D.Day 2011
Thursday, April 28th

08:30 Registration and mounting of posters

09:00 Professor Karine Lapouge
"What's there for lunch today:
How does Pseudomonas aeruginosa PA01 adapt to the menu?"

09:45 Meet your neighbors: selected talk from abstracts

Thierry Bouduban
"Role of lipid droplets proteins in hepatic insulin resistance in mice and human"

10:05 Poster session (odd numbers) around coffee and croissants

11:00 Meet your neighbor: selected talk from abstracts

Iole Pezzuto
"Differential miRNAs and target gene expression in embryonic stem cells lacking the Notch1 receptor"

11:20 Dr. Jeff Stevens
"The bounded rationality of cooperation"

12:05 Lunch

13:45 Meet your neighbors: selected talks from abstracts

Noemie Gardiol
"Role of canonical Wnt signaling in BCR-ABL induced leukemia"

Gaelle Billoud
"Endoglycan, a member of the CD34 family of sialomucins, is a E-selectin ligand expressed in membrane rafts of leukemia cells and a signaling molecule"

Irene Vassallo
"The Wnt Inhibitory Factor 1 (WIF-1) has tumor suppressing functions in glioblastoma potentially by inducing cellular senescence"

14:45 Poster session (even numbers)

15:45 Dr. Manuel Grez
"Gene therapy for Chronic Granulmatous Disease: Ups and Downs"

16:30 Poster Awards

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ABSTRACT LIST

Speaker Abstracts:

What's there for lunch today: How does Pseudomonas aeruginosa PAO1 adapt to the menu?

Prof.Karine Lapouge

Department of Fundamental Microbiology, University of Lausanne, Switzerland

Bacteria are indispensable components of our ecosystem. They have developed an extraordinary capacity to adapt to environmental changes by modulating cellular functions at the transcriptional and post-transcriptional levels. It has long been known that transcriptional regulation is crucial for this adaptation. However, translational regulation has recently been shown to be equally important, as it is rapid and effective at low energy cost. For transcriptional regulation depending on external signals, bacteria have developed sophisticated two-component regulatory systems. These systems sense and respond to environmental changes, such as nutrient availability, oxygen tension, osmolarity or cell population density. For post-transcriptional regulation mRNA stability and initiation of translation can be regulated mainly by small non-coding RNAs (sRNAs).
Our organism of interest is Pseudomonas aeruginosa PAO1, a versatile ubiquitous bacterium and opportunistic pathogen, which has a phenomenal capacity to adapt to different environments and utilizes a wide variety of organic molecules as carbon, nitrogen and energy sources. This is not surprising as its genome contains an unusually large number of genes for catabolism, nutrient transport and metabolic regulation. However, the regulatory systems involved in Pseudomonas aeruginosa PAO1 adaptation to environmental changes are not well known and we intend to investigate these mechanisms.
Therefore, the overall aim of our research is to study the mechanisms of transcriptional control by two-component systems and of translational control by novel sRNAs in nutrient uptake and catabolism in Pseudomonas aeruginosa PAO1.

Role of lipid droplets proteins in hepatic insulin resistance in mice and human (S01)

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Obese and/or diabetic patients often concomitantly develop a hepatic steatosis (fatty liver), an abnormal fat accumulation in the liver. The hepatic steatosis frequently induces hepatic insulin resistance, a main cause of diabetic fasting hyperglycemia. However how exactly hepatic steatosis leads insulin resistance has not been clearly defined.
To study molecular mechanisms of insulin resistance in hepatic steatosis, we have been using a mouse model of hepatic steatosis dissociated from insulin resistance. Mice without a gene of microsomal triglyceride transfer protein (Mttp-/-) in the liver develop a hepatic steatosis due to a defect of VLDL secretion. We found that Mttp-/- mouse liver induced series of genes coding proteins existing on the surface of lipid droplets such as "Cell-death Inducing DFFA-like Effector-C" (cidec), "Lipid Storage Droplet Protein-5" (lsdp5) and "Bernardinelli-Seip Congenital Lipodystrophy-2-Homolog" (seipin). In this study, we tested our hypothesis that lipid droplets proteins might protect the fatty liver against the insulin resistance.
We first studied whether these genes were involved in hepatic insulin resistance in Alfa Mouse Liver 12 (AML12) hepatocyte cell line. By downregulating cidec or seipin expression by siRNA technique, we found a defect in Akt phosphorylation under insulin stimulation. It strongly suggests that the lack of lipid droplets proteins induces insulin resistance in mouse hepatocytes.
We next analyzed their expression levels in liver biopsies from obese patients with diverse degrees of hepatic steatosis. We found that the expression of these genes was significantly increased in fatty liver. More interestingly, we found that diabetic patients significantly decreased the expression of lsdp5 and seipin compared to the non-diabetic patients with the same degree of hepatic steatosis. Our results strongly argue that the lipid droplet proteins are tightly involved in human hepatic steatosis and play an important role in the development of insulin resistance.

Differential miRNAs and target gene expression in embryonic stem cells lacking the Notch1 receptor (S02)

Iole Pezzuto, Mohamed Nemir, Thierry Pedrazzini

CHUV

Cardiomyocytes derived from differentiated embryonic stem (ES) cells represent an attractive source of cells for cell-replacement therapies in cardiac disease. However, cardiogenic differentiation of ES cells requires a complete understanding of the complex molecular mechanisms controlling the differentiation process. We demonstrated that differentiation of ES cells into cardiomyocytes is favored by inactivation of the Notch1 receptor. In addition some components of the Notch pathway in ES cells are under control of miRNAs, which, therefore, play an important role in cardiogenesis. So, we aim to investigate whether the increased cardiogenic potential in ES cells lacking Notch1 could rely on the expression of particular miRNAs and the modulation of their target genes. Alternatively, Notch1 could directly induce the modulation of particular target genes independently of miRNAs. As a first approach, we use microarray analysis to identify modulated miRNAs in wild-type and Notch1-deleted ES cells. In parallel, we determined the transcriptome in both cell lines. We found approximately 20 miRNAs, which were differently expressed in Notch1-deleted ES cells as compared to wild-type ES cells. Moreover, 100 genes, predicted to be targets of the identified miRNAs using computer assignment, were also modulated in the Notch1-deleted ES cells. We confirmed the differential expression of some target genes by quantitative methods. Together, these data demonstrate that Notch1 deletion induces specific miRNAs and gene expression patterns that could regulate the commitment of ES cells toward the cardiac lineage. Ongoing experiments evaluate the importance of these genes in the mesodermal and cardiogenic commitment of ES cells. Furthermore, we will also determine whether controlled modulation of these genes can be used to force ES cell to adopt a cardiogenic commitment.

The bounded rationality of cooperation

Dr. Jeff Stevens

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Animals often aid others without gaining any immediate benefits. Although these acts seem to reduce the donor's fitness, they are only apparently altruistic. Donors typically help because they or their kin receive future benefits or avoid costly punishment. Reciprocity – alternating the roles of donor and recipient – has been a well-studied form of cooperation among non-kin because of its intuitive appeal in explaining human cooperation. Models of reciprocity, however, have not considered the psychological mechanisms needed to implement the proposed decision strategies. A bounded rationality approach emphasizes the need to build realistic assumptions about cognition into models of cooperation.

Role of canonical Wnt signaling in BCR-ABL induced leukemia (S03)

Noemie Gardiol, Werner Held

UNIL

Canonical Wnt signaling is crucial for embryonic development as well as for the homeostasis and the self-renewing of adult tissues. Deregulation of this signaling pathway is implicated in the development of epithelial cancers of the colon, breast or skin.
The binding of Wnt proteins to cells induces the intracellular stabilization of beta- and gamma-catenin, the two known mediators of canonical Wnt signaling. These mediators can then associate in the nucleus with DNA binding TCF/LEF family factors to induce the transcription of target genes. The role of canonical Wnt signaling in normal hematopoiesis is currently unclear as hematopoiesis is normal in the combined absence of beta- and gamma-catenin.
Leukemias are often caused by oncogenic chromosomal translocation products, such as BCR-ABL. The latter is a constitutively active kinase that induces two types of leukemia in human, chronic myeloid leukemia (CML) and B-cell acute lymphoid leukemia (B-ALL). Recently, it was shown that BCR-ABL induced CML cells present an increased level of beta-catenin. It was further demonstrated that beta-catenin is an essential player in CML development and drives CML cancer stem cells. However, absence of beta-catenin does not impact B-ALL development.
Here we show that BCR-ABL induced B-ALL is strongly reduced in absence of gamma-catenin, the other canonical Wnt mediator. Furthermore, we show that induction of B-ALL using committed B cells is dependent on gamma-catenin. Using a Wnt reporter, we also show that B-ALL cells display Wnt signals, also partially dependent on gamma-catenin. In addition, we show that absence of both catenins essentially blocks the development of CML and B-ALL in mice.
Collectively, we find that beta- and gamma-catenin are dispensable for normal hematopoiesis but essential for the development of BCR-ABL induced leukemias. These findings suggest that the canonical Wnt pathway may represent a promising target for the therapy of leukemia.

Endoglycan, a member of the CD34 family of sialomucins, is a E-selectin ligand expressed in membrane rafts of leukemia cells and a signaling molecule (S04)

Gaelle Billoud [1], Bénédicte Baïsseagushi [2], Olivier Spertini [2]

UNIL [1], CHUV [2]

Selectins initiate leukocyte recruitment during inflammation and play an important role in cancer cell dissemination. Endoglycan (EGC), a sialomucin of the CD34 family, is a selectin ligand whose role remains unclear. Immunophenotypic and western blot analyses disclosed EGC on peripheral blood monocytes and leukemia cell lines. Under flow conditions, EGC/heavy chain IgG chimera efficiently supported E-, P- and L-selectin-dependent rolling. EGC immunoadsorbed from U937 cells strongly supported E-selectin-dependent rolling but weakly P-selectin-dependent rolling suggesting that EGC is mainly a ligand for E-selectin. Immunoblotting showed that EGC, like PSGL-1, is localized in membrane rafts isolated from U937 cells. As membrane rafts are signaling plateforms, EGC may be involved in cell signaling. Different activation experiments showed that EGC is a signaling molecule probably involved in different pathways by activating Erk, Akt, p38 and Syk. By deleting EGC intracellular domain, we observed that the cytoplasmic tail in not required for activating MAPK and PI3K pathways. Additional experiments will be performed to investigate the functional roles of EGC and to identify its binding partners in signal transduction.

The Wnt Inhibitory Factor 1 (WIF-1) has tumor suppressing functions in glioblastoma potentially by inducing cellular senescence (S05)

Irene Vassallo [1], Wanyu Lambiv [1], Mauro Delorenzi [2], Tal Shay [3], Annie-Claire Diserens 1, Anastasia Murat [1], Eugenia Migliavacca [2], Davide Sciuscio [1], Marie-France Hamou [1], Roger Stupp [1], Monika Hegi [1]

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Glioblastoma multiforme is the most aggressive form of human glioma and despite recent progress in therapy the prognosis remains dismal with a median survival of 15 months. Expression based prediction of gene alterations identified Wnt inhibitory factor I (WIF1) as a new candidate tumor suppressor gene involved in glioblastoma. WIF1 encodes a secreted Wnt antagonist and it was strongly down-regulated in most glioblastoma as compared to normal brain, implying deregulation of Wnt signaling. Silencing of the WIF1 gene was found to be mediated by deletion and WIF1 promoter hypermethylation. Ectopic expression of WIF1 in glioblastoma cell lines revealed a dose dependent decrease of Wnt pathway activity. To further dissect the biological effects of WIF1 re-expression, we established WIF1 overexpressing glioblastoma cell lines. We observed that WIF1 re-expression inhibited cell proliferation in vitro and strongly reduced anchorage independent growth. The ability of forming colonies in soft agar was reduced to less than 11% of the control. Moreover, the expression of WIF1 was able to completely abolish tumorigenicity in a respective xenograft model in nude mice. Interestingly, WIF1 overexpression in glioblastoma cells induced a senescence-like phenotype characterized by the appearance of enlarged flattened and multinucleated cells positive for the presence of beta-galactosidase, a late marker of senescence. These results provide evidence that WIF1 has tumor suppressing properties in glioblastoma, hence, the implication of a deregulated Wnt pathway may render glioblastoma sensitive to Wnt signaling inhibitors, potentially by diverting the tumor cells into a senescence-like state.

Gene therapy for Chronic Granulomatous Disease: Ups and Downs

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Chronic granulomatous disease (CGD) is a rare inherited immunodeficiency characterized by recurrent, often life threatening bacterial and fungal infections due to a functional defect in the microbial-killing activity of phagocytic neutrophils. In our gene therapy trial for X-CGD patients we demonstrated reconstitution of oxidative burst capacity and elimination of preexisting severe infections. However, an unexpected expansion of gene marked myeloid progenitors occurred five months after transplantation, which was triggered by insertional activation of MDS1/EVI1, PRDM16 or SETBP1.
We monitored patients for clinical health, hematological reconstitution, phagocyte function, gene marking, clonal fluctuation, cytogenetic and DNA methylation status. After the initial resolution of bacterial and fungal infections, both patients developed a myelodysplastic syndrome (MDS) caused by insertional activation of MDS1/EVI1 followed by clonal progression and the loss of chromosome 7. Also the level of superoxide production by gene corrected cells decreased constantly with time. Quantitative RT-PCR analysis over time revealed a transcriptional down regulation of gp91phox expression due to CpG methylation at the viral LTR promoter. P1 died 27 months after gene therapy of MDS in combination with severe septicemia, the latter resulting from loss of bacterial killing activity in transduced cells. P2 underwent allogeneic stem cell transplantation. Forced overexpression of MDS1/EVI1 or EVI1 in human cells disrupted normal centrosome duplication, linking MDS1/EVI1 activation to the development of genomic instability.
Gene therapy for X-CGD can provide considerable clinical benefits. Advances in vector design that avoid proto-oncogene activation and promoter methylation will enable the safe and effective application of this strategy for the long-term correction of X-CGD.

Poster Abstracts:

P 1

DNA extraction from Chlamydiales: a tricky mission

Sébastien Aeby, Antony Croxatto [1], Gilbert Greub

CHUV

The order Chlamydiales includes the Chlamydiaceae, Parachlamyliaceae, Waddliaceae, Simkaniaceae, Criblamydiaceae and Rhabdochlamydiaceae families. Members of the Chlamydiales order are obligate intracellular bacteria that replicate within eukaryotic cells of different origins including humans, animals and amoebae. Chlamydiales are characterized by a biphasic development cycle comprising infectious metabolically inactive elementary bodies (EB) and non-infectious metabolically active and replicating reticulate bodies (RB). These two developmental forms are characterized by profound differences in membrane composition and structure that can play an important role in the efficiency of bacterial lysis during DNA extraction procedures. This is an important issue considering that the growth of Chlamydiales bacteria within host cells is usually monitored by genomic DNA extraction and real-time PCR. Several lysis protocols compatible with the Promega Wizard SV Genomic DNA Purification kit have been evaluated on samples collected at different times corresponding to different developmental stages observed during the growth of several Chlamydia-related bacteria. These experiments showed that a specific lysis protocol has to be followed to ensure efficient lysis of both EBs and RBs in order to prevent the collection of incorrect data due to an experimental artefact caused by incomplete lysis of EBs.

P 2

RasGAP317-326-mediated inhibition of cell migration: a potential way to inhibit metastasizing

David Barras, Christian Widmann

UNIL

Metastases are the main cause of death in cancer patients. A first step in the metastasizing process is the escape of cancer cells from the primary tumor site. This involves increase in cell motility and a concomitant ability to clear a path through the extra-cellular matrix. From a therapeutic point of view, inhibiting cell migration is a logical approach to develop anti-metastatic drugs. We showed earlier that a cell permeable peptide derived from a caspase-generated fragment of the RasGAP protein, called TAT-RasGAP(317-326), efficiently and specifically sensitizes cancer cells to chemotherapy-induced cell death. We recently discovered that this peptide was also able to inhibit cell migration and to increase cell adherence without affecting other key cellular processes such as proliferation. These effects were not seen if the peptide lacked the cell-permeable sequence indicating that it exerted its effects from within cells. This was confirmed by transfecting cells with plasmids encoding the 317-326 sequence of RasGAP. The ability of TAT-RasGAP(317-326) to increase cell adherence did not require transcription or translation. Further work on this compound should define whether it could be used in vivo as an anti-metastatic therapy tool.

P 3

The Waddlia genome: a window in Chlamydial biology

Claire Bertelli [1], François Collyn [1], Antony Croxatto [1], Christian Rückert [2], Adam Polkinghorne [3], Carole Kebbi-Beghdadi [1], Alexander Goesmann [2], Lloyd Vaughan [4], Gilbert Greub [1]

Institute of Microbiology, University Hospital Center and University of Lausanne, Switzerland [1], Center for Biotechnology (CeBiTec), Bielefeld University, Germany [2], Institute of Health and Biomedical Innovation, Queensland University of Technology, Australia [3], Institute of Veterinary Pathology, University of Zurich, Switzerland [4]

Growing evidences suggest that a novel member of the Chlamydiales order, Waddlia chondrophila, is an agent of miscarriage in humans and abortion in ruminants. Due to the lack of genetic tools to manipulate chlamydia, genomic analysis is proving to be the most incisive tool in stimulating investigations into the biology of these obligate intracellular bacteria. 454 and Solexa technologies were thus used to sequence and assemble de novo the full genome of the first representative of the Waddliaceae family, W. chondrophila. The bacteria possesses a 2'116'324bp chromosome and a 15'593bp low-copy number plasmid that might integrate into the bacterial chromosome. The Waddlia genome displays numerous repeated sequences indicating different genome dynamics from classical chlamydia which almost completely lack repetitive elements. Moreover, W. chondrophila exhibits many virulence factors also present in classical chlamydia, including a functional type III secretion system, but also a large complement of specific factors for resistance to host or environmental stresses. Large families of outer membrane proteins were identified indicating that these highly immunogenic proteins are not Chlamydiaceae specific and might have been present in their last common ancestor. Enhanced metabolic capability for the synthesis of nucleotides, amino acids, lipids and other co-factors suggests that the common ancestor of the modern Chlamydiales may have been less dependent on their eukaryotic host. The fine-detailed analysis of biosynthetic pathways brings us closer to possibly developing a synthetic medium to grow W. chondrophila, a critical step in the development of genetic tools. As a whole, the availability of the W. chondrophila genome opens new possibilities in Chlamydiales research, providing new insights into the evolution of members of the order Chlamydiales and the biology of the Waddliaceae.

P 4

Genetic structure of cannabis sativa: from population genomics to forensic applications

Friederike Bienert

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Hemp (Cannabis sativa) was domesticated over 7,000 years ago as a source of fibre, food, and medicines. Besides its agroeconomical importance, C. sativa is also used as a recreational drug (i.e. marijuana or hashish), due to the presence of tetrahydrocannabinol (THC), a cannabinoid compound with psychoactive properties. Morphology and cannabinoid content vary extensively between populations and individuals. As a result, the differentiation between licensed fibre and prohibited drug varieties of C. sativa poses a major impediment to the development of the industrial and therapeutic potential of this controversial species.
Hence, in this study we want to understand the genetic effects of human domestication and selection on patterns of genetic diversity within and among populations. Moreover, we analyze the potential for creating an effective forensic tool to distinguish between drug and agricultural varieties based on genetic differentiation.
Therefore, we genotyped 24 agricultural Cannabis populations (839 individuals) and 25 drug varieties (507 individuals) for 13 microsatellite loci. Clustering analyzes revealed the presence of two distinct genetic clusters separating drug and crop varieties. Further assignment tests of 120 known Cannabis samples showed that each of them has been correctly assigned to either the drug or fibre cluster. Genetic diversity analyzes also demonstrated that allelic richness was significantly lower for drug (2.19 $p<0.001$) than for crop varieties (3.44 $p<0.001$). In addition, we found higher genetic differentiation among drug populations (Fst : 0.424 $p<0.001$) than among agricultural populations (Fst : 0.147 $p<0.001$). This might be due to an intense selection for specific phenotypes (bottleneck) and the use of clonal reproduction (especially for drugs).

P 5

The TRAF interacting protein (TRAIP) regulates keratinocyte proliferation

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UNIL - CHUV / Service de Dermatologie

TRAIP is an E3 ubiquitin ligase which undergoes auto-ubiquitination and is reported to interact with TNF-receptor associated factors (TRAF) and two tumor suppressors (CYLD, SYK). TRAIP is necessary for mice development since TRAIP knock-out mice die in utero at day 6.5 caused by aberrant regulation of cell proliferation and apoptosis. Physiological substrates of TRAIP are not known. Over-expression of TRAIP in 293T cells inhibits TNF-alpha-induced NFkB activation. We showed that TRAIP mRNA expression was strongly down-regulated in cultured primary human keratinocytes undergoing differentiation triggered by high cell density or high calcium. Short-term phorbol-12-myristate-13-acetate (TPA) treatment or inhibition of phosphatidylinositol-3-kinase signaling in proliferative keratinocytes suppressed TRAIP transcription. Inhibition by TPA was protein kinase C dependent. Keratinocytes undergoing KD of TRAIP expression by lentiviral short-hairpin RNA (shRNA; T4 and T5) strongly reduced proliferation rates compared with control shRNA. Furthermore, cell-cycle analysis demonstrated that TRAIP-KD caused growth arrest in the G1/S phase. Keratinocytes with TRAIP-KD resembled differentiated cells consistent with the augmented expression of differentiation markers keratin 1 and filaggrin. Luciferase-based reporter assays showed no increase in NFkB activity in TRAIP-KD keratinocytes, indicating that NFkB activity in keratinocytes was not regulated by TRAIP. TRAIP mRNA expression was increased by ~2-fold in basal cell carcinomas compared with normal skin. To understand the mechanisms in which TRAIP is implicated, dynamics of TRAIP protein expression and subcellular localization during the cell cycle was examined. In HeLa cells and human epidermal keratinocytes transiently over-expressing a TRAIP-GFP fusion protein, the fluorescent protein localized mainly to the nucleolus. In summary, these results underline the important role of TRAIP in the regulation of cell cycle progression and the tight linkage of its expression to keratinocyte proliferation.

P 6

Coronary MRI: when is the best time to collect the data?

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UNIL [1], CHUV [2]

Purpose: Cardiovascular MRI data acquisition is commonly performed ECG triggered during several consecutive cardiac cycles. Several studies have been performed to identify the best position within the RR interval with lowest coronary velocity; however the precision of the coronary artery repositioning in subsequent cardiac cycles remains unknown. The aim of this study was therefore to measure the beat-to-beat irregularity of coronary position on x-ray coronary angiograms and to test the hypothesis that intervals with more precise geometric repositioning of the coronary arterial system do exist.
Methods: Routine diagnostic cine breath-hold x-ray angiograms of 12 patients who underwent elective coronary angiography for diagnostic purposes were retrospectively analyzed. Informed consent for cardiac catheterization was obtained from all patients. Images were acquired at 15fps with a 0.32mm isotropic spatial resolution and the ECG was recorded simultaneously. Mid left anterior descending coronary artery and left coronary circumflex bifurcations were identified on the x-ray angiograms over multiple heartbeats using a computer algorithm. Time resolved bifurcation coordinates of one cardiac cycle were compared to those of the subsequent cycles to quantitatively evaluate beat-to-beat irregularity of coronary repositioning.
Results: Beat-to-beat irregularity of coronary motion on x-ray coronary angiograms shows a distinctive time pattern which was similar in all patients and the average remains always below 1.4mm. Local minima at both end-systole and at mid-diastole are observed with an average beat-to-beat irregularity as low as 0.66mm. The irregularity of these distinct time windows is significantly lower than that measured immediately after the R-wave of the ECG ($p<0.05$ student's t-test).
Discussion: Quantitative beat-to-beat irregularity of coronary motion measured on x-ray coronary angiograms is lowest at end-systole and during mid-diastole. Since RR variability primarily affects the duration of diastole, we posit that end-systolic imaging will improve detail visibility, image quality and the diagnostic value of coronary MRI in general.

P 7

Sustained iNKT cell activation by CD1d fusion proteins leads to efficient tumor inhibition

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Invariant Natural Killer T cells (iNKT) are potent activators of Natural Killer (NK) cells, dendritic cells (DC) and T lymphocytes, and their anti-tumor activity has been well demonstrated. A single injection of the superagonist CD1d ligand alpha-galactosylceramide (alphaGalCer) leads to strong iNKT cell activation, followed however by a long-term anergy, limiting the therapeutic use of this ligand. As a promising alternative, we demonstrated that when alphaGalCer was loaded on recombinant soluble b2m-CD1d molecules (alphaGalCer/sCD1d), repeated injections led to sustained iNKT cell activation associated with continued cell proliferation and IFNgamma secretion. The capacity of recombinant CD1d fusion proteins to keep iNKT cells reactive was associated with attenuated PD-1 upregulation even after six injections, in contrast to high PD-1 expression reached already after one injection of alphaGalCer as a free drug. Similarly, human iNKT cells could be activated and expanded by alphaGalCer/sCD1d fusion proteins independently of the presence of CD1d-APCs. Moreover, lower PD-1 upregulation was also observed when compared with human iNKT cell activation by alphaGalCer-pulsed APCs. Importantly, the retained reactivity of iNKT cells allowed prolonged antitumor activity against HER2- or CEA-expressing mouse tumors, when the alphaGalCer/sCD1d protein was fused to an anti-HER2 or anti-CEA scFv antibody fragments. In order to optimize the efficacy of iNKT cells in the immunosuppressive tumor environment, the importance of MDSCs is currently being addressed. First, recombinant CD1d immunotherapy is tested in a mouse model with myeloid specific Arginase 1 deletion, as this enzyme is one of the main mechanisms used by MDSCs to suppress T cell function. Second, several chemotherapeutic agents known to deplete MDSCs are evaluated in combination with the CD1d-antitumor treatment. Altogether, promoting the sustained activation of iNKT cells at the tumor site, while decreasing the immunosuppressive environment, appears as a promising antitumor strategy.

P 8

Human primary auditory cortex follows the shape of Heschl's gyrus

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Tonotopic maps have been difficult to measure in the human auditory cortex because map details are just below the spatial resolution of standard functional imaging techniques. As a result, the exact number and location of auditory cortex subfields in the human (including primary and secondary regions) remains unknown. Here, using ultra-high field strength fMRI (7T) with voxel volumes as low as 1.7mm3, we have measured tonotopic maps in 10 human subjects, and provide the clearest measures of human tonotopy to-date. The results in 20 out of 20 hemispheres clearly and consistently demonstrate that iso-frequency lines run parallel to the long-axis of Heschl's gryus, thus settling a long standing debate about the orientation of the primary maps. The primary tonotopic subfields are oriented along a posterior-to-anterior axis in the human, as in the macaque, and not rotated as previously proposed. Furthermore, the low frequency union of two mirror-symmetric tonotopic maps (border between primary areas A1 and R) is consistently located on the crown of Heschl's gyrus. This suggests a striking and previously unknown parallel with the visual cortex where the union of mirror-symmetric retinotopic maps (the V1/V2 border) also occurs on a gyrus. In summary, our results significantly clarify the organization of human auditory cortex, indicate homology with the macaque, and also suggest a common organizational pattern with early-visual cortex.

P 9

The role of AKAP-Lbc signaling complex in the activation of Nuclear Factor-kappa B during cardiomyocyte hypertrophy

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UNIL [1], UNIL-UNIBA [2]

In response to various pathological stresses, the heart undergoes a remodeling process that is associated with cardiomyocyte hypertrophy and to the progression to heart failure. Our earlier work indicates that AKAP-Lbc, an A-kinase anchoring protein (AKAP) with an intrinsic Rho-specific guanine nucleotide exchange factor activity, is critical for activating RhoA and transducing hypertrophic signals downstream alpha1-adrenergic receptors (a1-ARs). To identify the effector proteins linking AKAP-Lbc to the activation of cardiomyocyte hypertrophy, we followed a proteomic approach to determine the signaling molecules associated with the AKAP-Lbc signaling complex. Analysis of AKAP-Lbc immunoprecipitates using mass spectrometry identified the nuclear factor-kappa b (NF-kB) activating kinase IKKb as an AKAP-Lbc interacting protein. This raises the hypothesis that AKAP-Lbc might promote cardiomyocyte growth by maintaining a signaling complex that promotes the activation of the pro-hypertrophic transcription factor NF-kB. Interestingly, our results indicate that AKAP-Lbc is an important mediator of NF-kB activation as shown by the fact that suppression of AKAP-Lbc expression by infecting cells with lentiviruses encoding AKAP-Lbc-specific short hairpin RNAs strongly reduces the transcriptional activation of NF-kB induced by a1-ARs. Moreover, overexpression of a mutant form of AKAP-Lbc displaying constitutive Rho-GEF activity promotes NF-kB activation in HEK-293 cells. Importantly, AKAP-Lbc mediated NF-kB stimulation involved RhoA, Rho kinase and IKKb since it is inhibited by the dominant negative T19N mutant of RhoA, Rho kinase inhibitors, and the dominant negative mutant K44M of IKKb. Mapping analysis of the interaction between AKAP-Lbc and IKK indicates that IKK interacts with a region located within the Dbl homology domain of AKAP-Lbc. Altogether these results indicate that in HEK cells AKAP-Lbc can organize a transduction pathway including RhoA, Rho-Kinase and the IKK complex that is required for the activation of NF-kB. Future experiments will assess whether this complex mediates the hypertrophic responses induced by a1-ARs.

P 10

Transgenic Animal Facility - TAF

Marie-Laure Dénéréaz, Damien Maison, Anne-Marie Merillat, Edith Hummler

UNIL

The Transgenic Animal Facility (TAF) is now in its 6th year of activity. The platform generates genetically-engineered mouse models for biomedical research. In principle, the Platform generates transgenic mice using BACs and mini-genes, knock-out and Knock-in mice and performs rederivation of established mouse strains. Additionally, we offer thawing of frozen embryos. The TAF actively participates in the continued formation and offers thereby a platforme open to the local scientific community in order to answer questions concerning the generation and analysis of these mice.
Since August 2010, the Transgenic Animal Facility is part of the technical platforms of the NCCR Kidney :CH (Kidney Control of Homeostasis) and will develop complex engineered rat and mouse animal models using novel techniques like zinc finger nuclease technology.

P 11

Immunotherapeutic strategy to induce vaccine-specific CD8 T cells in bladder and regress orthotopic bladder tumors

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Service d'urologie - CHUV

Non musculo-invasive bladder cancer (NMIBC) can respond to immunomudolation as demonstrated by intravesical treatments with Bacillus Calmette-Guerin (BCG) after transurethral resections, though many patients fail to respond and/or suffer from side effects. Here, aiming to develop therapeutic vaccines, we have examined how to target vaccine-specific CD8 T cells to the bladder. Using an adjuvanted human papillomavirus oncogene (E7) vaccine as a model we have compared different routes of immunization.
Both subcutaneous and intravaginal vaccination induced similar number of TetE7+CD8+T cells in the bladder, while subcutaneous immunization induced higher responses in the spleen and intranasal immunization did not induce detectable responses in bladder. Bladder tumor regression was evaluated in an orthotopic model with tumor-cells co-expressing E7 and luciferase by bio-imaging. A single immunization by either routes conferred full tumor protection in a prophylactic setting. In a therapeutic setting, intravaginal vaccination efficiently regressed established tumors in 80% of mice, whereas subcutaneous vaccination led to only 40% surviving mice and intranasal immunization had no effect. Tumor regression correlated with vaccine-specific CD8 T cell tumor-infiltration with decreased regulatory T cells. To increase vaccine-specific CD8 T cells in bladder, intravesical BCG, CpG, Poly(I:C) or live attenuated bacteria were used after subcutaneous vaccination. This led to a 5-30 fold increase in number of vaccine-specific CD8 T cells in bladder, while systemic responses were not affected.
Our data shows that both subcutaneous and intravaginal vaccination can efficiently induce vaccine-specific CD8 T cells able to both prevent and regress small established tumors in bladder. Subcutaneous vaccination followed by intravesical application of immunostimulants significantly increased vaccine-specific CD8 T cells in the murine bladder. We anticipate that this will result in regression of larger bladder tumors, as we have shown in the case of murine genital tumors. This may represents a valuable immunotherapeutic strategy to investigate in NMIBC patients.

P 12

TCRep3D : A new automated in silico approach to build TCRpMHC structures and study the properties of TCR repertoires

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UNIL [1], SIB [2]

TCRep 3D is an automated systematic approach for TCR-peptide-MHC class I structure prediction, based on homology and ab initio modeling. It has been considerably generalized from former studies to be applicable to large repertoires of TCR. First, the location of the complementary determining regions of the target sequences are automatically identified by a sequence alignment strategy against a database of TCR Valpha and Vbeta chains. A structure-based alignment ensures automated identification of CDR3 loops. The CDR are then modeled in an ab initio approach based on a simulated annealing protocol. During this step, dihedral restraints are applied to drive the CDR1 and CDR2 loops towards their canonical conformations, described by Al-Lazikani et. al.. We developed an automated algorithm that determines additional restraints to iteratively converge towards TCR conformations making frequent hydrogen bonds with the pMHC. We demonstrated that our approach outperforms the most popular scoring methods (Anolea, Dope and Modeller) in predicting relevant CDR conformations. Finally, this modeling approach has been successfully applied to experimentally determined sequences of TCR that recognize the NY-ESO-1 cancer testis antigen. This analysis revealed a mechanism of selection of TCR through the presence of a single conserved amino acid in all CDR3beta sequences. The important structural modifications predicted in silico and the associated dramatic loss of experimental binding affinity upon mutation of this amino acid show the good correspondence between the predicted structures and their biological activities. To our knowledge, this is the first systematic approach that was developed for large TCR repertoire structural modeling.

P 13

Activity of daptomycin, levofloxacin, clindamycin and rifampicin against Propionibacterium acnes in planktonic and biofilm state

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UNIL [1], CHUV [2]

Objectives: Propionibacterium acnes is a low-virulent, facultative anaerobe organism, causing infections associated with implants. These infections are difficult to eradicate due to reduced antimicrobial susceptibility in biofilms. We investigated the antimicrobial activity of daptomycin, levofloxacin, clindamycin and rifampicin against P. acnes in planktonic form by conventional tests and in biofilms on glass beads by measuring growth-related heat production (microcalorimetry).

Methods: The activity of daptomycin, levofloxacin, clindamycin and rifampicin against P. acnes (ATCC 11827) was tested. The minimal inhibitory concentration (MIC) was determined by Etest, the minimal bactericidal concentration (MBC) by macrobroth dilution (MBD) in reduced Brain Heart Infusion (rBHI) at 48 h. P. acnes biofilm was formed on porous sintered glass beads in rBHI supplemented with 0.5% glucose (rBHI+g) at 37°C under anaerobic conditions without agitation. After 72 h, beads were washed in sterile normal saline trice and incubated in rBHI+g containing serial dilution of antimicrobials for 24 h. Beads were then washed as described above and placed in 4 ml rBHI+g. Recovering bacteria were detected by measuring heat production at 37°C for 72 h. The minimal biofilm inhibition concentration (MBIC) was defined as the lowest antimicrobial concentration inhibiting heat production during 72 h. All experiments were repeated three times.

Results: Against planktonic bacteria, rifampicin was the most active antimicrobial, whereas clindamycin was not bactericidal. Against biofilm bacteria, considerably higher concentrations of daptomycin (256x) and rifampicin (8000x) compared to MIC were required to inhibit growth of P. acnes biofilm during 72 h. Neither levofloxacin nor clindamycin were inhibiting P. acnes biofilms up to 1024 mg/L.

Conclusions: The activity of daptomycin and rifampicin was reduced in P. acnes biofilm, whereas levofloxacin and clindamycin showed anti-biofilm activity only at very high concentrations. Microcalorimetry allowed the investigation of most promising antibiotic agent eradicating biofilms for in vivo experiments in a foreign-body infection animal model.

P 14

The Protein Modeling Facility

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SIB Molecular Modeling Group

Protein Modeling Facility (PMF) is a platform at the University of Lausanne providing the researchers of Faculty of Biology and Medicine of Unil with a competence center for protein structure prediction, protein structure modeling, and drug design fields.
The in silico approach enables studying the macromolecular structure, interactions and dynamics at the level being often out of reach for experimental techniques, leading to more detailed understanding of the biological phenomena.
Since its creation in 2007 PMF contributed to many scientific projects mainly in the homology modeling and small ligand docking areas.
Few examples of typical projects conducted by the PMF will be presented.

P 15

Cardiomyocyte protection mediated by RasGAP-derived fragment N

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UNIL

The irreversible end point in heart failure induced by longstanding hypertension, myocardial infarction and oxidative stress is the apoptotic death of heart contractile units (cardiomyocyte). Apoptosis plays an important role in cardiotoxicity as a consequence of Doxorubicin-induced oxidative stress, which is at least in part mediated by the generation of peroxynitrite (PN) a nitrating and oxidant mediator formed in various pathological situations. Hypertrophy, growth in size without myocyte proliferation is compensatory mechanism in order to improve the contractile function. Apoptosis also plays a role in hypertrophic conditions where chronic hypertrophy will end by cardiomyocyte cell death. Increasing the survival potential of cadiomyocytes would be beneficial during episodes of myocardial hypertrophy and oxidative stress. We have determined that the N-terminal fragment of RasGAP (fragment N) can efficiently protect a variety of cell types against many different stresses. Fragment N protects primary cardiomyocytes from cell death induced by peroxynitrite-dependent oxidative stress. In addition, we investigate the heart activity in an in vivo mouse model where a mutation renders RasGAP caspase resistant (D455A KI mice); these KI mice are no longer able to generate fragment N. Heart parameters in two different experimental models were studied. In the first model, pressure overload through Trans aortic constriction (TAC), which induces stress leading to hypertrophy, resembles longstanding hypertension over the left ventricle, heart parameters were measured by echocardiography. In the second model mice were injected with a high dose of doxorubicin, which induces cardiotoxicity and cardiomyocyte apoptosis, Heart parameters were measured by Millar catheter. Our results show that RasGAP cleavage and fragment N generation play a role in preserving the contractile function of the heart upon stress. D455A KI mice TAC-stressed showed an increased expression of the hypertrophy gene markers, a marked reduction in cardiac function.

P 16

Control of cell polarity by the microtubule-associated protein Tea4

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Cell polarity is an essential property of most cell types and is a prerequisite for defining cell shape. Rod-shaped fission yeast cells grow by extension at cell tips and divide medially. Cdc42, a Rho-family GTPase, is localized at growing tips and essential for polarized growth. Cdc42 is activated by the Guanine Exchange Factor Gef1 and inactivated by the Rho GTPase Activating Protein Rga4. In fission yeast, microtubules define the sites of polarized cell growth. This is mediated by Tea4, which is transported to cell tips on microtubules and necessary for correct cell morphogenesis and bipolar growth. We asked the question of how the microtubule-associated Tea4 recruits Cdc42 for polarized cell growth. Tea4 contains a conserved SH3 domain (Src Homology domain 3). We show that this domain is essential for Tea4 function in vivo, as point mutations in this domain produce aberrant cell shapes and monopolar growth. Biochemical analysis of Tea4 complexes demonstrates that the SH3 domain is necessary for interaction with a type I phosphatase, Dis2. We show that Tea4 is an instructive signal for polarized growth: ectopic recruitment of Tea4 to the cell middle promotes growth at this site and recruits Dis2. Active Cdc42 is present at this ectopic site, as well as its activator Gef1. In contrast, Rga4, the Cdc42 inactivator, is either dispersed or excluded from this ectopic growth site. Disrupting the interaction between Tea4 and Dis2 or deleting Rga4 or Gef1 prevents ectopic growth. Localization studies indicate that Gef1 is not required for Rga4 exclusion, while Gef1 fails to be recruited in absence of Rga4, suggesting that Tea4 functions upstream of Rga4. Tea4 may regulate Rga4 localization by detaching it from the cell cortex through Dis2-dependent de-phosphorylation to allow local Cdc42 activation. Our results suggest a molecular pathway for how microtubules promote cell polarization and morphogenesis.

P 17

Astrocytic perisynaptic processes on neurons born in the adult hippocampus

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UNIL

Neurogenesis occurs in the adult brain and results in the production of neurons which integrate in the synaptic network. Our aim is to examine the development of astrocytic perisynaptic processes on synapses of neurons born in the adult hippocampus. Astrocytic processes are closely associated with synapses and actively modulate synaptic function and plasticity. Thus, our hypothesis is that the establishment of perisynaptic processes is of great importance for the synaptic and functional integration of newborn neurons.
Here, we used viral-mediated labeling of newborn neurons into the mouse brain and serial-section electron microscopy and confocal microscopy, to identifiy newborn neurons and analyze the development of perisynaptic processes in the molecular layer of the dentate gyrus.
We show that, one month after adult-born neuron formation, astrocytes already partially unsheath dendritic spines. Interestingly, dendritic spines from adult-born neurons recruit astroglial processes which are already involved in perisynaptic processes of dendritic spines from more mature neurons contacting the same axon terminal.
These results indicate that newborn neurons are contacted by astrocytes which form proper perisynaptic processes. Our observations suggest that new astrocytes are not created for the accommodation of new neurons, but instead new neurons recruit astrocytic processes from neighboring synapses. This astrocytic plasticity is relevant to the function of adult-born neurons and may participate to their integration into the adult brain

P 18

Molecular basis and regulation of insecticidal activity in plant root-associated pseudomonads

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UNIL [1], ETH Zurich [2]

Fungal diseases and insect pests cause major damage to agricultural crops and they are very difficult to control, in particular when below-ground plant parts are affected. Pseudomonas fluorescens are well-known disease suppressive bacteria in the rhizosphere of various plant species. The plant-beneficial P. fluorescens strain CHA0 produces many secondary metabolites which protect the plant roots against fungi and was recently discovered to also exhibit potent systemic and oral insecticidal activity.
The anti-insect action of CHA0 depends on the production of a novel large protein toxin termed Fit, for P. fluorescens insecticidal toxin, and additional yet unidentified bacterial factors. While non-toxic Escherichia coli can be rendered lethal to insects by transgenic expression of the toxin gene, Fit toxin-negative mutants of P. fluorescens are less virulent to insect larvae. The Fit toxin is part of a virulence cassette coding for regulators and a type I protein secretion system predicted to function in toxin export.
We try to identify mechanisms and signals that control the production, secretion and biological activity of the novel toxin and accessory virulence factors, and to understand their ecological role in the plant root environment. To answer our major research questions we make use of fluorescent proteins, toxin-specific antibodies, epifluorescence and electron microscopy, qRT-PCR, various insect and microanimal models, plant assays, and further techniques of molecular biology.

P 19

Sleep loss alters DNA-binding activity of circadian transcription factors

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We have previously shown that sleep deprivation (SD) alters the expression of clock genes in the forebrain suggesting that clock genes are not only involved in circadian rhythms, but also in sleep homeostasis [Franken P & Dijk DJ, Eur.J.Neurosci.2009]. Here, we test the hypothesis that SD alters clock genes expression by modifying the specific DNA-binding of the three core-clock transcription factors BMAL1, CLOCK, and NPAS2 to E-box or E?-box containing sequences of their target clock genes Per1, Per2, Cry1, and Dbp.

First, we verified if the DNA-binding of BMAL1 and CLOCK to targeted clock genes varied in function of time-of-day in the cerebral cortex of C57BL/6J mice using chromatin immunoprecipitation (ChIP) at ZT0, -6, -12, and -18 (ZT0 = light onset). DNA enrichment of sequences was measured by qPCR. We observed that BMAL1 and CLOCK binding to Per1, Per2, Cry1, and Dbp genes varied with time-of-day with maximal binding reached around ZT6-12. We then sleep deprived mice from ZT0 to ZT6 to assess the effects of sleep loss. We found that SD significantly and specifically decreased DNA-binding of CLOCK to Dbp, of NPAS2 to Per2, and of BMAL1 to both these target genes.

Our results show that the changes in the expression of specific clock genes with sleep pressure, notably that of Dbp and Per2, could result, at least in part, from changes in the DNA-binding activity of the core clock proteins BMAL1, CLOCK, and NPAS2.

P 20

Complex genetic influences on division of labor in social insects

Romain Libbrecht, Laurent Keller

UNIL

One of the hallmarks of insect societies is the division of labor. Several studies reported that increased genetic diversity (through multiple queens per colony and/or multiple mating per queen) facilitate the division of labor among workers. However none of these studies focused on the influences of the paternal and maternal genetic backgrounds and the genetic compatibility effects between parental genomes. In this study we investigate such genetic influences on two nursing tasks: feeding and gathering brood. We conducted controlled crosses in the Argentine ant Linepithema humile and established single-queen colonies to investigate the influences of both parents on worker nursing abilities. The time to gather pupae was affected by the maternal genotype while the ability to feed larvae was influenced by the interaction between parental genomes. First these results reveal that different worker nursing abilities can be differently influenced by the parental genotypes. Second they suggest complex effects of the genetic architecture on ant behavior. Such effects are likely to play an important role in the division of labor among workers in insect societies.

P 21

Development of a new Chlamydiales-specific real-time PCR and its application to respiratory clinical samples

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UNIL [1], HUG [2]

Originally composed of the single family Chlamydiaceae, the Chlamydiales order has extended considerably in the last decades. Chlamydia-related bacteria were added and classified in 6 different families and family-level lineages: the Criblamydiaceae, Parachlamydiaceae, Piscichlamydiaceae, Rabdochlamydiaceae, Simkaniaceae and Waddliaceae. While several members of the Chlamydiaceae family are known pathogens, recent studies showed diverse associations of Chlamydia-related bacteria with human and animal infections. Some of these latter bacteria are preoccupying since, given their ability to replicate in free-living amoebae, they may also replicate efficiently in other phagocytic cells, including cells of the innate immune system. Thus, a new Chlamydiales-specific real-time PCR targeting the conserved 16S rRNA gene was developed. This new molecular tool can detect at least 5 DNA copies and show very high specificity without cross-amplification from other bacterial clade DNA. The new PCR was validated with 128 clinical samples positive or negative for Chlamydia trachomatis or C. pneumoniae. Among 65 positive samples, 61 (93.8%) were found positive with the new PCR. The 4 discordant samples, re-tested with the original test, were negative or below detection limits. Then, the new PCR was applied to 422 nasopharyngeal swabs taken from children with and without pneumonia: 48 (11.4%) samples were positive, of which 45 were successfully sequenced. The majority of the sequences corresponded to Chlamydia-related bacteria and especially to members of the Parachlamydiaceae family.

P 22

The anemonefish adaptive radiation

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UNIL

Adaptive radiation is the process by which a single ancestral species diversifies into many descendants adapted to exploit a wide range of habitats. Despite its postulated importance for organism diversification, only few species groups have been thoroughly described as adaptive radiations and this stands even more strongly for marine organisms. In this study we show that following the development of obligate mutualism with sea anemones, the anemonefish adaptively radiated across most of the Indian and Pacific oceans reef habitats. Anemonefish vary in host usage and present local assemblages of host generalists and specialists that differ among various morphological traits such as body shape or gill rakers number. The development of the mutualism was followed by an increase in diversification as well as in phenotypic evolutionary rates as expected under the ecological theory of adaptive radiation. The comprehensive approach we used adds evidence validating hypotheses on the role of molecular evolution and dispersal in adaptive radiations suggested by previous theoretical work. More generally we propose the anemonefish, a group already well studied because of features such as sex reversal, increased longevity, complex social system or agonistic sound production, as a new model study group for adaptive radiation.

P 23

Correlate of protection in a human papillomavirus pseudovirions genital challenge murine model

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The available virus-like particles-based prophylactic vaccines (Gardasil® and Cervarix®) against specific HPV types, including the most prevalent types HPV16/18, afford close to 100% protection against the associated lesions and disease. According to PV animal models, protection is provided through HPV type-specific neutralizing antibodies that transude from serum to genital tract. However, a correlate of protection could not be established by the clinical trials because few disease cases had occurred and because some vaccinee had appeared to be seronegative with in vitro assays used to determine HPV-neutralizing antibodies.
Here, we determined minimal amounts of passively transferred HPV-neutralizing antibodies that are necessary to prevent in vivo genital infections, using the mouse genital Pseudovirion (PsV) challenge model. After transfer of known amounts of HPV16 neutralizing monoclonal antibody H16.V5 or different volumes of Gardasil-immunized mice serum, recipient mice were challenged with PsV encoding luciferase (PsV-luc). Protection against in vivo PsV16-luc and/or 18-luc genital challenge was compared to HPV16 or 18 neutralizing antibodies titers measured in serum using in vitro PsV neutralization assay. Our data show that serum antibody levels more than 100-fold lower that those detectable by in vitro neutralization assay are sufficient to confer protection against PsV genital infections in this model. This demonstrates that in vivo mouse genital challenge assay is at least 100 fold more sensitive than in vitro neutralization assay. Finally, for the first time, a correlation is drawn between serum HPV-neutralizing antibodies titers and the ability to prevent in vivo genital challenge in mice, these results may help to establish a correlate of protection in vaccinated women.

P 24

Cambinol, an inhibitor of Sirtuin 1 and Sirtuin 2, inhibits innate immune responses

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Background: Mammalian histone deacetylases (HDACs) are represented by HDAC1-11 and sirtuins (Sirt1-7). Sirt1-7 display deacetylase and/or ADP-ribosyl transferase activities, and have been involved in the control of cell metabolism, proliferation and survival. Pharmacological modulators of sirtuins are currently being evaluated for the treatment of metabolic, neurogenerative and oncologic disorders. We have recently shown that inhibitors of HDAC1-11 strongly inhibit innate immune responses and protect from mice from septic shock. The aim of the study was to analyze the impact of cambinol, an inhibitor of Sirt1-2 on innate immune responses in vitro and in vivo.
Methods: Bone marrow-derived macrophages (BMDMs), RAW 264.7 macrophages and human whole blood were incubated for 1 h with cambinol and stimulated with microbial products (LPS, Pam3CSK4, CpG DNA, E. coli and S. aureus). TNF and IL-6 mRNA and protein levels were quantified by RT-PCR, bioassay and ELISA. The activation of MAPK and NF-kB intracellular signalling pathways was analyzed by Western blotting. Mice were injected with LPS (LPS 17.5 mg/kg i.p.) and treated with cambinol (10 mg/kg). Blood was collected after 3h, and mouse survival followed over 6 days.
Results: Cambinol dose-dependently inhibited the secretion of TNF and IL-6 by macrophages and whole blood stimulated with microbial products. In agreement, cambinol inhibited LPS- and Pam3CSK4-induced cytokine mRNA expression in macrophages. Cambinol strongly interfered with the phosphorylation of ERK1/2 and p38 MAPKs but did not affect NF-kB nuclear translocation. In vivo, cambinol reduced TNF blood levels (P=0.05) and increased survival (from 8% to 46%; P=0.03) of mice injected with LPS.
Conclusion: Cambinol has potent anti-inflammatory activity in vitro and in vivo. Although the molecular mechanisms by which cambinol interferes with innate immune responses remain to be fully characterized, our data suggest that drugs targeting sirtuin activity could represent promising adjunctive therapy for the treatment of acute or chronic inflammatory disorders.

P 25

Activity of fluconazole against planktonic and biofilm candida albicans by microcalorimetry

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UNIL

Objectives: Candida albicans implant-associated infections are difficult to eradicate due to reduced antimicrobial susceptibility in biofilm. We investigated the activity of fluconazole against planktonic and biofilm C. albicans by measuring the growth-related heat production (microcalorimetry).

Methods: The activity of fluconazole was tested against a standard strain of C. albicans (ATCC 90028). The minimal inhibitory concentration (MIC) was determined by microbroth dilution according to the EUCAST guidelines (EDef 7.1). Planktonic C. albicans was evaluated by adding 5x10^5 CFU in 3 ml Saubouraud Dextrose broth (SDB) containing serial dilution of fluconazole (0.5-256 μg/ml). C. albicans biofilm was formed on porous sintered glass beads (diameter 4 mm, pore size 60 μm) in SDB at 37°C. After 24 h, beads were washed and incubated in SDB containing serial dilution of fluconazole (1-1024 μg/ml) for 24 h. Beads were then washed and placed in 3 ml SDB. Recovering bacteria were detected by measuring heat production at 37°C in a microcalorimeter.

Results: The MIC for fluconazole was 0.25 μg/ml. Fluconazole inhibited heat production of planktonic C. albicans at 1 μg/ml (~50% decrease of heat flow peak), whereas no inhibition of biofilm heat production was observed up to fluconazole concentration of 1024 μg/ml.

Conclusions: Microcalorimetry showed that the activity of fluconazole was reduced >1000x in C. albicans biofilm compared to planktonic growth. Additional antifungal drugs (alone or in combination) can be evaluated by microcalorimetry to determine the optimal strategy for eradication of C. albicans biofilms.

P 26

Contribution of glucocorticoid signaling to the brain molecular changes related to sleep homeostasis

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UNIL

Sleep is an essential and complex behavior controlled by two main processes: a homeostatic process, activated by the loss of sleep, and a circadian process which determines the time-of-day sleep occurs. Sleep homeostatic mechanisms are primarily studied by depriving subjects of sleep. Sleep deprivation (SD), however, not only prolongs wakefulness but also induces stress through activation of the hypothalamic-pituitary-adrenal axis which, in mice, is evident from the increase in corticosterone secretion.
Our previous study has shown that corticosterone greatly impacts the brain gene expression during SD. In particular, expression of clock genes and genes implicated in synaptic plasticity were affected, suggesting that glucocorticoid signaling contributes to both the circadian and homeostatic regulation of sleep. Moreover, among the many transcripts in the brain that are affected by SD, changes in microRNAs (mi-RNAs) have been reported. The aim of this study is to define the contribution of gluococorticoids to the brain molecular changes that are associated with the loss of sleep.
In a first experiment, whole brain mi-RNA levels were determined and compared in mice that were either sleep deprived for 6h (starting at light-onset) or left undisturbed, using micro-arrays. We found several miRNA significantly affected by SD and currently are confirming these transcripts by quantitative PCR. Moreover, to quantify the contribution of glucocorticoids to these SD-induced changes in miRNA expression, we will repeat the experiment in adrenalectomised and sham-operated mice.
The preliminary data show that miRNA play a role in sleep regulation through sleep homeostasis and that gluococorticoids seem to be implicated in regulating the molecular changes correlated to sleep homeostasis.

P 27

Anti-inflammatory properties of secretory IgA on intestinal epithelial cells during infection by Shigella flexneri

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The intestinal immune system has the complex task to protect the sterile core of the organism against invasion. Shigella flexneri, by invading intestinal epithelial cells (IEC) and inducing inflammatory responses of the colonic mucosa, causes endemic worldwide bacillary dysentery. The mechanism of entry of this bacterium is still a matter of debate. M cells participating in sampling antigens from the gut lumen through Peyer's patches are commonly considered as the primary site of entry of the bacteria. Once in the lamina propria, Shigella can invade IEC via their basolateral pole and spread from cell-to-cell leading to massive tissue destruction. More recently, data are accumulating demonstrating that bacteria can also enter the lamina propria directly via IEC, underscoring IEC as another gate of entry. In addition, the protective role of secretory IgA (SIgA) produced by plasmocytes of the lamina propria has been established in shigellosis context but few is known about its role in maintaining IEC monolayer integrity. Here, the impact of the bacterium was studied using polarized Caco 2 cell monolayer apically infected with a virulent strain of S. flexneri either alone or complexed with a cognate SIgA. Parameters associated with the infection process including cytokine measurements (IL-8, IL-18) and laser scanning confocal microscopy detection of Zonula Occludens-1, a tight junction (TJ) protein were studied. We demonstrate that bacteria are able to infect IEC through their luminal-like pole as well, inducing the complete disruption of TJ and the destruction of the whole reconstituted Caco-2 cell monolayer. SIgA upon neutralization of bacteria led to the maintenance of TJ supporting IEC integrity, and the reduction of cytokine release. Together with anti-inflammatory properties of SIgA, the fact that apical bacteria can damage the IEC without the intervention of other cells strongly suggests new mechanisms of invasion that can be involved in shigellosis.

P 28

Two is better then one

Davide Merulla, Jan Vandermeer

UNIL

Contamination with arsenic is a recurring problem in both industrialized and developing countries [1]. Of particular concern is the contamination of potable water sources by arsenic in Southeast Asia (Bangladesh, Vietnam). In order to provide alternative measurement tools for detection arsenic contamination, our group has developed a number of bioassays with so-called reporter bacteria [2,3]. These bacteria synthesize an easily measurable protein (such as green fluorescent protein) in response to arsenic. The response is proportional to the amount of arsenic applied to the cells. But the question is: how proportional? The kinetic of response of such a system turned out to be extremely interesting in the sense that can be tuned, and different strains responding only above specific concentrations of pollutant produced. A family of differently responsive whole cell bioreporters could give an array of yes/no answers and the concentration of pollutant in the sample inferred by simply looking at the array without the need of expansive and complicated devices.

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P 29

Adaptive statistical iterative reconstruction for pediatric cardiac CT examinations

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To evaluate the benefits of the adaptive statistical iterative reconstruction (ASIR) method on the diagnostic image quality of pediatric cardiac CT examinations and radiation dose reduction.

Four pediatric radiologists evaluated ten low-dose pediatric cardiac examinations (80 kVp - CTDIvol (4.8-7.9 mGy) - DLP (37.1-178.9 mGy·cm)). The average age of the cohort studied was 2.6 years (1 day-7 years). Acquisitions were performed on a 64-MDCT scanner. All images were reconstructed at various ASIR percentages (0%-100%). For each examination, radiologists scored 19 anatomical structures using the relative visual grading analysis method. To estimate the potential for dose reduction, acquisitions were also performed on a Catphan phantom and a pediatric phantom.

The best image quality for all clinical images was obtained with ASIR 20% and 40% whereas above ASIR 50%, image quality significantly decreased. With ASIR 100%, a strong noise-free appearance of the structures reduced image conspicuity. A potential for dose reduction of about 36% is predicted for a 2-3 year-old-child when using an ASIR 40% rather than the standard filtered back projection (FBP) method.

A mixture of 20% to 40% of ASIR slightly improved the conspicuity of various pediatric cardiac structures in newborns and children with respect to conventional reconstruction (FBP) alone. This result is in good agreement with clinical studies performed in adults.

P 30

Understanding the protective mechanisms induced by the RasGAP derived fragment N

Nieves Peltzer, Christian Widmann

UNIL

RasGAP, a regulator of the Ras and Rho small G proteins, is cleaved by caspase-3 into two fragments in low stress conditions. The N-terminal fragment, called fragment N, displays potent anti-apoptotic properties, protecting a variety of cell types against many death stimuli. Fragment N protects cells by activating the Ras-PI3K-Akt pathway. Which of the many Akt effectors is participating in fragment N-induced protection is unclearly defined at the moment, although we have recently observed that survivin, a member of the inhibitor of apoptosis (IAP) family, is induced by fragment N in an Akt-dependent manner.

Moreover we observed that when survivin is silenced, even though fragment ?N is produced in the cell under a mild stress, cells are no longer protected against apoptosis suggesting that indeed survivin is a key mediator in fragment N - mediated protection.

Survivin is an antiapoptotic protein but also a chromosomal passenger and so it is highly regulated at different levels, suffering from transcriptional to post-translational modifications. In this study we would like to characterize the mechanism of activation of survivin by fragment N and try to understand better this antiapoptotic pathway as a whole.

P 31

Secretory IgA: an actor of the interplay between the commensal flora and the intestinal immune system

Nicolas Rol, Blaise Corthesy

CHUV

In the gastro-intestinal tract, Peyer's patches have been described as a major inductive site for mucosal secretory IgA (SIgA) responses directed against pathogens. The classical view is that SIgA serves as the first line of defense against microorganisms by agglutining potential invaders and faciliting their clearance by peristaltic movements, a mechanism called immune exclusion.
Our laboratory has shown that SIgA is not only able to be "retrotransported" into Peyer's patches via the associated M cells, but also to deliver sizeable cargos in the form of SIgA-based immune complexes, resulting in the onset of non-inflammatory type of responses.
Such a novel function raises the question of the possible role of mucosal SIgA in the interplay with commensal bacteria and the contribution of the antibody in bacterial homeostasis. To address this question, Lactobacillus rhamnosus (LPR) was administered, in association or not with SIgA, into a mouse ligated loop comprising a Peyer's patch. After 2 hours of incubation in the loop, LPR bacteria are found more abundantly in the subepithelial dome (SED) region when they are coated with SIgA than LPR administered alone. Herein, it is shown that LPR-SIgA complexes enter into PPs via M cells, where they are engulfed by the dendritic cells of the subjacent SED region. Interestingly, LPR bacteria are found coated by the endogenous natural SIgA present in mice intestinal secretions, confirming the requirement of SIgA for this type of entry. Finaly, based on the expression of CD40, CD80 and CD86, it is shown that isolated Peyer's patches DCs cultured with LPR or LPR-SIgA both result in low maturation of these cells.
This work gives new evidences about the involvement of SIgA in the mechanism by which the intestinal immune system permanently checks the content of the intestine.

P 32

The role of NOX proteins in Chlamydial growth in the model organism Dictyostelium discoideum

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CHUV

Different members of the chlamydial family vary considerably in their ability to grow in phagocytic cells. Waddlia chondrophila multiplies logarithmically within macrophages, whereas Parachlamydia acanthamoebae may infect macrophages, leading to rapid apoptosis of the host cell. Macrophages are refractory to Chlamydia pneumoniae infection. Since D. discoideum may be used to study the phagocytic machinery, we investigated the role of the Nox genes in the outcome of infection using various nox gene mutants. Dyctiostelium discoideum encodes three members of the NADPH oxidase (Nox) family. NoxA and noxB are homologous to the phagocytic NOX2, an enzyme responsible for the respiratory burst. NoxC is homologous to NOX5 that contains two EF-hand domains. Preliminary results show that W. chondrophila is able to replicate in D. discoideum, even in the absence of nox genes. The growth of the bacteria depends on the density of the host cells and the available nutriments. Under these poor conditions W.chondrophila tends to form large aberrant bodies and arrest its replication.
P. acanthamoebae was not able to replicate in D. discoideum independently of the presence or absence of Nox genes. The bacteria were not even able to de-differentiate into reticulate bodies. However, the bacteria persisted in a viable state, since re-infection of D. discoideum cultures on P. acanthamoeba's natural host Acanthamoebae castellanii resulted in a productive infection. Further studies will be done to determine if the cohabitation of P. acanthamoebae with D. discoideum will be deleterious to the latter one.

P 33

Annual radiation dose of the Swiss population from diagnostic procedures

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UNIL [1], OFSP [2]

National radiation dose surveys are recommended to follow the trends in population exposure and ensure radiation protection safety. The last national survey was conducted in 1998 and the annual dose was estimated to be 1 mSv/caput. The purpose of our study was to follow the trends in diagnostic radiology between 1998 and 2008 in Switzerland and determine the contribution of different modalities and types of examinations to the total collective dose from medical X-rays. All users of X-ray units in the country were asked to participate in the survey. More than 225 examinations, divided into groups according to different modalities, were included in the survey. For this reason, an online database (www.raddose.ch) was developed. The effective dose for each examination was re-evaluated, taking into account the introduction of digital detectors and differences in radiological techniques. Differences between participants (radiology institutes, hospitals, general practitioners etc) were also examined. Data from more than 3,500 users (42%) were collected. Between 1998 and 2008 the number of radiographies, CT, mammographies, interventional procedures and bone densitometry examinations has increased. The 2008 survey showed that the total collective dose was 1.2 mSv/capita.

P 34

St3gal6, a new regulator of adipose tissue development

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UNIL

In order to better understand the molecular events leading to obesity, we are interested in the biology of adipocyte, the major component of adipose tissue and particularly in adipogenesis, the process by which new mature adipocytes are created.
The aim of the initial study was to identify new genes involved in adipose tissue development. We started from a microarray analysis comparing lean and obese mice to select genes whose expression in white adipose tissue is correlated with increasing body weight. We chose to focus our attention on one gene encoding for an enzyme, the alpha-galactosidase alpha 2,3-sialyltransferase 6 (St3gal6), involved in sialylation, a post-translational protein modification.
First we had shown that St3gal6 is expressed at the same level in white and brown adipose tissues and that it is expressed in the adipocyte fraction. Its expression is linked to adipose tissue expansion as it is increased during differentiation of preadipocytes and in adipose tissue of obese mice (genetic obesity and diet induced obesity). Moreover, St3gal6 belongs to a family of 20 enzymes and it appeared that St3gal6 is the sialyltransferase the most highly expressed in white and brown adipose tissue and then it suggests a potential role in adipose tissue development.
To address the issue of the role of St3gal6 in adipocyte differentiation, first we downregulated the gene expression in vitro in white and brown preadipocyte models that can be induced to differentiate. We found that the downregulation of St3gal6 by shRNA strategy decreases adipocyte differentiation. To go further, we are overexpressing St3gal6 in preadipocyte models to observe the effect of overexpression on adipocyte differentiation.
In order to test a role for St3gal6 activity in adipogenesis we are also testing the use of an alpha 2-3 sialyltransferase inhibitor and assess the impact of such a treatment on adipogenesis.

P 35

Integration of new neurons in the adult hippocampus : in vitro synaptogenesis

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UNIL/FNS

New neurons are continously integrated into existing neural circuits in the adult dentate gyrus of the mammalian brain. What is the function of these new cells and how are they integrated? Nowadays, the role of adult hippocampal neurogenesis is still unclear even though increasing evidence suggests its involvement in hippocampus-dependent learning and memory. The integration of these newly generated neurons represents a unique form of plasticity in the hippocampus and result in a drastic remodeling of the adult circuitry. We recently showed , at the electron microscope level, that adult-born neurons preferentially contact pre-existing synapses, thereby forming multiple synapse boutons (MSB). To better understand how newborn neurons integrate in the hippocampus at the synaptic level, we will explore the morphological mechanisms of synatogenesis in vitro and focus on MSB formation, stability and dynamics.
First, to observe long-term synapse formation and stability we will use a co-culture system of primary neurons and adult hippocampal stem cell-derived neurons, tagged fluorescently with pre- and post-synaptic marker, and time-lapse imaging. Then, to explore the role of neuronal activity in MSB dynamics, we will use a technique of light-driven control of neuronal excitability based on Channelrhodopsin-2 expression. The combination of these techniques will enable us to study the role of neuronal activity on synaptic integration, MSB formation and new neurons maturation and survival.

P 36

Spatio-temporal sequence of cross-regulatory events in root meristem growth

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A central question in developmental biology is how multicellular organisms coordinate cell division and differentiation to determine organ size. In Arabidopsis roots, this balance is controlled by cytokinin-induced expression of SHORT HYPOCOTYL 2 (SHY2) in the so-called transition zone of the meristem, where SHY2 negatively regulates auxin response factors (ARFs) by protein-protein interaction. The resulting down-regulation of PIN-FORMED (PIN) auxin efflux carriers is considered the key event in promoting differentiation of meristematic cells. Here we show that this regulation involves additional, intermediary factors and is spatio-temporally constrained. We found that the described cytokinin-auxin crosstalk antagonizes BREVIS RADIX (BRX) activity in the developing protophloem. BRX is an auxin-responsive target of the prototypical ARF MONOPTEROS (MP), a key promoter of vascular development, and transiently enhances PIN3 expression to promote meristem growth in young roots. At later stages, cytokinin induction of SHY2 in the vascular transition zone restricts BRX expression to down-regulate PIN3 and thus limit meristem growth. Interestingly, proper SHY2 expression requires BRX, which could reflect feedback on the auxin responsiveness of SHY2 because BRX protein can directly interact with MP, likely acting as a cofactor. Thus, cross-regulatory antagonism between BRX and SHY2 could determine ARF activity in the protophloem. Our data suggest a model in which the regulatory interactions favor BRX expression in the early proximal meristem and SHY2 prevails because of supplementary cytokinin induction in the later distal meristem. The complex equilibrium of this regulatory module might represent a universal switch in the transition toward differentiation in various developmental contexts.

P 37

Doxorubicin plus quercetin combination in human breast cancer cells

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Purpose Doxorubicin is a first-line chemotherapeutic for breast cancer; however, it is associated with severe side effects to non-tumoral tissues. Thus, it is necessary to develop new therapeutic combinations to improve doxorubicin effects at lower concentration of the drug associated with protective effects for non-tumoral cells. In this work, we evaluated whether the plant-derived flavonoid quercetin may represent such an agent.
Methods The effects of doxorubicin and quercetin as single agents and in combination were evaluated on cell survival, DNA and protein synthesis, oxidative stress, migratory potential and cytoskeleton and nucleus structure in highly invasive and poorly invasive human breast cancer cells in comparison with non-tumoral human breast cells. Results In human breast cancer cells, quercetin potentiated antitumor effects of doxorubicin specifically in the highly invasive breast cancer cells and attenuated unwanted cytotoxicity to non-tumoral cells. Quercetin interfered with cell metabolism, GST activity, cytoskeleton and invasive properties specifically in breast tumor cells compared with non-tumoral breast cells. Doxorubicin induced DNA damage in tumor and non-tumor cells; however, quercetin reduced this damage only in non-tumoral cells, thus offering a protective effect for these cells. Quercetin also induced polynucleation in aggressive tumor cells, which was maintained in combination with doxorubicin. Conclusions By combining quercetin with doxorubicin, an increase in doxorubicin effects was obtained specifically in the highly invasive breast cancer cells, while in nontumoral cells quercetin reduced doxorubicin cytotoxic side effects. Thus, quercetin associated with doxorubicin demonstrated very promising properties for developing chemotherapeutics combinations for the therapy of breast cancer.

P 38

Regulation of ICEclc transfer in pseudomonas knackmussii B13

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Background
Genomic islands (GEI) are a large family of potentially mobile DNA elements and play an important role in the dissemination of virulence factors, antibiotic resistances or toxic compound metabolism. Despite detailed information on coding capacities of GEIs, little is known about the regulation of GEI transfer.
The 103-kb self-transmissible GEI of P. knackmussii B13 (ICEclc) carries 3-chlorobenzoate (3-CBA) degradation genes and is capable of self-transfer to various hosts belonging to Beta- and Gammaproteobacteria. Self-transfer is initiated by an excision of the element from its chromosomal location, followed by conjugation to a new host cell and reintegration. Notably, only a small percentage (3-5%) of the population is able to transfer the ICEclc.
Objectives
Microarray studies revealed a highly expressed 14-kb gene cluster on ICEclc (orf68241 - orf81655), comprising mostly genes with unknown functions and most highly upregulated during stationary phase. Here, we focus on understanding the regulation of expression of this gene cluster.
Methods
In order to study gene expression of orf81655 at an individual cell level, transcriptional and translational gfp fusion reporter strains were constructed. In addition, Fluorescence In Situ Hybridization (FISH) was carried out to detect orf81655 mRNA in individual cells.
Conclusions
Interestingly, both GFP reporters and mRNA-FISH show that the orf81655-cluster is expressed in a few percent of cells in a population. This suggests it is part of the bistability regulon driving ICEclc activation and transfer. The fact that orf81655 mRNA can be detected by FISH in individual cells indicates that it is an extremely highly expressed gene.

P 39

The role of single-trial, episodic multisensory learning in unisensory object discrimination

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Single-trial multisensory experiences can influence the ability to accurately discriminate image repetitions during a continuous recognition task. Pairing visual objects with their corresponding sounds can enhance subsequent visual discrimination, whereas pairing visual objects with an identical pure tone has been shown to impair subsequent visual discrimination compared with performance with objects only encountered visually. Despite their opposing polarity, these effects indicate incoming visual stimuli access multisensory memory traces established through single-trial learning. One open issue is the role of semantic versus episodic multisensory experiences, because prior work was confounded by pairing different visual objects with an identical pure tone. Here, we determined the role of episodic multisensory experiences by pairing (on their initial encounters) visual objects with meaningless, but unique sounds. Subjects discriminated initial from repeated presentations of images of common objects. Half of the initial presentations of images were presented in a unisensory visual manner. Each of the remaining half of the images was paired on its initial presentation with a distinct but meaningless sound in a multisensory context. All repeated presentations were exclusively unisensory visual. Accuracy in recognition of repeated images was impaired for those that had been initially presented in a multisensory context. This decrement was dissociable from performance during initial image presentations, ruling out explanations in terms of attention or direct transfer from encoding to retrieval. Instead, the results indicate that the direction of the impact of single-trial multisensory memories on visual object discrimination is linked to the semantic versus episodic contingencies between the senses.

P 40

C4-dicarboxylate transport system in P. aeruginosa

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Pseudomonas aeruginosa is a versatile ubiquitous Gram-negative bacterium which has a phenomenal capacity to adapt to different environments and utilizes a wide variety of organic molecules as carbon and energy sources. Its large genome contains numerous genes for catabolism, nutrient transport and metabolic regulation. P. aeruginosa preferentially utilizes tricarboxylic acid (TCA) cycle intermediates such as the C4-dicarboxylates malate, fumarate and, in particular, succinate as carbon and energy sources.
To better understand the bacterium adaptation to its environment, we studied the C4-dicarboxylate transport (Dct) system in PAO1.
We showed that the growth of a PAO1ΔdctA mutant was impaired in minimal media supplemented with C4-dicarboxylates, indicating that DctA is the major C4-dicarboxylate transporter. However, residual growth of the dctA mutant in these media suggested the presence of additional C4-dicarboxylate transporter(s). Therefore, we performed Tn5 insertion mutagenesis of the ΔdctA mutant to identify a second Dct system. This strategy allowed the discovery of the DctPQM transporter, belonging to the tripartite ATP-independent periplasmic (TRAP) family of carriers. To investigate the redundancy of the Dct transport we carried out competition experiments. Our data showed that the efficiency of DctPQM and DctA transport diverged in presence of μM or mM concentrations of succinate.
Furthermore, using mutational and reporter fusion experiments, we discovered a novel two-component system regulating the expression of the C4-dicarboxylic acids transporters. This is the first time that the Dct system is unraveled in PAO1.

P 41

Melanic color-dependent anti-predator behavior in barn owl nestlings

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DEE, UNIL [1], EPFL [2]

The arms race between predators and prey has led to morphological and behavioral adaptations. Different anti-predator strategies can coexist within a population if each strategy is the result of a trade-off with competing demands. Anti-predator behavior can be associated with morphological traits, like color patterns, either because in the context of sexual selection coloration signals the ability to avoid predators or because coloration is a naturally selected trait useful in avoiding predators. Because in the barn owl (Tyto alba) heritable eumelanic plumage coloration is associated with the glucocorticoid-dependent response to stress, we tested whether anti-predatory behavior is also related to this trait. Compared to small-spotted nestlings, individuals displaying larger black spots hissed more intensely in the presence of humans, feigned death longer, had a lower breathing rate and were more docile when handled. Cross-fostering experiments showed that the covariation between spot size and the duration of feigning death was inherited from the biological mother. Our results confirm that melanin-based coloration is associated with suites of behavioral traits, a property that is under both genetic and environmental influence. Coloration can thus evolve as a direct or indirect response to predation, but it can also be a signal of anti-predator strategies to potential mates. Finally, the link between color and anti-predator strategies is phenotypically plastic.

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In vivo fluorine-19 MR angiography in a mouse model

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CIBM-CHUV [1], CIBM-EPFL [2], Uni Düsseldorf [3], Cardio-CHUV [4]

INTRODUCTION
Magnetic resonance angiography (MRA) is commonly performed using gadolinium (Gd), which might be dangerous to people with renal insufficiency, or using time-of-flight (TOF)-derived imaging, which is susceptible to distortions due to the flowing blood it highlights. Therefore, a flow-independent angiographic technique without Gd may be most valuable. We therefore propose MR imaging of a compound that, after intravenous injection, is only present in the blood-pool. The perfluorocarbons perfluoro-15-crown-5-ether (CE) is an excellent candidates for this lumen imaging: it is chemically inert, non-toxic and is in several phase-3 FDA trials [1], which also opens up the outlook for translation into the human setting. For these reasons, we have developed a fluorine-19 (F19) MRA methodology using CE, have tested it in vitro and have, for the first time, explored its utility for angiography in vivo.
METHODS
All experiments were performed in a 9.4 T horizontal-bore animal spectrometer. The study was approved by the local animal-ethics committee. Preparation of a 10% CE emulsion was carried out as previously described [2]. Male balb/c mice (n=9) were anesthetized and injected with 12 ul/g of the CE emulsion. After acquisition of anatomic H1 gradient echo (GRE) images (30x30x2 mm3), F19 GRE Imaging at the same anatomical level was repeated (512 averages, acquisition time = 21 min).
RESULTS AND DISCUSSION
The F19 images clearly, selectively and exclusively visualized the blood pool at different anatomical levels with high contrast. These F19 images consistently co-registered with the corresponding anatomy on the anatomical images.
CONCLUSION
Intravenously administered F19 is well-suited for a selective and exclusive visualization of the vasculature and the heart chambers in vivo. The high CNR and long intravascular containment makes 19F MRI a promising alternative flow-independent angiographic MRI technique. Since perfluoro-15-crown-5-ether is safe, a translation into the human setting may be possible.
[1]Ruiz-Cabello et al.,NMR Biomed(2010) [2]Flögel et al.,Circ (2008)

P 43

Reassessing the pathogenic role of staphylococcus aureus fibronectin-binding protein A (FnBPA) in a realistic model of infective endocarditis using prolonged low-grade bacterial inoculation

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Background: Recurring low-grade bacteremia following tooth brushing, or even mastication, is likely to cause infective endocarditis (IE) in patients at risk. This was recently confirmed in a new animal model of IE, in which prolonged low-grade inoculation of S. aureus was as infective as bolus injection of large bacterial numbers (> 104 CFU; Entenza et al. ICAAC 2009). Since this model mimics more closely the human situation, it is relevant to use it to reassess the role of S. aureus virulence factors involved in IE. Here used it to study the role of FnBPA, which was expressed individually in surrogate Lactococcus lactis.

Methods: Rats with sterile aortic vegetations (Veg) were inoculated with 106 or 107 CFU of L. lactis WT (lacking surface adhesins) or recombinant L. lactis expressing FnBPA (Que et al. IAI 2001; Que et al. JEM 2005). Identical inoculum sizes of each strain were given by continuous i.v. infusion, at a rate of 0.0017 ml/min over 10 h. Bacteremia levels 2 h after inoculation [expressed as mean (range) CFU/ml of blood] and Veg infection 24 h later were determined.

Results: Results:A CI of 106 CFU resulted in 4 of 14 (28%) and 9 of 12 (75%) infected vegetations for L. lactis pIL253 and L. lactis FnbpA, respectively (P< 0.05).Increasing the inoculum size to 107 CFU infected 9 of 18 (50%) and in 18 of 19 (95%) vegetations for L. lactis pIL253 and L. lactis FnbpA, respectively (P< 0.05).

Conclusions: Recombinant L. lactis expressing FnBPA were more infective parent L. lactis WT in this realistic low-grade inoculation model. This confirms FnBPA as a critical virulence factor in IE, and as potential target for andi-adhesin strategies. Reassessing the role of other virulence factors, such as that of fibrinogen-binding protein (ClfA), is currently in progress.

P 44

Evaluation of a PCR-RFLP assay for dermatophytes identification in situ

Julie Verrier, Olympia Bontems, Marina Fratti, Karine Salamin, Michel Monod

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Background: Dermatophytes are the main cause of superficial mycoses. These fungi have the capacity to invade keratinized tissue of humans or animals to produce infections that are generally restricted to the corneocytes of the skin, hair, and nails.
Statement of the problem: It is common to obtain negative results from fungal cultures of dermatological specimens where direct mycological examination showed fungal elements (30-40%). However, correct identification of the isolated dermatophytes from Tinea is important to choose the appropriate treatment.
Objectives: To develop a rapid polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) assay based on 28S rDNA that is able to identify dermatophytes species in positive dermatological samples.
Results: PCR-RFLP identification of dermatophytes in skin or hair allowed validation of the results obtained in culture. It was also possible to identify the infectious dermatophytes when direct hair/skin mycological examination showed fungal elements, but negative results were obtained from fungal culture.
Conclusion: PCR methods may provide significant benefits in the rapid diagnosis of Tinea. First, there is an increase in sensitivity of dermatophytes identification when enough material is available. Secondly, identification of the infecting agent can be obtained in 24h with PCR-RFLP or sequencing, whereas results from fungal cultures can take 3-4 weeks.
Keywords: dermatophytes, identification in situ, PCR-RFLP assay

P 45

Discovery of a NtrC dependent non-coding small RNA in Pseudomonas aeruginosa PAO1

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The ubiquitous bacterium Pseudomonas aeruginosa strain PAO1 is able to use a wide range of different carbon and nitrogen sources as nutrients. Some of these nutrients provide more energy or are easier to assimilate, consequently these preferred nutrients are always consumed in priority by this microorganism. To ensure an optimal coordination of the nutrient utilization, this bacterium has evolved very efficient regulation systems. Indeed, the genome of P. aeruginosa encodes for many transcriptional regulators and two-component systems which are involved in the sensing of nutrients availability and in the regulation of nutrient uptake and catabolism genes. The CbrA/B and NtrB/C two component systems play a major role in these sensing/regulation systems and operate with the alternative sigma factor RpoN (sigma54). The NtrB/C system is specialized in nitrogen utilization, while the CbrA/B system is involved in both carbon and nitrogen utilization.
Nutrient utilization is also regulated at the post-transcriptional level by regulatory non-coding small RNAs (sRNAs).
Here we characterize a new sRNA which is regulated by the NtrB/C cascade and by RpoN. The function of sRNA2913 in P. aeruginosa PAO1 remains unknown and is currently being studied.

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Mining bioisosteric molecular replacements

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During the course of the discovery of new medicines, a considerable amount of time is spent in a phase commonly called lead optimisation. In this process, compounds that have been identified to be bioactive against the chosen target are modified in order to improve potency and/or other relevant criteria, such as bioavailibility, solubility or counter-target selectivity. One fundamental tool medicinal chemists possess to drive this process is “bioiososteric replacements”, i.e. fragments that are commonly believed to be interchangeable without affecting the overall properties of the molecule. The current knowledge of such replacements is captured in the literature as well as in dedicated databases, but, as the replacements are usually only annotated for single targets, the accessible data are often more of an anecdotal nature than generalisable. In order to identify and appropriately judge those replacements, we analysed data from the ChEMBL database [1] of the EBI, paying special emphasis on those that occur in various targets and target classes. “Matched molecular pairs” (molecules that differ only in a small substructure) with reported activity against the same target were identified with the help of a highly efficient algorithm adapted from the literature [2] and stored in a dedicated database. This set of currently 3’955’771 molecular replacements enables not only the analysis from all possible angles but aims to become a tool directly applicable in computer-aided drug discovery. It will be made accessible as part of the public SIB web services.

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[2] Hussain, J., Rea, C. (2010) Computationally Efficient Algorithm to Identify Matched Molecular Pairs (MMPs) in Large Data Sets. J. Chem. Inf. Model , 50, 339-348

P 47

KCM, a novel model for codon evolution

Maryam Zaheri, Nicolas Salamin

UNIL

KCM: a novel mechanistic codon model with increased control over codon substitution rates:
Models of codon evolution have attracted particular interest because of their unique abilities to detect selection forces acting on protein coding sequences. Here, we present a novel approach to model codon evolution using a matrix operator called Kronecker product. In particular, the 61 by 61 transition rate matrix of codon models is implemented using Kronecker product of three 4 by 4 nucleotide transition weigh matrices, which are the building blocks of codons. This mechanistic model generalizes current models of codon evolution while restricting the parameter space to 19 parameters (3 times 6 for each nucleotide transition matrix and one selection parameter) through the use of Kronecker product. AIC measures showed that our models had a better fit than current codon models on several data sets from mammals and plants. It is capable to better explain the biological complexity of protein coding gene evolution, and in particular can take into account multiple substitutions per codon. Finally, we applied this new model to the detection of positive selection and we show that the model is more accurate in estimating selection pressure when compared to previous codon models.

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AtPHO1 expression in guard cells influence the response of stomata to abscissic acid

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UNIL

In plants, stomatal opening and closure is driven by ion fluxes that cause fluctuations in cell turgidity, a process that is in turn regulated by the phytohormone absicisic acid (ABA). We report genetic evidence in Arabidopsis thaliana that stomatal movements in response to ABA are influenced by AtPHO1 expression in guard cells. PHO1 is a mediator of phosphate export that has thus far been associated with phosphate export into xylem tissue. Consequently, the pho1 mutant has very low phosphate in leaves. Gene expression analysis using microarray and qPCR techniques revealed specific expression and induction of PHO1 in guard cells following treament with ABA. The pho1 mutant was unaffected in its stomatal response to white light, blue light, and fusicoccin. However, the stomatal response to ABA treatment, both in terms of induction of closure and inhibition of opening, was severely reduced in this mutant. Normal shoot growth and Pi content was observed following a micrograft of pho1 shoots onto wild type roots, but the stomatal response to ABA treatment was only partially restored. Specific expression of AtPHO1 in guard cells of pho1 mutant plants resulted in partial complementation and reestablishment of ABA sensitivity. In agreement with this result, specific expression knockdown of AtPHO1 in guard cells of wild type plants using RNAi caused a reduced stomatal response to ABA treatment. Combined, these results imply a role of AtPHO1 as a transporter and/or signaling component influencing the ABA-mediated stomatal response.

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