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RÉSUMÉS DES COMMUNICATIONS PAR AFFICHE

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The polyphenol curcumin inhibits *in vitro* angiogenesis and cyclic nucleotide phosphodiesterases (PDEs) activities similarly to PDE inhibitors

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VEGF, by stimulating endothelial cells to migrate, proliferate and differentiate, plays a major role in angiogenesis. Increase in intracellular cAMP is known to inhibit basal as well as VEGF-induced endothelial cell proliferation. Cyclic nucleotide phosphodiesterases (PDEs) play a key role in signal transduction by hydrolyzing specifically cyclic nucleotides.

Our team has previously reported that PDE2 and PDE4 up-regulations (activity, protein and mRNA) in human umbilical vein endothelial cells (HUVECs) are implicated in VEGF-induced angiogenesis and that inhibition of PDE2 and PDE4 isozyme activities prevents the development of angiogenesis by increasing cAMP level, by inhibiting cell proliferation, cell migration and cell cycle progression (Favot et al., Thromb Haemost 2003, 90: 334 and 2004, 92: 634). On another hand, we have shown that polyphenols inhibit PDEs (Orallo et al., Naunyn Schmiedebergs Arch Pharmacol 2004, 370: 452; Planta Med 2005, 71: 99; Alvarez et al., Br J Pharmacol. 2006, 147: 269).

The polyphenol curcumin, isolated from the plant *Curcuma longa*, is present in curry powder, and is known to have anti-inflammatory, anti-oxidant and anti-cancer properties. The anti-carcinogenic properties of curcumin have been demonstrated in animals by its ability to inhibit tumor initiation and tumor progression. Therefore, this study was aimed to investigate the participation of PDEs in anti-angiogenic properties of curcumin.

The effect of curcumin on PDE activities was assessed by the determination of IC_{50} values on the five isozymes PDE1-PDE5 purified from vascular tissues. Curcumin was able to inhibit PDE1, PDE2, PDE3 and PDE4 with IC_{50} values in the range of 10 to 20µM, and PDE5 with an IC_{50} value of 35µM.

Curcumin at a concentration of 10µM inhibited both basal and VEGF-stimulated HUVEC proliferation (58% and 54% respectively, P=0.003). Furthermore, 10 µM curcumin inhibited significantly (52%, P=0.001) VEGF-induced HUVEC migration similarly to the selective PDE2 inhibitor (0.1µM BAY-60-7550, 69%, P=0.003) and the selective PDE4 inhibitor (10µM rolipram, 41%, P=0.006).

These results, showing for the first time that curcumin inhibits PDE activities, suggest that curcumin present in food might inhibit angiogenesis at endothelial cell level by inhibiting PDE activities.

The methyl cytosine base flipping induced by the UHRF1 SRA domain is involved in the tumor suppressor gene silencing inheritance

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Hypermethylation of the promoters of tumor suppressor genes is associated with the development of cancer. Although DNA methyltransferase 1 (DNMT1) catalyzes the methylation reaction of newly-synthesized daughter strands, it requires the E3 ligase UHRF1 to replicate these patterns with high fidelity. UHRF1 contains a SRA domain (Set and Ring Associated), which exhibits binding for hemi-methylated DNA and DNMT1. Structure studies show that the SRA domain of UHRF1 flips the methyl cytosine out of the CpG duplex DNA, burying it in a pocket in the protein. The recognition of the hemi-methylated DNA by the UHRF1 SRA domain subsequently allows DNMT1 to be recruited to the target sites and finally allows the faithful duplication of the DNA methylation patterns. Epigallocatechin gallate (EGCG) is the main polyphenol found in green tea extracts and is known to up-regulate tumor suppressor genes. UHRF1 has been described to down-regulate tumor suppressor genes among which *RB1* and *p16INK4A*.

The goal of the present study was to see whether and how the mechanism of action of EGCG involves a down-regulation of UHRF1 and DNMT1. We also investigated if over-expression of UHRF1 re-silences tumor suppressor genes and if it is dependent upon the methyl cytosine base flipping mechanism.

Our results showed that the down-regulation of UHRF1 by EGCG is redox-sensitive and that EGCG causes a time-dependent phosphorylation of Src, Akt, JNK and p38 MAPK. Furthermore, we have demonstrated that UHRF1 overexpression induces down-regulation of tumour suppressor genes ($p16^{INK4A}$ and *p73*) in EGCG-treated Jurkat cells. Interestingly, over-expression of UHRF1 overcomes EGCG-induced cell cycle blockade and inhibits EGCG-induced apoptosis. Overexpression of SRA-mutated-UHRF1 showed that the SRA domain is involved in $p16^{INK4A}$ and *p73* repression.

These results show that EGCG is able to down-regulate UHRF1 and DNMT1 expression. The mechanism of down-regulation probably involves Src, Akt, Erk and JNK activation via phosphorylation. Our results show that it is the down-regulation of UHRF1 and DNMT1 that is involved in the EGCG-induced up-regulation of tumor suppressor genes. Furthermore, these results support the idea that the methyl cytosine base flipping mechanism is involved in the tumor suppressor gene silencing inheritance

Bruton's tyrosine kinase is involved in miR-346-related regulation of IL-18 release by LPS-activated rheumatoid fibroblast-like synoviocytes

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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 and THP-1 cells were transfected with the Clear-miR™ miRNA inhibitors or with miR-346 mimic respectively, using the Human Dermal Fibroblast Nucleofector™ kit and Cell Line Nucleofector Kit V from Amaxa. To test that miR-346 could play a direct role in IL-18 and Btk post-transcriptional regulation, we generate luciferase reporters plasmids for IL-18 and Btk. IL-18 was evaluated in cell supernatants by ELISA.

Results. As assessed by microarray analysis, 15 miRNAs were overexpressed more than two fold in LPS-activated FLS and among them, we identified one miRNA, miR-346 that had partial sequence complementarity within the 3'-UTR region of the IL-18 mRNA. We then used antisense oligonucleotides molecules to block miR-346 activity; transfection of the inhibitor targeting miR-346 reestablished IL-18 release by LPS-activated FLS. Moreover we also showed that transient transfection of miR-346 in THP-1 cells, inhibited IL-18 secretion by these cells in response to LPS. Using a luciferase assay we showed that miR-346 does not target directly IL-18 mRNA. In search for another target that could explain the effect of miR-346 on IL-18, we also demonstrated that miR-346 inhibited Btk expression at the transcriptional level in LPS-activated RA FLS and that inhibition of Btk expression regulated negatively IL-18 release.

Conclusion: These results suggest an important role of miR-346 in the control of IL-18 synthesis and indicate that its expression may be critical to prevent an excessive inflammatory response.

Synthesis, Configurational Study and Anti-Toxoplasma gondii Activity of New 4-thiazolidinone Substituted Derivatives

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Parasitic diseases, as toxoplasmosis, affect millions of people, and they are responsible for some of the most important and prevalent diseases of humans and domestic animals. The toxoplasmosis is caused by an intracellular parasite, *Toxoplasma gondii*, and is associated with severe pathologies, including pneumonia, myocarditis and pulmonary necrosis. In order to investigate the anti-*T. gondii* activity of new structural analogues, we describe the synthesis, configurational study and in vitro biological activity of two series of 2-[(Phenylmethylene)hydrazono]-3-phenyl-4-thiazolidi-none-5-substituted.

The synthesis of 2-[(Phenylmethylene)hidrazono]-3-phenyl-4-thiazolidinone derivatives (**2a-h**) was performed by condensation of Benzaldehyde 4-phenyl-3-thiosemicarbazone substituted (**1a-h**) with ethyl chloroacetate. 2-[(Phenylmethylene)hidrazono]-3-phenyl-5-(4-nitro-phenyl)methylene-4-hiazolidi-none derivatives (**3a-h**) were synthesized by the reaction of 2a-h with ethyl 2-cyano-3-(4-nitrophenyl)-acetate. All final compounds were purified by recrystallizations, giving yields ranging between 36-100%. Compounds 3a-h exists as potential *E* and *Z* geometrical isomers. The configuration of the 5 exocyclic C=C double bond was assigned on the basis of NMR ^{1}H spectroscopy. As previously described, the spectra showed only one kind methine proton (7.77-7.98 ppm) characteristics for *Z* geometrical isomers. This result was supported by Non-decoupled NMR ^{13}C spectra.

The synthesized 4-thiazolidinones were submitted to incubation for 24 h in cultures of Vero cells infected with *T. gondii*. After drug treatment, the cultures showed decreasing percentage of infection. As a consequence the mean number of normal tachyzoites decreased. In the concentration of 1 mM, some compounds were highly toxic because very few or no infected cells and intracellular parasites could be observed. For higher concentrations, the parasitophorous vacuole was enlarged and contained distorted parasites.

It can be concluded that 4-thiazolidinones provide interesting lead for anti-*T. gondii* drugs discovery. Modifications to improve the potency for these derivatives by structural diversification and to examine the mechanism of action of such compounds are currently in our laboratory, in order to fully understand their anti-*T. gondii* activity.

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Redox-sensitive activation of endothelial Nitric Oxide synthase by catechins: role of Hydroxyl moieties

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Objectives: Several rich sources of polyphenols have been shown to strongly increase the endothelial formation of nitric oxide (NO), a potent vasoprotecting factor, via the redox-sensitive activation of the PI3-kinase/Akt pathway leading to the phosphorylation of endothelial NO synthase. The purpose of the present study was to investigate the molecular mechanisms underlying the stimulatory effect of catechins on the endothelial formation of NO using different catechins (flavan-3-ols).

Methods: Vascular reactivity studies were performed using porcine coronary artery rings, which were suspended in organ chambers for the measurement of changes in isometric tension. All experiments were performed in the presence of indomethacin (an inhibitor of cyclooxygenases), and the combination of apamin and charybdotoxin (two inhibitors of endothelium-derived hyperpolarizing factor-mediated effects) to assess only the NO component of the relaxation. Cultures of porcine coronary artery endothelial cells (P1) were used to determine the phosphorylation level of Akt and endothelial NO synthase by Western blot analysis. Both natural and synthetic catechins were evaluated.

Results: (-)-Epigallocatechin-3-*O*-gallate (EGCg) induced potent endothelium-dependent relaxations in porcine coronary artery rings. The EGCg-induced relaxation was inhibited by MnTMPyP (a membrane permeant analogue of superoxide dismutase, SOD) whereas extracellular SOD had no effect, indicating a major role of the intracellular formation of ROS. Relaxations to EGCg were minimally affected by rotenone (an inhibitory of the mitochondrial respiratory chain), sulphenazol (an inhibitor of cytochrome P450), apocynin (an inhibitor of NADPH oxidase) or allopurinol (an inhibitor of xanthine oxidase). The replacement of all hydroxyl groups of EGCg by *O*-methyl groups resulted in the total loss of the relaxing activity whereas partial replacement decreased the relaxing activity.

Conclusions: EGCg caused endothelium-dependent relaxations of coronary arteries via the redox-sensitive formation of NO in endothelial cells. The stimulatory effect does not involve major intracellular sources of ROS including the mitochondrial respiratory chain, xanthine oxidase, NADPH oxidase and cytochrome P450 but is critically dependent on the presence of hydroxyl groups possibly leading to auto-oxidation of the polyphenol.

Endothelial NO synthase activation by a red wine phenolic extract: Isolation of active components by bioguided fractionation

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Objectives: Previous studies have shown that Red Wine Phenolic extracts induce nitric oxide (NO)-mediated vasoprotective effects, mainly by causing a redox-sensitive activation of endothelial NO synthase (eNOS). However, Red Wine Phenolic extracts are complex mixtures of a several hundreds of phenolic compounds. Therefore, the aim of the present study was to isolate active phenolic compounds using multi-step bioguided fractionation of the red wine extract.

Methods: Red Wine Phenolic Extract was submitted to a bioguided fractionation using chromatographic methods. The fractions obtained at each step were tested for their potential to induce the activation of eNOS in endothelial cells. Cultures of porcine coronary artery endothelial cells (P1) were used to determine the phosphorylation level of Akt and endothelial NO synthase by Western blot analysis. Identification of phenolic compounds in each active fraction was performed by MALDI-TOF and HPLC-MS techniques.

Results: The first step of fractionation on lipophilic Sephadex® yielded 9 fractions of which 4 of them significantly increased the phosphorylation level of Akt and eNOS in endothelial cells. The active fractions contained mainly procyanidins and some anthocyanins compounds. The fractionation of one of the active fractions by preparative reverse-phase HPLC yielded 11 sub-fractions; all of these sub-fractions significantly increased the phosphorylation level of Akt and eNOS. The analysis of the phenolic compounds indicated that these sub-fractions contained mixtures of procyanidin dimers or conjugated anthocyanins.

Conclusions: The red wine extract contains several types of phenolic compounds, which are able to enhance the activity of NO synthase in endothelial cells including procyanidins dimers and oligomers as well as several conjugated anthocyanins.

A structural region within p53 core domain controls E6-dependent p53 cellular degradation processes

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Worldwide, cervical cancer is the second cause of cancer mortality in women. The principal ethiologic agents are the high risk Human Papilloma Viruses (HPV), in particular HPV16 and 18. The high-risk oncoprotein E6 participates in synergy in the oncogenic process. In fact, E6 induces the cellular degradation of the tumour suppressor p53 via formation of a trimeric complex comprising E6, p53 and the ubiquitin ligase E6-AP. HPV16E6 (16E6) binds to at least two distinct sites on p53. One site, located in the p53 C-terminal region, recognised by both high- and low-risk HPVE6s. The second site is situated within the core domain of p53, and is only recognised by high-risk HPV E6s associated with E6-AP, but this site was not identified precisely.

Based on previous observations (Gu et al. 2001, Bech-Otschir et al. 2001**)** and the selection by phage display of E6 binding peptides, we studied p53 mutants protected from 16E6 mediated degradation. We focused our studies on the core domain 97-112, 155 and 265 amino-acids. We tested the resistance of p53 mutants in vitro and in animal cell models. Moreover, we determined the conformation “native or mutant” of the different p53 mutants using NMR-HSQC, p21 transactivation and immunoprecipitation assay.

We will present our observations on the relation between p53 mutant conformation and resistance to 16E6. In conclusion, we propose a structural region of the core domain of p53 as the target of the E6/E6-AP complex.

Interaction du récepteur nucléaire PPARα avec le métabolisme des xénobiotiques dans les voies respiratoires normales

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Le métabolisme des xénobiotiques implique des biotransformations en plusieurs étapes, dont la première est une étape de fonctionnalisation catalysée par la famille des cytochromes P450. Un membre important de cette famille est le cytochrome P450 1A1 (CYP1A1). En effet, cette enzyme induite par la présence de polluants, comme les Hydrocarbures Aromatiques Polycycliques (HAP) ou les dioxines, est responsable de la bioactivation des HAP en composés génotoxiques. Le récepteur alpha activé par les proliférateurs de peroxisomes (PPARα) est un récepteur nucléaire contrôlant la transcription de nombreux gènes cibles. Ses ligands naturels sont des acides gras et ses ligands synthétiques font partie de la famille des fibrates, connue pour son action hypolipémiante. PPARα est impliqué dans le métabolisme des acides gras et le contrôle de l'inflammation, mais il interfèrerait également avec le métabolisme des xénobiotiques.

Nous nous sommes donc intéressés au rôle de PPARα dans la régulation de CYP1A1 dans les voies respiratoires chez la souris. Des souris C57BL/6 ou déficientes en PPARa (PPARa KO) ont été traitées par du 3-méthylcholanthrène (3-MC) ou du benzo(a)pyrène (B(a)P), forts inducteurs de cyp1a1, ou par leur véhicule. Une cinétique d'induction sur 24 h a tout d'abord été réalisée chez la souris C57BL/6 afin de déterminer les conditions optimales pour l'analyse de la transcription de l'ARNm de cyp1a1 et la mesure de l'expression et de l'activité de la protéine. L'ARNm de cyp1a1 a été quantifié par qPCR. L'expression de la protéine a été analysée par Western Blot. Son activité a été déterminée par la mesure de l'activité 7-éthoxyrésorufine O-dééthylase (EROD). La cinétique d'induction chez la souris C57BL/6 a montré que l'expression (ARNm et protéine) et l'activité de cyp1a1 sont nulles à l'état basal dans le poumon. La transcription de l'ARNm est maximale 4 h après l'administration de 3-MC, puis diminue jusqu'à des niveaux proches du niveau de base à 24 h. La protéine et son activité sont détectées à partir de 8 h et augmentent progressivement jusqu'à 24 h. Dès lors, les temps de 4 h et 24 h ont été retenus pour étudier l'inductibilité de l'ARNm et de la protéine Cyp1a1, respectivement, chez la souris PPARa KO. L'expression de cyp1a1 est également nulle à l'état basal dans le poumon de souris PPARa KO. Après traitement par le 3-MC ou le B(a)P, l'expression de cyp1a1 est augmentée dans le poumon des souris PPARα KO par rapport aux souris sauvages, aussi bien au niveau de l'ARNm (+37%, $p<0,05$), que de la protéine (+55%, $p<0,05$) et de son activité (+47%, $p<0,05$).

Ainsi, nos travaux suggèrent que les activateurs de PPARα, largement utilisés en thérapeutique, pourraient avoir un impact sur le métabolisme des substrats du cyp1a1 dans le poumon.

Activity guided fractionation methodology applies to halophytic plants biological sceening

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Halophytes lives in a saline biotope like coastal sand dunes and suffer of various environmental stresses (salinity, drought, heat/cold, luminosity, wind…)[1]. These abiotic aggressions may trigger oxidative stress and thus reach plant morphological adaptation that can be anatomical, physiological or biochemical resulting for the last one in the synthesis of original molecules (osmolites, defenses molecules…)[2]. Reactive oxygen species (ROS) produce in these stress condition are destructive for plant vital metabolic structure or biological molecules, such as DNA, proteins or lipids leading to cell and tissue injuries. In human these oxidative damages can cause cancer and are initiation factors of inflammation[3]. Consequently, we investigate the antioxidant and anti-inflammatory capacity in halophytic species from Brittany (North-West France) littoral.

The characterization of bioactive metabolites in complex plant mixtures requires efficient and chart approach like activity guided fractionation. This methodology is preceding by a screening and activities selection of crude extracts, which will be fractionated using various phytochemical methods until the isolation of active molecule(s). Each step of fractionation go with activity test and phytochemical analysis, in order to select the fraction(s) biologically active and permit to avoid time-consuming isolation of known constituents or non active molecules.

The aim of this study is to screen different biological targets for anti-inflammatory and/ or antioxidant activities. Thus, for inflammation we used two assays: the inhibition of TNFα (tumor necrosis factor alpha) production by THP1 cells (Human acute monocytic leukemia cell line), and nitric oxide ($NO^{\cdot}$) production by macrophages RAW 267.4 cells. For radical scavenging activities, the superoxide radical ($O_2^{\bullet -}$) and hydroxyl radical ($^{\bullet}OH$) inhibition models are used.

Our preliminary results showed potent antioxidant activities of the crude extract of a selected halophyte (61.8% against $^{\bullet}OH$ and 68.8% against $O_2^{\bullet -}$ for 2µg/mL and 25µg/mL respectivement). Liquid/liquid extractions permit to concentrate this activity in the ethyl acetate extract with 73.4% and 97.2% d'inhibition for 1µg/mL and 5µg/mL respectively against $^{\bullet}OH$. Interestingly we observed also an anti-inflammatory activity of the concentrated cyclohexane and dichloromethane extracts in the inhibition of the nitric oxide production at 25 and 12.5µg/mL. Fractionation and phytochemical chromatographic analysis permit to concentrate successfully the two kinds of activities and allow believing that biological activities are bind to different kind of molecules.

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SPR label-free ranking of small molecule negative modulators of adrenomedullin

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Surface Plasmon Resonance (SPR) is one of the most informative technologies for generating binding data (kinetic, affinity, thermodynamic parameters, binding stoechiometry). Weak affinity interactions can be detected and quantified because complex formation is monitored in real-time. SPR is thus a promising tool not only for screening libraries of chemical compounds, but also for structure-activity relationship studies which require ranking of a series of related compounds for their binding properties.

Adrenomedullin (AM) is a 52 amino-acid peptidic hormone, whose dysfunction is related to several diseases, such as diabetes, hypertension, and cancer. A Surface Plasmon Resonance (SPR) biosensor (Biacore T100®, GE Healthcare Biacore) was used to screen against AM, a collection of 21 synthetic compounds generated from a previously identified AM negative modulator.

AM was immobilized on a sCM5 sensor chip surface. Compounds were injected over AM and reference surfaces at concentrations ranging between 25 and 200 µM. Binding data were obtained after reference subtraction, DMSO correction and molecular weight adjustment. Equilibrium SPR responses were low (between 1 and 14 resonance units), corresponding to binding affinities (Kd) in the 50-500 microM range.

The data generated were used to derive a three-dimensional quantitative structure-activity relationship (3D-QSAR) model which was useful to identify relevant features for an effective binding to AM. These compounds have potential interest as anti-angiogenic and anti-tumour agents.

Peptide-based fluorescent ratiometric sensors for direct detection of proteins

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A biosensor is a tool in which a receptor (recognition element) is coupled to a transducer able to convert the receptor-target recognition event into a measurable signal. Biosensors therefore allow the detection of receptor-target interactions as they take place. The recognition elements are mostly biological compounds (ligands) such as enzymes or antibodies. But it is theoretically possible to use any type of ligand as long as binding affinity for the target to 'sense' is sufficient.

Here we present the design, synthesis and functional evaluation of two peptide-based fluorescent constructs for wavelength ratiometric biosensing of antibody fragments. The ratiometric fluorescent sensors were obtained by solid-phase synthesis of peptides singly labeled with a 3-hydroxychromone (3HC) derivative, an environmentally sensitive fluorophore with a two-band emission. The peptides contain the binding sites recognized by the antibody fragments.

The presence of the dye only marginally affected the kinetic and affinity parameters of the interaction between the antibodies and respective peptides, as measured by surface plasmon resonance. Upon complex formation, the two peptidic sensors showed up to 40 and 56 % change in the ratio of their emission bands, with a detection limit of 10 nM demonstrated in one of the systems.

This work demonstrates that efficient fluorescent sensors can be readily designed and synthesized based on peptide binders coupled to a single environment-sensitive fluorescent label of 3HC family. With the perspective of further improvement of dye properties, these sensors represent promising tools for the quantitative detection of protein targets.

Regulation of α5β1 expression by caveolin-1 in human glioblastoma

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Caveolin-1 is a structural protein of caveolae that plays a checkpoint function in the regulation of processes often altered in cancer. We previously showed that caveolin-1 plays a critical role in the aggressiveness (proliferation, clonogenicity and invasion) of glioblastoma multiforme (GBM) through the regulation of the expression of α5β1 integrin. Caveolin-1 was able to repress α5β1 integrin at the transcriptional level so that the expression of both proteins was always inversely correlated. We also identified α5β1 integrin as the mediator of caveoline-1 effects in GBM.

This study focused on the molecular mechanism by which caveolin-1 regulates α5β1 integrin expression. FAK, p53, p38, SAP/JNK or β-catenin/Tcf/Lef signaling pathway were not affected by the modulation of caveolin-1 expression indicating that these pathways were not involved in the regulation of α5β1 integrin. In contrast, an increase of p42-44 MAPK phosphorylation was observed after depletion of caveolin-1 while a diminution of p42-44 MAPK phosphorylation was shown in cells expressing high level of caveolin-1. Inhibition of the MAPK activity using UO126 (a MEK inhibitor) prevented the induction of α5 and β1 expression observed after caveolin-1 depletion in U87-MG. Data suggest the involvement of MAPK in the regulation of α5β1 integrin expression by cav-1. Additionally, an increase of Akt phosphorylation was observed in cells depleted in caveolin-1. The inhibition of the PI3-K/AKt pathway using a specific inhibitor of PI3-K, LY294002, seems to abolish the induction of α5 and β1 integrin subunits observed after caveolin-1 depletion in U87MG. As p42-44 MAPK and PI3-K/Akt might contribute to the TGFβ/Smad signaling pathway, the expression of TGFβR1 and the activation of Smad2 was explored. Depletion of caveolin-1 lead to an increase of TGFβR1 expression and Smad2 phosphorylation. Conversely, a diminution of TGFβR1 expression and Smad2 phosphorylation was observed in cells overexpressing caveolin-1. Inhibition of TGFβR1 activity using SB431542 (a specific inhibitor) and/or downregulation of Smad2/3 using siRNA might help to understand the involvement of the TGFβ/Smad pathway in the regulation of α5β1 integrin by cavéoline-1.

Data underlight a new pathway by which caveolin-1 regulates α5β1 integrin expression to promote GBM aggressiveness.

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In vivo manipulation of skeletal muscle to characterize the mechanisms underlying centronuclear myopathies

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Myotubularin (MTM1), dynamin 2 (DNM2), and amphiphysin 2 (BIN1) define a novel pathway necessary for normal function of skeletal muscles. This pathway is mutated in Centronuclear myopathies (CNM) and Charcot-Marie-Tooth neuropathies (CMT). The aim of this project is to characterize this pathway in skeletal muscle.

Aim 1: Modulate the myotubularin-dynamin pathway using Adenovirus-Associated Vector (AAV) transduction to understand its role in normal muscle function

The normal skeletal muscle function of MTM1, DNM2 and BIN1 is barely characterized. We will modulate this pathway by transducing wild-type mouse skeletal muscle with shRNA or dominant negative AAV constructs and look for centralized nuclei, fiber atrophy, and increased type 1 fibers; the three main features in CNM patients, using histological staining of muscle sections, and a novel confocal macroscope to visualize nuclei position in live mice.

Aim 2: Characterize the pathological mechanisms leading to centronuclear myopathies

To characterize the pathological mechanisms that cause CNM, we will transduce wild-type MTM1, DNM2 and BIN1 AAV constructs into MTM1 KO skeletal muscle, and BIN1 muscle-specific KO mice currently being developed, to attempt to rescue the CNM phenotype. In addition, MTM1 KO mice exhibit abnormal calcium homeostasis. As calcium stimulation activates the calcineurin pathway resulting in increased type 1 fibers, we will analyze this pathway, initially by performing calcineurin activity assays in these mice.

Aim 3: Examine the molecular relationship between myotubularin, dynamin 2 and amphiphysin 2, and how mutations in these genes lead to CNM

MTM1, DNM2 and BIN1 have been associated with phosphoinositide signaling and membrane remodelling in other tissues, however this has not been studied in skeletal muscle. Using phosphoinositide probes, we will identify the expression and localization of phosphoinositides associated with these proteins, to characterize their normal role in muscle. As BIN1 has been associated with T-tubule biogenesis, we will analyse T-tubule structure in the available mouse models by immunofluorescence analysis.

Proposed outcomes: Our goal is to discover novel functions for MTM1, DNM2 and BIN1 in skeletal muscle. This project will help decipher the pathological mechanisms leading to CNM, and may suggest possible therapeutic approaches.

How does the LIN-12/Notch pathway allow a cellular reprogramming event in *Caenorhabditis elegans*?

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Various examples of cellular identity changes have been reported resulting in the reprogramming of a commited cell (aka transdetermination) or of a differenciated cell (aka transdifferentiation). More recently, it has been shown that a differentiated cell can be reprogrammed to a stem cell state by forced expression of defined factors. However, the mechanisms allowing a differenciated cell to change its identity are poorly understood. Understanding these mechanisms is important to improve our knowledge of developmental and pathological processes.

To answer this question, we have established the nematode *Caenorhabditis elegans* as a powerful *in vivo* model to study reprogramming events in a physiological context. We have focused on one transdifferentiation event, that happens in a stereotyped manner during wild type development. In the posterior region of *C. elegans*, one of the 6 epithelial cells forming the rectum in a young larva, named «Y», migrates away later during development and, in absence of cell division, becomes a moto-neuron named «PDA».

We are interested in the early events that specifically allow this rectal cell, and not its neighbours, to change its identity. We have shown that the LIN-12/Notch pathway is necessary just after the birth of «Y» to give it the competence to transdifferentiate. The activation of the LIN-12/Notch pathway is the earliest such signal we have uncovered. Furthermore, ligands of the LIN-12/Notch pathway appear to be expressed in cells close to the rectum around «Y» birth, and could represent the signal that activates the LIN-12/Notch pathway in the «Y» cell. We are assessing the importance of these ligand-expressing cells and if the canonical LIN-12/Notch pathway is involved. We are also developping strategies to identify target genes of the activated LIN-12/Notch pathway. We will report on our progresses at the meeting. We expect that our study of an *in vivo* cellular reprogramming event will allow us to better understand the mechanisms that allow a cell to change its identity, and to be of interest to a wide audience ranging from the developmental biology and the stem cell and reprogramming to the cancer biology and regenerative medicine fields.

Tumour initiating cells and Gliomas: Screening for new molecules affecting cancer stem cell proliferation

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Malignant high-grade astrocytomas (glioblastomas) are highly aggressive brain tumours without available curative treatment. Cancer cells with stem cell properties have been identified in these tumours. These cells, named cancer stem cells or tumour initiating cells and representing a fraction of the whole tumour, have been proposed to play critical roles in tumour initiation, progression, metastasis and resistance to conventional therapies (chemotherapy and radiotherapy) due to their ability to express high levels of Multi-Drug Resistance (MDR) genes and proteins involved in DNA repair. The new paradigm in cancer therapy prones targeting of, not only cancer cells, but also the more resistant cancer stem cells.

In order to find molecules active on cancer stem cell proliferation, TG1 cells isolated from a human glioma and presenting properties characteristic of stem cells were screened using the Prestwick chemical library. The compounds were assayed for their effect on TG1 cell viability. TG1 cells were tested either in their proliferative or quiescent state.

Compounds acting specifically on TG1 cells in their proliferative or quiescent state were found. The mechanism of action of the chemical compounds and their specificity for tumour cells is under investigation.

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Inhibiting B-cell activation by skin fibroblasts and pulmonary epithelial cells: a new treatment for Systemic Sclerosis?

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Systemic sclerosis (SSc) is a rare autoimmune disease characterized by a localized or systemic hardening of the skin due to excessive fibrosis associated with musculoskeletal, gastro-intestinal, renal, cardiac and pulmonary complications. Pulmonary fibrosis happens to be the most frequent SSc-related cause of death. Three types of cells, resident cells (fibroblasts), endothelial cells and immune cells, especially macrophages, T- and B-cells are involved in SSc pathogenesis. A crucial component in the pathogenesis of SSc is the persistent and unregulated activation of genes encoding various collagens in fibroblasts, which leads to fibrosis of the target organ. B-cells also play an important role in the pathogenesis through the secretion of numerous auto-antibodies (anti-topoisomerase I, anti-centromere, anti-fibrillin 1), the release of pro-inflammatory cytokines and T-cells activation. More recently, the role of B cells in the development of fibrosis was demonstrated.

The aim of this study was to determine if skin fibroblasts and B cells could collaborate in fibrosis induction and auto-antibodies production. We showed using quantitative RT-PCR and ELISA that skin fibroblasts isolated from SSc patients synthesized and released the B-cell activating factor (BAFF), which plays a crucial role in B-cell survival as well as in antibody synthesis, in response to the stimulation of innate immune receptors such as TLR3 (Poly I:C) and TLR4 (LPS). Interestingly BAFF release by skin fibroblasts seemed to be disease specific as this cytokine was not detected in normal skin fibroblasts culture supernatants activated with LPS or poly I:C. We also found that broncho-alveolar lavages and lung lysates isolated from bleomycin-treated mice (the bleomycin-treated mice is an experimental model of pulmonary fibrosis) contained high concentrations of BAFF. Maximum effect was observed at day 14.

Taken together, these data suggest that pulmonary and skin residents cells could collaborate with B-cells and stimulate the pro-fibrotic activity of B-cells through BAFF release. To test this hypothesis, we are currently investigating the therapeutic effect of BAFF inhibitors (BAFF-R-Ig) in the development of pulmonary fibrosis in the bleomycin mouse model.

Conception, synthèse et évaluation de ligands de la chimiokine CXCL12

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La chimiokine CXCL12 est une petite protéine impliquée dans les phénomènes inflammatoires et de migration cellulaire (réf.1 & 2).

Récemment, le criblage de la chimiothèque patrimoine nous a permis d'identifier le premier ligand de cette chimiokine. Cette molécule qui appartient à la famille des chalcones est capable d'inhiber l'interaction entre la chimiokine et son récepteur naturel, CXCR4 (réf. 3). Les résultats préliminaires obtenus *in vivo* chez la souris ont démontré l'activité de la molécule dans un modèle d'inflammation pulmonaire.

Afin d'améliorer à la fois l'affinité et la solubilité de la molécule tête de série, nous avons entrepris une étude des relations structure-activité autour du motif chalcone. Ainsi, 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 une extrémité lipophile et apolaire d'une part, et une extrémité aromatique et polaire d'autre part.

Les données de modélisation moléculaire ont permis de nous guider dans la conception de nouveaux ligands originaux à fort potentiel thérapeutique.

Une des principales pistes explorées est l'augmentation de la solubilité des ligands potentiels. Ainsi, des dérivés de type pro-drogue de notre ligand de référence ont montré une excellente activité anti-inflammatoire *in vivo* sur le modèle murin d'inflammation pulmonaire.

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Self-Assembled Nanostructures From Organic Bolaamphiphiles For Applications As Gene Delivery Vectors

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The success in gene therapy relies strongly on new efficient gene delivery vectors. Non-viral vectors are highly attractive, since they can deliver large quantities of genetic information and are low immunogenic. In this respect, bolaamphiphiles, which are amphiphiles bearing two polar head groups connected by a hydrophobic spacer, are an attractive building block for vector design, as they can form supramolecular nanostructures (nanovesicles and nanotubes) that are much more stable than those from lipids.

The aim of the present work is to develop new gene delivery vectors based on bolaamphiphiles capable to form supramolecular nanotubes hosting a single molecule of DNA. For this purpose, we synthesized a number of bolaamphiphiles containing neutral (carbohydrate) and positively charged (ammonium) head groups connected by a hydrophobic peptide linker. Their self-assembly was studied by Atomic Force Microscopy (AFM), Dynamic Light Scattering (DLS), Electron Microscopy (EM) and fluorescence techniques. The DLS data suggested that the assembly of bolaamphiphiles takes place within 30 min-1 hour. Our AFM data showed that the bolaamphiphiles form monolayer sheets of ca 3.5 nm height on the surface of mica (Fig. 1). This suggests a parallel assembly of the molecules, with their positively charged head group attached to the negatively charged mica surface and the neutral group exposed to the solution. In EM microscopy, we observed ribbons and tubes (Fig. 1) of ca 40 nm in the diameter. The self-assembly depends on the nature of the bolaamphiphile, the presence of DNA, the sample preparation and the substrate.

The evaluation of the transfection ability and cytotoxicity of the obtained complexes with plasmid DNA are in progress.

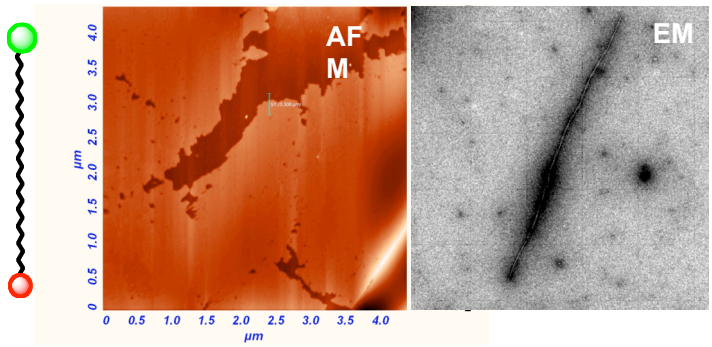

Fig. 1. Schematic presentation of a bolaamphiphile containing two head groups connected by a hydrophobic linker. Self-assembled sheets (monolayers) on a mica surface (AFM) and nanotubes on a graphite surface (EM).

Tif1β associates with the C-terminus of AID and is required for efficient class switch recombination

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Class switch recombination (CSR) and somatic hypermutation (SHM) are initiated by DNA damage induced by activation induced cytidine deaminase (AID). B cells expressing C-terminal truncations in AID display a selective defect in CSR, suggesting that the C-terminal domain of AID is required for CSR but not SHM.

To identify proteins binding to the C-terminal domain of AID and potentially involved in mediating CSR, we established B cell lines expressing epitope tagged-AID. We identified co-precipitating proteins by tandem affinity purification and mass-spectrometry experiments.

We found that the transcription factor Tif1β binds to the C-terminus of AID, suggesting a role for Tif1β in AID targeting and/or in CSR. Tif1β is a transcriptional repressor that has been recently implicated in the DNA damage response. Tif1β is phosphorylated by ATM in response γ-irradiation and its knockdown results in the loss of DNA break-induced chromatin decondensation and in hypersensitivity to double stranded DNA break-inducing agents. Here we have examined the role of Tif1β in responding to programmed DNA breaks induced by AID during CSR. We find that Tif1β is phosphorylated in an AID-dependent manner in B cells undergoing CSR. Furthermore, we show that conditional inactivation of Tif1β results in a B cell intrinsic defect in CSR (50% reduction to all isotypes tested) that is independent of abnormalities in proliferation, switch region transcription or AID expression.

Thus, we define Tif1β as a novel DNA repair protein that associates to the C-terminal domain of AID and that is involved in responding to AID-induced DNA damage during CSR.

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Influence of inflammation on the expression and activity of MDR1 and MRP2 in human intestinal Caco-2 cells

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Membrane transporters play a critical role in absorption, distribution and elimination of both endogenous and xenobiotic substrates. Defects in transporter function can lead to altered drug availability including loss of efficacy or toxicity as in chronic inflammatory diseases. To mimic inflammation, we investigate here the effect of TNF-α on the expression and activity of two multidrug resistance transporters (MDR1 and MRP2) in human intestinal Caco-2 cell line.

Methods: Cells grown on Transwell plates are treated with TNF-α for 24 hours.

For expression studies, specific antibodies for P-glycoprotein (MDR1) and M2 III-6 (MRP2) are used. A labelled secondary antibody Alexa488 is then applied. Immunofluorescent images are acquired on an inverted Leica Laser Scanning Confocal Microscope.

For tranporter's activity studies, rhodamine 123 and PSC 833 (respectively as the substrate and the specific inhibitor of MDR1) and carboxy dichlorofluorescein diacetate and Probenicid (respectively as the substrate and the specific inhibitor of MRP2) are used. Intracellular rhodamine 123 and 5 CDF are assessed by capillary flow cytometry.

Results*:* In Caco-2, TNF-α treatment increases IL-8 secretion level. Moreover, TNF-α treatment causes significant induction of MDR1 and MRP2 expression with modulation in activity of these tranporters.

Conclusion: Clearly, the impact of changes in transporter expression and activity by inflammation can have consequences in term of pharmacokinetic distribution of drugs. A better knowledge of these mechanisms could be of interest for optimizing new therapeutic strategy in intestinal inflammatory diseases such as Crohn's diseases.

Role of cutaneous inflammation induced by different *Borrelia burgdorferi* pathotypes of Lyme disease manifestations in a murine model.

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EA 3432: Physiopathologie et médecine translationnelle : borréliose de Lyme

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Lyme borreliosis is the most frequently reported arthropod borne disease in the northern hemisphere. The disease corresponds to a multisystemic disorder caused by spirochetes of the *Borrelia burgdorferi* sensu lato group. The skin is an essential interface for a successful transmission of the pathogens. Thus, erythema migrans (EM) is the primary manifestation of Lyme disease corresponding to a cutaneous lesion at the site of the tick bite. Then, secondary manifestations corresponding to spirochete dissemination via blood circulation occurs in distant organs including central nervous system, skin, heart and joints. Mechanisms responsible of this specific dissemination are not known.

A possible explanation for *Borrelia* organotropism might be peculiar events occurring in the skin, at the site of initial bacteria inoculation. In this study we postulate that a different cutaneous innate immune response could be involved in the bacterial organotropism. In a mouse model, we studied the cutaneous inflammation induced by different *Borrelia burgdorferi* sensu stricto strains isolated from various human clinical manifestations. Part of skin innate immunity is constituted by the secretion of antimicrobial peptides (AMPs). Two families of AMPs are well-represented in the skin: the cathelicidin (CRAMP) and the defensin (mBD3 and mBD14). The role of these AMPs, of the proinflammatory cytokine TNF-α and of the chemokine MCP-1 has been assessed.

We challenged C3H/HeN mice with spirochetes from *B. burgdoferi* sensu stricto strains initially isolated from human primary skin lesions (EM), from disseminated skin lesions (multiple EM), from cerebrospinal fluid of a patient with neuroborreliosis. The borrelia strain N40 isolated from an *Ixodes* tick was used as a reference strain. Bacteria were inoculated by syringe. We compared skin innate immunity for each strain. Gene expression was analyzed by quantitative RT-PCR. Our preliminary results show a strain-specific immune response in the skin that could explain the bacterial dissemination to specific organ.

Development of new fluorescent membrane probes for apoptosis and raft domains

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Fluorescent dyes are of great importance for investigation of biological processes. Recently, we developed the first ratiometric fluorescent probe for apoptosis detection (F2N12S).[1] This probe, being bound to the outer leaflet of the cell plasma membrane, detects the loss of transmembrane lipid asymmetry that occurs on apoptosis.

The aim of the present work was to develop improved fluorescent apoptosis probes that will overcome the drawbacks of the first probe F2N12S. It includes two research directions: 1) better understanding the mechanism of the response of F2N12S to apoptosis and 2) synthesis of new potential apoptosis probes. Using different model and cellular membranes, we found that membrane hydration and phase state drive the probe response to apoptosis. Importantly, apoptosis increased the hydration of the outer leaflet of the cell plasma membrane, which suggested a loss of the liquid ordered phase (rich in sphingomyelin and cholesterol) at this leaflet. This was confirmed by cholesterol extraction, which results in similar changes in the fluorescence spectra and ratiometric images, as those on apoptosis.

These data suggested a new direction in the design of probes for apoptosis, based on the use of probes sensitive to the membrane hydration and the phase state. In a next step, we synthesized ten new probes, which could be classified in into three types. One type of dyes preserves their high sensitivity to the negative surface charge and to the phase state of the lipid bilayers, similarly to F2N12S. The second type showed nearly no sensitivity to the phase state, while its sensitivity to the surface charge was much higher (>2-fold) than that of F2N12S. The third type, based on the Nile Red fluorophore was sensitive only to the phase state of lipid bilayers. Surprisingly, only some of the new dyes showed response to apoptosis. Indeed, the probes that were sensitive only to the surface charge did not respond to apoptosis, while the other two types of probes that were sensitive to the phase state showed significant response to apoptosis. Moreover, the response of the Nile Red-based probe on cholesterol extraction was similar to that observed on apoptosis. This confirmed that apoptosis may result in the loss of the liquid ordered phase at the outer leaflet of the cell plasma membrane.

Thus, apoptosis can be investigated based on two independent membrane properties: the surface charge and lipid order. Moreover, the probes sensitive to the membrane phase represent a new tool for studies of cholesterol-rich domains and quantification of cholesterol content in cell membranes.

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Phytochemical Study and Antiprotozoal Activity of Extracts and Compounds Isolated from *Thalia geniculata L.*

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Thalia geniculata L. (Marantaceae) is an African medicinal plant traditionally used in Benin to treat malaria and other parasitic diseases. The phytochemical analysis of the aerial parts of this plant led to the isolation of five compounds, identified as sitoindoside I [β-sitosterol-(6-*O*-hexadecanoyl)- 3-*O*-β-D-glucoside] (**1**), daucosterol β-sitosterol-3-0-β-D-glucoside (**2**), stigmasterol (**3**), β-sitosterol (**4**), and geranylfarnesol (**5**).

The structural elucidation was achieved using spectrometric methods and by comparison with the literature. Biological activity was evaluated against *Plasmodium falciparum (K1* multidrug-resistant strain*), Trypanosoma rhodesiense (*STIB 900 strain*), Trypanosoma cruzi (*Tulahuen C4 strain*),* and *Leishmania donovani (*MHOMET-67/L82 strain*).* Geranylfarnesol (**5**) showed significant antiprotozoal activity against *P. falciparum* and *L. donovani,* with IC_{50} values of 12.7μM and 13.2 μM, respectively.

Keywords: Antiprotozoal activity, geranylfarnesol, Marantaceae, *Thalia geniculata.*

Novel "SPOrT" resins to facilitate the labelling of biomolecules: application to the synthesis of the first fluorescent, non-peptidic and selective vasopressin ligand.

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Fluorescent techniques have proven to be powerful tools to probe the structure and function of proteins. On the other hand, Fluorescence Resonance Energy Transfer (FRET) or fluorescence polarization based methods are extremely useful for binding studies and can advantageously be used for high throughput screening of drug-like compounds based libraries.[1] In addition to the sensitivity and specificity that can be achieved, these techniques represent a safe and versatile alternative to radioligand-based methods.

Nevertheless, the prerequisite for the application of such techniques is to design and to prepare fluorescent ligands which retain a good affinity for the receptor. In this context, two novel « SPOrT (Solid Phase Organic Tagging) » resins were synthesized to facilitate the labelling of fluorescent peptides or small-molecule-based ligands. This is a rapid and straightforward method which combines the advantages of the solid-phase chemistry with those of the Cu(I)-catalyzed 1,3-dipolar cycloaddition referred to as "click" chemistry. To highlight the efficacy of the two novel resins, models fluorescent 2,3,4,5-tetrahydro-1*H*-benzo[b]azepine (1-benzazepine) and tripeptide were synthesized in high yields and purities.

In addition, the "SPOrT" resin was successfully applied to prepare the first non-peptidic and selective fluorescent compound with nanomolar affinity for human vasopressin V_2 receptor (V_2R) subtype.[2] This molecule will find useful applications in receptor structural study but also in the discovery of original ligands by using polarization or fluorescence resonance energy transfer (FRET) techniques.

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ALPHA5BETA1 INTEGRIN EXPRESSION LEVEL AND P53 PROTEIN STATUS BOTH DETERMINE THE CHEMOTHERAPY OUTCOME OF HUMAN GLIOBLASTOMA

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Scientific background - Gliomas are the most abundant adult brain tumors nowadays treated with conventional therapies (surgery, radiotherapy and/or chemotherapy). These therapeutic strategies have demonstrated only modest survival benefits. Recent progress in the field of glioma molecular biology helped to identify new potential therapeutic targets. Among them, α5β1 integrin has recently attracted interest as it appears overexpressed in Glioblastoma (GBM). We have shown recently that SJ749, a specific α5β1 integrin antagonist, inhibits proliferation of GBM cell lines. We studied here the implication of this integrin in the chemotherapy answer of GBM in relation with the p53 protein status of the tumors.

Material and Methods – U87MG (p53WT) and U373 (non functional mutated p53) cell lines were treated with ellipticine (a DNA intercalating agent and topoisomerase II inhibitor). α5β1 integrin was inhibited by SJ749. Apoptosis and senescence were quantified by FACS analysis/ PARP cleavage and acidic β galactosidase staining respectively. P53 activation was assessed by western blot with anti phosphor-ser15 p53 specific antibodies and by quantification of p53-targeted gene transcription. U373 cells were genetically manipulated to over-express the α5 integrin subunit.

Results – Ellipticine mainly induced premature senescence in U87MG cells through activation of p53 pathway. In contrast, ellipticine triggered p53-independent apoptosis in U373 cells. Inhibition of α5β1 integrin by SJ749 antagonist reversed the ellipticine-induced senescent phenotype in U87MG cells and led to cell apoptosis by inhibiting the p53 activation. In contrast, no increase in apoptosis after ellipticine and SJ749 cotreatment was detected in U373 cells. Intriguingly, we observed that overexpression of the α5 subunit in U373 cells restored the cell capability to senesce either in absence or in presence of ellipticine and inhibited apoptosis. In these conditions, SJ749 was unable to cause apoptosis but rather increased the senescent cell population.

Conclusions – Our data reveal a crucial role of the α5β1 integrin in the control of the balance between apoptosis and senescence after chemotherapy in glioblastoma. The impact of the integrin is clearly dependent of the p53 status of the tumour. Although p53 status has hardly been demonstrated as a predictor of chemotherapeutic answer in glioblastoma, concomitant screening of tumors for α5β1 integrin and p53 status may be warranted in patients with brain cancer resistant to chemotherapy.

Structural and functional analysis of the complex between HIV-1 Integrase and cellular partners (LEDGF, INI1)

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Helped by several cellular and viral proteins, INtegrase (IN) inserts the reverse transcribed cDNA of HIV-1 (Human of Immunodeficiency Virus 1) in the host cell genome. Integrase is, with the reverse transcriptase and the protease, one of the three viral enzymes strictly required responsible for the replication of HIV-1, constituting thereby a relevant target for antiretroviral drug. Before integration, the viral cDNA is in complex with viral and cellular proteins, forming the pre-integration complex (PIC). In our lab we solved the first structure of a complex between IN and a cellular partner in the PIC (LEDGF) with and without DNA (1). This structure enables us to propose the first model for the HIV-1 cDNA integration in the human genome.

In this work presented here we try to elucidate the role of a second cellular partner of IN in the PIC, INI1 (INtegrase Interactor 1) which is a part of the mammalian chromatin remodeling ATP-dependent complex SWI-SNF and is the first cellular protein that has been identified to interact with IN.

First we determined the limits of the structural domains of INI1 around the Integrase Binding Domain (IBD) by bioinformatics studies. These domains where cloned, expressed in bacteria, purified and used to form stable complex between HIV-1 integrase and INI1 fragments for crystallization assays for high resolution studies. In parallel, using the full length wild type proteins, we solved the low resolution structure of ternary supra-molecular complex between HIV-1 integrase, LEDGF and INI1 IBD by negative stained electro-microscopy. In perspective, we will perform functional tests by fluorescence anisotropy to elucidate the functional role of INI1 in the viral cDNA integration in the human genome.

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Structure of the heme outer membrane transporter ShuA from the human pathogen *Shigella dysenteriae*

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Iron is an essential element to most of living organisms[1] and is an important determinant of bacterial virulence. In humans, iron is bound to iron storage proteins such as lactoferrin, ferritin or transferrin and its free concentration in biological fluids is very low (10^{-18} M), and less than that required for bacterial growth (10^{-7} M to 10^{-8} M)[2].

Shigella dysentriae and other Gram negative human pathogens are able to use iron from heme bound to hemoglobin for growing. We solved at 2.6 Å resolution the 3D structure of ShuA, the TonB-dependent heme/hemoglobin outer membrane receptor from *S. dysenteriae*. ShuA binds to hemoglobin and transports heme across the outer membrane. ShuA is folded in a 22 transmembrane β-barrel, the lumen of which is filled with the N-terminal plug domain. One distal histidine ligand of heme is located at the apex of the plug, exposed to the solvent. His86 is situated 9.86 Å apart from His420, the second histidine involved in the heme binding. His420 is in the extracellular loop L7.

The heme coordination by His86 and His420 involves extracellular conformational changes of ShuA. Structural comparisons with the hemophore receptor HasR of *Seretia marescens* suggest an extracellular induced fit mechanism for the heme binding. The loop L7 contains hydrophobic residues which could interact with the hydrophobic porphyrin ring of heme. The required energy to the transport by ShuA is derived from the proton motive force after interactions between the periplasmic N-terminal Ton-Bbox of ShuA and the inner membrane protein, TonB. In ShuA, the TonB-box is buried and cannot interact with TonB. Structural comparisons with the hemophore receptor HasR bound to HasA-heme suggests its conformational change upon the heme binding for interacting with TonB. The signaling of the heme binding could involve an hydrogen bond network observed from His86 to the TonB-box.

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Interaction between simvastatin, a HMG CoA reductase inhibitor, and two components of grapefruit juice: *in vitro* characterization and *in vivo* relevance

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Introduction: Grapefruit juice contains various components, in majority flavonoids such as naringenin (NRG) and furanocoumarin such as bergamottin (BG). It is able to modify the pharmacokinetic parameters of many drugs in particular, it increases oral bioavailability of simvastatin (SV), a HMG CoA reductase inhibitor. SV is a prodrug characterized by a low oral bioavailability imputable to an important first pass effect (CYP450 enzymes implication). However many membrane transporters, in particular, efflux transporters such as Pgp or MRP-2 can also be involved in this low oral bioavailability. Our objective was then to characterize and quantify the mechanisms of interaction between two components of grapefruit juice and SV in the intestine and in the liver.

Methods: The intestinal transcellular transport of SV was evaluated by the apparent permeability coefficient (Papp) in Caco-2 cells in presence or in absence of NRG and BG. To explain the mechanisms, SV and these two polyphenols were incubated in presence of specific membrane carrier inhibitors such as P-gp, MRP2 and OATP inhibitors. Moreover, the modulation of interaction of membrane transporters by NRG and BG was investigated by flow cytometry measuring the intracellular fluorescent substrates release of treated cells (Rhodamine 123 for P-gp and 5(6)-carboxy-2',7'-dichlorofluorescein for MRP2). The metabolic interaction between SV and these two polyphenols in the liver was quantified by determination of inhibitory constant by using rat and human hepatocytes and liver microsomes.

Results: In presence of NRG and BG, the metabolism of SV was inhibited by CYP3A4 with the same inhibiting capacity in human liver (Ki ≈ 28.29 ± 8.58µM). The intestinal Papp from apical to basolateral side of SV was increased with NRG and BG (5 and 2 times by NRG and BG respectively). Moreover, NRG and BG enhanced intestinal permeability of SV by inhibition of P-gp and MRP2 efflux transporters (IC_{50}=1.15 ± 0.04µM for NRG and 0.02 ± 0.004µM for BG).

Conclusion: NRG and BG were able to modify intestinal absorption and liver metabolism of SV contributing to increase oral bioavailability and plasma concentrations of SV, raising its potential for adverse effects. These properties should be taken into account to adjust doses when SV is co-administered with grapefruit juice.

Inhibition effects of four antifungal triazoles (itraconazole, fluconazole, voriconazole and posaconazole) on specific activities of CYP3A4 in human liver microsomes

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Triazoles are inhibitors of various cytochrome P450 (CYP) isoenzymes such as CYP3A4, CYP2C9 and CYP2C19. Their inhibitory potencies are vastly different and also differ for the various isoforms. Itraconazole inhibits CYP3A4 stronger than fluconazole and voriconazole, in human liver microsomes. Few data exist in regards to the comparative inhibitory effects of posaconazole on CYP3A4.

Objective: To compare in vitro the inhibition effects of 4 antifungal triazoles (itraconazole, fluconazole, voriconazole and posaconazole) on specific activities of CYP3A4 via Ki values under identical experimental conditions.

Methods: The inhibition effects of the four antifungal drugs were assessed on cytochrome CYP3A4-mediated midazolam oxidation activities in human liver microsomes. Ki values were estimated from Dixon plots using the appropriate enzyme inhibition model by nonlinear regression. These studies were performed by using midazolam, a specific substrate of CYP3A4 in presence and in absence of these antifungal drugs after incubation of liver microsomes and regenerating system after dosage of midazolam. The inhibition model was evaluated with ketoconazole as a positive control of CYP3A4 inhibition. At 25 µM and 75 µM ketoconazole, midazolam metabolism from 5 µM midazolam was inhibited by 46% and 87% respectively.

Results: The Ki values (+/- SD) of itraconazole, fluconazole, voriconazole and posaconazole were 16 +/- 2 µM, 221 +/- 2 µM, 151 +/- 4 µM and 215 +/- 14 µM, respectively. The inhibitory effect on CYP3A4 is the strongest for itraconazole (itraconazole >>> voriconazole > fluconazole = posaconazole) (p value = 0.024). Itraconazole was also determined to be a competitive inhibitor whereas fluconazole, posaconazole and voriconazole seem to induce a non-competitive or mixed-type inhibition.

Our in vitro results show that posaconazole is a weak CYP3A4 inhibitor comparable in intensity to fluconazole as opposed to itraconazole or voriconazole, which induce a much stronger inhibitory effect. When compared with other extended-spectrum triazoles, this suggests that posaconazole may have a decreased potential of drug-drug interactions via CYP3A4 inhibition.

"High Content Screening" (apoptose, cytokines) par cytométrie capillaire sur cellules adhérentes.

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Les maladies inflammatoires chroniques sont accompagnées d'activation constitutive du facteur de translocation NF-κB et par conséquent les cellules sont soumises à l'expression continue des gènes de survie [(1)]. Bien que salutaire pour la survie des cellules pendant l'effort inflammatoire, l'activation continue de NF-κB peut poser un risque : les cellules avec un phénotype de survie peuvent devenir des cellules sans interruption de prolifération et ainsi être tumorigènes. La progression vers un phénotype malin de ces cellules impliquera très probablement des changements additionnels de l'expression des gènes régulés par N-F-κB, par exemple un décalage dans l'équilibre des gènes pro- et anti-apoptotique vers un phénotype anti-apoptotique. Pour remédier à cette cancérisation possible il faut pouvoir induire une action anti-inflammatoire accompagné d'une activité pro-apoptotique.

Notre travail de chimiogénomique a pour originalité d'utiliser une technique de pharmacologie inverse, par criblage de chimiothèque [(2)], afin de rechercher des molécules à activité inductrice d'apoptose (sur lignées cancéreuses intestinales) mais également inhibitrice de la production de cytokines proinflammatoires (par des cellules mononuclées humaines) afin d'étudier leur(s) mécanisme(s) d'action et identifier de leur(s) cible(s) moléculaire(s).

Le criblage anti-inflammatoire sur la lignée monocytaires humaines THP-1 de la chimiothèque de la plateforme PCBIS a été mené en parallèle à un criblage pro-apoptotique sur 3 lignées cancéreuses intestinales (Caco-2, HT29, SW-620). Ainsi 400 molécules (substances naturelles et dérivés) ont été testées et leurs activités confrontées grâce à une nouvelle génération de logiciel (Simplicity®, Guava Technologies, CA, USA). Ce logiciel permet de comparer de manière dynamique avec les cytogrames (portes logiques et/ou histogrammes) les 4 activités biologiques dans une matrice 96 puits. Ainsi il nous a été possible de comparer les activités proapoptotiques et anti-inflammatoires des 400 molécules en parallèles.

La suite de ce travail utilisera les molécules révélées par le criblage comme autant d'outils pharmacologiques pour rechercher les mécanismes moléculaires de production de ces médiateurs inflammatoires et ceux de la régulation de l'apoptose afin de mieux comprendre les mécanismes d'action mis en jeu.

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Posttranscriptional regulation of inflammatory response in rheumatoid arthritis by miRNA

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Rheumatoid arthritis (RA) is a chronic autoimmune disease of which the main characteristic is irreversible joint destruction. An excessive stimulation of resident cells such as fibroblast-like synoviocytes (FLS) seems to be the primary fundamental event. This activation is in part due to microbial agents that release PAMPs (Pathogen Associated Molecular Patterns) that activate cells by interacting with Pattern Recognition Receptors (PRRs) such as Toll like receptors (TLRs). Their activation triggers the transcription of numerous genes coding for pro-inflammatory cytokines and metalloproteases implicated in cartilage and bone destruction.

MicroRNAs (miRNA) are small non-coding RNA that have emerged as key players in the regulation of translation and degradation of target mRNAs. We demonstrated recently that one miRNA, miR-346 is involved in the regulation of IL-18 release by rheumatoid FLS (Alsaleh et al. J. Immunol. 2009). The aim of this work was to evaluate their potential involvement in the control of TLR expression by RA FLS.

By quantitative RT-PCR and western blotting, we first showed that RA FLS expressed constitutively TLR2, TLR3 and TLR4 and that TLR2 and TLR3 expression is upregulated in response to BLP, poly I:C and LPS which are the ligands of TLR2, TLR3 and TLR4, respectively. TLR4 expression was not modified. To identify miRNA targeting TLR2 and TLR3 mRNA, RA FLS were activated with BLP, LPS and poly I:C for 6 hours and miRNA microarray analysis were then performed by Eurogentec using the mirAnalyser microarray which contains calibrated human miRNA oligonucleotides from the Sanger miRbase, Fourteen miRNAs were down regulated as compared to the control and among them 7 were predicted to target various TLR: miR-let-7a, miR-15a, miR-203, miR-21, miR-24, miR-620 and miR-19b. Their down regulation was confirmed by quantitative RT-PCR. To assess a potential correlation between miRNA modulation and TLR expression, transfection of RA FLS with miRNA analogs was performed. Preliminary experiments performed with a mimic of miR-15a, did not modify TLR expression. Further experiments are now in progress to evaluate the potential implication of others selected miRNA.

Ratiometric fluorescent peptide labels for monitoring interactions with biomembranes

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Interaction of proteins or peptides with biomembranes changes the conformation, properties and functions of these proteins and peptides. Fluorescence spectroscopy is one of the best tools to study such interactions. In this respect, numerous non-covalent solvatochromic membrane probes were proposed to monitor changes in the membrane properties upon protein binding. Of special interest are two-color ratiometric probes that provide concentration-independent response on environment properties in heterogeneous systems like cellular and model membranes. Notably, 3-hydroxyflafone (3HF)-based probes have been successfully applied for monitoring membrane polarity, hydration and electrostatic properties. These dyes show two emission bands due to the excited-state intramolecular proton transfer reaction that strongly depends on the environment properties.

Our objective was to develop a 3HF-based label for covalent labeling to proteins and test its ability to sense protein-membrane interactions, using a model cationic peptide synthesized by solid phase synthesis and N--terminally labeled by the constructed probe. Upon interaction with membranes, the labeled peptide shows an about 10-fold increase of its fluorescence quantum yield and two well-separated emission bands, allowing a clear discrimination between the free and membrane-bound form of the peptide. Fluorescence emission spectra of the labeled peptide were found to depend on the composition in phospholipids of LUVs, taken as model membranes. The peptide distinguishes neutral from negatively charged membranes due to strong differences in the binding affinity. The labeled peptide was shown to bind at the bilayer surface with a probable anchoring of the peptide N-terminus at the level of the phospholipids heads. Interaction of the peptide with DNA strongly increases the ability of the peptide to interact with neutral membranes. Formation of the ternary protein-DNA-membrane complexes was confirmed using AFM imaging.

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Utilisation de sondes de relaxation paramagnétiques pour l'étude de l'interaction PDZ1-peptide E6c(11)

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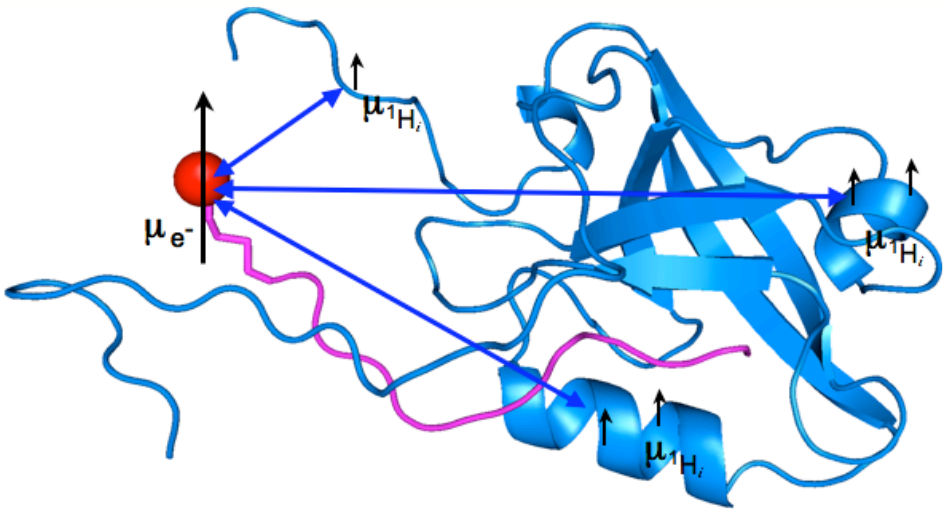
La protéine E6 du papillomavirus humain HPV16 est responsable du cancer du col de l'utérus. Le mécanisme de l'oncogenèse induite par cette protéine implique la fixation de son extrémité C-terminale sur des domaines appelés PDZ. Une de ces interactions implique le domaine PDZ1 de MAGI-1 qui reconnaît la séquence C terminal RSSRTRRETQV de E6 nommé E6CT11.

Dans notre laboratoire, a été déterminée par RMN la structure du complexe PDZ1/E6CT11. Afin d'obtenir plus d'informations sur la dynamique du complexe nous avons mis en ouvre la méthode de marquage de spin (Battiste 2000) qui consiste à mesurer sur les spectres de RMN de la protéine, la relaxation induite par le spin électronique (Fig. 1).

Nous avons introduit le radical PROXYL dans le peptide E6CT11, de façon systématique tous les deux résidus et étudié la relaxation paramagnétique induite lors de la formation des complexes. Les spectres HSQC ^{1}H-^{15}N des complexes ont été enregistrés avant et après la réduction du radical PROXYL (pour le complexe de chaque peptide). Nous présentons l'information structurale issue de ces expériences de RMN ainsi que la préparation des peptides utilisés dans celles-ci.

Figure 1. Representation schématique de la relaxation induite par le spin électronique du PROXYL sur les spins des protons du PDZ1.



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Activation and Internalization of the Delta Opioid Receptor *Ex Vivo*

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In the Central nervous system, the delta opioid receptor (DOR) is involved in nociception but also in the control of emotions, in particular stress and anxiety. It is expressed in some neurons were its activation leads to inhibition of the neuron activity. DOR belongs to the G protein coupled receptor (GPCR) family for which agonist stimulation results in G protein activation and receptor phosphorylation. This leads to G protein uncoupling and receptor internalization.

Our study aimed at establishing a correlation between DOR trafficking and the associated change in neuronal activity, as monitored by electrophysiological recordings.

For this aim, we took advantage of a Knock-in mice generated in the laboratory which express endogenous DOR fused to eGFP at a physiological level (DOR-eGFP KI mice) to look at DOR subcellular localization directly *Ex Vivo* in acute brain slices.

Experiments revealed that:

(1) Classical acute slice preparation protocol induce spontaneous internalization of the receptor.

(2) Decreasing sodium dependant excitatory currents in the whole slice by the use of sodium free aCSF during the slice preparation was efficient to block DOR spontaneous internalization, suggesting implication of excitotoxcicity in spontaneous internalization.

(3) Using this preservative slice preparation protocol both internalization of the receptor and change in neuronal activity could be observed following Agonist (Deltorphin II) bath application.

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

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Gankyrin is a seven ankyrin-repeat protein overexpressed in hepatocellular carcinomas (HCC) and was initially identified as a component of the 19S regulatory complex of the 26S proteasome. This liver oncoprotein has been reported to have an early function in HCC pathogenesis and to be an important player in the regulation of the tumor suppressors Rb and p53 by enhancing their proteasomal degradation [1]. Several strategies, such as RNA interference, have been explored to knock down the gankyrin oncogenic activity, but it is not clear which domain of gankyrin is involved in tumorogenicity. In a previous work, we performed the construction and validation of a large synthetic human single chain antibody fragment (scFv) library based on a unique framework and optimized for cytoplasmic expression [2]. Recombinant antibody fragments have been shown to be valuable tools for blocking specifically protein interactions and offer a new experimental mean to analyse the functions of the targeted protein. For this reason, we decided to isolate scFvs binding the whole human gankyrin protein from the large synthetic human scFv library we constructed in our laboratory. The selection of binders, among the 10^9 independent clones present in this library, was performed by phage display against the human gankyrin protein expressed in the cyanobacterium *Anabaena sp*. PCC 7120 [3]. 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. We confirmed the high level of soluble expression of these scFvs in the cytoplasm of *E. coli* (100 mg protein by liter of bacterial culture). We also found that selected anti-gankyrin scFvs are soluble and active under conditions of intracellular expression in HeLa cells and, when fused to specific cellular localization sequences, they are capable of relocalizing gankyrin to specific subcellular compartments in these cultured cancer cells. The isolated anti-gankyrin scFvs represent thus promising candidates for testing the reduction of cellular proliferation by restoration of the tumor suppressors p53 and Rb levels in hepatocellular carcinoma cells.

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PARP-1 and PARP-2 respond to AID-induced DNA damage to facilitate repair through alternative NHEJ and suppress IgH/c-myc translocations during immunoglobulin class switch recombination

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Immunoglobulin class switch recombination (CSR) is initiated by DNA breaks triggered by activation-induced cytidine deaminase (AID). These breaks activate DNA damage response proteins to promote appropriate repair and long-range recombination. Aberrant processing of these breaks however, results in decreased CSR and/or increased frequency of illegitimate recombination between the IgH locus and oncogenes like c-myc.

Here, we have examined the contribution of the DNA damage sensors Parp1 and Parp2 in the resolution of AID-induced DNA breaks during CSR. We find that while Parp enzymatic activity is induced in an AID-dependent manner during CSR, neither Parp1 nor Parp2 are required for CSR. We find however, that Parp1 favours repair of switch regions through a microhomology-mediated pathway and that Parp2 actively suppresses IgH/c-myc translocations. Thus, we define Parp1 as facilitating alternative end-joining and Parp2 as a novel translocation suppressor during CSR.

Mechanism of the chaperone properties of Hepatitis C Virus core protein

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The core protein of Hepatitis C virus (HCV) is a multifunctional protein involved in many viral processes. It has been recently shown that the core protein exhibits chaperone properties similar to those of the nucleocapsid protein, NCp7 from Human Immunodeficiency Virus (HIV).

In the present study, we characterized the mechanism of the chaperone properties of the core protein, by monitoring the annealing of TAR, and its DNA analogue dTAR, promoted by a peptide (E) that represents the second and third highly basic (2BD) clusters of the N-terminal domain of the HCV core protein. The annealing of dTAR with cTAR involves two second-order kinetic components that are activated by at least three orders of magnitude by Peptide E. The peptide E-promoted activation of cTAR-dTAR annealing was correlated with its ability to destabilize the lower half of dTAR stem, in order to expose the single-stranded complementary regions for nucleating the duplex structures. The two kinetic components have been assigned to two pathways. The rapid one is connected with the annealing of the terminal bases of cTAR to dTAR. On the other hand, extended duplex formation follows a slower pathway, limited kinetically by the nucleation of residues located mainly within the central double-stranded segments of cTAR and dTAR stems.

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Cryo-EM structure of the 30S translation initiation complex

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Initiation of protein synthesis is a vital cellular mechanism that proceeds through tightly regulated steps. In bacteria, the concerted action of three initiation factors (IF1, IF2, IF3), the mRNA and the initiator fMet-tRNA^{fMet} contribute to the functional assembly of the 30S initiation complex (30SIC). This assembly allows setting the correct reading frame of the mRNA with respect to the initiator tRNA, before the 50S subunit joins to form the 70S initiation complex[1] ready for starting protein synthesis. While the structures of individual domains of IF3 and IF2, and the structure of IF1 bound to the 30S have been determined to atomic resolution, the structure organization of the 30SIC has remained elusive.

Using cryo-electron microscopy and advanced particle separation techniques we report the structure of the 30S initiation complex containing mRNA, fMet-tRNA^{fMet} and initiation factors IF1 and GTP-bound IF2, thus addressing the localization and interaction of the factors and initiator tRNA within the complex[2]. The architecture of the 30SIC assembly shows that the initiator tRNA is stabilized within the complex by anchoring the decoding stem in the 30S peptidyl (P) site, whereas the free acceptor stem is hold by the C-terminal domain of IF2. The exposed position of the G-domain of IF2 observed in the present cryo-EM structure, rationalizes the rapid activation of GTP hydrolysis that occurs upon 30SIC-50S joining and 70S formation.

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Binding and chaperone properties of the HIV-1 Tat protein

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Tat is a highly conserved retroviral regulatory protein, that is composed of 86 or 101 amino acids, depending on the viral isolate. Tat appears to play multiple roles in the viral life cycle, notably as a transactivator of transcription and a regulator of mRNA capping and splicing. Tat likely belongs to the family of intrinsically disordered proteins, which should facilitate the interaction of Tat with multiple targets. Moreover, this property and the basic nature of the protein are susceptible to provide it with nucleic acid chaperone abilities.

Characterization of chaperone properties of full length Tat protein and of its derivatives was the aim of the present work. Binding of full length Tat protein and mutants thereof to the DNA analogues of HIV-1 TAR sequence was investigated. We found that the interaction between the full length protein (Tat (1-86)), the arginine-rich domain (Tat (44-61)) and domain (48-86) is governed by similar equilibrium association constants ($\approx 10^8\ M^{-1}$). We found also that non-ionic interactions are critical in complex formation and likely contribute to the binding specificity. Then, we investigated the ability of Tat and of its fragments to promote the hybridization of complementary sequences. We found that Tat (44-61), Tat (1-86), and Tat (48-86) exhibit nucleic acid chaperones properties *in vitro* whereas Tat (1-20) and Tat (21-43) (cysteine reach domain) show no such properties. The Tat (44-61)-accelerated formation of the 55 bp cTAR/dTAR extended duplex (ED) may proceed *via* a two step mechanism in agreement with the following scheme:

$$\mathrm{cTAR} + \mathrm{dTAR} \underset{k_{diss}}{\overset{k_{ass}}{\rightleftharpoons}} \mathrm{ED}^* \xrightarrow{k_2} \mathrm{ED}$$

where an intermediate complex ED* precedes the formation of the final Extended Duplex. Based on the comparable kinetic rates obtained with Tat (44-61), Tat (48-86), and Tat (1-86), we concluded that the region (44-61) is critical for promoting cTAR/dTAR hybridization.

Finally, kinetic investigation of the Tat (44-61)-promoted annealing reaction using cTAR and/or dTAR mutants showed that interactions between cTAR and dTAR in the presence of Tat (44-61) are likely initiated at the bottom end of the TAR stem.

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High throughput mutations screening for the identification of novel genes in centronuclear myopathies

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For rare monogenetic disorders, identification of the mutated gene is the compulsory first step towards molecular diagnosis, creation of animal models, and characterization of the pathological mechanisms, and is a prerequisite for most therapeutic approaches. Apart from a few diseases where the defective protein could be guessed from metabolic studies (eg. mutation in an enzyme synthesizing the missing metabolite), most disease genes are identified through a laborious positional cloning strategy requiring extensive informative families. Although such approach was successful, it cannot be applied to rare disorders without informative families that now represent the usual case. We aim to provide a proof-of-principle that sequence capture and high throughput sequencing can be used to screen thousands of candidate genes in parallel, and could be applied to gene identification in myopathies. We will focus on centronuclear myopathies (CNM), a group of rare muscle diseases typically presenting an abnormal positioning of nuclei in muscle fibers. We have previously been implicated in the identification of the three genes known to be associated to CNM. Mutations in the MTM1 gene encoding a phosphoinositides phosphatase lead to the severe neonatal myotubular myopathy. Mutations in BIN1 (encoding Amphiphsyin 2) and DNM2 (Dynamin 2) induce autosomal recessive and dominant forms respectively. A number of patients were not found to carry mutations in the known genes despite classical CNM features. It is conceivable that other genes are implicated in the development of the disease, and this is backed up by our preliminary linkage analysis data. For the first part of the project we have selected more than 2000 candidate genes based on functional and positional criteria. We aim to screen the exons and adjacent sequences of those genes in 10 DNA samples of patients. Starting from genomic DNA, we will select the sequences of interest to screen by sequence capture through hybridization of the fragmented DNA on 5 Mb chips. Next, we will perform the mutation screen using the innovative large-scale parallel pyrosequencing. The second part will involve 30 Mb chips covering all exons of the human genome and therefore not requiring further selection of candidate genes. We will apply several consecutive bioinformatics filters to discard any variation that is not linked to the disease, and will sequence the best candidate genes through Sanger method in additional patients from our large panel of CNM patients. Finally, we aim to investigate the functional impact of the identified sequence variation(s) by biochemical analysis and cell-based assays. This approach, if successful, could be applied to any rare (and frequent) genetic disorders, both for molecular diagnosis and novel gene identification.

When Chemistry meets Biology: the PCBIS pathway

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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), 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. Moreover we give access to state of the art apparatus to researcher interested in miniaturized fluorescence or luminescence technologies.

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

Epigenetic regulation of nuclear receptor mediated transcription - the role of arginine methylation of CBP

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The specific positional modifications of histones play a key role in regulation of DNA-related processes as transcription, DNA repair and DNA replication. Recently, it has been shown that non-histone proteins are modulated by "epigenetic" enzymes. In this sense CBP (CREB binding protein) has been shown to be methylated by CARM1 in different domains *in vitro* and it has been suggested that CBP methylation plays an important role for its coactivator function by modulating protein-protein interactions.

The aim of this study is to understand the role of arginine methylation at specific sites of the transcriptional coactivator CBP *in vivo*. To address this issue, we have developed ChIP-grade antibodies for unmodified as well as for methylated versions of the residues R714 and R2151, and antibodies against the methylated forms of R742 and R768. ChIP analysis revealed that R2151 methylation is apparently required for CBP recruitment to the endogenous pS2 promotor but not necessarily R714 methylation. This results suggest that methylation of R2151 of CBP has a different function compared to methylation of R714 and possibly R742 and R768. In order to evaluate this observation in a global manner we performed ChIP assays combined with massive parallel DNA sequencing (ChIP-seq).

Preliminary results strongly support the hypothesis that specific positional methylation on CBP might regulate distinct transcriptional programs.