BAFF synthesis by rheumatoid synoviocytes is positively controled by integrin α5β1 stimulation and negatively regulated by TNF-α and TLR ligands.

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The B cell-activating factor (BAFF) is known to play a central role in the maturation and survival of B cells as well as in antibody synthesis. BAFF is produced as a membrane form or secreted by cells of hematopoïetic origin, essentially monocytes, dendritic cells, macrophages and neutrophils.

Recently, it was demonstrated that fibroblast-like synoviocytes (FLS) isolated from rheumatoid arthritis (RA) patients express BAFF transcripts that are strongly up-regulated by inflammatory cytokines like TNF-α, and IFN-γ. We then evaluated whether bacterial components which are ligands of TLR4, TLR2, TLR9 and integrin α5β1, were able to induce BAFF expression and release by RA FLS.

Results indicated that neither TNF-α, LPS, BLP nor CpG induce BAFF *de novo* synthesis and release by FLS converse to IFN-γ. Priming of cells with IFN-γ does not have a synergistic effect on BAFF synthesis by FLS stimulated with PAMPs. Moreover, we demonstrated that IFN-γ-induced BAFF synthesis is inhibited by simultaneous stimulation with either TLR ligands or TNF-α. We also showed that interplay between TLRs, TNF receptors and IFN-γ signaling induces expression of Socs1 and 3 and reduces IFN-γ-dependent Stat1 phosphorylation, which might explain this inhibition. Conversely, we demonstrated that stimulation of integrin α5β1 can induce BAFF synthesis and release *per se* and that stimulation of this pathway, has no inhibitory effect on IFN-γ-induced BAFF synthesis.

These findings indicate that BAFF secretion by resident cells of target organs of autoimmunity is tightly regulated by innate immunity, with positive and negative controls depending on receptors and pathways triggered.

Grape juice causes direct endothelium-dependent relaxation via a redox-sensitive Src- and Akt-dependent activation of eNOS and a redox-sensitive increased-expression of eNOS

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Objectives: An enhanced endothelial formation of nitric oxide (NO) is thought to contribute to the protective effect of moderate consumption of red wine on coronary diseases. This study examined whether Concord grape juice (CGJ), a rich source of polyphenols, is able to up-regulate the endothelial formation of NO, and if so to examine the underlying mechanism.

Methods: Porcine coronary artery rings were suspended in organ chambers for the measurement of changes in isometric tension. The formation of NO was assessed by electron spin resonance spectroscopy, and the phosphorylation of Src, Akt and eNOS by Western blot analysis in cultured endothelial cells. The fluorescent NO-sensitive-probe diaminofluorescein-2 diacetate (DAF-2DA) was used to determine NO formation in coronary artery sections. eNOS expression was assessed by real-time polymerase chain reaction (PCR) and Western blot. CGJ (2.3 g/l polyphenols) was provided by Welch Food Inc. (USA).

Results: Endothelium-dependent relaxations to CGJ were significantly reduced by L-NA, not affected by charybdotoxin (CTX) plus apamin (APA, two inhibitors of EDHF-mediated responses) whereas they were abolished by the combination of L-NA, CTX plus APA. CGJ stimulated the formation of reactive oxygen species and the N^{ω}-nitro-L-arginine-, PP2- and wortmannin-sensitive formation of NO in endothelial cells. The formation of NO was associated with a redox-sensitive and time-dependent phosphorylation of Src, Akt and eNOS. CGJ induced a sustained phosphorylation of eNOS and formation of NO (up to 6 h). CGJ upregulated the expression of eNOS mRNA and protein. The stimulatory effect of CGJ was inhibited by MnTMPyP and PEG-catalase.

Conclusions: CGJ induced endothelium-dependent relaxations of coronary arteries, which are predominantly mediated by NO. The CGJ-induced NO formation involves the redox-sensitive activation of Src kinase with the subsequent PI3-kinase/Akt-dependent phosphorylation of eNOS. In addition, CGJ caused the redox-sensitive up-regulation eNOS resulting in a sustained formation of NO.

Friedreich ataxia neurological mouse model demonstrates caspase-independent cerebellar granule cell death with autophagy and cathepsins induction.

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Friedreich ataxia (FRDA), the most common recessive ataxia, is a progressive neurodegenerative disease associating degeneration of the large sensory neurons and spinocerebellar tracts, and cardiomyopathy. It is caused by severely reduced rate of frataxin, a mitochondrial protein involved in iron-sulfur cluster biosynthesis. Mechanisms of neuronal cell death execution are however unknown because of good neurological model. Through a spatio-temporally controlled conditional gene targeting approach, we have generated two neurological mouse models which develop progressive mixed cerebellum and sensory ataxia. One model develops a progressive loss of the cerebellar granule cell (CGC) in addition to the degeneration of the sensory neurons of the dorsal root ganglia (DRG), spinocerebellar tract and posterior column. We previously identified an autophagic process as the causative pathological mechanism in the DRG. We now report the characterization of the events leading to frataxin-deficient CGC. Ultrastructural studies show morphological evidences that cannot be classified into a single cell-death category (necrosis, autophagy, apoptosis). Furthermore, expression studies demonstrate a combined activation of the cathepsins and autophagic markers, with no caspase activation. Several reports demonstrate the involvement of cell cycle reactivation in the process of neurodegeneration, we are investigating the expression of different cell cycle proteins. Our preliminary results suggest that prior to the massive cell loss, cell cycle specific markers are expressed. To verify these results, we proposed to develop a cellular model from CGC of our neurological mouse models.

Contribution to the determination of geographic origin of chocolates based on volatile profiles

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Chocolate is a worldwide consumed confectionery, which special taste and flavour, highly appreciated, are directly linked to its volatile content. It may be influenced by the cocoa bean variety, the geographic origin (soil, climate, ripening degree) and the processing steps undergone during chocolate manufacturing: fermentation, drying, alkalisation, roasting grinding and conching. Some authors showed interest for the geographic origin of cacao beans, but few studies were done on the final product: chocolate.

The isolation of volatiles from major components of chocolate (such as lipids, sugars, proteins, polyphenols, alkaloids and other non-volatile and potentially extractable substances in the matrix) and their analysis may provide useful information about their geographical origin.

Volatiles of 52 chocolate samples of different origins have been isolated by hydrodistillation, before being extracted by isooctane and separated on a polar column by gas-chromatography. For each sample, the resulting chromatogram provided the volatile profile. Principal Component Analysis (PCA) was carried out and each sample was assigned coordinates by the three major principal components P1, P2, P3. The selection of the peaks of major interest (32) allowed the best separation. Even if chocolates from Africa and those from Madagascar were not entirely separated from those from Venezuela, they tended to form two groups. In addition, Caribbean chocolates (Trinidad, Jamaica and Dominican Republic) appeared to be clearly separated from the other chocolate sets.

Fluorescence spectroscopy study of the basic domain of Tat free and bound to TAR RNA

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The Human Immunodeficiency Virus (HIV) transactivator of transcription (Tat) is a small peptide essential for efficient transcription of viral genes and for viral replication. The 101-amino acid Tat protein, with residues 1-72 encoded by a first exon and residues 73-101 encoded by a second exon, can be arbitrarily considered as containing several "domains". This protein can bind a structured RNA element of the HIV promoter: TAR (TransActivation Responsive region), a 59 nt stem-loop structure located at the 5'-end of all nascent HIV-1 transcripts. This Tat-TAR interaction results in the recruitment of positive transcription elongation factors that increase the processivity of RNA polymerase II. The interaction of Tat with TAR mainly involves the basic domain of Tat which encompasses the Gly44-Gly61 residues of the protein. This domain is also thought to exhibit nucleic acid chaperone properties that direct nucleic acids to their more stable conformation. To characterize the nucleic acid binding and chaperoning properties of this basic domain, an 18 amino acid peptide corresponding to this domain was synthetized by solid phase synthesis. In this peptide, the native Tyr47 residue was substituted by a Trp residue to get an environment-sensitive intrinsic fluorescent probe. It was checked that this mutation does not affect the binding constant of the peptide with TAR. Both the maximum emission wavelength and the inhibition of Trp fluorescence by acrylamide indicated that the Trp residue in the free peptide was fully accessible to the solvent. Interaction with cTAR was found to induce a large decrease in the Trp47 fluorescence intensity. Time-resolved measurements indicated that the binding of the peptide to cTAR is heterogeneous. In the major fraction of bound peptides, Trp47 was found to be stacked with the oligonucleotide bases. Nevertheless, significant populations of bound peptides do exhibit only a partial stacking of their Trp residue or no stacking at all. Finally, we found out that cTAR can accommodate about Tat (44-61) peptides with an affinity of about 10^6 M^{-1} in the presence of 30 mM NaCl. Experiments are in progress to characterize the chaperone properties of the peptide.

Identification and Characterization of new Calmodulin ligands by Fluorescence Polarization High-Throughput Screening

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The ability to discover chemical compounds for novel therapeutic targets, is the first critical step in drug discovery programs.

The aim of our work is to develop a screening assay for soluble proteins using fluorescence methods, specially fluorescence polarization/anisotropy (FP) which is widely used in the study of protein-ligand interactions because of its inherent sensitivity, and the fact that they can be implemented at true equilibrium conditions. FP is a very useful tool to study association equilibrium when free- and bound-species implicated in the equilibrium have different rotational speeds.

The calcium ion (Ca^{2+}) is an ubiquitous second messenger that is crucial for the regulation of a wide variety of cellular processes implicating many Ca^{2+}-binding proteins, among which calmodulin (CaM) is the best studied in the eukaryotic cell. CaM binds to a variety of cellular proteins regulating their activities and is thus implicated in many pathological processes of disease such as inflammation, ischemic neuronal death, Alzheimer, cancer, diabetes, cardiomyopathy and changes in behaviors.

We have developed a FP-high-throughput screening (FP-HTS) assay for CaM, using synthetic CaM (SYNCAM)[1] and specific mutants[2] (namely Tryptophan and electrostatic mutants). Screening a fluorescent chemical library composed of 1328 lissamine-tagged compounds allowed us to retrieve four probes that bind to CaM in a Ca^{2+}-dependent manner. This primary FP-HTS provides us with a tool for screening the patrimonial library (5920 compounds) by FP-based competitive binding assay in order to isolate small specific molecules[3], which were characterized by anisotropy titration (IC_{50}<1µM).

The selected molecules could be starting points to develop new therapeutic drugs acting on calmodulin.

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Dysfunction of huntingtin-protein interactions in the pathogenesis of Huntington's Disease

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Our group is interested in pathomechanisms involved in Huntington's Disease (HD), a neurodegenerative disorder caused by an expansion of CAG repeat coding for a polyglutamine stretch (polyQ) in the huntingtin (htt) protein. PolyQ expansion confers toxic properties to mutant htt, which displays abnormal interaction with its partners, and forms nuclear aggregate structures in neurons. We previously showed that these anomalies induce neuronal stress and gene deregulation, leading to dysfunction prior to cell death. Mutant htt displays aberrant interactions with two regulators of the endoplasmic reticulum Ca2+ stock, Hap1 and the Ca2+ release channel-receptor InsP3R1. As a consequence, this Ca2+ channel becomes more sensitive to its ligand InsP3 - produced upon glutamate stimulation - leading to intracytoplasmic Ca2+ overload. Using a bioinformatic approach, we recently found that another Ca2+ release channel-receptor, RyR (ryanodine receptor), possesses a domain, with a strong sequence homology to the domain of InsP3R1 that interacts with htt and Hap1. Neurons express 3 different RyRs that are also indirectly regulated by glutamate. Mutant htt might thus interact with and alter the function of RyRs.

The project aims at determining whether functional deregulation of RyRs is involved in the pathogenesis of HD. As a first step the interaction properties between htt, Hap1 and RyRs will be studied in vitro using recombinant proteins and techniques, such as classical GST pull down and gel filtration chromatography. Importatly, the influence of polyQ length of htt in its interaction with partners will be studied by surface plasmon resonance on purified proteins.

These first steps will provide adequate background to study subsequent physical and functional interactions in vivo between htt, Hap1 and RyRs and to determine if mutant htt causes deregulation of RyR function leading to neuronal death induced by Ca2+ overload.

Comparison of the chaperone activities of the HIV-1 and the MoMuLV nucleocapsid proteins.

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The retroviral nucleocapsid proteins (NCs) are small basic proteins that contain either one or two copies of a highly conserved C-X_2-C-X_4-H-X_4-C zinc finger motif. The two-zinc-fingers-containing HIV-1 NCp7 protein chaperones the two obligatory strand transfers during reverse transcription. The first DNA strand transfer relies on the destabilization by NCp7 of the double-stranded segments of the transactivation response element, TAR sequence, and the complementary cTAR sequence. NCp7 activates the transient opening (fraying) of cTAR terminal base pairs, leading to a partial melting that propagates up to the middle of cTAR stem. To further understand the structural determinants of the chaperone activity of NC proteins, our aim was to compare, by fluorescence spectroscopy and fluorescence correlation spectroscopy (FCS), the binding and destabilizing activities of NCp7 with those of the one-zinc-finger-containing NCp10 of Moloney murine leukaemia virus. NCp10 was found to promote the melting of cTAR to a larger extend than NCp7, and to increase more importantly than NCp7 the kinetics of cTAR fraying. The chaperone activity of NCp10 was found to be mediated by the unique zinc finger motif and part of its flanking domains. This is in contrast to NCp7 where the chaperone activity relies only on the central zinc finger domain. In the future, new drugs against HIV-1 will be designed based on a single finger model.

Endothelium-dependent relaxations to acetylcholine are impaired whereas those to red wine polyphenols are maintained in the aorta of PPARalpha KO mice: role of reactive oxygen species

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Objective: The endothelium plays a key role in the maintenance of vascular homeostasis, in part, by the release of nitric oxide (NO), which prevents pro-inflammatory and pro-atherogenic responses and helps to maintain blood fluidity. Peroxisome proliferator activated receptor alpha (PPARalpha) regulates the expression of several proteins involved in inflammation, atherogenesis, and thrombosis. Therefore, the aim of the present study was to evaluate the role of the PPARalpha in the endothelial formation of NO

Methods: PPARalpha knockout (KO) and wild type (WT) male (12-14-week old) mice were studied. Aortic rings were suspended in organ chambers for the measurement of changes in isometric tension. The in situ formation of reactive oxygen species (ROS) by aortic segments was assessed using dihydroethidine (DHE) by confocal microscopy.

Results: The concentration-dependent relaxations of intact rings to acetylcholine, were significantly reduced in PPARalpha KO mice compared to WT mice, whereas those to red wine polyphenols and sodium nitroprusside assessed in the presence of NG-nitro-L-arginine were similar. Relaxations to acetylcholine were not affected by indomethacin but significantly increased by MnTMPyP plus PEG-catalase in PPARalpha KO mice. An increased DHE staining was observed in aortic sections from PPARalpha KO mice compared to WT mice, and this signal was abolished by MnTMPyP and by PEG-catalase.

Conclusions: These results indicate that acetylcholine-induced endothelium-dependent relaxations are impaired in PPARalpha KO mice and that this is due to an excessive vascular formation of reactive oxygen species.

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Insights into a 55 nucleotides stem-loop structure and dynamics using a fluorescent structural probe

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Local changes in the structure and dynamics of DNA are assumed to guide the protein-DNA interactions involved in many cellular processes. Probing DNA structures and dynamics is thus of major interest to further characterize DNA-proteins molecular interactions. Fluorescence spectroscopy offers an elegant opportunity to probe in detail these localized and highly-dynamic behaviours. 2'-deoxyribosyl-2-aminopurine (2AP), a fluorescent nucleoside-analogue, highly sensitive to its micro-environment and which minimally disturbs the structure and functions of native DNA, can substitute adenosines at selected positions in oligonucleotides. The 55 DNA nucleotide cTAR, the DNA complementary sequence of TAR in HIV-1, has been selectively modified in different positions with 2AP in order to investigate its stem-loop structure and dynamics. The thermodynamic stability of the different double-stranded segments of the stem was ascertained from their temperature-induced melting transitions, providing a detailed map of the local stabilities of cTAR stem. This also allowed evidencing the unexpected "looped-out" distal bulged conformation. Time-resolved experiments revealed an heterogeneous environment of all the different 2AP positions along the stem with four lifetimes ranging from <50ps to ~10ns. The latter were interpreted as due to distribution of partially stacked structures with varying dynamics.

Based on these data, this low-perturbing structural fluorescent probe clearly appears to be an interesting tool in the study of DNA-proteins molecular interactions. One obvious application could be the HIV-1 viral RNA-chaperone protein NCp7 which exhibits a highly dynamic behaviour while binding.

Prokineticin receptor-1 (GPR73) promotes cardiomyocyte survival and angiogenesis

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Prokineticins are potent angiogenic factors that bind to two G protein-coupled receptors to initiate their biological effects. We hypothesize that prokineticin receptor-1 (PKR1/GPR73) signaling may contribute to cardiomyocyte survival or repair in myocardial infarction. Since we showed that prokineticin-2 and PKR1 are expressed in adult mouse heart and cardiac cells, we investigated the role of prokineticin-2 on capillary endothelial cell and cardiomyocyte function. In cultured cardiac endothelial cells, prokineticin-2 or overexpression of PKR1 induces vessel-like formation without increasing VEGF levels. In cardiomyocytes and H9c2 cells, prokineticin-2 or overexpressing PKR1 activates Akt to protect cardiomyocytes against oxidative stress. The survival and angiogenesis promoting effects of prokineticin-2 in cardiac cells were completely reversed by siRNA-PKR1, indicating PKR1 involvement. We thus, further investigated whether intramyocardial gene transfer of DNA encoding PKR1 may rescue the myocardium against myocardial infarction in mouse model. Transient PKR1 gene transfer after coronary ligation reduces mortality and preserves left ventricular function by promoting neovascularization, and protecting cardiomyocytes without altering VEGF levels. In human end-stage failing heart samples, reduced PKR1 and prokineticin-2 transcripts and protein levels implicate a more important role for prokineticin-2/PKR1 signaling in heart. Our results suggest that PKR1 may represent a novel therapeutic target to limit myocardial injury following ischemic events.

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Optimisation de composés de la famille des triazines comme inhibiteurs de l'entrée du VIH-1 dans les cellules hôtes

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Les méthodes actuelles de traitement du SIDA visent à inhiber la réplication du virus après qu'il ait infecté les cellules et intégré son patrimoine génétique au génome de l'hôte. Ces traitements, bien que très efficaces, ont cependant des capacités limitées pour éliminer des réservoirs viraux qui persistent. L'expérience montre que le bénéfice thérapeutique optimal est obtenu par l'action en synergie de plusieurs agents thérapeutiques agissant à des niveaux différents du cycle viral dans la cellule. Un traitement axé sur l'inhibition de l'entrée du virus dans les cellules hôtes représente une voie de choix pour compléter l'arsenal actuel.

Nous avons criblé une collection de 10.000 molécules parmi lesquelles nous avons pu identifier une famille de composés « triazine » dont les membres sont soit inhibiteurs dans un essai de fusion cellulaire qui mime le processus d'infection par le virus, soit agonistes du co-récepteur CCR5 du VIH ou encore se lient au récepteur CXCR4 et empêchent de cette manière la liaison de la chimiokine naturelle CXCL12. Le motif chimique identifié se distingue des autres familles de molécules non-peptidiques déjà connues, ciblant les corécepteurs CCR5 et CXCR4. De plus, nos molécules présentent une propriété originale pour des composés non peptidiques : celle d'activer le récepteur CCR5 et donc probablement de pouvoir provoquer son internalisation, ce qui conduirait à la disparition du récepteur de la surface cellulaire.

Nos travaux actuels consistent à optimiser ces molécules pour en faire des agents pharmacologiques originaux capables de provoquer l'internalisation de l'un et/ou de l'autre des deux co-récepteurs du VIH (CCR5 et CXCR4). Si de plus, nous parvenions à leur faire perdre complètement ou partiellement leur activité agoniste sur ces récepteurs, il sera possible de minimiser les effets indésirables que pourraient provoquer ces composés. Nous obtiendrions ainsi des molécules qui, faisant disparaître le co-récepteur de la surface cellulaire, opposeraient au processus d'infection par le VIH, un obstacle supplémentaire.

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Identification and caracterization of amphiphyin 2 splicing isoforms in myotonic dystrophic patients

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With one individual affected out of 8000, myotonic dystrophies (DM) are the most common myopathies in adults.

DM patients suffer from muscle weakness, myotonia, cardiomyopathies, cataracts, insulin resistance and mental disorders.

These diseases are due to expanded CTG or CCTG repeats in the DMPK and ZNF9 loci. When transcribed, the expanded CUG or CCUG repeats fold and aggregate into nuclear foci, which sequester the MBNL splicing factor.

Consequently, myotonic dystrophic patients show alternative splicing misregulation of some targets responsible for the disease condition. Notably, aberrant alternative splicing of the chloride channel and insulin receptor, lead to myotonia and insulin resistance respectively. However, the cause of dystrophy is not known yet.

Using a microarray approach (Exon array, Affymetrix), we have found another mRNA target, amphiphysin 2, which splicing is misregulated in DM patients.

Amphiphysin 2 is implied in the biogenesis of T-tubules which are invaginations of the cytoplasmic membrane of muscle cells, essential for muscle contraction. Furthermore, an amphiphysin 2 knock-out mouse model shows a dystrophic phenotype.

By RT-PCR, we confirmed an alteration of amphiphysin 2 exon 7, 11 and 17 alternative splicing in a cohort of DM patients.

We are currently performing an *in cellulo* study of amphiphysin 2 alternative splicing, and finding the binding sites of some splicing factors by UV cross-linking to understand the causes of splicing alteration. Finally, we are leading functional studies to understand the consequences of amphiphysin 2 alternative splicing misregulation in muscle cells from DM patients.

Analysis of the mechanisms of notch pathway activation in T lymphomas from Ikaros-deficient mice.

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Ikaros is a transcription factor exclusively expressed in the hematopoietic system. It has been implicated in the regulation of chromatin remodeling between active and inactive states. Ikaros is also a tumor suppressor: by three months of age all mice homozygous for a hypomorphic mutation in Ikaros ($Ik^{L/L}$) develop thymic lymphomas. The data obtained in our laboratory suggest a fundamental role for the notch pathway in the leukemogenesis of $Ik^{L/L}$ mice. Upon Notch receptor binding to Notch ligands expressed on neighboring cells, the intracellular fragment of Notch (NIC) is cleaved by metalloproteases and γ-secretase, and translocates to the nucleus to induce target gene expression. All $Ik^{L/L}$ tumors exhibit strong activation of the Notch pathway, including high levels if NIC. Importantly, we have found C-terminal truncations of Notch1 similar to those found in human T-ALL, suggesting a conserved mechanism of Notch1 mediated oncogenesis between humans and mice. This truncation contributes to the stability of Notch1.

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Role of Chromatin Remodelling Factors During Trans-Differentiation in *C. Elegans* : an *in vivo* Approach

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Trans-differentiation is the irreversible conversion of a terminally differentiated cell type into another (1) with or without an intermediate dedifferentiated stage or cell division. This aspect of cell plasticity is poorly understood and has been studied mostly *in vitro,* primarily because of the lack of a simple *in vivo* model. Trans-differentiation occurs in nature in a few specific cases like during organ regeneration in Amphibian, but tools to systematically address the mechanisms involved are lacking. Here we describe a novel *in vivo* system for unraveling the molecular mechanisms underlining this process using *C. elegans* as a model. Because the cellular lineage of the worm has been determined (2), and genetic screens can be performed targeting a single specific cell, the nematode represents an ideal system to study this process.

We primarily focus on 1 example of trans-differentiation that happens in absence of cell division : the case of the Y cell. The Y cell is an epithelial cell that forms part of the rectum, a vital organ, at the end of the embryogenesis. During the normal development of the worm (precisely during the second larval stage called L2), this Y epithelial cell migrates and transforms into a motor neuron named PDA.

We have postulated that certain genes activities can be a priori predicted to affect such cellular plasticity event. As epigenetic modifications have been involved in the maintenance of the cellular identity, we decided to conduct a targeted RNAi screen against the *C. elegans* chromatin remodelling genes. We first built a list of candidates using the available litterature and performing protein domain analysis. This led to the identification of 304 candidates. From 259 genes screened so far by Nomarsci optics for a defect in the Y to PDA identity change, 30 positive hits were found. We now are in a process of confirming them in a secondary RNAi screen using a PDA::GFP marker, characterising the mutant phenoype obtained and examine their potential interactions, as well as their known partners. We will report on our progress at the meeting.

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AMP-activated protein kinase is involved in both NO- and EDHF-mediated endothelium-dependent relaxations to red wine polyphenols

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Introduction : Several epidemiological studies have shown that regular consumption of moderate amounts of wine, in particular 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, which are potent vasodilators and have anti-thrombotic properties. Polyphenols have been shown to induce pronounced endothelium-dependent relaxations of arteries by causing the redox-sensitive PI3-kinase-dependent 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)-induced endothelial formation of NO and EDHF.

Methods : Rings of aorta and mesenteric arteries from 12 to 14 weeks-old male Wistar rats, and of porcine coronary arteries were suspended in organ chambers for recording of changes in isometric tension. Cultured porcine coronary artery endothelial cells were used to study the phosphorylation level of endothelial NO synthase (eNOS) at serine 1179, and AMPK at the threonine 172 by Western blot analysis. RWPs were prepared from a french red wine from the southwest containing 2.9 g/L polyphenols expressed as gallic acid equivalents.

Results : 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^G-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. Moreover, RWPs induced the phosphorylation of AMPK at threonine 172 and eNOS at serine 1179 in endothelial cells within 10 minutes.

Conclusion : The present findings indicate that RWPs cause both NO and EDHF-mediated relaxations in several types of isolated arteries and that these effects are dependent on the activation of the AMP-activated protein kinase pathway.

A Series of Fluorescent Acylcholine Analogues with Optimized Pharmacological and Spectroscopic Properties to Probe the Mechanism of *Torpedo* Nicotinic Acetylcholine Receptor

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We have synthesized a series of fluorescent acylcholines with different linker length and structure between the acetylcholine unit and the environment-sensitive fluorophores 7-diethylaminocoumarin-3-carbonyl (DEAC) or N-(7-nitrobenz-2-oxa-1,3-diazol-yl) (NBD). Pharmacological, binding and fluorescence properties of all these analogues are investigated and results are compared to already described fluorescent acylcholine analogues[1; 2]. Their action as agonist depends on the length and on the structure of the linker and increases with increasing linker length. One particular analogue, DEAC-Gly-C6-choline, shows partial agonist behavior (about 50 %), which is at least 20 times higher as observed for described fluorescent Dansyl[1]- and NBD[2]-acylcholine analogues. Binding and inhibitor constants are in the nano- and micromolar range for the high and low affinity states of the receptor, respectively as determined by fluorescence intensity and radio-detection. Upon binding of DEAC-Gly-C6-choline to *Torpedo* nicotinic acetylcholine receptors (nAChR) a 10-fold enhancement of fluorescence is observed. Both, pharmacological and spectroscopic properties of this analogue indicate great potential use in studying the mechanism of *Torpedo* nAChR and in competitive drug screening of chemical libraries.

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Conception et Synthèse de Nouveaux Anti-inflammatoires et Analgésiques

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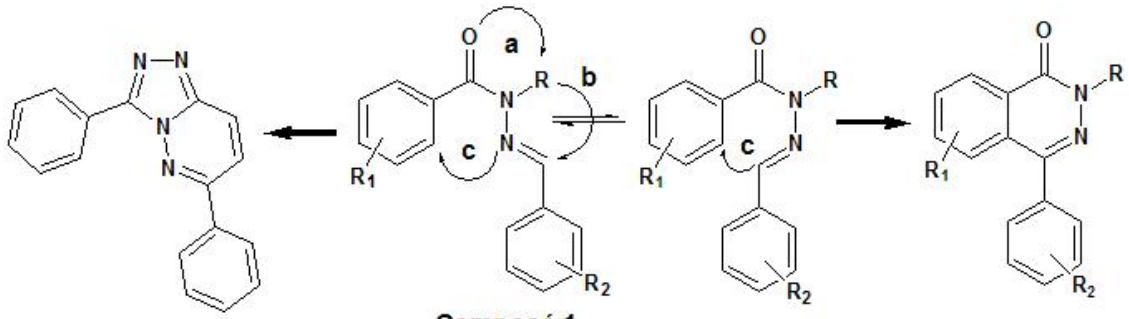
Parmi les nombreux axes thérapeutiques, la recherche de nouveaux anti-inflammatoires constitue un vrai problème de santé publique aujourd'hui, en particulier pour une population vieillissante et de plus en plus sensible aux problèmes d'allergie et de sensibilisation à la pollution environnante. Il y a en particulier un fort intérêt pour de nouvelles cibles intégrées dans une cascade d'évènements moléculaires localisés en surface ou à l'intérieur des cellules du système immunitaire. Parmi les nouvelles cibles étudiées de manière intensive actuellement, les inhibiteurs d'AMPcyclique phosphodiestérases de type 4 (PDE4) figurent parmi les classes thérapeutiques les plus prometteuses pour le traitement de l'asthme, de l'arthrite rhumatoïde ou la maladie de Crown. De plus, lorsque les composés ont un tropisme central, ces composés pourraient avoir des propriétés neuro-anti-inflammatoires importantes et permettre d'envisager le traitement de maladies comme la sclérose en plaque. Notre stratégie générale consiste à conservoir et synthétiser des inhibiteurs puissants et sélectifs de PDE4 qui pourraient aussi agir sur une autre cible impliquée dans la réponse inflammatoire des cellules concernées, et se traduisant par la production de cytokines inflammatoires, en particulier le TNFα.

Ce projet de recherche de nouveaux anti-inflammatoires s'inscrit dans le cadre d'une collaboration entre l'Université de Rio et celle de Strasbourg. A Rio nous avons développé une chimiothèque ciblée autour des N-acylhydrazones (composé 1). Certains de ces composés ont montré une activité analgésique intéressante sur le modèle de la douleur induit par l'acide acetique chez la souris. Ces dérivés ont constitué nos chef de file et nous avons conçu et synthétisé de nouveaux types hétérocycles susceptibles de mimer les N-acylhydrazones (schéma 1). Ces constructions font appel aux techniques de couplage palladium et l'utilisation du micro-ondes.



Composé 1

Inflammation cutanée et Borréliose de Lyme : (2) analyse de l'immunité innée des cellules résidentes de la peau dans deux modèles *in vitro*.

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La borréliose de Lyme est une infection bactérienne, transmise à l'homme par piqûre de tiques du genre *Ixodes* spp. La maladie sévit principalement dans l'hémisphère Nord où elle représente la maladie à transmission vectorielle la plus fréquente. Après une inflammation cutanée locale au point de piqûre de la tique, elle se manifeste par des atteintes disséminées principalement neurologiques, cutanées et articulaires. En Europe, la maladie est due à plusieurs espèces bactériennes : *Borrelia burgorferi* sensu stricto (arthrites), *B. afzelii* (manifestations dermatologiques) et *B. garinii* (manifestations neurologiques).

L'immunité innée joue un rôle essentiel au niveau de la peau dans le contrôle des infections. La sécrétion de peptides antimicrobiens (AMPs), petites molécules cationiques à large spectre antimicrobien, est clairement démontrée lors d'infections cutanées bactériennes, mais n'a pas été étudiée dans la borréliose de Lyme. Nous avons mis au point deux modèles *in vitro* de kératinocytes humains primaires d'une part, et de fibroblastes humains primaires d'autre part, afin d'analyser l'interaction de *B. burgdorferi* avec ces cellules résidentes. L'induction de molécules de l'immunité innée, notamment des cytokines (IL-6 et IL-8) et des peptides antimicrobiens, défensine et cathélicidine, a été mesurée par ELISA et RT-PCR afin de quantifier la réponse inflammatoire.

Dans ce travail, nous mettons en évidence la sécrétion d'IL-6, d'IL-8, de défensine et à un moindre degré de cathélicidine par les kératinocytes. La réponse inflammatoire est beaucoup moins importante pour les fibroblastes. Puis nous avons analysé si une protéine majeur de surface de *Borrelia*, OspC, essentielle pour l'initiation de la maladie chez l'hôte vertébré, pouvait être responsable de cette inflammation. Ce lipopeptide induit une réponse inflammatoire similaire à la bactérie entière.

L'inflammation cutanée locale pourrait être inhibée localement par la salive de tique, connue pour ses propriétés immunomodulatrices, expliquant ainsi la multiplication des bactéries au niveau cutané puis leur dissémination chez l'hôte infecté, expliquant ainsi les manifestations cliniques systémiques.

Inflammation cutanée et Borréliose de Lyme : (1) analyse du rôle immunomodulateur de la salive de tique sur l'immunité innée de souris C3H.

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Les maladies infectieuses à transmission vectorielle représentent un problème majeur de santé publique. La transmission des micro-organismes pathogènes par piqûre hématophage d'acarien ou d'insecte, est un élément clef de la maladie se traduisant le plus souvent par une inflammation cutanée locale avant les premiers symptômes cliniques systémiques. Nous avons pris pour modèle la borréliose de Lyme afin d'analyser l'inflammation cutanée au point de piqûre du vecteur. Cette maladie est une infection due à des bactéries du groupe *Borrelia burgdorferi*, sévissant principalement dans l'hémisphère Nord et transmise à l'homme par piqûre de tiques du genre *Ixodes* spp.. Lorsqu'une tique infectée par *Borrelia* pique l'hôte vertébré, il se développe dans la plupart des cas, au point de piqûre une inflammation cutanée, l'érythème migrant. Cette réaction inflammatoire cutanée initiale joue un rôle essentiel dans le développement de la maladie qui se manifeste ensuite par différentes atteintes, notamment articulaires (*B. burgorferi* sensu stricto), cutanées (*B. afzelii*) et neurologiques (*B. garinii*).

La tique, est connue pour jouer un rôle immunosuppresseur sur l'immunité de l'hôte, notamment sur l'immunité adaptative (inhibition de la prolifération lymphocytaire). Son effet sur l'immunité innée est moins bien documenté. Plusieurs études ont montré que la salive de la tique ou du moustique augmente le pouvoir de virulence des pathogènes inoculés. La modulation de l'immunité innée par la salive de la tique pourrait constituer une stratégie efficace mise en place par la bactérie afin d'échapper à la réponse immunitaire de l'hôte vertébré.

Afin d'élucider la nature de l'inflammation cutanée au point de piqûre et afin d'analyser le rôle de l'immunité innée, nous avons établi plusieurs modèles expérimentaux d'infection chez des souris C3H : inoculation de *Borrelia* à la seringue ou via des tiques infectées. Par RT-PCR quantitative en temps réel, nous avons analysé la réponse inflammatoire au point de piqûre au cours du temps. Certains effecteurs importants de l'immunité innée ont été sélectionnés pour cette étude dont les peptides antimicrobiens, défensine (mBD3) et cathélicidine (CRAMP).

Is *Fusarium venenatum* a candidate for phytase production?

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Phytate (inositol hexaphosphate) is the stocking molecule in plants. Unfortunately, monogastric animals (pigs, poultry and fishes) are unable to use untransformed phytate to fit their phosphate requirements. Furthermore phytate is rejected in large quantities by these animals in environment, leading to soil enrichment and eutrophysation. In human populations with exclusively plant-based alimentation, the propensity of phytate to bind many ions may cause severe deficiency in these essential elements.

Addition of phytases (enzymes hydrolysing phosphoester bonds) in the food ration is the most common way used to reduce these poor assimilation problems. Phytases are produced by bacteria and fungi. There are several classes essentially depending on the phosphoester bond cut first. Phytases may hydrolyse successively several bonds or not. For better efficiency, it would be interesting to find an enzyme able to rapidly liberate the six phosphate groups or to produce a mix of phytases as efficient.

Fusarium graminearum is the model fungus studied in the laboratory. Its genome sequence has been released and allows the characterisation of seven putative phytases, apparently belonging to several classes. As *F. graminearum* is a phytopathogenic fungus (class II), it is not recommended for food processes. This is the reason why, a very close non pathogenic relative, *Fusarium venenatum*, was investigated for phytase production.

F. venenatum was cultivated on several media where phytate was the only phosphate and/or carbon source. Phytase activities were detected in several culture supernatants. Concentrated supernatants loaded on SDS-PAGE display complex profiles. In order to identify the proteins in supernatants, mass spectrometry experiments were performed. To date, one peptide, belonging to a protein similar to one of the seven putative phytases from *F. graminearum*'s genome was found. Data analysis is under way to determine the actual ability of *F. venenatum* to secrete phytases.

Variation in the activity of cyclic nucleotide phosphodiesterases (PDEs) in rat left cardiac ventricles (LV) after chronic treatment by angiotensin II (Ang II)

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The renin-angiotensin system actively participates to the development of heart failure. PDEs regulate cardiac function by specifically hydrolysing cyclic nucleotides. The objective was to evaluate the implication of PDEs in heart failure induced by chronic treatment of rats with Ang II.

4 male rats of 12-week age were treated during 14 days by Ang II (0.4 mg/kg/d) using osmotic mini-pumps. Control group (6 rats) received saline solution. LV were removed, frozen, powdered individually in liquid nitrogen. Resulting homogenates were kept at -80°C. Protein concentration and enzymatic activity were measured on LV aliquots.

The cAMP- and cGMP-PDE isozyme activities were measured by a radioenzymatic assay developped in our laboratory using the following specific inhibitors : nimodipine (PDE1), EHNA (PDE2), and DMPPO (PDE5) for cGMP-PDE activity; cilostamide (PDE3) and rolipram (PDE4) for cAMP-PDE activity. Results are expressed as specific activity (pmol/min/mg of protein). Statistical analysis was performed using Student two-tailed-*t* test, with $P<0.05$ considered as significant.

Ang II induced the development of cardiac hypertrophy ($P=0.017$) and arterial hypertension (+54 mm Hg, $P=0.001$), without tachycardia. cAMP-PDE activity was 2.6 times higher than cGMP-PDE activity in control rats. These activities were mainly and respectively due to PDE4 and PDE2.

In Ang II-treated rats, PDE4 activity was increased (+20%, $P=0.0004$). A specific inhibitor of PKA, H89 (1μM), did not affect cAMP-PDE activity in control rats, but decreased the activity in Ang II-treated rats (-22%, $P=0.005$). cGMP-PDE1 (+167%, $P=0.012$), PDE2 (+85%, $P<0.0001$) and PDE5 (+100%, $P=0.002$) activities were increased significantly.

Cardiac hypertrophy induced by Ang II is mainly associated with increased PDE2 and PDE4 activities. Increased PDE4 activity could result of a PKA-dependent phosphorylation. These findings suggest that cAMP and cGMP are implicated in the cardiac effects induced by Ang II.

In vitro Flavonoids effect on an intestinal inflammatory cellular model.

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Background and Objective: Drug pharmacokinetics parameters are dependent on membrane transporters expressed at the apical and basolateral side of epithelial cells in various tissues. In intestinal epithelial cells, several efflux transporters are expressed e.g. ABCB, ABCC, ABCG2 as well as several uptake influx transporters such as OATP2B1 and PEPT1. Our objective here is to evaluate a new *in vitro* cellular model of intestinal inflammation for the:
i) Impact of intestinal inflammatory diseases on the absorption mechanism across the intestinal epithelial cells implying influx and efflux membrane carriers
ii) Anti-inflammatory effect of a few flavonoids in Caco-2 inflammation model and their impact on Crohn's disease (CD).
Design: Caco-2 cells were activated by lipopolysaccharide from E.Coli (LPS) at 10µg/ml during 24h cell culture. We examined the drug intestinal transport on this inflammatory model by incubating test molecules through Caco-2 cells from apical (A) to basolateral (B) side during 120 min followed by liquid chromatography determination. The passive transcellular transport was evaluated by incubating propranolol. The P-gp activity was studied by incubating Rhodamine 123 (Rho123), a P-gp specific substrate, with or without P-gp inhibitors (verapamil or PSC833).
We studied anti-inflammatory properties of 4 flavonoïds (quercetin, naringenin, ellagic and gallic acids) on this *in vitro* model by incubating them at different concentrations (10µM at 100 µM) after LPS activation during 24h.
Main Outcome Measures: The inflammation phenomenon was evaluated by ELISA measurement of an interleukin IL-8. The intestinal transport was estimated by computing the apparent permeability coefficient (Papp).
Results: First, our results emphaze that intestinal inflammation increases the drug passive transport and the ABCB dependent efflux transport. Secondly, at 50 µM, the 4 flavonoïds decreased IL8 production in Caco-2 cells induced by LPS.
Conclusion: Flavonoids anti-inflammatory effect (i.e. inhibition of IL-8 production) associated to their antioxidant properties should be of interest in Crohn disease. Moreover, the understanding of the ability of these molecules to normalize the expression or the activity of membrane carriers (altered in inflammatory diseases) will help to get a better knowledge of those mechanisms and so help to develop new therapeutic strategies in CD.

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Interaction between two statins (simvastatin and pravastatin) and food flavonoids in intestinal absorption: *in vitro* study and clinical relevance

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Background and Objective: Statins, i.e. HMG CoA reductase inhibitors, are used to lower elevated cholesterol. They are characterized by low oral bioavailability imputable to an important intestinal or hepatic first pass effect. However many membrane transporters, in particular, efflux transporters such as Pgp (ABCB1) or MRP-2 (ABCC2), are also involved in such low oral bioavailability. Flavonoids are natural substances found in abundance in plants, fruits and vegetables. They are known to interfere with cytochrome P-450 isoenzymes involved in the drug metabolism (CYP1A2, CYP2C9, CYP2E1, CYP3A...) and to modulate some membrane transporters involved in drug pharmacokinetics. The objective of our work was to study *in vitro* the modulation by different flavonoids on intestinal permeability and bioavailability of these two statins and their possible influence on their clinical effect.
Design: We examined the transcellular transport of two statins from apical (pH 6.5) to basal side (pH 7.4) by incubation with human colonic carcinoma cell line (CaCo2) in presence or absence of various flavonoids belonging to different chemical classes (naringenin, quercetin, luteolin, gallic and ellagic acids) at 100µM and this during 30, 60 and 90 min.To explain the mechanisms involved, the two statins were incubated in presence of membrane transport proteins inhibitors such as P-gp inhibitors (PSC 833 and verapamil), MRP2 inhibitor (probenecid) and OATP inhibitor (Estrone 3-sulfate).
Setting: The Caco-2 cells were seeded in Transwell support on artificial membrane. The transcellular transport was evaluated by measuring by liquid chromatography the statin concentrations after incubation in different conditions.
Main Outcome Measures: The results are expressed by computing the apparent permeability coefficient from apical to basolateral side (Papp AB).
Results: The Papp AB of two statins was increased with flavonoids: Gallic and Ellagic acid increased both statins Papp 3 times. Simvastatin intestinal permeability was increased 5 times by naringenin and 4 times by quercetin. However, pravastatin Papp was increased 5 times by quercetin and only 3 times by naringenin. Moreover, Simvastatin is a Pgp and MRP substrate but not OATP substrate like pravastatin.
Conclusions: The 5 tested flavonoids all increased intestinal permeability of statins. These observations should be taken into account when statins are co-administered with vegetal polyphenols in order to modify pharmacokinetics and, consequently, statins safety profiles. Our results emphasize and support a new model of intestinal drug absorption and mechanism of food drug interaction.

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Bioactive constituents of sea fennel (*Crithmum maritimum* L.)

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Crithmum maritimum L. (Apiaceae) is a perennial littoral halophyte, thriving on rocky coasts. Besides its obvious interest as a naturally salt-tolerant plant, the species shows considerable economical and medicinal potentials.

Salt-tolerance mechanisms are quite complex, including osmotic adjustement, compartmentation of toxic ions, metabolite accumulation, ion homeostasis, redox control and scavenging of activated oxygen species (AOS) [1, 2]. Previous studies has demontrated that the salt stress increased the activities of antioxidant enzymes (superoxide dismutase, catalase and peroxidase) present in *C. maritimum* [3].

Our aim is to investigate its bioactive constituents which can also explain its exceptionnal salt-tolerance.

Our *in vitro* studies results shown that compounds obtained from dichloromethane and ethyl acetate extracts derived from ethanol 60% aerial part extract of *C. maritimum* exhibit good anti-inflammatory and radical scavenging activities, respectively.

The identification by HPLC UV, NMR, MS of the constituents present in the active extracts shown that they consist mainly of phenolic acids and flavonoids in the ethyl acetate extract, and coumarins, terpenes and polyacetylenes in the dichloromethane extract.

Thus, the great diversity of these bioactive substances could explain the adaptability of sea fennel to hostile biotopes.

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Search for nitric oxide release inhibitors from plant extracts: Development of a miniaturized model

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During inflammatory reactions, nitric oxide (NO) produced by inducible nitric oxide synthase (iNOS), in cells such as macrophages, hepatocytes, and renal cells act as a defense and regulatory molecule with homeostatic activities. However, when excessively produced, NO, as a reactive radical, directly damages functional normal tissues[1]. Moreover, it can also react with superoxide anion radical to form the even stronger oxidant peroxynitrite ($ONOO^-$) which is more toxic to biological systems than $O2^-$ or NO by causing a modification of proteins or nucleic acids[2]. So, the inhibition of their over expression may be of therapeutic interest.
Our laboratory is interested in understanding the role of bioactive plant compounds on the inhibition of NO production by macrophages. We aim to screen such compounds to find new anti-oxidants.

For this purpose we set up the experimental conditions in a cell culture model using a 96-well plate format compatible with a robotised platform.
This poster will show our first results.

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A Forward Genetic Screen to Identify Genes Affecting the Trans-Differentiation of an Epithelial Cell into a Motor-Neuron

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It is widely accepted that after fertilization cells are persuaded to make series of decisions in relation to external signals that progressively restrict their developmental options until they are finally differentiated. The conversion of a differentiated cell into another kind of differentiated cell, called "trans-differentiation" is less understood. Indeed, no trans-differentiation event occurring during natural developmental programs has been clearly demonstrated so far, eventhough trans-differentiation events have been shown to occur in different organisms (for example during the regeneration of mutilated appendages in amphibians).

The lineage of the nematode *C. elegans* has identified a few cells that change their fates as the worm develops, but this interesting observation has never been studied further. The most striking example relays in a epithelial cell named "Y". This cell forms part of the rectum since the worm hatches but, during the larval stage 2 (L2), it becomes a neuron called PDA without cell division. We have first demonstrated that Y is a fully differentiated epidermal cell. This allows us to affirm that the change in fate of Y has the hallmarks of a natural trans-differentiation process.

Our goal is to understand the cellular and molecular mechanisms that control this trans-differentiation process *in vivo*. We propose to identify and analyze the genes and the genetic cascades that allow the trans-differentiation of the Y cell. For that purpose we have created transgenic animals expressing Green Fluorescent Protein (GFP) in PDA. Using these strains we have performed genetic screens. We have selected animals in which the fluorescent PDA was not observed. This strategy allowed us to identify mutant worms in which the Y to PDA trans-differentiation does not take place. We are currently mapping these mutations in order to identify the key players of this trans-differentiation process.

Anthraquinones from *Vismia laurentii (Guttiferae)* induce apoptosis in colon cancer cells

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The group of Dr C.D. Muller has recently developped a fast and accurate high content screening assay for monitoring apoptosis, investigating the biological activity of molecules on living adherent cell lines [1]. Apoptosis (programmed cell death) 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, ischemia, neurodegeneration and cancer. Specific targets and pathways are known to be involved in this process. 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 assay is dramatically efficient for drug discovery and development.

We evaluate here the capacity of plant extracts containing bioactive natural substances to induce apoptosis and/or necrosis on the human colon (Caco-2) carcinoma cell line. One of the hit obtained, isolated from an apolar extract of the stem bark of *Vismia laurentii*, shows at 25 µg/ml up to 95% apoptosis induction on Caco-2 cells. Moreover the bioguided isolation of its constituents led to the identification of a new tetracyclic triterpene, (20-ethylnortirucalla-7,24-dien-3-one) vismiaturucallone (1), along with eight known compounds namely : 3-geranyloxyemodin (2), vismiaquinone A (3), vismiaquinone B (4), bivismiaquinone (5) epifriedelinol (6), betulinic acid (7), tirucalla-7,24-dien-3-one (8), and stigmasta-7,22-dien-3-ol (9). Compounds (2), (3) and (4) in particular, showed strong inhibition of cancer cell proliferation with micromolecular IC50 values comparables to previously described Anthraquinones [2].

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Hight throughput mutation screening in different forms of centronuclear myopathies.

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Centronuclear myopathies (CNM) are rare genetic diseases. Patients usually suffer from muscle atrophy, muscle weakness and sometimes breathing distress. At the histological level, muscle cells have nuclei in central position whereas they are normally in periphery, along the cellular partition. Several forms of CNM exist: the form linked to the X chromosome, different recessive autosomal forms, and the dominant autosomal form. Up-to-now, two genes have been implicated in the CNM: the MTM1 gene, which codes for the phosphoinositides phosphatase myotubularin, and DNM2, which codes the dynamin 2 large GTPase.

CNM are genetically heterogeneous diseases implicating several genes, and the different forms can sometimes display similar clinical severity. Lot of PCR and sequencing could be necessary for one patient to know his genotype. Moreover, new genes remain to be identified, especially for the autosomal recessive forms. We wish to implement a high throughput screening 1) to determine which of the known genes is mutated in patients and 2) to identify new implicated genes among a list of functional and positional candidates. Indeed, a low throughput approach is use to sequencing the DNM2 gene for the moment. This approach consists of the PCR amplification of all exons of the gene which are going to be sequencing. This work is time-consuming and detected mutations are rare because a lot of genes are implicated in CNM. The high throughput screening permits to decrease the number of sequences and the time of handling for each patient. Indeed, the goal of this approach is to automate all the protocol of mutation identification. Robots will be used to make the PCR pipetting, the amplification will be done with a fluorochrome, the LC Green. Then, a high resolution thermal denaturation will be used to screen PCR fragments; only the samples which present a variant will be sequenced.

In this way, we will be able to determine the molecular defect causing the different CNM forms. Furthermore, this high throughput protocol will be applicable to other genetic diseases.

Phosphorylation de NFκB par la kinase MSK1: implication dans l'expression du facteur de croissance des mastocyes, le SCF

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Introduction. L'expression du facteur de croissance des mastocytes, le stem cell factor (SCF), est augmentée dans l'asthme. *In vitro*, l'augmentation d'expression du SCF par l'IL-1β implique les voies des MAP kinases p38 et ERK1/2 ainsi que le facteur de transcription NFκB. L'activité transcriptionnelle de NFκB est régulée par phosphorylation de sa sous-unité p65 (RelA) sur de nombreux résidus sérine, dont la sérine 276. Nous avons étudié l'implication d'une kinase activée par p38 et ERK1/2, MSK1 (mitogen- and stress-activated protein kinase-1), dans l'activation de NFκB conduisant à l'expression du SCF.

Méthodes. L'activité promotrice du SCF a été mesurée dans des fibroblastes pulmonaires humains en conditions inflammatoires mimées par l'Il-1ß après transfection du promoteur du SCF en amont d'un gène codant pour une luciférase. L'implication de MSK1 a été étudiée avec son inhibiteur, le composé H89 (10μM, 1h), par transfection d'un siRNA spécifique de MSK1 ou transfection d'une forme inactive de MSK1 (MSK1 KD). La phosphorylation de p65 a été étudiée par western blotting ou par transfection d'un vecteur p65 sauvage ou muté sur la sérine 276 (S276C). La liaison de NFκB, de MSK1 et du coactivateur CBP au promoteur du SCF a été quantifiée par immunoprécipitation de la chromatine (ChIP).

Résultats. Un prétraitement par l'inhibiteur de MSK1 (H89) réduit l'activité promotrice du SCF induite par l'IL-1ß de 83±14% après 2,5h. La transfection de siRNA spécifique de MSK1 ou la transfection de MSK1-KD réduit l'expression de la protéine du SCF de 67±14% et 63±4% (à 5h), respectivement. Ceci indique que MSK1 est impliqué dans l'expression du SCF induite par l'IL-1ß. De plus, H89 inhibe la phosphorylation de p65 sur la sérine 276 ainsi que la liaison de p65, MSK1 et CBP à l'élément κB du promoteur du SCF. Enfin, l'expression d'une protéine p65 mutée en S276C induit une diminution d'expression du SCF induite par l'IL-1β de 78±2% comparativement à une p65 sauvage. Ceci indique que la phosphorylation de p65 en Ser276 par MSK1 est nécessaire à sa liaison à l'élément κB du promoteur du SCF et à l'augmentation d'expression du SCF en conditions inflammatoires.

Conclusion. Nos résultats montrent clairement que la kinase MSK1 contrôle l'expression du SCF en conditions inflammatoires par phosphorylation de la sous-unité p65 de NFκB sur sa sérine 276 permettant la liaison de NFκB et de son coactivateur CBP au promoteur du SCF.

Analysis of the human stem cell differentiation using DNAchip and quantitative PCR

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As a part of the MYOTmutES research program this work is focused on identifying biomarkers related to the expression of the mutated dystrophin myotonin protein kinase (DMPK) gene occurring in the rare monogenic disease Myotonic Dystrophy (DM1). This study is based on the concept of detecting any molecular, biochemical, functional or morphological markers, for which the expression or activity could be quantitatively or qualitatively altered in cells expressing a mutant gene as well as on the characterization of the cell phenotype(s) and stage(s) of differentiation in which these biomarkers are the most informative.

Therefore a large- scale differential screening of the transcriptome using Affymetrix DNA chips "Human Genome U133 plus 2.0 array" and a transcriptomic analysis concentrated on the subfamily of endo G- protein coupled receptors using quantitative PCR (qPCR), has been carried out for human embryonic stem cells (hES) as well as for Mesenchymal (MSC) and neural precursor cells (NP) in the native and mutated state.

To obtain the most reliable gene expression values as possible, the results of several algorithms available for probe level microarray analysis have been compared to the more sensitive gene expression values received by qPCR, the "gold- standard" in gene expression measurement. All array data were preprocessed in the statistical language 'R' using Bioconductor and nine of the most widespread algorithms (dChip.pm, dChip.mm, Farms, gcRMA, MAS5, mmgMOS, Plier, RMA, VSN). After removing all expression values detected as absent by the MAS5 software the Affymetrix and qPCR gene expression values showed a relatively good correlation for all nine algorithms with a correlation coefficient between 0.66 and 0.72. Besides, the correlation coefficient between the expression values of the nine algorithms varies between 0.92 and 0.99. Afterwards a moderated t-test was applied to the expression values to detect genes differently expressed between native and mutated cells in different stages of differentiation. The number of genes detected as differently expressed is related to the p-value of the t- test, the preprocessing algorithm used, the differentiation stage of the analyzed cell and changes visibly by changing those factors.

In vivo Analysis of the Role of EMT and Cell Migration in one Trans-Differentiation Event in C. Elegans

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Our aim is to address the role of genes involved in the expression of the epithelial phenotype or in the epithelial-to-mesenchymal transition (EMT) as well as genes involved in cell migration during one example of cell plasticity in *C. elegans*. The work is focused on an epithelial cell, 'Y' that is part of the rectum early in larval development, then migrates and trans-differentiates to a motor neuron, 'PDA'.

A list of candidate genes known to be involved in cell migration, polarity, adhesion or specifically expressed in the Y cell was prepared using available online resources like pubmed, wormbase.org, textpresso.org. Cell specific markers were identified and transgenic lines where either Y or PDA is labeled with fluorescent proteins have been developed. A targeted RNAi screening strategy was then designed and tested, in order to examine the potential involvement of our candidates in the early steps of the Y to PDA identity change. 214 candidate genes were thus screened by RNAi by feeding the worms with bacteria expressing specific double stranded RNAs. 4 candidates were found to play a role in the early steps of this trans-differentiation event. 2 of the genes are transcription factors while the other 2 are members of a signaling pathway playing a role in sex myoblast migration in *C. elegans*.

We are in the process of identifying interactors of the genes we have identified using RNAi and mutant analysis, to their mutant phenotype with respect to the morphology, identity and position of the Y cell, using molecular markers. We also plan to examine the time and cell specific requirement of the genes activity.

Understanding the molecular mechanisms of trans-differentiation and the requirements of the cellular microenvironment has implications for the possible development of therapeutic strategies to treat debilitating diseases.

Ikaros regulates B cell immunoglobulin class switching

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In both mice and humans, infections are countered with an early burst of IgM isotype antibodies, followed by the prolonged production of IgG isotype antibodies. When B cells cannot produce IgG antibodies, individuals suffer from recurrent and severe infections. The antibody isotype expressed by a B cell depends entirely upon the structure of the immunoglobulin heavy chain (IgH) locus. The process by which B cells stop making IgM, and start making IgG antibodies, is called class switch recombination and involves the reorganization of this locus though the excision of large portions of genomic DNA (60-200kb). Transcription of heavy chain constant region genes (the determinants of isotype) in the IgH locus is a crucial first step for class switching, though the transcription factors control this transcriptional activation is not well understood. We have studied the role that the zinc finger transcription factor, Ikaros, plays in class switching using a mouse line with a hypomorphic mutation in the Ikaros gene ($Ik^{L/L}$). We have found that Ikaros regulates class switching to IgG isotypes by directing the transcription of IgH constant region genes. Our data suggest that this regulation may be due to Ikaros binding in the IgH 3' transcriptional enhancer.

Etk is implicated in the cross talk between MyD88 and Focal adhesion kinase pathways.

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In a previous study, we demonstrated that MyD88 plays a major role in FAK-dependent protein I/II-induced cytokine release by human fibroblast like-synoviocytes (FLS). We also showed that MyD88-dependent IL-6 secretion in response to LPS, requires the presence of FAK, confirming that MyD88 and FAK pathways are interlinked. The precise nature of this cross talk is not presently well defined. FAK possesses neither a death domain nor a TIR domain for direct interaction with MyD88, but FAK may participate in MyD88 activation by recruiting another kinase. Kinases from the Tec family such as Btk have been implicated in LPS signaling in myeloid cells. Btk interacts with the TIR domain of TLRs as well as with MyD88, MAL, and IRAK-1 . Because FAK has been demonstrated to be an upstream activator of Etk, another member of the Tec family which is expressed in fibroblasts, Etk is an interesting candidate to link FAK to MyD88. Based on these observations, we investigated the possibility of a role of Etk in the cross talk between the FAK and the MyD88 pathways.

We first showed, that stimulation of FLS with LPS or protein I/II resulted in an increasing amount of phosphorylated Etk which was detectable within 5 min and remained elevated for at least 60 min. We then tested the role of Etk in cytokine release from activated FLS. Using RNA interference, we showed that LPS or protein I/II-induced IL-6 release from activated FLS was strongly inhibited in transfected cells. These results demonstrated the involvement of Etk in both pathways. To investigate whether Etk interacted with FAK and MyD88 directly, we examined their association. Lysats from LPS or protein I/I-activated FLS were immunoprecipitated with anti-FAK antibodies and coimmunoprecipitation of Etk and MyD88 was examined by Western blotting. Etk was detected in FAK immune complexes by anti-Etk immunoblotting. Similarly Mal and MyD88 were detected in FAK immune complexes by anti-Mal and anti-MyD88 immunoblotting. We therefore postulated that Etk interacts with MyD88 and FAK. Taken together these results indicated that Etk plays a role in the cross talk between TLR and integrin signalling pathways.

CD47 and integrins mimic GPCRs in mediating mast cell exocytosis induced by basic secretagogues

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Connective tissue mast cells are known to be activated by two distinct pathways, the well characterized IgE/FcεRI receptorial pathway and a G protein-dependent pathway induced by basic secretagogues like mastoparan and compound 48/80. However, the implication of heptahelical G protein coupled receptors remains controversial. CD47 (or IAP, integrin-associated protein) is a ubiquitously expressed glycoprotein having five transmembrane segments. CD47 is involved in cell-cell and cell-extracellular matrix interactions (Brown & Frazier, 2001). Whether CD47 might play a role role in activation mast cells has never been addressed. We report here that CD47 is implicated in mast cell exocytosis induced by basic secretagogues.

Purified peritoneal mast cells were obtained from male Wistar rats. Exocytosis of mast cells elicited by 4N1K, a specfic peptide agonist of CD47, or by mastoparan was quantified fluorimetrically by measuring released histamine (Ferry et al., 2001).

Exocytosis induced by 4N1K was rapid (maximal effect obtained within 10-20 s) and dose-dependent (reaching 60% of total histamine content at 30 μM). Pre-incubation of mast cells with an anti-CD47 antibody (1 μg/mL) caused 50% inhibition of exocytosis induced by both 4N1K and mastoparan. Pre-incubation with antibodies against β integrin subunits resulted in 30-40% inhibition of exocytosis induced by both 4N1K and mastoparan, indicating that β1, β2 and β3 integrin subunits are implicated in mast cell activation. Pre-inbubation with pertussis toxin (50 ng/mL) or an antibody against protein Gβγ resulted in essentially total inhibition of 4N1K-induced exocytosis.

Our data allow to propose CD47, in cooperation with integrins, as a putative membrane receptor mediating exocytosis stimulated by basic secretagogues in mast cells. Interestingly, our data are consistent with the hypothesis of Brown & Frazier (2001) whereby the CD47/integrin/G-protein complex mimics classical heptahelical G protein coupled receptors.

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Transgenic myocardial overexpression of prokineticin receptor-1 increases epicardial derived progenitor cell differentiation and coronary capillary networks

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Prokineticins are potent angiogenic and pro-inflammatory factors. They exert their biological activity by binding to two cognate Gq protein coupled-receptors, which their role in cardiovascular function was not widely studied. We generated transgenic mice overexpressing prokineticin receptor-1 (PKR1) specifically in the cardiomyocytes, to investigate the pathological potential of excess PKR1 signaling in heart. Histomorphological and functional analysis on transgenic hearts revealed no sign of cardiac hypertrophy or functional abnormalities. Transgenic mice demonstrated increased systolic function in response to isoproterenol, indicating an improved myocardial contractile function due to increased vascularization. Increased capillary density and coronary arteriole numbers in transgenic hearts were demonstrated by PECAM-1 and alpha-smooth muscle actin staining. Transgenic mice heart exhibit increased Epicardin positive endocardial derived cell (EDC) niches indicating that chronic activation of PKR1 in cardiomyocytes enhanced the contribution of epithelial-derived progenitor cells to myocardial neovascularization. We show *in vitro* that PKR1 signaling induces significant outgrowth from mouse epicardial explants restoring pluripotency and triggering differentiation of smooth muscle cells and endothelial cells from epicardially derived precursor cells.

The present findings for the first time demonstrate an important role of cardiomyocyte-mediated PKR-1 signaling in the differentiation and growth of the ventricular capillary network via activating EDC.

Plate-forme de Chimie Biologique Intégrative de Strasbourg

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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) 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.

Red wine polyphenols prevent angiotensin II-induced postischemic neovascularization in the rat hindlimb

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Introduction − Angiogenesis plays a key role in the development of atherosclerotic lesions. Epidemiological studies have indicated that regular intake of red wine is associated with a reduced risk of coronary diseases. We hypothesize that the protective effect of red wine on atherothrombosis might be in part through an anti-angiogenic effect. Therefore we examined whether intake of red wine polyphenols (RWPs) affects the enhancement of ischemia-induced formation of new blood vessels by a non-hypertensive dose of angiotensin II (Ang II) in the rat hindlimb.

Methods − Unilateral hindlimb ischemia was induced, in the four groups of treatment, by occlusion of the right femoral artery. Ang II (0.1 mg/kg/day) was infused for 21 days with osmotic mini-pumps implanted subcutaneously, in Ang II and Ang II + RWPs groups. Control and Ang II-treated rats received either solvent (vehicle and Ang II groups) or RWPs (RWPs and Ang II + RWPs groups) at the dose of 25 mg/kg/day in the drinking water for 28 days beginning 7 days before the ligature and Ang II infusion.

Results − Ang II infusion enhanced the ischemia-induced neovascularization as assessed by microangiography and capillary density in the hindlimb by 2.2- and 2.1-fold, respectively. This effect was associated with an increased formation of reactive oxygen species (ROS) as assessed with dihydroethidine, an increased expression of endothelial NO synthase (eNOS), vascular endothelial growth factor (VEGF), nitrated proteins and hypoxia inducible factor-2 (HIF-2) as assessed by immunofluorescence and western blotting. Intake of RWPs prevented the Ang II-induced neovascularization, ROS formation, protein nitration and expression of eNOS, VEGF and HIF-2 whereas RWPs alone were without effect. Ang II-induced neovascularization in the ischemic hindlimb was prevented by apocynin (25 mg/kg/day), a known antioxidant.

Conclusions − The present findings indicate that RWPs prevent the stimulatory effect of Ang II on ischemia-induced neovascularization most likely by preventing oxidative stress and the subsequent expression of pro-angiogenic factors.

ShcA – LRP1 signalling pathway : Control of the cell proliferation and Protection against atherosclerosis

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Low density lipoprotein receptor-related protein 1 (LRP1) protects against atherosclerosis by controling Vascular Smooth Muscle Cells (VSMC) proliferation. The association of LRP-1 with the receptor inhibits PDGF signaling pathway (1). ShcA is an adaptator protein interacting with growth factors receptors to activate Shc :Grb2 :Sos :Ras :Erk signaling pathway inducing cell proliferation (2). ShcA can bind to the phosphorylated cytoplasmic tail of LRP1 but the function of this interaction is still unknown.

Goal: to determine if ShcA is essential for LRP1 cell proliferation control and the importance of ShcA in the development of atherosclerosis lesions.

Methods: we used IGF-1-stimulated Mouse Embryonic Fibroblast (MEF) lacking LRP1 (LRP1-/-) or not (LRP1+/+) and techniques of immunoprecipitation, cell fractionation and metabolic [P32] assay.

Results: After IGF-1 stimulation, there is a lack of tyrosine phosphorylation of ShcA p66 isoform in MEF LRP1-/- cells associated with a decreased of Grb2 recruitment and Erk phosphorylation. In VSMC, LRP-1 and IGF-I-R coimmunoprecipitate.

Conclusion: LRP1 and the IGF-I receptor associate in a complex which controls ShcA-Erk1/2 signaling pathway. Ongoing experiments : phenotypic analysis of VSMC ShcA deficient mouse.

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Fluorescence Polarization High-throughput Assay applied to Death-Associated Protein Kinase

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Death-associated protein kinase (DAPK) a protein of the serine threonine protein kinase family that phosphorylates multiple substrates. It plays a major role in apoptosis and is nowadays one of the main targets for cancer therapy. Our aim is to find new molecules interacting with the enzyme that could constitute potential pharmacological tools or therapeutic agents. The objective of the present work is to set up a non-functional assay that can be used for high throughput screening (HTS) of chemical libraries.

Fluorescence polarization (FP) is one of the most highly sensitive and widely used HTS methods for studying interaction between proteins and small molecules. For FP based HTS, one needs a small fluorescent compound with affinity for DAPK serving as first ligand. The screening assay is then based on the displacement of this first fluorescent ligand (monitored by its change in fluorescence polarization) by chemical compounds of the screened libraries. To set up our assay, 1520 small molecules tagged with the fluorescent probe lissamine were screened. One of these molecules exhibited relatively high affinity for DAPK (Kd = 6.1 μM). The molecule and one analog were re-synthesized as para- and ortho- isomers and used to titrate DAPK. Binding curves analysis give para-isomers as the most interesting compounds with dissociation constants values for DAPK of 6.5μM for the molecule and 5.1μM for the analog. These two compounds will now be used in the competitive FP-HTS assay to screen non-fluorescent compound libraries and namely the Prestwick Chemical library and the Strasbourg academic library.

The result of our experiment not only has clinical potential, but also has methodological significance as the developed strategy may be applied for screening any kinase or any soluble protein.

Novel Hydrophobically Modified Low Molecular Weight Chitosan Derivatives as Potential Nonviral Vectors

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Non-viral delivery systems for gene therapy are increasingly proposed as alternatives to viral vectors. Chitosan is considered to be a good candidate for this purpose since it is already known as a biocompatible, biodegradable, and low toxic material with high cationic charge potential. However, the use of chitosan for gene therapy is limited due to its low transfection efficiency. In order to enhance its transfection efficiency, we synthesized novel low molecular weight chitosans grafted with N-/2(3)-(dodec-2-enyl)succinoyl groups with mean molecular mass ~5 kDa, degree of acetylation ~3% and degree of tetradecenoyl substitution (TDC) from 3 to ~20 mol%. We investigated by quasielastic light scattering the size and the surface charge of their complexes with calf thymus DNA at different pH, salt concentrations and N/P ratios (expressed in charged units of chitosan amines to DNA phosphates). Moreover, gene _expression in vitro and in vivo was studied using a luciferase reporter gene and a beta-galactosidase reporter gene respectively. We found that the smallest and most positively charged complexes were obtained at pH 5.8 and N/P=5 in the absence of salt: a condition where the chitosan derivatives were fully protonated and in excess over the DNA phosphate groups. Moreover, the 3 % TDC chitosan/DNA complexes gave higher levels of gene expression compared with the higher percentage TDC chitosan/DNA complexes in vitro and in vivo. We conclude that the 3 % TDC chitosan is more attractive as a gene delivery system than the conventional low molecular weight chitosans.