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RÉSUMÉS DES COMMUNICATIONS ORALES

Modality of spindle positioning and orientation in colon epithelial cell

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The APC (Adenomatous Polyposis Coli) tumour suppressor protein is mutated in the vast majority of sporadic colon cancers. The earliest lesions related to colon cancer are Aberrant Crypt Foci (ACF), which are thought to arise by perturbation of tissue architecture and deregulation of crypt fission. Mutations in apc seem to be involved in these processes. Beside its major role in the regulation of the Wnt signaling pathway, APC is also implicated in different aspect of fundamental cellular processes like migration and mitosis. Recent reports indeed revealed an involvement of APC in microtubule network regulation, and in the orientation of the spindle in the crypts of $APC^{Min+/-}$ mice (one of the mice models of colon cancer carrying a mutation in apc). Two processes regulate spindle positioning in an epithelial mitotic cell: the planar positioning of the spindle relative to the substrate and the orientation of the spindle along the tissue axis. One major question is weather a global mechanism governs the spindle orientation in the colon epithelium and what is the relation of this mechanism with the Wnt/Planar Cell Polarity (PCP), a signalling pathway that can dictate a global axis of division on a group of cells. Furthermore could a mutation in apc be sufficient to perturb this mechanism or is a second hit needed?

To answer these questions we examined the spindle orientation in healthy wild type mouse crypts to see if there is a preferred orientation with the lonthat carry a mutation in the apc gene. Previous studies on such questions have been performed using histological slices, which we thought could be a limitation in the precision and thus the conclusions of such studies. We therefore used intact entire fixed crypt that were processed with an adapted immunofluorescence protocol. The need of a large data set implied the use of a fast scanning confocal microscope for the acquisition (SP5, RIO IGBMC). We were eventually able to perform precise measurement of angles in 3D to determine spindles orientation.

Our results show that spindles aligned with the long axis of the crypt in a non random fashion. We hypothesize that this occurs via a process of OCD (Oriented Cell Division), which in some systems is regulated by the Wnt/PCP pathway. We show moreover that this OCD is functional in healthy crypts of the two mouse models carrying an apc mutation. We want to test whether the OCD is already perturbed in healthy crypt of $APC^{Min/+}$ mice, which express full length and a dominant negative form of APC, and also if OCD is still functional or not in ACF. Our next goal is to examine if the OCD observed relies on the Wnt/PCP pathway.

PARG activity controls mitotic progression and cell survival in irradiated cells

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Poly(ADP-ribosyl)ation is a post-translational modification of proteins mediated by the Poly(ADP-ribose) Polymerases (PARPs). PARP family is composed of 17 PARPs which have functions in many cellular processes such as DNA repair, mitotic segregation, telomere homeostasis, transcription and cell death. The most studied PARPs are the DNA damaged dependent PARP-1 and PARP-2 which play key roles in the maintenance of genome integrity. The functions of the poly(ADP-ribose) (PAR) synthesized at a DNA interruption is to signal the lesion and to facilitate its repair by opening the chromatin structure and recruiting DNA repair factors at the site of damage.

Poly(ADP-ribosyl)ation is a transient and reversible reaction. This reversibility is provided by Poly(ADP-ribose) Glycohydrolase (PARG), the enzyme responsible for PAR degradation. Whereas the PARP family consists of 17 proteins coded by 17 genes, there is a single *PARG* gene encoding for 4 isoforms displaying different subcellular localizations. PARG was reported to play a critical role in the control of life-and-death balance following DNA insult. PARG knock-out in mouse is embryonic lethal, whereas a hypomorphic mutation allowing the expressing of truncated form of PARG is viable but the mice are sensitive to ionizing radiation and alkylating agents.

We have generated a cell line constitutively expressing a shRNA directed against PARG mRNA.The expression of PARG isoforms has been efficiently down regulated. We have shown that in this cell line, PAR accumulates spontaneously and even more dramatically following DNA damage, and persists longer, demonstrating the invalidation of PARG activity.

The absence of PARG leads to a defect in the repair of DNA single and double strand breaks, revealed by the COMET assay and the immunodetection of γH2Ax, respectively. The recruitement of the DNA repair factor XRCC1 at laser micro-irradiation-induced DNA lesions was altered. In addition, down regulation of PARG expression increased radiosensitivity. X-irradiated PARG-deficient cells displayed a strong cell cycle alteration : a G2/M block 24 hours after low doses of irradiation and the accumulation of polyploid cells 48 hours post-irradiation. Looking more deeply into the mitotic progression of irradiated PARG deficient cells, we revealed numerous mitotic aberrations, a defect in kinetochore checkpoint and the accumulation of multipolar mitosis. Finally, we observed an increase in cell death by mitotic catastrophe. Taken together, our results indicate that PARG is required for cell survival and mitotic progression following irradiation.

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Red wine polyphenols intake prevent tumor growth in a syngenic model of colon cancer

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Introduction – The growth of a tumor and its ability to develop metastases is angiogenesis-dependent. Epidemiological studies have indicated that a moderate and regular consumption of red wine is associated with a reduced risk of cancer. Our group have previously shown that Red Wine Polyphenols (RWPs) have anti-angiogenic properties *in vitro* and *in vivo*. In the present study, we hypothesize that RWPs could prevent tumor growth by controlling tumor angiogenesis.

Methods – C26 cells, derived from colon carcinomas (chemically induced in BALB/c mice), were subcutaneously injected in each flank of 9 weeks-old BALB/c mice. Two days after the injection, RWPs or vehicle were given in the drinking water at the dose of 100 mg/kg/day for the following 26 days. At the end of the treatment period, we investigated the macrovessel density in tumors by high definition microCT system using a radio opaque silicon rubber and the microvessel density by immunohistochemistry (anti-CD31) on frozen sections. In parallel, we measured an index of proliferation (Ki67) and apoptosis (TUNEL, activated caspase-3) and we determined the expression level of pro-angiogenic factors (VEGF, MMPs) and tumor suppressor genes (p21, p16, p73 and p53) on paraffin sections.

Results – After one month of treatment by RWPs, tumor size was significantly reduced by 30% compared to control mice. Vessel density, assessed by microCT and immunohistochemistry, was reduced by 40% and by 67%, respectively, in the RWPs-treated group. We observed a concomitant decrease for VEGF and MMP-2,9 expression levels. Moreover, RWPs treatment induced 61% reduction of proliferating index (Ki67) and 81% increase of apoptotic index (TUNEL) associated with high caspase-3 activity. RWPs induce also the expression of tumor suppressor genes such as p16, p21, p73 and p53.

Conclusions – RWPs reduce tumor growth by preventing tumor neovascularisation and inducing tumor cells apoptosis. Further studies are necessary to highlight the anti-cancer properties of RWPs in a real model of colon cancer.

Dissection *in vivo* de la voie Notch dans un modèle murin de leucémie

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La voie Notch joue un rôle majeur dans les leucémies aiguës lymphoblastiques T (LAL-T). Le gène Notch1 est la cible de mutations activatrices dans plus de 50% des LAL-T, et pourrait donc jouer un rôle prépondérant. Cependant, Notch3 est également exprimé dans les LAL-T, et les rôles respectifs de ces récepteurs restent obscurs. J'étudie le rôle de la voie Notch dans un modèle murin de leucémie T, chez des souris déficientes pour le facteur transcriptionnel Ikaros. Ces souris développent des leucémies T, qui présentent toujours une activation de la voie Notch. Mes données pharmacologiques et génétiques montrent que cette voie joue un rôle important dans le développement de ces leucémies *in vivo*.

Mécanismes de la mutagenèse induite par les ADN polymérases translésionnelles chez l'homme

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La synthèse translésionnelle (TLS) permet à la cellule de répliquer l'ADN malgré la présence de lésions qui bloquent la progression des fourches de réplication. La synthèse d'ADN se fait alors grâce à l'intervention d'ADN polymérases spécialisées (les ADN polymérases translésionnelles) capables de franchir ces obstacles. Ce mécanisme est potentiellement mutagène du fait de l'altération de la capacité codante des bases endommagées et de l'absence d'activité de correction de lecture de ces polymérases. La TLS doit donc être strictement régulée afin de limiter l'apparition de mutations.

L'organisation des échanges entre les différentes ADN polymérases, la régulation de leur activité ainsi que leur interaction avec le complexe réplicatif constituent les thématiques principales abordées dans notre équipe. En particulier nous étudions l'implication de Proliferating Cell Nuclear Antigen (PCNA). En effet, PCNA interagit à la fois avec les ADN polymérases réplicatives et l'ensemble des ADN polymérases translésionnelles et pourrait être impliquée dans leur échange . De plus, PCNA est monoubiquitiné par le complexe Rad6/Rad18, en réponse à des agents qui bloquent la fourche de réplication. Les évènements moléculaires qui déclenchent la monoubiquitination de PCNA ne sont pas connus et le rôle exact de cette modification dans le processus de TLS est controversé. Dans ce contexte, notre travail consiste à élucider le mécanisme d'activation de Rad6/Rad18, et à comprendre le rôle de la monoubiquitination de PCNA dans la TLS.

Pour cela, nous utilisons un test *in vitro* où la synthèse d'ADN en face de la lésion est analysée grâce à des expériences d'extension d'amorces radiomarquées dans différents extraits de cellules humaines. Le mécanisme par lequel PCNA est monoubiquitiné sera présenté (Schmutz *et al.*, 2007) ainsi que l'efficacité de TLS dans les extraits où la monoubiquitination de PCNA par le complexe Rad6/Rad18 est aboli.

Schmutz V, Wagner J, Janel-Bintz R, Fuchs RP, Cordonnier AM. (2007). Requirements for PCNA monoubiquitination in human cell fre extracts. DNA Repair . 6, 726-1731.

Incorporation of Photo-activable Unnatural Amino Acids into Proteins by Nonsense Suppression

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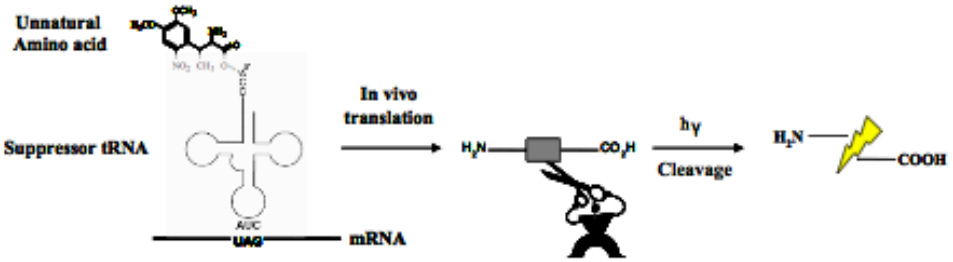
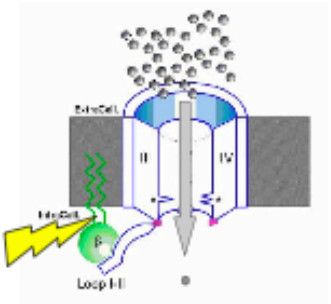
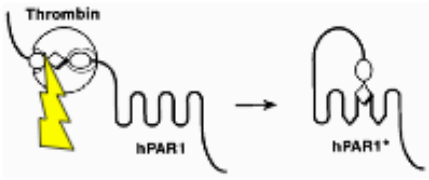
Incorporation of non-natural amino acids into proteins has raised considerable interest among chemists and biologists since the early description of the methodology by S. Hecht [1] followed by the dense work of the groups of P. Schultz[2] and D. Dougherty / H. Lester[3], respectively. This methodology leads to fascinating protein engineering developments which were not accessible by other conventional methods.

Position-specific incorporation of unnatural amino acids into proteins has been achieved with expanded genetic codes by using an amber codon. This method involves site directed mutagenesis to introduce an *amber* stop codon (UAG) at a specific site in a gene of interest. The *amber* nonsense codon is not recognized by any of the common tRNAs involved in protein synthesis. A truncated suppressor tRNA is then chemically aminoacylated with the desired unnatural amino acid and is added to an in vitro transcription-translation system optimized for the incorporation of unnatural amino acids into proteins.



The present project focuses on an amino acid, which when incorporated into a protein, allows a targeted photochemical cleavage of the protein. Such photo-proteases allow either a controlled release of a functional fragment from a protein precursor or an induced inactivation in a spatio-temporal controlled manner. Both types of applications will be presented in the present project by studying *in vivo*, (xenopus oocyte), photochemical inactivation of the calcium channel as well as activation of a G protein-coupled thrombin receptor.





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A Unique Step Economical Synthesis of [4.6.4.6]Fenestradienes and [4.6.4.6]Fenestrenes based on an 8π-6π-Cyclization-Oxidation Cascade

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The **[m.n.p.q]fenestranes** are highly strained organic molecule containing a tetracoordinate carbon.[1-3] These rare compounds of synthetic and theoretical interest are a class of tetracyclic compounds (Figure 1), defined as doubly α,α'-bridged spiroalkanes. The **lauren-1-ene 1** remains the only known naturally occurring compound with an intact fenestrane ring system (Figure 2).

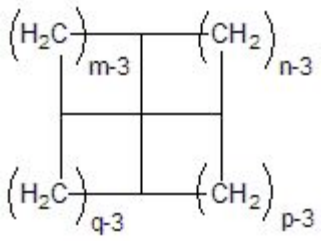


Figure 1. *[m.n.p.q]fenestranes*

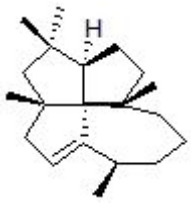
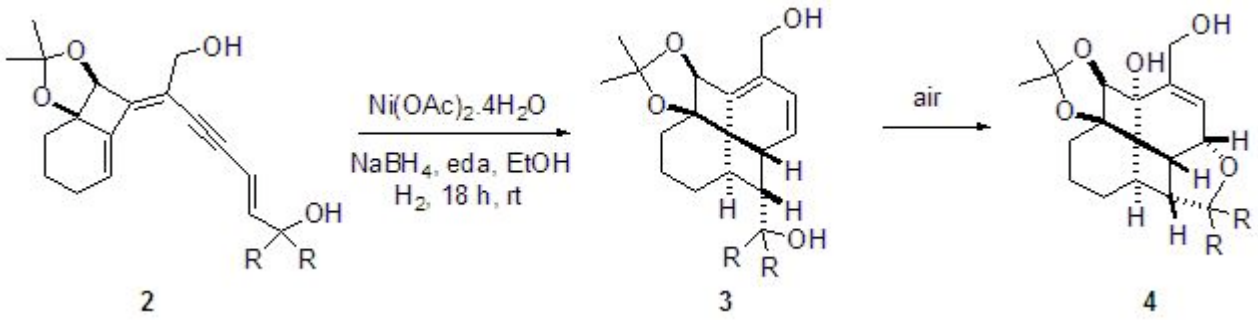


Figure 2. *Lauren-1-ene **1***

Our study was focused on the synthesis of new and original **[4.6.4.6]fenestradienes 3** and **[4.6.4.6]fenestrenes 4** (Scheme 1).[4] Our approach implied the formation of these tetracyclic structures by a reaction cascade, based on four consecutive transformations starting from the trienyne **2**: an initial soft hydrogenation using a P-2 Nickel catalyst at room temperature, followed by a conrotatory 8π electrocyclization, a disrotatory 6π electrocyclization, and a final spontaneous oxidation of fenestradienes **3** with molecular oxygen.



Scheme 1. *Synthesis of [4.6.4.6]fenestradienes **3** and [4.6.4.6]fenestrenes **4***

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Regulation of the hypoxic response, implication of Net in the HIF1α signalling network

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Ets proteins are attractive targets for the development of chemotherapeutic intervention. We have focussed on one of the Ets transcription factors: Net. Net has an important role in physiological and pathological processes, including wound healing, cell migration, angiogenesis and tumorigenesis. Interestingly, all these functions are also regulated by hypoxia. Hypoxia is defined as a decrease in available oxygen. It's an important feature of most solid tumours.

The ternary complex factor Net is downregulated in hypoxia and participates in the induction by hypoxia of several genes, including c-fos, VEGF and egr-1 (Gross at al, MCB, 2007). However, the global role of Net in hypoxia remains to be elucidated. We have identified, in a large-scale analysis of RNA expression using microarrays, more than 370 genes that are regulated by Net in hypoxia. In order to gain insights into the role of Net in hypoxia, we have analysed in parallel the genes regulated by HIF-1α, the classical factor involved in the response to hypoxia. We identified about 190 genes that are regulated by HIF-1α in hypoxia. Surprisingly, when we compare the genes induced by hypoxia that require either Net or HIF-1α, the majority are the same (75%), suggesting that the functions of both factors are closely linked. Interestingly, in hypoxia, Net regulates the expression of several genes known to control HIF-1α stability, including PHD2, PHD3 and Siah2, suggesting that Net regulates the stability of HIF-1α. We found that inhibition of Net by RNAi leads to decreased HIF-1α expression at the protein level in hypoxia (Gross et al., oncogene, 2007). These results indicate that Net participates in the transcriptional response to hypoxia by regulation of HIF-1α protein stability.

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The ternary complex factor net is downregulated by hypoxia and regulates hypoxia-responsive genes. Mol Cell Biol. 2007 Jun;27(11):4133-41. Epub 2007 Apr 2.

Functional convergence between Ikaros and the Notch pathway in T cells

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The Notch signalling pathway plays a major role in controlling fundamental aspects of multicellular life including proliferation, stem cell maintenance, differentiation and death. It has a central role in the haematopoietic system and the requirement for Notch activation in thymocyte differentiation is well established. However, the mechanisms that could regulate the Notch pathway are still poorly understood. One factor that may be important for regulating Notch pathway is Ikaros. Ikaros is a zinc finger transcription factor, predominantly expressed in haematopoietic cells and it works as a transcriptional repressor Interestingly, Ikaros has also been implicated in early thymocyte differentiation. We study the role of Ikaros in the extinction of Notch signalling in T lymphocytes. Indeed, the data of my lab show that Ikaros-deficient mice develop T lymphomas, which are associated with an early activation of the Notch pathway. The molecular basis of this repression could be due to the similarity between the sequences recognized by Ikaros and RBPJk, the transcription activating partner of Notch. Indeed, Ikaros and RBPJk both bind to sequences having the TGGGAA motif. Our previous data show that Hes1, a well-known Notch target gene, is expressed at a high level in thymocytes of Ikaros-deficient mice, and that in the Hes1 gene promoter, these two factors compete for repression (Dumortier, MCB, 2006).

Our hypothesis is that Ikaros competes with RBPJk to repress a set of common target genes, and that this mechanism plays a central role in silencing Notch target genes during T lymphopoiesis. Furthermore, the loss of Ikaros would cause a deregulation of the Notch target genes. This could lead to a new molecular way of the regulation of the Notch pathway by Ikaros to extinct the Notch target genes during T cell development.

A Dumortier, R Jeannet*, P Kirstetter*, E Kleinmann*, M Sellars*, NR dos Santos, C Thibault, J Barths, J Ghysdael, JA Punt, P Kastner and S Chan. Notch activation is an early and critical event during T cell leukemogenesis in Ikaros-deficient mice. Mol Cell Biol 26:209-220. (2006)

Tex19.1, a germline specific gene potentially involved in pluripotency.

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Embryonic stem cells (ES) constitute a very promising tool for cell replacement therapy, as they can indefinitely self-renew and give rise to all tissues of the adult. The complex network underlying pluripotency properties, involving extracellular signals like LIF and BMP, and a fine tuning of the level of expression of some crucial genes, namely Oct4, Nanog and Sox2, begins to be understood. However it's still important to identify new genes involved in ES cells pluripotency or proliferation.

In this context, we identified Tex19.1 as a potential new pluripotency actor in mouse ES (mES) cells. Initially Tex19 was identified by substractive cloning, as being expressed in the mouse testis (Wang et al., 2001). We showed that many homologs exist, only in mammals, comprising human. Multiple sequence alignment showed two highly conserved domains, which present no homology with any known domain. Tex19 is duplicated in mouse and rat, we named these genes Tex19.1 and Tex19.2 (Kuntz et al., 2007). Tex19.2 expression appears late during development (approximately 16,5dpc) and is limited to somatic cells of the gonad. Expression profile of Tex19.1 in mouse and in pluripotent stem cells is very close to the one of key pluripotency genes such as Oct4. In particular, we showed that Tex19.1 is maternally inherited, expressed in the ICM of the blastocyst, and later is germ cell specific. Moreover, many pluripotent cell lines, namely mES, human embryonic stem cells (hES), mouse embryonic germ cells (mEG) and mouse (P19 et F9) and human (N-Tera2) teratocarcinomas cell lines express Tex19.1 in the undifferentiated state, but loose its expression upon differentiation (Kuntz et al., 2007). The nuclear localization of Tex19.1 protein and its ability to link nucleic acids make this gene a good candidate as a transcriptional regulator. These elements suggest a possible role of Tex19.1 in mES cells pluripotency.

The precise function of this protein in self-renewal or pluripotency maintenance, its eventual targets or partners, and the pathway(s) in which it is involved in mES cells are under investigation.

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Membrane efflux transporter modulation by flavonoids

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Introduction. Flavonoids have been the focus of research for many years as they can help to prevent diseases. They belong to a large group of naturally occurring polyphenols in plants consumed daily and in large amounts. Such compounds are principally found in teas, cacao, and fruits such as apples, berries and grapes, these later are particularly rich in flavanols and procyanidins. Some of these molecules were described as inhibitors of metabolic enzymes (CYP1A2, CYP2C9, CYP2E1, CYP3A) and as modulators of several membrane carriers implicated in drug pharmacokinetic.

Our investigations using the human Caco-2 cell model shows here that flavonoids like quercetin, nargenin and luteolin increase simvastatin intestinal permeability and so its bioavailability. In order to explain the mechanisms involved we evaluated firstly the transport of flavonoids in presence of inhibitors of efflux membrane transporters MDR1 and MRPs. Secondly, we demonstrated by capillary cytometry that all tested flavonoids were able to modulate MDR1 transporter.

Methods. Flavonoids transcellular transport was studied by incubating Caco-2 cells in presence of flavonoids with or without inhibitors: PSC833 for MDR-1, probenecid for MRPs and estrone 3 sulfate for OATP. Transcellular transport was evaluated during 90 min from apical (pH 6.5 and pH 7.4) to basolateral side (pH 7.4). Capillary cytometry monitoring of rhodamine 123 efflux permitted to evaluate modulation of MDR 1 transporters by flavonoids.

Results. For both tested pH, apparent permeability coefficient of flavonoids from basolateral to apical side (Papp BA) is found 3 fold higher than the opposite way (Papp AB). Therefore the transport of flavonoids could be mediated by an active efflux transport. In presence of MDR-1 and MRPs inhibitor, the Papp AB of flavonoids is increased 5 times pointing out flavonoids as MDR and MRP substrates. Flavonoids Papp AB is decreased in presence of Estrone-3 sulfate suggesting that flavonoids could at as OATP substrate. Rhodamine 123 is retained by cells in presence of quercetin and luteolin (100 μM).

Conclusion. Our work shows that flavonoids are not only transported through intestinal membrane via OATPs, MDR-1 and MRPs transporters but that flavonoids are as well MDR-1 modulators. Our results should be taken into account when flavonoids are co-administered with drugs knowing they could influence their pharmacokinetic and so change the safety profiles. On the contrary our results can help to develop new therapeutic strategies to enhance drug bioavailability.

Mechanism of NRSF upregulation in Huntington's Disease

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Huntington's disease (HD), Spinocerebellar ataxia type 7 (SCA7) and 7 other inherited neurodegenerative disorders are caused by the expansion of a polyglutamine stretch (polyQ) in 9 otherwise unrelated proteins. The molecular mechanisms responsible for polyQ-induced neurodegeneration remain elusive. Several lines of evidence indicate that maintenance of neuronal phenotype might be impaired in PolyQ disorders. To test this hypothesis, we are investigating the role of a critical regulator of neuronal terminal differentiation, the neuron-restrictive silencer factor (NRSF), in the context of HD. Downregulation of NRSF is required for expression of several genes implicated in neuron specific functions. In cell and mouse models of HD, we found that NRSF was upregulated. To understand the underlying mechanism we studied the three alternative promoters of NRSF, which contain several putative response elements for factors, such as AP1, NF-kappaB and Sp1, which are known to be altered in HD. Using luciferase reporter assay and a set of deletions and point mutations of the promoters, we could exclude a role for AP1 and NF-kappaB in the NRSF upregulation, and narrow down the minimal active promoter to GC rich regions, which contain several Sp binding sites. We then show that Sp1 can activate the NRSF promoter, while Sp3 acts as a repressor, suggesting a dual role of these factors in the pathogenesis of HD.

Functional Conservation of Human, Yeast and *Plasmodium falciparum* RNA Polymerase subunits

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The RNA polymerase II (RNAPII) is the nuclear enzyme that is responsible for the transcription of the main part of the eukaryotic genome. This multiprotein complex consists of 12 subunits ranging from 220 to 7 kDa. The structure of the yeast (*Saccharomyces cerevisiae*) RNAPII has been established at 3Å resolution (Cramer et al 2000). Although the detailed structure of the human enzyme is presently unknown, genetic studies suggest that both the functions and the main structural features of the twelve subunits have been conserved throughout evolution.

The intracellular parasite *Plasmodium falciparum* (P fal) has been subjected to a strong evolutive pressure, yielding a eukaryotic cell that exhibits several distinctive features. The sequencing of the full genome of 3D7 strain of P fal (Gardner et al. 2002) enabled us to recover a complete set of genes sequences encoding the putative RNAPII subunits of P fal. although this enzyme has not yet been biochemically characterized.

We investigated the functional conservation of the P fal. RNAPII subunits using a genetic test in the yeast *Saccharomyces cerevisiae*. The recovery of the P fal subunits coding sequences turned out to be technically more difficult than initially anticipated and we had to reconstruct several sequences. We shall present the current state of our project.

The investigation of the RNAPII of P fal. is important as it may be a potential target for drug screening. Indeed, the mammalian RNAPII is selectively targeted by the mushrom toxin alpha-Amanitin, which suggests that the –selective- inhibition of this enzyme may be an interesting novel anti parasite strategy.

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Towards a better understanding of human frataxin maturation

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Frataxin is a 210 amino acids nuclear encoded protein targeted to mitochondria. A genetic deficiency in frataxin causes Friedreich ataxia, a progressive neurodegenerative disorder associating spinocerebellar ataxia and cardiomyopathy. The exact function of frataxin is still unknown but it is closely linked to iron metabolism. It is suggested that frataxin is an iron chaperone needed for iron-sulfur clusters and heme biosynthesis and/or iron storage. The mitochondrial import and maturation of frataxin has been known for several years to involve two independent N-terminal cleavages to generate the mature form of the protein. However, many controversies still remain about the exact structure and start site of mature frataxin. Indeed, three different forms have been described, the first being a protein starting at amino acid 56 (m-fxn$_{56\text{-}210}$), and two other more recently described forms beginning at amino acid 78 and 81, respectively (m-fxn$_{78\text{-}210}$ and m-fxn$_{81\text{-}210}$). Our project aims at identifying the mature form of frataxin and at dissecting each step of its maturation. By mass spectrometry and mutational analysis, we determined that human frataxin begins at amino acid 81. Interestingly, we show that m-fxn$_{56\text{-}210}$ and m-fxn$_{78\text{-}210}$ can be generated when the normal maturation process of frataxin is impaired. A cell survival test indicates that all isoforms are able to rescue cell growth, however m-fxn$_{81\text{-}210}$ appears to be more efficient. Finally, we show that frataxin is only present in the mitochondrial compartment in human cells and provide evidences that cells expressing only a cytosolic frataxin are unable to grow. This is in contradiction with recently published data which described the existence of an extramitochondrial pool of human frataxin, which would be able to protect cell from stress. Our results are important in the light of finding therapeutic approaches for Friedreich ataxia based on frataxin replacement.