibler A.P., Bec N., Larroque C., Saven J., Courtête J., Weiss E. & Martineau P. (2007). *BMC Biotechnology*; 7:81.

2. Desplancq D., Rinaldi A.S., Hörzer H., Niergarten H., Atkinson A.R., Kieffer B. & Weiss E. (2008). *Biotech. Appl. Biochem*, under review.

Poster number :**27**

Conception, synthèse et évaluation de ligands de la chimiokine SDF-1

Rohmer, F.[1]; Bonnet, D.[1], Marsol, C.[1], Hachet-Haas M.[2], Galzi, J.-L.[2], Hibert, M.[1]

Institut Gilbert Laustriat, UMR 7175-LC1 ULP/CNRS

[1]Département de Pharmacochimie de la Communication Cellulaire
Faculté de Pharmacie de Strasbourg
74 Route du Rhin, BP 24, 67401 Illkirch, France

[2]Département Récepteurs et Protéines Membranaires
Ecole Supérieure de Biotechnologie de Strasbourg
Boulevard Sébastien Brant, Illkirch, France

francois.rohmer@pharma.u-strasbg.fr

La chimiokine SDF-1 est une petite protéine impliquée dans les phénomènes inflammatoires et de migration cellulaire.

Nous en avons récemment découvert un ligand en criblant par la technique de FRET son récepteur naturel CXCR4 avec la chimiothèque du laboratoire. Notre composé de référence de la famille des chalcones est capable d'empêcher l'interaction protéine-récepteur. Les résultats préliminaires obtenus *in vivo* chez la souris ont montré l'activité de la molécule sur un modèle d'inflammation pulmonaire. Aussi, nous avons entrepris l'exploration des paramètres nécessaires à la liaison de cette molécule à SDF-1 en synthétisant des analogues structuraux qui ont été testés *in vitro*.

Plusieurs séries de molécules ont permis d'évaluer les rôles respectifs des substituants des cycles benzyliques ainsi que l'importance de la fonction cétonique α,β-insaturée. Les premiers résultats montrent qu'il est primordial de conserver un système insaturé qui soit pratiquement plan et nécessaire de conserver deux extrémités lipophile et apolaire d'une part, hétéroaromatique et polaire d'autre part.

Les données de modélisation moléculaire ont permis de rationaliser nos études de relation structure activité, amenant à affiner notre stratégie quant aux possibilités de modification de notre structure de référence et conduisant à la conception de nouveaux ligands plus solubles et « drug-like » appartenant à la famille des pyrimidines.

Poster number :**28**

Methodological developments at SBGP for eukaryotic proteins production

Didier Busso[*°], Pierre Poussin-Coumontagne[*°], Loubna Salim[*°], Matthieu Stierlé[*°], Edouard Troesch[*°], Christophe Romier[°], Arnaud Poterszman[°], Karine Pradeau[°], Robert Drillien[°], Dominique Desplancq[#], Etienne Weiss[#], Jean-Claude Thierry[°], Patrick Schultz[°] and Dino Moras[°]

[*]Structural Biology and Genomics Platform, IGBMC – CEBGS, 1 rue Laurent Fries, Illkirch 67404, France

[°]Structural Biology and Genomics Department (CNRS – UMR 7104), IGBMC, 1 rue Laurent Fries, Illkirch 67404, France

[#]Biology of molecular interactions (CNRS – UMR 7175), ESBS, Boulevard Sébastien Brandt, Illkirch 67412, France

The production of sufficient quantities of homogenous protein is an essential prelude for structural studies but represents a rate limiting step of the structure determination process for many human proteins. To circumvent this bottleneck, the Structural Biology and Genomics Platform (SBGP) as been implemented. We have developed high-throughput (HTP) methodologies from cloning to crystallization, and we focus on new developments, in particular in the field of protein production. Selected examples illustrate recent developments and their impact on the structural biology of human protein: (i) vector development for parallel cloning and (co-)expression of multiple constructs; (ii) (co-)expression screening in bacteria and insect cells to determine conditions resulting in soluble production of targets and further production optimization; (iii) implementation of original expression systems.

Busso D. et al. (in press) Methods Mol. Biol. 426: 523-536
Romier C. et al. (2006) Acta Cryst. D62: 1232-1242
Perrakis A. and Romier C. (in press) Methods Mol. Biol. 426: 247-256
Busso D. et al. (in press) Methods Mol. Biol. 426: 173-184
Busso D. et al. (in press) Biotechniques
Hebben M. et al. (2007) Protein Expr. Purif. 56: 269-278
Desplancq D. et al. (submitted) Biotechnol. Bioeng.

Identification of ligands for mammalian RF-amide receptors from a chemical library of RF-amide derivatives

Christophe Wersinger, Frédéric Bihel, Martine Schmitt, Jean-Jacques Bourguignon & Frédéric Simonin
UMR7175, LC1, ESBS, Illkirch cedex
Poster, Thématique Pharmacologie

Neuropeptide FF (NPFF), Neuropeptide VF (NPVF), Prolactin-releasing peptide 31 (PrRP31), QRFP26 (26-RFa) or Metastin (Kisspeptin), belong to the familly of RF-amide bioactive peptides, which all share a common carboxy-terminal Arg-Phe-NH_2 sequence. They are implicated in various physiological and pathological processes, including pain modulation, opioid tolerance, circadian rythms and obesity. Their receptors belong to the G protein coupled receptors (GPCRs) superfamilly of transmembrane proteins : hNPFF1 receptor for NPVF, hNPFF2 for NPFF, hGPR10 for PrRP31, hGPR54 for Metastin, and hGPR103 for 26RFa. Pharmacological tools or drugs that would help define physiological roles for these peptides are still missing. Recently, we have reported the identification and characterization of a novel potent and selective NPFF receptor antagonist derived from the RF-amide sequence, called RF9 [Simonin et al. 2006 PNAS 103(2): 466-471]. These results prompted the establishment and design of a chemical library of RF-amide derivatives by the group of J.J. Bourguignon (Fac de Pharmacie, Strasbourg). Screening of this chemical library was undertaken in our group by competition binding assays, on membrane protein preparations of CHO cells stably expressing the five RF-amide receptors. These cell lines were first established and selected by fluorescence activated cell sorting (FACS) for the presence of Flag-tagged GPCRs. Functionality of the GPCRs was verified by agonists-stimulated intracellular calcium mobilization with the fluorescent calcium sensor Fluo3, or in GTPgamma^{35}S binding assays. Binding conditions for each GPCR were defined. Approximately 40 compounds out of 250 showed similar or better competition activity than RF9 (Ki < 50 nM) for the specific binding of [^{3}H]FFRFamide to either hNPFF1 or hNPFF2 receptors. Thirteen compounds from our chemical library were able to significantly compete with the specific binding of [^{125}I]PrRP20 to hGPR10 receptors, with Ki values ranging from 0,93 to 3,42 microM. Approximately 25 compounds were competitors for the specific binding of [^{125}I]QRFP43 to hGPR103 receptors, with Ki values expected to be in the 500 nM to 1 microM range. Competition binding assays for the specific binding of [^{125}I]Metastin 45-54 to hGPR54, are currently underway. Our results fully confirm our hypothesis that this library of RF-amide derivatives contains ligands for all mammalian RF-amide receptors.

Poster number :**30**

ShcA – LRP1 signaling pathway: IGF signaling modification and atherosclerosis protection

WOLDT E., MATZ R.L., BOUCHER P.

LC1, UMR CNRS 7175, Département de Pharmacologie et de Physico-Chimie, Université Louis Pasteur, Strasbourg.

Vascular smooth muscle cell (VSMC) proliferation and migration are important events in the development of atherosclerosis. The low-density lipoprotein receptor-related protein (LRP1) has a pivotal role in protecting vascular wall integrity and preventing atherosclerosis by controlling platelet-derived growth factor (PDGF) (1) and transforming growth factor-beta (TGF) signaling (2). The tyrosine located in the LRP1 cytoplasmic domain is phosphorylated by Src, which can serve as a docking site for the protein ShcA (3). This protein is an adaptor protein which mediates the recruitment and the activation of Grb2/Sos/ERK to promote cell proliferation.

It was shown that Insulin-like growth factor-I (IGF-I) is a potent stimulant of VSMC migration and proliferation and that it is implicated in the development of experimental atherosclerotic lesions.

In the present study we investigated the mechanisms by which the LRP1-ShcA pathway controls IGF-1 signaling and protects from atherosclerosis.

We report that in mouse embryonic fibroblasts (MEF) and upon IGF stimulation, the activated IGF receptor (IGFR) forms a complex with LRP1. This complex recruits and directly phosphorylates ShcA on tyrosine residue. Phosphorylation of ShcA recruits Grb2 initiating Ras and Erk1/2 activation. By contrast, in MEF LRP1-/-, IGF-I stimulation no longer recruits ShcA to the membrane and its tyrosine phosphorylation is abrogate. However we observe an increase of phospho-Akt and subsequent mTor activation.

These results suggest that LRP1 function as a molecular switch between to major physiological pathways: It recruits ShcA at the plasma membrane an events that activates Grb2/Ras/ERK phosphorylation and cell proliferation, while absence of LRP1 leads to phosphorylation of Akt and mTOR, a pathway known to lead to cell differentiation.

Poster number :**31**

Construction of Gateway -based vectors for high-throughput cloning and (co-)expression screening in Escherichia coli

Didier Busso, Loubna Salim, Edouard Troesch, Bénédicte Delagoutte-Busso, Jean-Claude Thierry and Dino Moras
Structural Biology and Genomics Department (CNRS – UMR 7104)
IGBMC, 1 rue Laurent Fries, Illkirch 67404, France

We describe the construction of a 10 Gateway-based vector set applicable for high-throughput cloning and for expressing recombinant proteins in Escherichia coli. Plasmids bear elements required to produce recombinant proteins under the T7 promoter control and encode different N-terminal fusion(s). A sequence encoding a 6 histidine tag has been inserted to be in frame with the cloned Open Reading Frame (ORF) either at its N-terminusor at its C-terminus, giving the flexibility of choosing the 6 histidine tag location for further purification. Since the vector set is derived from a unique backbone, a consistent comparison of the impact of fusion partner(s) on protein expression and solubility is easily amenable. Moreover, the presence of
the T7 promoter facilitates parallel expression screening using auto-inducible media (Studier, F.W. (2005) Prot. Expr. Purif. 41: 207-234). Following the same strategy, we are now constructing a new Gateway-based compatible vector set for co-expressing multi-protein complexes directly in E. coli.

Poster number :**32**

When Chemistry meets Biology: the PCBIS pathway

P. Villa[1]; C. Valencia[1]; B. Didier[1,2]; J.-L. Galzi[1,2]; M. Hibert[1,2]; J. Haiech[1,2]
[1] : PCBIS Institut Fédératif de Recherches 85, Illkirch ; [2] : UMR7175 LC1, Illkirch
pascal.villa@pharma.u-strasbg.fr

High Throughput Screening (HTS) is the technology which best facilitates the search of new molecules with the potential of becoming the drugs of tomorrow. Until recently this expensive technology was only available in pharmaceutical companies. The 'Plate-forme de Chimie Biologique Integrative de Strasbourg' (PCBIS) of the "**Institut Fédératif de Recherches 85" (IFR85)**, has the expertise and equipment necessary for new drug discovery. One of our main goals is to offer our expertise to laboratories aiming to find new drugs to cure rare and/or neglected diseases.

We will show the different tools that PCBIS can propose to the scientific community.