Abstracts of the LS2 Annual Meeting 2017

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This booklet will not be printed.

The printed meeting booklet on-site contains the poster number/title/authors/affiliations.

Poster categories indicated by the authors are listed alphabetically

1
Biochemistry

Impact of light on the circadian clock and mood-related behavior

Olejniczak, Iwona
Olejniczak, Iwona (1); Mansencal-Strittmatter, Laureen
(1) Fribourg University, Biology

As much as one in twenty people in Switzerland may be suffering from major depressive syndrome right now. While a number of successful therapies are on our disposal, most of them target reuptake inhibitors for neurotransmitters, and are burdened with slow onset and many side effects. An alternative may be the use of light, in the form of bright light therapy (BLT), which has been shown to affect mood. Unfortunately, the molecular mechanism of BLT remains elusive, although it is very likely to involve circadian component per1 induction and circadian clock resetting.

I observed that mice after light treatment in late night show less depressive-like behavior in Porsolt forced swim test, while per1 knock-out mice are characterized by higher immobility times and no response to light treatment. Also, I detected per1 induction after light pulse in late night in Lateral habenula (LHb), a nuclei in the brain involved in reward processing. LHb has been observed to be hyperactive in major depressive syndrome, and also receives direct signal from retinal ganglion cells after light stimulation.

I want to show that per1 induction in the LHb is essential for anti-depressive effect of light treatment in mice, provide a molecular mechanism of action, and consequently legitimize the use of BLT as treatment for major depressive syndrome.

2
Cancer Biology

Tracking the Clonal Origin and Chemotherapy Resistance of Malignant Pleural Mesothelioma

Selected for talk

Oehl, Kathrin
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Aims:
Malignant Pleural Mesothelioma (MPM) is a rare but aggressive neoplasm with a median life expectancy around 12 months, despite multimodal treatment including chemotherapy and surgery. So far, no effective second-line treatment could be established. To investigate the mechanisms of MPM development leading to treatment resistance, the objective of this study was to track the clonal origin and to find genetic markers that predict resistance to chemotherapy.
Methods:
A cohort of MPM patients undergoing treatment with Cisplatin and Pemetrexed followed by surgery were chosen for analysis. DNA was isolated from FFPE samples taken at the treatment-naive biopsy, at surgery after combined induction chemotherapy and at relapse. Afterwards, we performed targeted amplicon sequencing. For this purpose, based on a systematic literature review, a custom-designed MPM sequencing panel was established, targeting the 30 most frequently mutated genes in MPM. Analysis was conducted following internal guidelines for diagnostic sequencing.
Results:
Sequencing revealed a high frequency of mutations occurring in BAP1 and NF2. They are already present at a high allele frequency in the diagnostic biopsy, indicating an early clonal origin. Additionally, in most cases, they lead to a termination or frameshift, suggesting a high impact on protein function. Furthermore, BAP1 mutations showed a strong association to chemotherapy resistance.
Conclusions:
Sequencing analysis revealed a high inter- and intratumoral heterogeneity in MPM. Most of the mutations were found in BAP1 and NF2; they occured early in tumor evolution and are possible drivers of MPM. Especially BAP1 seems to be involved in the innate resistance of MPM to chemotherapy.

3
Cancer Biology
TGFβ mediates the pro-tumor effect in innate immune cells

Stefanescu, Cristina
Stefanescu, Cristina* (1); Roblek, Marko (1); Heikenwälder, Mathias (2); Borsig, Lubor* (1)
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Tumor microenvironment contains different types of immune cell infiltrates which contribute to cancer progression. Inflammation has been linked to formation of a metastatic microenvironment that supports the tumor cell extravasation and colonization of distant organs. TGFβ is a cytokine that is implicated in cancer progression, both as a tumor suppressor and a tumor promoter. These two functions are exerted by both a cell-autonomous TGFβ signaling and inducing communications within the tumor microenvironment. Thus, TGFβ effects on cancer progression are cell context-dependent. We studied the role of TGFβ using a mouse model with deletion of TGFβ receptor II in myeloid cells (LysMCre/TR2fl/fl). Experimental metastasis of colon and lung carcinoma, and melanoma cells were attenuated in these mice, indicating that TGFβ signaling in myeloid cells has pro-metastatic activity. IHC analysis of lungs of tumor injected mice showed a reduced size of metastatic foci at 4 and 7 days, but no difference in leukocyte (Ly6G, F4/80, CD3, B2.20) recruitment and association with cancer cells. We observed also decreased spontaneous lung metastasis in LysMCre+ mice. Analysis of lungs 28 days after subcutaneous tumor cell injection revealed increased numbers of B220 B-cells and CD3 T-cells in the lung metastatic lesions and an increase in pro-inflammatory cytokines in the lung at 14 and 20 days. We test the hypothesis that TGFβ signaling in myeloid cells affects tumor outgrowth at metastatic sites by modulating both the adaptive and the innate immunity.

4
Cancer Biology

The Role of Hypoxia and Inflammation in the Tumor Microenvironment of Colon Carcinoma

Selected for flash talk

Glaus Garzon, Jesus Francisco

Glaus Garzon, Jesus Francisco (1); Jurisica, Igor (2); Heikenwälder, Mathias (3); Hottiger, Michael O. (4); Wenger, Roland H. (1); Borsig, Lubor* (1)
(1) University of Zurich, Institute of Physiology; (2) Princess Margaret Cancer Centre; (3) German Cancer Research Centre; (4) University of Zurich, Institute of Veterinary Biochemistry and Molecular Biology

Colorectal carcinoma is one of the most frequent cancers in the world with increased mortality due to liver and lung metastasis. Changes in the tumor microenvironment increase cell aggressiveness and immune evasion that promote tumor progression. Inflammation and hypoxia are key factors that modulate the tumor microenvironment. Inflammation recruits and stimulate immune cells that influence tumor development. Hypoxia induces HIF stabilization, which mediates transcriptional gene activation required for angiogenesis, proliferation and invasiveness. We used a syngeneic orthotopic murine model of colorectal cancer to evaluate the role of an impaired tumor-specific hypoxic response (MC-38-HIF-1a-KD) in leukocyte recruitment, angiogenesis and tumor progression. HIF-1a-KD tumors were smaller with reduced infiltration of granulocytes but increased recruitment of inflammatory monocytes. RNA sequencing data of sorted tumor cells revealed downregulation of pro-angiogenic molecules and upregulation of vessel-stabilizing factor in HIF-1a-KD cells. TRAF6 was predicted to interact with HIF-1a in silico. Role of TRAF6 in tumor progression was evaluated in vivo. MC-38-TRAF6-KD cells showed a reduced metastatic potential. Increased infiltration of myeloid cells with anti-tumorigenic phenotype was observed in TRAF6-KD tumors, which suggests an intact tumor-specific inflammatory response is required for modulation of the tumor microenvironment. Hypoxia-inflammation crosstalk should be considered for development of cancer therapies.

5
Cancer Biology

L-selectin on myeloid cells mediates development of metastasis in a tissue-specific manner

Protsyuk, Darya

Protsyuk, Darya (1); Glaus Garzon, Jesus (1); Borsig, Lubor* (1)
(1) University of Zürich, Institute of Physiology

Bone marrow-derived myeloid cells have been identified as promoters of metastasis. L-selectin is a leukocyte homing receptor that is important for the initial capture of leukocytes to the activated endothelium. We sought to determine the function of L-selectin in metastatic progression. L-selectin-dependent monocyte recruitment to the metastatic emboli promoted experimental lung metastasis. Lower numbers of L-sel-/- myeloid cells correlated with reduced levels of cytokines during lung seeding. While a depletion of circulating monocytes diminished metastasis both in WT and L-sel-/- mice, adoptive transfer of monocytes restored lung metastasis. WT monocytes facilitated trans-endothelial migration of tumor cells, L-sel-/- monocytes showed no effect in vitro. We demonstrated that injection of tumor-conditioned media rescued experimental lung metastasis in L-sel-/- mice and that spontaneous lung metastases in the absence of L-selectin were not affected. Interestingly, spontaneous liver metastasis was increased in L-sel-/- mice. Similarly, increased experimental liver metastases were observed in the absence of L-selectin. Among differences in leukocyte population within the liver, a significant reduction in the number of F4/80+ cells was observed in tumor-bearing L-sel-/- mice when compared to WT controls. Expression of anti-tumor cytokines (e.g. IFNγ, IL-12, IL-2) was reduced in the livers of L-sel-/- mice, thus more protumorigenic environment was likely promoting metastatic growth. Although L-selectin facilitates tumor cell extravasation and metastasis in the lungs through the recruitment of F4/80+ cells, the very same cells limit the tumor outgrowth in the liver changing the tissue microenvironment.

6
Cancer Biology
MitoModules as cancer biomarkers

Sajic, Tatjana

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Biomarkers are molecules that are able to distinguish between disease and the healthy state of the body. Although in general practice biomolecules are usually observed and measured as independent clinical markers of a specific disorder, in real biological systems these molecules are not independent; rather, they are part of physical and functional interaction networks. For example, mitochondrial protein complexes play a role in cancer bioenergetics, survival, and proliferation. Here, we hypothesized that those mitochondrial functional modules with severe metabolic alterations in cancer tissues can be detected by an accurate quantitative approach that measures the abundance of mitochondrial proteins. To challenge our hypothesis in a diethylnitrosamine (DEN)-chemically induced mouse liver cancer model that causes distinctive metabolic perturbations, we proceeded, in parallel, with two proteomic experiments: one from total cellular liver lysate, the other from enriched mitochondrial fractions. We identified and quantified over 2700 proteins in both experiments. From the total cellular lysate and from the mitochondrial fraction, we detected the subset of liver proteomes that changes significantly between the normal and the cancerous liver tissue. Furthermore, concerning the detection of the cancer state, particularly informative was the combination of two proteome data sets (i.e., lysate and mitochondrial fraction) since it allowed us to observe protein abundance in relation to its subcellular localization.

7
Cancer Biology

Proteomic characterization of high-grade serous ovarian carcinoma using SWATH-MS

Friedrich-Grube, Betty

Thomas, Stefani (1); Friedrich-Grube, Betty (2); Aebersold, Ruedi* (3); Zhang, Hui* (1)
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High-grade serous ovarian carcinoma (HGS-OC) is a highly aggressive subtype of ovarian cancer that accounts for 70% of ovarian cancer deaths. Although approximately half of these tumors exhibit deficiency in homologous recombination, which is associated with chemotherapy treatment sensitivity, a basic understanding of the origin and development of these tumors is still lacking.
In this study we characterized the proteomic landscape of 103 HGS-OC tumors previously analyzed by The Cancer Genome Atlas (TCGA) (The Cancer Genome Atlas Research Network, Nature 2011) as well as the Clinical Proteomic Tumor Analysis Consortium (CPTAC) (Zhang H et al., Cell 2016).
We analyzed the samples with the massively parallel targeted, data-independent mass spectrometry method SWATH-MS (Gillet L et al., Molecular Cell Proteomics 2012), thus generating a proteomic data set that is orthogonal to the data generated initially by CPTAC.
We confidently quantified about 3000 proteins using the open-source software OpenSWATH (Röst HL et al., Nature Biotechnology 2014).
Clustering analysis revealed 4 molecular subtypes with high overlap in the corresponding analysis of the original CPTAC study. The orthogonal SWATH-MS approach verified the original discovery phase findings and these results will be pursued to reveal new functional insights into HGS-OC biology and to assess the performance of different proteomics methods in a clinically relevant research setting.

8

Cancer immunology

Formation and function of tertiary lymphoid structures in lung squamous cell carcinoma

Silina, Karina

Cancelled

9
Cellular Immunology
Dictyostelium discoideum cell-intrinsic immunity.

Raykov, Lyudmil

Raykov, Lyudmil (1); Soldati, Thierry (1)
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The social amoeba Dictyostelium discoideum is a recognized model phagocyte to study processes of cell-intrincic defences, which are conserved in professional phagocytes of the animal immune system, such as macrophages or neutrophils. We use D. discoideum as a host cell for the pathogenic bacterium Mycobacterium marinum, a close relative of Mycobacterium tuberculosis, to study the molecular mechanisms of the host-pathogen interactions during infection. Following uptake by the host cell, M. marinum inhibits host defense mechanisms such as autophagy and lysosomal-dependent degradation, to establish a permissive niche with endosomal features, where it replicates. Studies in animals showed a group of interrelated host factors involved in stress signaling transduction and pathogen detection. These proteins are the E3-type ubiquitin ligases TNF-associated factors (TRAFs), the guanylate binding proteins (GBPs, a family of cytokine-induced large GTPases), the tripartite motif (TRIM) proteins known to play a dual role as receptors and regulators of autophagy, and finally the signal transducers and activators of transcription (STATs). However, little is known about the upstream sensors, recognizing the pathogen and promoting activation and recruitment of these factors, and the downstream actors, relaying the stress signal and leading to differential expression of defense genes. Therefore, to identify relevant relationships and potential partners in these immune pathways, we are exploiting and characterizing the, TRAF, GBP, TRIM and STAT orthologs in D. discoideum. We are monitoring by live microscopy the intracellular localization of all these proteins, previously tagged with fluorescent proteins, in normal and stress conditions. In addition, we are analysing mycobacterial survival within D. discoideum knockout cells lacking these factors. Finally, we aim at identifying the targets of the infection-relevant STAT transcription factors and at screening for relevant interaction partners by applying the ChIP (chromatin immunoprecipitation) and APEX2 (proximity labelling with ascorbate peroxidase) techniques, respectively.

10
Computational Biology

Onctopus: Subclonal reconstruction of cancer samples based on single nucleotide variants and copy number aberrations

Selected for talk

Sundermann, Linda

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Cancer samples are often genetically heterogeneous, harboring subclonal populations (subpopulations) with different mutations such as copy number aberrations (CNAs) or single nucleotide variants (SNVs). Information about such mutations in the subpopulations can help to identify driver mutations or to choose targeted therapies. Sequencing of bulk tumor samples is current standard practice because single–cell assays are yet not well established due to high cost and limited resolution. Recently, several methods that attempt to infer the genotype of subpopulations using CNAs, SNVs, or both have been published. Here, we present Onctopus, a new approach to jointly model and reconstruct the subclonal composition of a bulk tumor sample utilizing CNAs and SNVs. Given haploid average copy numbers of segments affected by CNAs and variant counts of SNVs, Onctopus assigns a frequency, CNAs and SNVs to N subclonal lineages. Each of these lineages is defined through the CNAs and SNVs that arose in this lineage. We build a joint likelihood model and model the tumor as consisting of a mixture of lineages. We choose subclonal lineages to avoid ambiguous solutions that can occur when copy numbers are determined for subpopulations. We developed a linear relaxation of our model as a mixed integer linear program that can be solved with state–of–the–art solvers.

11
Computational Biology

HAMAP - leveraging Swiss-Prot curation for the automated annotation of protein sequences

Pedruzzi, Ivo

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(1) SIB Swiss Institute of Bioinformatics, Swiss-Prot Group

HAMAP (High-quality Automated and Manual Annotation of Proteins) is a rule-based automatic annotation system for the functional annotation of protein sequences. HAMAP can be used for the annotation of individual protein sequences or complete microbial proteomes via our HAMAP-Scan web interface at http://hamap.expasy.org. HAMAP consists of a collection of family profiles for determining protein family membership, and their associated annotation rules for attachment of functional annotation to member sequences. As well as the annotations themselves, HAMAP rules also specify the conditions under which these annotations may be applied, such as taxonomic constraints or a requirement for key functional residues (identified by structural or other experimental studies), thereby achieving high specificity by coupling predictions to presence of specific residues. Both HAMAP family profiles and annotation rules are created and maintained by experienced curators using experimental data from expertly annotated UniProtKB/Swiss-Prot entries. Originally developed to support the manual curation of UniProtKB/Swiss-Prot records describing microbial proteins, the scope and content of HAMAP has been continually extended to cover eukaryotic and lately also viral protein families. Part of the UniProt automatic annotation pipeline, HAMAP now routinely provides annotation of Swiss-Prot quality for millions of unreviewed protein sequences in UniProtKB/TrEMBL.

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Computational Biology

Fresh, high quality, local and global: The UniProt protein resource

Gasteiger, Elisabeth

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The Universal Protein Resource (UniProt) is a comprehensive resource for protein sequence and functional data. The centerpiece of UniProt is the knowledgebase (UniProtKB) which is composed of the expert curated UniProtKB/Swiss-Prot section and its automatically annotated complement, UniProtKB/TrEMBL. Swiss-Prot contains over 550,000 sequence entries that combine manually verified sequences with experimental evidence derived from biochemical and genetic analyses, 3D-structures, mutagenesis experiments, information about protein interactions, post-translational modifications and disease associations. TrEMBL provides a further 70 million sequences that have been largely derived from high throughput sequencing of DNA and are annotated by our rule-based automatic annotation systems. UniProt contains data for over 30,000 species with completely sequenced genomes, organized into "proteomes". UniProtKB is complemented by the UniProt Reference Clusters (UniRef) that cluster protein sequences at different levels of sequence identity to speed up sequence similarity searches, and the UniProt Archive (UniParc) which provides a complete set of known sequences, including historical obsolete sequences. All these databases are available on the UniProt website at http://www.uniprot.org, where they can be browsed and queried seamlessly, both by interactive users and by programmatic access. The website was designed using a user-centric approach, and also includes services such as similarity search (BLAST), multiple sequence alignment, identifier mapping, and exact peptide search.

13
Computational Biology

Hybrid *de novo* assembly of highly heterozygous allotetraploid cereal genome assisted by its parental diploid genome

Hatakeyama, Masaomi

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Heterozygosity or polyploidy is one of the difficulties on de novo genome assembly. The heterozygosity or polyploidy of genome causes a lot of homologous sequenced reads and it makes complicated to concatenate short reads in most cases of graph based de novo assembly method. Typically domesticated crops such as rice, wheat, and millet are mostly polyploid plants, and it is said that about 30%-80% of living plants are polyploid. A long read sequencing technology such as PacificBio sequencer is improving this situation, since long sequenced read can solve the homologous short read concatenation with long range sequence information. However, long reads normally have high error rate and the sequencing costs relatively expensive. We have been developing a hybrid de novo assembly method for polyploid genome using shallow long and deep short sequenced reads assisted by its parental diploid genome. The pipeline consists of 1) normal de novo assembly of parental diploid genome, 2) homeolog separation of polyploid reads using diploid assembled genome, 3) normal de novo assembly of polyploid using short reads, 4) scaffolding and gap closing using long reads. The pipeline will be integrated with the existing application framework currently used at the Functional Genomics Center Zurich. It is expected that the total sequencing cost will be reduced by using the smaller parental diploid genome and the accuracy of assembly will be improved by homeolog separation, and furthermore the assembled genome reference will be applicable to gene expression or population genetics analysis of each homeolog separately.

Computational Biology

Metagenome annotation with Distributed Reference Graphs

Kahles, Andre

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The accurate and comprehensive annotation of microbial communities becomes an increasingly relevant task in clinical research. Currently established methods for the analysis of microbiota are limited by incomplete taxonomies and annotation biases and, importantly, waste a large fraction of the raw sequence data that cannot be assigned to existing references. To address these shortcomings, we have implemented a new, highly sensitive approach to combine, represent and identify the microbial and functional composition of a large set of metagenome samples with a major focus on taking previous knowledge into account. Building on techniques from genome assembly and text compression we use succinct data structures to efficiently represent all sequence information in a k-mer based assembly graph, capturing both inter- and intra-species variability and achieving compression rates of over 70%. Structured as a dynamic self-index, the graph allows for efficient extension and can be used for alignment and annotation of reads arising from metagenome sequencing experiments. Furthermore, the graph allows for extension with new sequences without re-indexing. We also developed a concept to distribute the index over a set of computers for faster alignment. The nodes of the graph are colored using compressed annotation vectors, encoding information such as species or other associated metadata. The graph leverages information from known genomes as well as from the many previous studies, providing access to rare observations not yet present in reference databases, integrating information over many samples. It will have a greater sensitivity to detect unseen or rarely seen species and inherently represents nearest neighbors with less bias towards species overrepresented in existing databases.

15
Drug Discovery

Experimental verification of in silico predicted protein binder to the FOXO4 transcription factor

Taus, Petr

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During the last decade, in silico prediction of protein-protein interactions became a widely used method in primary research as well as in drug design. However, the predicted structures are still far from fitting to their in vivo behaviour of the respective biomolecules. Experimental data are essential for the improvement of the prediction algorithms. In our project we chose the mouse PDZ domain as a suitable structure for rational design. We identified the key interface residues in the complex of the PDZ domain and a biologically relevant target – transcription factor FOXO4 and we selected ten mutations in the PDZ domain resulting in higher interaction affinity between the two molecules. To determine protein stability of the predicted PDZ mutant variants and their binding affinity to FOXO4 we used nano differential scanning fluorimetry technology and microscale thermophoresis technology respectively. We observed no significant changes in the PDZ protein stability and determined higher binding affinity to FOXO4 for mutant versions of PDZ compared to wild type. Therefore we proved that PDZ domain structure is suitable for rational design and we verified our choice of these mutant variants. In the follow-up experiments, we will analyze PDZ/FOXO4 binding interface by two complementary experimental methods HDX-MS and NMR. Experimentally obtained data will be used for the improvement of algorithms for in silico p-p interaction prediction.

16
Genetics

Functional Mapping of Yeast Genomes by Saturated Transposition

Michel, Agnès

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Yeast genetic screens have been absolutely instrumental in our understanding of cell biology. Yet they remain tedious and oftentimes incomplete. Next generation sequencing on the other hand is fast and exhaustive. I have implemented a transposon-based approach combined with deep sequencing to define the complete set of genes that are essential for growth in a particular condition, in one go. The idea is to saturate the yeast genome with independent transposon insertions. Transposons cannot insert in genes that are essential in a given condition. Millions of clones, each bearing one insertion, are easily generated and collected as a pool. Deep-sequencing of the transposon-genome junctions identifies the locations that tolerate the presence of the transposon and allows to deduce those that do not, revealing the corresponding set of essential genes. The strength of the method lies on the fact that it interrogates the entire genome at once and is readily amenable to multiple growth conditions for comparison. When used to compare different genetic backgrounds, it reveals genetics interactions. When applied to compare drug-treated to untreated cells, it reveals the set of genes conferring resistance or sensitivity to the drug. In addition to identifying essential genes, this method also generates informative alleles. For instance, transposon insertions can yield truncations of essential genes, allowing to map functional protein domains.
Our method thus allows to screen the yeast genome with an unprecedented throughput and resolution.

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Genetics

Analysis of smad4 gene expression pattern during zebrafish embryonic development

Scepanovic, Jelena

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Background
The Smad4 protein is a highly conserved mediator of many developmental processes in vertebrates and it is associated with malignant tumor progression. As zebrafish (Danio rerio) is rapidly becoming a superior vertebrate model system for cancer research, it could be used for investigation of the Smad4 role in tumorigenesis. The goal of this study was to examine the expression pattern of smad4 gene in zebrafish early embryogenesis and to compare it to the expression pattern during development in other vertebrates.
Method
Zebrafish embryos were maintained at 28°C and collected at different developmental stages: 0, 6, 24 and 48 hours post-fertilization (hpf). For each time point, total RNA was extracted from 20 embryos using TRIzol reagent and subjected to cDNA synthesis. The relative level of smad4 gene expression was analyzed by real-time PCR using SYBR Green chemistry, with β-actin as the reference gene.
Results
The smad4 gene expression was found to be significantly increased at 6 hpf to 207% relative to 0 hpf (baseline, 100%). The expression level gradually decreased to 71% at 24 hpf, and further to 31% at 48 hpf.
Conclusion
Our results show that smad4 gene was expressed at all examined zebrafish developmental stages, which is consistent with the essential role of Smad4 in early embryonic development. The obtained smad4 expression pattern in zebrafish was similar to the pattern observed in other vertebrates, including mammals. Therefore, zebrafish can be considered as a suitable model system for research on the role of Smad4 in carcinogenesis.

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Infectious Diseases

Cellular communication between Plasmodium falciparum-infected red blood cells and neutrophils via extracellular vesicles

BABATUNDE, ADEBAYO

BABATUNDE, KEHINDE-ADEBAYO (1); Fellay, Isabelle (1); Ghiran, Ionita (2); Filgueira, Luis (1); Walch, Michael (1); Mantel, Pierre-Yves (1)
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Extracellular vesicles (EVs), including exosomes and microvesicles, are small membrane vesicles derived from multivesicular bodies or from the plasma membrane. EVs play important roles in intercellular communication, both locally and systemically, as they transfer their contents, including proteins, lipids, and RNAs, between cells. EVs are involved in numerous physiological processes, and vesicles from both non-immune and immune cells have important roles in immune regulation. We have shown that Plasmodium falciparum-infected red blood cells (iRBCs) release vesicles, which not only regulate parasite development but also mediate the host-pathogen interactions. EVs contain RBC-derived miRNAs that regulate gene expression in the recipient cells including endothelial cells and neutrophils. We are investigating the contribution of EVs in the regulation of the pathogenesis during malaria. Our data suggest that miR451a contained in EVs is an important regulator of the immune function of neutrophils.

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Infectious Diseases

Extracellular vesicles in the regulation of neutrophil dysfunction during malaria

Selected for talk

Mantel, Pierre-Yves

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People infected with Plasmodium falciparum the parasite responsible for malaria are more susceptible to bacterial infections. Indeed bacterial infections are often a fatal complication of malaria infection. In particular, the neutrophil bactericidal activity is impaired, leading to deficient resistance mechanisms. The factors causing neutrophil dysfunction remain unknown. Extracellular vesicles (EVs) are small vesicles released by infected red blood cells (iRBCs). These vesicles contain proteins, lipids and nucleic acids that are derived from the RBC as well as the parasite. Recent works demonstrated that EVs play important roles in intercellular communication, both locally and systemically, as they transfer their contents, including proteins, lipids and RNAs, between cells. EVs are involved in numerous physiological processes, and vesicles from both non-immune and immune cells have important roles in immune regulation. We demonstrated that iRBCs release vesicles, which regulate parasite development and mediate the host-pathogen interactions. EVs contain RBC-derived miRNAs that regulate gene expression in the recipient cells. We are investigating the contribution of EVs in regulating neutrophil function. Our data show that EVs are rapidly taken up by neutrophils and inhibit reactive oxygen species production, which results in a reduced bactericidal activity. Remarkably, EVs deliver functional miR451a from iRBCs to neutrophils. After transfer, miR451a control the transcriptional response to microbes. Indeed, following EV uptake, several miR451a-target genes are downregulated upon subsequent LPS treatment. In conclusion, we found that EVs released by iRBCs act as regulators of the neutrophil response to pathogens. Therefore, EV-mediated transfer of regulatory RNA species might be responsible for the deficient resistance to bacterial infections.

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Infectious Diseases

Antagonistic manipulation of autophagy by the Mycobacterium marinum ESX-1 secretion system

Cardenal Muñoz, Elena

Cardenal-Muñoz, Elena (1); Arafah, Sonia* (1); López-Jiménez, Ana Teresa (1); Kicka, Sébastien (1); Falaise, Alexandra (1); Bach, Frauke (2); King, Jason S. (3); Hagedorn, Monica (2); Soldati, Thierry* (1)
(1) University of Geneva, Biochemistry; (2) Bernhard Nocht Institute for Tropical Medicine, Parasitology; (3) University of Sheffield, Biomedical Sciences

The autophagy pathway is a catabolic process that eukaryotic cells use to digest and recycle cytoplasmic components. Under stress conditions, such as nutrient starvation or oxidative stress, autophagy is induced and generates double-membrane autophagosomes that mature in autolysosomes, where damaged organelles and misfolded protein are degraded to be finally recycled into the cytoplasm. This well conserved pathway also participates in cell-autonomous defence. Thus, intracellular pathogens are engulfed by autophagosomes, where, after fusion with lysosomes, they are killed and digested. However, some pathogenic bacteria are capable to subvert autophagy and benefit from its machinery. We demonstrate that Mycobacterium marinum, a close relative of M. tuberculosis, antagonistically induces an early autophagic response while repressing its autophagic digestion by the host cell. This antagonistic control of autophagy by M. marinum is dependent on the function of the mycobacterial ESX-1 secretion system, known to secrete a membrane-damaging factor that perforates the M. marinum-containing vacuole (MCV). This membrane damage activates the formation of autophagosomes and their recruitment to the MCV, but very likely it also impedes the lysosomal fusion and subsequent degradation inside autolysosomes. In addition, we suggest that the manipulation of autophagy by M. marinum is orchestrated via TORC1, the major kinase complex regulating nutrient-sensing and cell metabolism in eukaryotes.

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Infectious Diseases

Understanding the role of vacuolins/flotillins in the biogenesis of the Mycobacterium marinum niche

Bosmani, Cristina

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(1) University of Geneva, Department of Biochemistry; (2) Bernhard Nocht Institute for Tropical Medicine, Hamburg

We use Dictyostelium discoideum as a host model to study mycobacterial infections, using Mycobacterium marinum, a close cousin of Mycobacterium tuberculosis. Dictyostelium has three vacuolins (A, B and C) that localize at Mycobacteria-containing compartments and are upregulated upon infection. Dictyostelium vacuolins are homologs of the metazoan flotillins, which can oligomerize and form microdomains at the membrane. Flotillins are involved in TfR recycling to the plasma membrane via interactions with the recycling machinery. We seek to understand whether vacuolins are involved in the establishment of a permissive M. marinum niche by altering endosomal recycling. We found that vacuolin C localizes to lysosomes, whereas vacuolins A and B are found on postlysosomes. In addition, we demonstrate that vacuolins, like flotillins, are strongly associated with membranes, are palmitoylated, and can be found in detergent resistant membranes. We found that a deletion of the vacuolin B and C genes dramatically impairs phagocytosis of several types of particles with different sizes. Moreover, the vacuolin BC double knock-out show a delayed reneutralization of the phagosome, suggesting that the retrieval of the v-ATPase is affected. In fact, GFP-Trap pulldown experiments indicated that vacuolins interact with multiple subunits of the v-ATPase as well as several Rab GTPases. Furthermore, we show that the vacuolin BC double knock-out confers partial resistance to infection, suggesting that vacuolins are important host factors that are manipulated by the pathogen to establish its permissive compartment. We are confirming whether vacuolins are involved in the trafficking of the v-ATPase, lysosomal enzymes, and plasma membrane receptors.

22
Infectious Diseases

Evaluation of a new, VLP-based vaccine against infectious laryngotracheitis (ILT)

Schaedler, Julia

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ILT is a respiratory disease of chicken. Depending on the virus strain symptoms vary from mild to severe forms which can even lead to death by asphyxiation. Mortality rates vary from 5-70%, but even reduced weight gain or decreased egg production cause massive economical losses. The most efficient way of protection is immunization with live attenuated vaccines. However, they pose a certain risk of mutating or recombining with field strains, resulting in new pathogenic variants. The need for a safe and efficient vaccine is obvious.
We developed a vaccine based on virus-like particles (VLPs) displaying ILTV glycoproteins on their surface. VLPs are neither infectious nor replication competent and offer the opportunity to present antigens in high density at their surface. In a first in vivo-study we tested for the tolerance of in ovo-delivered VLPs. Compared to the untreated control group, no differences in hatching rate and weight gain were observed. Thus, the VLPs do not seem to interfere with the embryo or chicken development. The current attempt consists of a dual approach which aims at evoking two different immune responses: Firstly, VLP-gG in combination with plasmid encoded chicken interleukin 2 (pChIL) will be delivered in ovo. This should lead to the formation of antibodies against gG, which are supposed to prevent the viral immune-evasion mechanisms initiated by gG. Secondly, protection against ILT is known to depend on cell-mediated immune responses. Thus, the Th1-enhancing pChIL-18 and maleylated VLP-gB will be delivered i.m. post-hatch.

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Infectious Diseases

Regulation of Cytoskeletal Dynamics by Post-Translational Glutathionylation: Implications for NET Formation

Stojkov, Darko

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Neutrophils are the most abundant cells in blood and their antimicrobial defense capabilities are defined, at least partially, by their formation of neutrophil extracellular traps (NETs). For the past decade, efforts have been made to elucidate the molecular mechanisms of NET formation. In this study, we demonstrate that inhibiting cytoskeletal dynamics using pharmacological inhibitors or in knockout mouse neutrophils having defects in genes regulating the actin and tubulin networks, prevents the degranulation and DNA release both required for NET formation. Wiskott–Aldrich syndrome protein (WASP)-deficient mouse (Was-/-) neutrophils, which are unable to polymerize actin, exhibit a block in degranulation and DNA release after stimulation. In addition, activation of mouse and human neutrophils with a genetic defect in NADPH oxidase failed to induce actin and tubulin polymerization or NET formation. Moreover, neutrophils deficient in glutaredoxin 1 (Grx1), an enzyme required for de-glutathionylation of actin and tubulin, were unable to polymerize either cytoskeletal network and failed to degranulate or release DNA. Taken together, cytoskeletal dynamics are achieved as a balance between ROS-regulated effects on polymerization, and glutathionylation on the one hand, and the Grx1-mediated de-glutathionylation that is required for NET formation, on the other. Thus, these findings inform us about the molecular mechanisms involved in NET formation and provide new potential strategies for increasing the anti-microbial activity of neutrophils in patients with defects in the innate immune system.

24
Infectious Diseases

Role of ESCRT in membrane repair during Mycobacterial infection

Selected for flash talk

Lopez Jimenez, Ana Teresa

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Mycobacterium tuberculosis is the causative agent of tuberculosis. In order to study mycobacterial pathogenicity and host defenses, we use the model system composed by Mycobacterium marinum (a closely-related species of M. tuberculosis) and Dictyostelium discoideum (a macrophage surrogate with a high degree of conservation of cell autonomous defence mechanisms). After phagocytic uptake by D. discoideum, M. marinum converts the phagosome into a permissive niche that supports M. marinum replication. The bacteria are later able to escape from the compartment in order to gain access to the cytosol thanks to the secretion of a membranolytic toxin called ESAT-6, encoded in the RD1 locus.
ESCRT is an ancient machinery that promotes membrane deformation and scission in multiple biological processes, including plasma membrane repair. Therefore, we wondered whether ESCRT would participate in repairing membrane damage caused by M. marinum ESAT-6. We have confirmed that different membrane damaging agents induce specific GFP-Vps32 structures. Besides, in cells infected with M. marinum, we have observed the recruitment of GFP-Vps32 at places where the membrane integrity of the compartment is lost. This recruitment is decreased when cells are infected with M. marinum lacking the RD1 locus. Mutants lacking some of the ESCRT components are more sensitive to M. marinum-induced damage, which enable the bacteria to escape earlier to the cytosol and become ubiquitinated. This leads to the recruitment of Atg8, one core protein of the autophagy pathway. We are currently investigating the crosstalk between these two pathways and their impact on intracellular bacterial replication.

25
Infectious Diseases

The role of neutrophils in the outcome of drug treatment against Leishmania (Viannia) panamensis

Regli, Ivo

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Background: Leishmania (Viannia) panamensis (L. (V.) p.) is the main causative agent of cutaneous leishmaniasis in Colombia. This disease is usually treated with either meglumine antimoniate (MA) or miltefosine (MIL). In recent years, there has been an increasing amount of evidence for the emergence of drug-resistance against these drugs. Data recently obtained show gene expression signatures in lesion biopsies that suggest enhanced neutrophil recruitment to lesions of patients that do not respond to anti-leishmanial treatment when compared to those who respond to treatment. Neutrophils are known to play an important role in immunity against Leishmania. They are the cells first recruited upon infection and are also found in chronic lesions. However, their role in the outcome of anti-leishmanial treatment has not been investigated yet.
Methods: Neutrophils from naïve mice or human blood neutrophils from healthy volunteers were challenged in vitro with laboratory lines and clinical strains of L. (V.) p. that are drug-susceptible or resistant to either MA or MIL. Neutrophil extracellular trap (NET) formation was analyzed by confocal microscopy and quantified by measuring the amount of extracellular dsDNA. The impact of the different L (V) p strains on neutrophil effector functions upon in vitro infection were further assessed by the analysis of surface activation markers and production of reactive oxygen species (ROS).
Results: MA and MIL resistant L. (V.) p. strains were shown to elicit significant increased NET formation in murine and human neutrophils when compared to drug susceptible L. (V.) p. strains. Furthermore, we will present recent data on the impact of both laboratory lines and clinical drug susceptible or resistant L. (V.) p. strains showing distinct impact on neutrophil activation phenotypes.
Conclusions: These results suggest that parasite drug susceptibility may influence neutrophil activation and function. Since neutrophils participate in the onset of infection as well as in the chronic phase, they should be considered as potential determinants of outcome of anti-leishmanial drug treatment.

26
Infectious Diseases

Reactive oxygen species contribute to the immune response of Dictyostelium discoideum to mycobacterial infection

Dunn, Joe Dan

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Reactive oxygen species (ROS) are key components of the immune response to intracellular pathogens. Deleterious mutations in the ROS-generating phagocyte NADPH oxidase (NOX) underlie chronic granulomatous disease, marked by severe, recurring bacterial and fungal infections. We are using Dictyostelium discoidium, a genetically tractable amoeba that preys on bacteria, as a model phagocyte to delineate the events leading to ROS production and to identify ROS contributions to the immune response to Mycobacterium infection.
Phagocyte NOX comprises Nox2, the catalytic subunit, and p22phox, which recruits cytosolic proteins required for activation. Dictyostelium expresses NoxA, CybA, and NcfA, homologs of Nox2, of p22 phox, and of the NOX regulator p67phox, respectively. We have observed that Dictyostelium produces ROS when exposed to microbial products such as lipopolysaccharide and that ROS production is decreased in mutants lacking NoxA or CybA.
Fluorescent protein fusions indicate that CybA localizes to the plasma membrane, macropinosomes, and bacteria-containing phagosomes. NcfA is cytosolic and is enriched at nascent macropinosomes and phagocytic cups via a Rac-binding domain that potentially couples cytoskeletal remodeling events such as migration and phagocytosis with NOX activation.
During infection, CybA co-localizes with Mycobacterium. In intracellular growth assays, Mycobacterium mutants lacking ROS-detoxifying enzymes exhibited decreased growth in wild-type Dictyostelium, and wild-type bacteria and mutants grew less well in a Dictyostelium mutant with elevated levels of ROS. Mycobacterium strains expressing redox-sensitive GFP will be used to assess the oxidative stress experienced by bacteria during infection; this will enable us to determine whether ROS function as signaling molecules or anti-microbial effectors.

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Infectious Diseases

Granzyme B acts as an innate immune barrier by specifically attenuating bacterial virulence

Selected for flash talk

Lopez Leon, Diego

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Pathogenic bacteria and the alarming increase in antibiotic resistance are a global major health threat. The human immune system evolved multiple innate and adaptive mechanisms to successfully cope with the constant threat of ubiquitous bacterial pathogens. Among those mechanisms, immune proteases were recently recognized to play an integral role in antibacterial immune defense. The immune serine proteases of cytotoxic T and natural killer cells – the granzymes (Gzms) – are well known to mediate killing of virus infected or malign transformed mammalian cells when delivered into the target cells by the pore forming protein perforin (PFN). We recently discovered that the Gzms of cytotoxic T cells, when delivered into bacteria by the prokaryotic membrane disrupting protein granulysin (GNLY), exhibit potent antibacterial activity by cleaving multiple vital bacterial protein substrates triggering rapid bacterial death (Walch et al. Cell, 2014). Comprehensive proteomics and bioinformatics analysis of bacterial GzmB substrates in the model pathogen Listeria monocytogenes revealed a highly targeted attack on protein networks that are specifically up-regulated during infectious growth in vivo. This analysis suggests an unexpected immune mechanism that specifically targets bacterial proteins directly related to virulence and pathogenicity. Our experimental evidence supports the hypothesis that GzmB, even in absence of GNLY, severely compromises bacterial virulence; not only of Listeria, but also of Salmonella and Mycobacteria spp. GzmB treatment limited bacteria survival in epithelial cells, as well as in human macrophages, that was mediated by the specific destruction of secreted bacterial virulence factors. Overall, these data provide an evolutionary insight of how to effectively kill bacterial pathogens and restrict infections.

28
Immunology

Neutrophils are protective in oral mucosal Candida albicans infection by releasing soluble mediators

Braunsdorf, Christina
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Candida albicans is a comensal of the mucosa in healthy individuals but it can cause superficial mucosal infections as well as disseminated systemic infections with high mortality rates. The host's epithelial barrier and the immune system limit fungal growth and dissemination with neutrophils as important players mediating antifungal protection. Studies so far focused on the phagocytic functions of neutrophils and their direct role as pathogen combatants. We investigated the immunological crosstalk between epithelial cells and neutrophils in C. albicans mucosal infection setting.
We used a three dimensional epithelial infection model of oral epithelial cells (RHOE) and assessed by LDH release and histology how neutrophils isolated from venous blood of healthy donors modulated the epithelial cell damage induced by C. albicans. Induction and release of immune mediators such as antimicrobial peptides (AMPs) and chemokines was investigated by qRT-PCR and ELISA. Immunofluorescence staining and microscopy was applied to confirm the presence of AMPs on protein level.
Our data show that neutrophils were protective in oral mucosal infection with C. albicans and the damage was reduced in the presence of neutrophils by effector functions, which in our experimental setup are exclusively mediated by soluble factors. We found that the AMP LL-37 (Cathelicidin) is released by neutrophils and at the same time they induce epithelial beta-defensin 3 (HBD3), which likely contributes to the observed protective effect.
Our findings suggest that neutrophils, besides their active role in immune defense, have important regulatory roles with impact on antifungal cutaneous immunity.

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Immunology

The role of neutrophil extracellular traps during systemic C. albicans infection

Guiducci, Eva

Guiducci, Eva* (1); LeibundGut, Salomé* (1)
(1) UZH

Neutrophils are critical for immunity against systemic C. albicans infection. They are recruited very rapidly to the site of infection where they control the fungus by phagocytosis, ROS production, and proteolitic enzymes. More recently, neutrophils have been shown to trap C. albicans hyphae by the expulsion of a meshwork of chromatin armed with antimicrobial proteins termed neutrophil extracellular traps (NETs). NET formation is regulated by peptidyl arginine deiminase, type IV (PAD4), which catalyzes histone H3 citrullination, by neutrophil elastase (NE)-mediated histone degradation and by myeloid peroxidases (MPO)-driven chromatin decondensation. PAD4 is expressed prominently in mature neutrophils and it has been shown to be central for NET formation, as PAD4 knockout mice show abolished induction of histone citrullination and fail to form NETs. NETs are efficiently induced by C. albicans hyphae, as in vitro studies have shown. However, the contribution of NETs to fungal control and host protection in vivo remains less clear. We found NETs to be strongly induced in the kidney of WT mice during systemic C. albicans infection. The abundance of citrullinated histones H3 increased over time, paralleling the increase in neutrophil numbers and the presence of fungal hyphae. Our results support a role for NETs in controlling fungal growth in the kidney during systemic C. albicans infection, which is further assessed by using PAD4-deficient mice.

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Microbiology

MULTIDRUG-RESISTANT BACTERIA AND ANTIBIOTIC TREATMENTS

Vitale, Alessandra

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In the past years, non-adapted usage of antibiotics led to the development of multi-drug resistant bacteria, which became a major problem in the world. Following the emergence of such dangerous pathogen, those bacteria were categorised into the ESKAPE group, referring to Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanni, Pseudomonas aeruginosa and Enterobacter species, for example Escherichia coli. It is a big challenge but crucial to find new compounds to fight these bacteria. In the chemistry group of Prof. John Robinson, a new family of peptidomimetic antibiotics was designed. Peptidomimetic antibiotics are synthetized by assemblage of amino acids and the 3-D structure (B-hairpin) is important for their effect. The mechanisms of action of those drugs still remains to be elucidated. In this study, we investigated one of the last drug candidate, POL999, which is very active against E. coli ATC25922. We successfully sequenced the genome of this strain using the Pacific Biosciences platform. We further analysed the expression of the proteins upon challenging with POL999 using shotgun proteomics to find potential targets. Around 4800 proteins were detected in both conditions and 108 were significantly changing in abundancy in the presence of the antibiotic. Finally, we were able to confirm the proteomics data using real-time polymerase chain reaction for wcaI (glycosyl transferase), wzc (exopolysaccharides synthesis), wza (polysaccharide export protein), which were up-regulated upon drug addition. lexA (SOS response), ompF (outer membrane protein), yhjA (putative cytochrome C peroxidase) and lamb (transport protein) were confirmed to be down-regulated under antibiotic treatment.

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Microbiology

A family of PX-domain lipid binding proteins as organizers of the endocytic organelle system in Giardia lamblia

Cernikova, Lenka

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Giardia trophozoites have a distinctly polarized cellular architecture and are able to attach to the small intestinal epithelium of the host via a unique ventral organelle. Compartments of the highly simplified endosome-lysosome organelle system, the peripheral vacuoles (PV), are arrayed in the dorsal cortical region on the opposite side of the cell at ~50 nm distance from the plasma membrane (PM). We have recently shown that giardial clathrin does not produce coated vesicles at the PM, but instead forms unique highly stable arrays at the PV membrane – PM interface (see also abstract Zumthor et al.). Analysis of the clathrin interactome confirmed that the previously described lipid binding giardial EpsinR homolog (Ebneter et al., 2014) was not a component of these arrays, but instead revealed two novel phosphatidylinositol-binding proteins containing conserved PX-domains. Using data-mining and ectopic expression of tagged variants we demonstrated that Giardia trophozoites express a total of 6 PX-domain proteins. All family members localize exclusively to PVs by confocal fluorescence microscopy, but comprehensive reverse co-immunoprecipitation experiments show that only 2 are integrated into stable clathrin arrays via their effector domains. Rosetta modelling confirmed the presence of characteristic phospholipid-binding domains showing structural conservation of the 6 PX-domains. Binding assays with purified recombinant PX-domains revealed distinct phospholipid binding profiles consistent with localization at the PM and membranes of endocytic organelles. Further characterization of this protein family includes direct interference with function to test the hypothesis that PX-domain proteins have evolved as organizers of the highly specialized endocytic system in Giardia.

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Microbiology

The large GTPase atlastin3/Sey1 promotes intracellular replication of Legionella pneumophila by remodeling endoplasmic reticulum morphology

Steiner, Bernhard

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Legionella pneumophila is an amoeba-resistant environmental bacterium, which upon inhalation of contaminated aerosols causes a potentially lethal pneumonia in humans. After uptake by host cells through macropinocytosis, L. pneumophila forms a replication-permissive compartment termed the Legionella-containing vacuole (LCV), which tightly associates with ER membranes. LCV formation requires the bacterial Icm/Dot type IV secretion system (T4SS) that secretes approximately 300 different "effector" proteins into the host cell, where they interfere with membrane traffic and other processes [1]. Our recent proteomics studies of intact LCVs purified from infected macrophages or amoeba identified a number of host factors, including the large dynamin-like GTPase atlastin3 (Atl3), an intrinsic ER membrane protein governing ER morphology.
Fluorescence microscopy showed that ER regions containing Atl3, or its functional orthologue, Sey1 [2], is closely attached to LCVs in macrophages or amoeba in an Icm/Dot-dependent manner. Perturbation of ER morphology by single or simultaneous depletion of Atl3 and the ER-tubule shaping proteins reticulon4a/b by RNA interference in human epithelial cells inhibits intracellular proliferation of L. pneumophila, indicating that the ER dynamics around LCVs might be of functional importance during the pathogen life cycle. Consistently, the overproduction in Dictyostelium discoideum amoeba of dominant-negative Sey1_K154A reduces intracellular growth of L. pneumophila, whereas Sey1 overproduction leads to enhanced intracellular replication. These findings indicate that the role of Atl3/Sey1 for LCV formation is evolutionarily conserved. Current work is directed at mechanistically understanding the role of Atl3/Sey1 for LCV formation and Icm/Dot-secreted effectors contributing to this process.

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[2] Anwar, K., Klemm, RW. et al. (2012) The dynamin-like GTPase Sey1p mediates homotypic ER fusion in S. cerevisiae. J Cell Biol 197:209-217.

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Microbiology

Characterization of Legionella effector proteins involved in the modulation of small GTPases

Swart, Leoni

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Legionella pneumophila is a ubiquitous water-borne bacterium and the causative agent of a severe pneumonia termed Legionnaires' disease. The opportunistic pathogen infects lung macrophages upon inhalation of contaminated aerosols. The bacterium produces the Icm/Dot type IV secretion system (T4SS), through which it injects approximately 300 effector proteins into the host cell. These proteins manipulate various host cell processes, like signal transduction and vesicular trafficking, thereby enabling the bacterium to form a distinct "Legionella-containing vacuole" (LCV) and preventing degradation of the pathogen via the bactericidal endolysosomal pathway.
Proteomic analysis of purified intact LCVs revealed the presence of hundreds of host proteins, including a number of small GTPases implicated in the secretory and endosomal vesicle trafficking pathway, as well as the small GTPase Ran and its effector Ran binding protein 1 (RanBP1). The L. pneumophila effector protein LegG1 has been found to activate Ran and thereby promote microtubule polymerization, LCV formation and motility, host cell migration as well as intracellular bacterial replication. The exact mechanism by which LegG1 increases the intracellular Ran(GTP) pool remains unclear. Using various cell biological, genetic and biochemical approaches, including fluorescence imaging and yeast studies, as well as protein production and bacterial genetics, this project aims to characterize L. pneumophila effector proteins that are involved in the modulation of small GTPases, with a focus on LegG1, as well as its paralogues and orthologues.

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Microbiology

The Legionella pneumophila effector RidL binds to host Vps29 via its N-terminal domain

Bärlocher, Kevin

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The environmental bacterium Legionella pneumophila naturally replicates in amoebae. By utilizing a similar mechanism, the pathogen also parasitizes macrophages, possibly leading to the severe pneumonia “Legionnaires’ disease”. Hereby, the formation of a replication-permissive compartment, the Legionella-containing vacuole (LCV), is a crucial process. LCV formation is dependent on the Icm/Dot type IV secretion system (T4SS), which translocates approximately 300 different \"effector\" proteins into the host cell, where they modulate cellular processes. The retrograde vesicle trafficking pathway recycles cargo receptors along the endosomal route back to the Golgi apparatus and restricts intracellular bacterial replication. A key mediator of retrograde trafficking is the retromer complex, consisting of the heterotrimeric cargo-selective subcomplex (Vps26, Vps29, Vps35) and heterodimeric membrane-deforming sorting nexins (SNX).

Recently, we have shown that the L. pneumophila T4SS substrate RidL (Retromer interactor decorating LCVs) binds to Vps29, interferes with the host retrograde vesicle trafficking pathway and is required for efficient intracellular replication. L. pneumophila arrests retrograde trafficking of cholera toxin at the endosomal stage in a ridL-dependent manner in macrophages, and ectopically produced RidL blocks the retrograde transport of Shiga toxin in HeLa cells.

In order to elucidate the mechanism of action, we aim at solving the high-resolution crystal structure of RidL. The structure of an N-terminal RidL fragment exhibits an unprecedented “foot-like” fold encompassing a protruding loop at its “heel”, which is critical for binding to the Vps29 retromer subunit. Ongoing functional and structural studies will provide further insights into the molecular mechanism of this novel bacterial effector.

Microbiology

The Legionella pneumophila Lqs and SinR regulatory network: implications for biofilm architecture and virulence

Hochtrasser, Ramon

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The causative agent of Legionnaires' disease, Legionella pneumophila, harbors a quorum sensing system called Lqs (Legionella quorum sensing). The Lqs system regulates the switch between the replicative and transmissive (virulent) state of the pathogen's biphasic life cycle. The response regulator LqsR and the transcription factor SinR are part of this regulatory network. L. pneumophila strains lacking lqsR or sinR show virulence defects, and the sinR mutant is compromised for biofilm formation. The wild-type strain forms a biofilm, where bacterial aggregates appear to tightly "clump", while the strain lacking sinR produces a rather "mat-like" biofilm. Furthermore, the interactions of L. pneumophila with amoeba in a biofilm appear to depend on SinR and LqsR. In the biofilm system, the migration of amoeba, as well as adherence and uptake of bacteria are currently studied in detail.
In order to study the novel output domain of LqsR, the molecular structure and function of the response regulator is analyzed. LqsR is monomeric in the non-phosphorylated state and forms dimers upon phosphorylation. We aim at solving the structure of the monomeric and dimeric forms by X-ray crystallography. Purified LqsR forms crystals, which diffract to a resolution of up to 2.1 Å and allowed to build a preliminary model of the monomeric regulator. Currently, the final modelling of the monomer structure as well as attempts to crystallize and elucidate the dimer structure of LqsR are ongoing.

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Microbiology

Characterization of M. marinum virulence factors involved in micronutrient acquisition

Knobloch, Paulina

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Mycobacterium marinum is a water-borne pathogen causing tuberculosis-like infections in aquatic animals and occasionally skin lesions in humans. It is also a close relative of Mycobacterium tuberculosis. These intracellular bacteria modulate the biology of their phagocytic host cells by secreting a range of effector proteins through type VII secretion systems (T7SS). Consequently, phagosomal maturation is blocked and the pathogen vacuole is transformed into a specific, replication-permissive niche, called the Mycobacterium-containing vacuole (MCV). Only a few mycobacterial effectors have been thoroughly studied to date.
Mycobacterium spp. require metal ions for both intra- and extracellular growth. As an example, the bacteria produce siderophores, called mycobactins, which bind Fe^{3+} with high affinity and provide the cells with iron through receptors/carriers. Iron availability during infection is even more restricted, as host cells actively pump Fe^{2+} out of the MCV, thus controlling metal ion concentration in this compartment. On the other hand, bacteria are predicted to counteract and promote the opposite flow of ions through the MCV membrane.

To determine the complex role of metal ions in free-living M. marinum and during their infection of phagocytes, we produced bacterial deletion mutants lacking genes involved in metal metabolism. We use RAW 264.7 macrophages, Acanthamoeba castellanii and Dictyostelium discoideum amoeba to unveil the role of M. marinum metal-related genes during infection and to determine the effect of host factors relevant for ion availability for the pathogen.

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Microbiology

The role of 6S-like RNAs in Streptomyces coelicolor gene expression

Burýšková, Barbora

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Homologue molecules in related species are not always encoded by similar sequences. The variety of bacteria and their genomes sometimes causes conservation to be displayed in secondary and tertiary structures. In the case of the regulatory 6S RNA, sequence homologues have been found in over 100 bacterial species so far. However, none were found in the genus Streptomyces. The unique genome of these soil-dwelling bacteria, known for their capacity to produce antibiotics, has a high G/C content and therefore cannot be compared with genomes of distantly related bacteria on the basis of sequence alignment. Yet in the non-coding 6S RNA it is the secondary structure that is crucial for its function. The 6S RNAs trap sigma factors by mimicking target promoter sequences in order to help with switching sets of expressed genes during developmental transitions. 6S-like RNA genes in Streptomyces have been computationally predicted by comparison of in silico modelled secondary structures of known 6S RNAs. The aim of our project is to verify the presence of 6S-like RNAs in Streptomyces coelicolor and consequently study their function and effects on gene expression. The experimental approach is based on co-immunoprecipitation of RNA bound to HA-tagged sigma factors expressed by recombinant strains of S. coelicolor, as well as RT-PCR from total RNA samples using specific primers designed for the in silico predicted 6S-like molecules. The outcomes of this project will aid the study of Streptomyces gene expression regulation and could shed light on exploiting cryptic gene clusters, encoding new antibiotics.

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Microbiology

Genome Wide Mutagenesis strategies in Dictyostelium discoideum and Mycobacterium marinum to decipher the conserved genetic basis of mycobacteria intracellular infections

Selected for talk

Lefrançois, Louise

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This study aims at understanding how Mycobacteria manipulate the fundamental processes of innate immunity, in particular the phagosome environment. Because genetic analysis of host factors is difficult in diploid mammalian cells, we propose to use Dictyostelium discoideum as a model phagocyte. Our plan is to develop the host-pathogen system D. discoideum-M. marinum as a powerful genetically tractable model.
To that purpose, we will apply genome-wide mutagenesis and high throughput sequencing in M. marinum and M. bovis (Transposon, Tn-Seq) and a similar approach in D. discoideum (Restriction enzyme mediated insertion, REMI-Seq). The precise identification and relative abundance of insertions before and after selection will allow us to quantitatively compare the compositions of pools with a high dynamic range.
As a proof of principle, we validated the approach by applying the Tn-Seq method for the first time to M. marinum. Then, we tested whether M. marinum can use similar carbon sources as Mycobacterium tuberculosis (fatty acids and sterols), by performing selections of pools in different media. In addition, we infect D. discoideum and macrophages with both M. marium and M. bovis Tn pools to identify genes required for survival and replication in these two phagocytes. Finally, infection of D. discoideum REMI pools with M. marinum will reveal host genes implicated in resistance and susceptibility to infection.
These promising and innovative approaches will allow a comprehensive definition of the host and pathogen genes important for the intracellular host-pathogen interactions during infection of macrophages and Dictyostelium.

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Molecular and Cellular Biosciences

A fat body-secreted small protein remotely controls lipid absorption by the gut.

selected for talk

Rommelaere, Samuel

Rommelaere, Samuel (1)
(1) EPFL, GHI

Fly hemolymph, the homolog of mammalian plasma, supplies tissues with nutrients but also transports signaling molecules involved in inter-organ communication.
Using proteomics and forward genetics, we molecularly and functionally characterize a small, secreted protein that we called fat body secreted protein (Fbsp).
We show that Fbsp KO flies harbor abnormal neutral lipid and cholesterol retention in the posterior midgut. Fbsp is specifically required in the fat body, the fly homolog of liver and adipose tissue. Its deficiency results in altered lipoprotein trafficking in the gut and the fat body.
Using affinity purification combined to mass spectrometry, we identify several Fbsp binding partners involved in lipid storage and transport. Interestingly, these different interactors are required in distinct organs, suggesting that Fbsp acts both locally and distally.
Taken together, our data show that Fbsp controls lipoprotein production and, therefore, remotely regulates lipid assimilation by the gut.

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Molecular and Cellular Biosciences

Recombinant antibodies for academia

Cosson, Pierre

Cosson, Pierre (1)
(1) University of Geneva, Faculty of Medicine

Biomedical researchers constantly need new antibodies recognizing their favorite proteins, and distinguishing various modifications of the core protein (alternative splicing, post-translational modifications...). Animal immunization remains the most common method to generate antibodies, although new recombinant technologies using phage display are much more powerful. Our University has developed an open-access service to develop and produce recombinant antibodies. Come and discover how YOU may obtain recombinant antibodies against your favorite protein.

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Molecular and Cellular Biosciences

Endosomal recruitment of ESCRTs induced by the lipid-binding protein ALIX

Larios, Jorge

Larios, Jorge (1); Gruenberg, Jean* (1); Roux, Aurélien (1)
(1) University of Geneva, Biochemistry

Introduction: Proteins belonging to the ESCRT complexes are known as membrane remodeling factors, which regulate events such as cytokinesis, virus budding, multivesicular endosomes (MVE) formation and membrane repair. Specifically, CHMP proteins (ESCRT III complex) are known as the key proteins in the membrane deformation process.
Results: Our previous results showed that the ESCRT-associated protein ALIX, a binding partner of CHMP4, is recruited to late endosomes by its interaction with LBPA (an unusual lipid found only in late endosomes) and facilitates membrane deformation. In the present studies, we show that ALIX recruits CHMP4 to late endosomal membranes containing LBPA. Furthermore, this recruitment is dependent on ALIX interaction with LBPA. These results suggest that in mammalian cells ALIX, together with its binding partner LBPA, induces the redistribution of CHMP4 from the cytosol to endosomal compartments. Additionally, these studies are combined with in vitro experiments to investigate CHMP4 polymerization, membrane interactions, and the role of LBPA/ALIX in this process.

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Molecular and Cellular Biosciences

Control of astral microtubule length in S. cerevisiae : All About Balance

Chen, Xiuzhen

Chen, Xiuzhen (1); Barral, Yves* (1)
(1) Institute of Biochemistry, Department of Biology

The microtubule cytoskeleton is a dynamic structure in which the lengths of the microtubules are tightly regulated. Many factors are involved in controlling the length of astral microtubules, but we currently do not have a comprehensive view of the microtubule length control in vivo. Here, we established a quantitative microscopy-based framework to study microtubule behavior in vivo, which allows us to study the effect of microtubule-associated proteins on microtubules individually and the interplay between them. We found that Kip3 destabilizes growing microtubules while stabilizes shrinking microtubules, meanwhile Kip2 stabilizes growing microtubules whereas destabilizes shrinking microtubules. The balancing effect of Kip2 or Kip3 alone on growing and shrinking microtubules proposes a self-controlling mechanism for microtubule length. In addition, Kip2 and Kip3 antagonize with each other in controlling microtubule length. Further more, we discovered that Kip2 and Kip3 regulate catastrophe process rather independently on growing microtubules. However, Kip2 inhibits Kip3's rescue promoting activity by inhibiting the tail of Kip3 on shrinking microtubules. We also identified the rescue promoting activity of Kip2, which is regulated by the phosphorylation status of Kip2 and is independent of Kip3. We propose that the balance between growth and shrinkage phases, which in turn is regulated by microtubule-associated proteins, controls the lengths of astral microtubules.

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Molecular and Cellular Biosciences

Seeing forces: an optical biosensor for mechanical stimuli

Selected for talk

Yaganoglu, Sine

Yaganoglu, Sine (1); Pantazis, Periklis* (1)
(1) ETH Zurich, D-BSSE

Cells are constantly influenced by biochemical and mechanical signals in their immediate environment. In vitro approaches provide important knowledge about the effects of such signals on the cell. There is, however, a need for complementary innovative approaches to study cells in their native environment to fully recapitulate their endogenous state within their niche. While biosensors for chemical signals are rapidly improving both in their diversity and sensitivity, the in vivo visualization and reporting of mechanical signals exerted on and by the cell remains challenging. We propose a new tool to address this challenge by generating a genetically encoded fluorescent biosensor for Piezo1, a stretch-activated channel known to be involved in sensing shear stress and neural lineage specification. This biosensor will allow non-invasive imaging of mechanosensation with relative technical ease in tissue as well as in living organisms. Not only the cells will be visualized in their native environment, but also the timing of the mechanical signals can be estimated, providing a comprehensive view on the process of mechanotransduction in vitro and in vivo. Moreover, the biosensor can be combined with other fluorescent markers to study the downstream effects of mechanical stimuli. We have recently established this biosensor in cell lines and characterized its response to mechanical stimuli. Next, we will introduce the biosensor into mouse stem cells as well as mouse and zebrafish embryos where we will be able to address the role of mechanical forces and specifically Piezo1 within developmental and disease-related contexts.

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Molecular and Cellular Biosciences

Deciphering a prototypical MAPK signaling network at the single cell level using an optogenetic circuit

Dessauges, Coralie

Dessauges, Coralie (1); Blum, Yannick (1); Dobrzynski, Maciej (1); Khammash, Mustafa (2); Pertz, Olivier* (1)
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Receptor tyrosine kinases (RTK) enable to convert extracellular INPUTs as growth factors into specific cellular OUTPUTs through the activation of dynamic signaling networks. Nowadays we have a good idea about the network components, but we still miss crucial information about how these components are wired in a coherent signaling network. Our lab has recently brought new insights into the feedback and feed-forward structures regulating the ERK-MAPK network by delivering dynamical growth factor INPUTs with a microfluidic device and recording single cell ERK activation dynamics with an ERK biosensor (1). In a next step, we propose to use a similar INPUT/OUTPUT approach together with system perturbations to identify the molecular players involved in the network regulation. To increase the experimental throughput, we propose to build a synthetic signaling circuit using (a) an optogenetically activatable FGF receptor allowing to activate the MAPK network with light INPUTs instead of growth factors and (b) a spectrally compatible ERK biosensor to record MAPK signalling OUTPUTs. Here, we describe the initial design and characterization of our experimental pipeline including the optoFGF receptor, a spectrally compatible ERK biosensor, and a computer vision platform that automates image analysis at the single cell level.

(1) Ryu et al., Frequency modulation of ERK activation dynamics rewires cell fate, Molecular Systems Biology, 2015

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Molecular and Cellular Biosciences

Molecular mechanisms regulating cellular proteostasis in response to misfolded proteins

Bergmann, Timothy

Bergmann, Timothy (1); Rinaldi, Andrea (2); Boersema, Paul (3); Bertoni, Francesco (2); Picotti, Paola (3); Molinari, Maurizio* (4)
(1) IRB, USI, ETHZ; (2) IOSI; (3) ETHZ, DBIOL; (4) IRB, USI, EPFL

Synthesis of membrane bound, secretory and organelle proteins occurs into the ER. Dedicated quality control allows the export into the secretory pathway only of properly folded proteins, while polypeptides that fail to achieve their proper conformation, are engaged by the ERAD machinery, retrotranslocated into the cytosol and ubiquitynated for degradation via proteasomes.
Equilibrated synthesis, export and degradation is crucial for maintaining ER homeostasis. Different physiological and pathological conditions (e.g. fluctuations in protein synthesis, accumulation of defective gene products, pathogens, …) can perturb the ER environment, leading to conditions of ER stress. Such stresses activate UPRs: adaptive, transcriptional and translational programs that induce the expression of ER chaperones, folding and ERAD components, increase ER size and reduce synthesis of cargo protein trying to restore proteostasis. We aimed at characterizing cellular responses on perturbation of ER homeostasis by expression of proteins with different chimico-physical properties or exposure to ER-stress inducing chemicals. To identify such responses and elucidate possible regulatory mechanisms involved in adaptation to misfolded protein accumulation, we performed label-free shotgun proteomics and genome-wide gene expression profiling. Results showed that at similar stress induction, misfolded proteins (in contrast to chemical compounds) did not affect cellular viability and proliferation and induced only a restricted subset of the UPR target genes/proteins. Additionally, the expression of proteins with different structural and chemical properties elicited different responses. Further analysis aim at confirming the differential activation of the Ire1 and ATF6 pathways of the UPR by ER-retained, misfolded proteins.

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Molecular and Cellular Biosciences

Towards Understanding the Transcriptional Network Directing Lipid Droplet Biogenesis

Scott, Cameron

Scott, Cameron (1); Vossio, Stefania (1); Gruenberg, Jean (1)
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While lipid droplets are omnipresent in most cells where they serve as the primary storage site of neutral lipids, the number and nature of these organelles varies greatly, both over time and between cell types. Although several stimuli have been identified to induce accumulation of lipid droplets, including activation of the Wnt signaling pathway, the proximal transcriptional regulators remain unclear. Therefore, we initiated a multi-pronged approach to identify the transcription factors directing the biogenesis of lipid droplets. Firstly, we performed a promoter analysis of known lipid droplet proteins to identify over-represented sequence motifs. We also performed RNAseq analysis of Wnt-treated cells to identify changes in gene expression linked to the phenotype of lipid droplet accumulation. Together, this examination of the transcriptional regulation lead to identification of several candidate factors that may serve as part of the “master” regulatory network controlling lipid droplet biogenesis and include members of the C/EBP family (know regulators of adipogenesis), FHL2 and CBP/P300 elements of the Wnt signaling pathway, and the TFAP2 family of transcription factors. Consistent with this notion, siRNAs to several of these transcription factors diminished the accumulation of lipid droplets in response to Wnt stimulation. Further, overexpression of TFAP2C alone was sufficient to induce lipid droplet accumulation in cells, confirming the role of this transcriptional network in directing lipid droplet biogenesis.

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Molecular and Cellular Biosciences

Biochemical and in-vivo investigations of a peroxidase from the social amoeba Dictyostelium discoideum

Nicolussi, Andrea

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(1) University of Natural Resources and Life Sciences, Vienna, Chemistry; (2) University of Geneva, Biochemistry; (3) University of Modena and Reggio Emilia, Chemistry and Geology

Heme peroxidases catalyze the hydrogen peroxide-mediated oxidation of various organic and inorganic one- and two-electron donors. Mammalian heme peroxidases (e.g. myeloperoxidase or lactoperoxidase) use halides as substrates, thereby producing antimicrobial hypohalous acids that play an important role in innate immunity. Recent phylogenetic analysis revealed the presence of homologous peroxidases also in prokaryotic and early eukaryotic organisms. Hence, the peroxidase from the social amoeba Dictyostelium discoideum (DictyPoxA) is of particular interest, since it shares a high sequence similarity to the mammalian lactoperoxidase (LPO). Here, we present the biochemical and biophysical characterization of recombinant DictyPoxA produced in Pichia pastoris together with in-vivo investigations of the expression and putative roles of this peroxidase in D. discoideum. We report (i) the thermal and conformational stability of DictyPoxA, (ii) the presence of one heme to protein linkage, (iii) the standard reduction potential of the Fe(III)/Fe(II) couple, (iv) the spectral features of DictyPoxA in various oxidation states as well as (v) the kinetics of formation and interconversion of relevant redox states. The kinetics of the two-electron oxidation of bromide, iodide and thiocyanate is presented and compared to data from the mammalian counterparts. The presented in-vivo investigations include comparative studies on wild-type D. discoideum and the DictyPoxA KO-mutant, with respect to differences in developmental stages and fruiting body formation. Furthermore, we present a detailed protein expression study of DictyPoxA throughout the development cycle of D. discoideum. Combined with the biochemical data we will propose a model for the putative functions of this peroxidase in the social amoeba.

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Molecular and Cellular Biosciences

Sensitive detection of GPCR-mediated Erk1/2 phosphorylations in various cellular models with the HTRF® Advanced Phospho-ERK1/2 (Thr202/Tyr204) kit

Lamarque, Anissa

Dupuis, Elodie (1); Vrastor, Angelique (1); Maurin, Fabrice (1); Pannequin, Julie (2); Roux, Thomas (1); Trinquet, Eric (1)
(1) Cisbio Bioassays, Codolet, France I; (2) Institut de Génomique Fonctionnelle, Montpellier, France

G protein-coupled receptors (GPCRs) are seven-transmembrane domain receptors and represent a high percentage of investigational drug targets because of their involvement in many diseases. GPCRs have two principal signal transduction pathways: the cyclic AMP (cAMP) and the phosphatidylinositol (IP). As a world leader of advanced homogeneous assays (HTRF®) and reagents for GPCR studies, Cisbio Bioassays offers a comprehensive and efficient line of HTRF® assays to study the GPCR activation pathways. This include cAMP and IP-One HTRF® platforms to measure second messenger accumulations as upstream readouts of reference in biochemical, cell-based assays.

As several receptors are not optimally coupled through the cAMP or IP pathways, and other effectors are involved in the GPCR signaling pathways, Cisbio Bioassays has developed a new highly sensitive kit to detect modulations of ERK1/2 phosphorylations, illustrating phosphorylated ERK1/2 proteins as downstream readouts of GPCR stimulations.
From now on Cisbio Bioassays offers the Advanced Phospho-ERK1/2 (Thr202/Tyr204) kit (ref catalogue 64AERPEH) as an excellent and versatile tool for studying the downstream effect of pharmacological compounds on GPCRs. This new powerful product is the linkage in the HTRF® GPCR product portfolio between the well-known upstream readouts (cAMP, IP) and the protein phosphorylation investigations as downstream readouts. This allows the use of a single sample-saving technology to characterize pharmacological compounds on several readouts.
In this poster we demonstrate that Advanced Phospho-ERK1/2 (Thr202/Tyr204) kit enables measurement of ERK1/2 protein phosphorylations in several cellular contexts of varying complexity (from stable cell lines to tumor patient-derived cells) when mediated by overexpressed or endogenous GPCRs. Finally, we remind users of experimental optimizations required to perform ERK1/2 phosphorylation experiments with the Advanced Phospho-ERK1/2 (Thr202/Tyr204) kit for GPCR investigations.

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Molecular and Cellular Biosciences

Investigation of the molecular mechanisms regulating recovER-phagy

Loi, Marisa

Loi, Marisa* (1); Fumagalli, Fiorenza* (1); Molinari, Maurizio* (1)
(1) Institute for Research in Biomedicine

The endoplasmic reticulum (ER) is an intracellular compartment where one third of the total proteome is synthesized. Correctly folded polypeptides leave the ER along the secretory pathway while misfolded proteins are retained. In response to an accumulation of un- or misfolded proteins in the ER lumen cells activate unfolded protein response (UPR) programs to maintain ER homeostasis by decreasing protein translation, by degrading misfolded proteins and by increasing the production of molecular chaperones and enzymes involved in protein folding. After resolution of ER stress, the intracellular concentration of stress-induced ER chaperones and enzymes must return to physiological levels. Autophagy programs re-establish pre-stress ER size and content by clearing excess ER material. Selective autophagy targets specific proteins, aggregates or whole organelles into lysosomes via specialized autophagy receptors.
We identified a novel function of the translocon component Sec62, as an ER-resident autophagy receptor, which selectively delivers ER fragments to autolysosomes for clearance during recovery from ER stress in a series of events that we name recovER-phagy.
Sec62 contains a LC3-interacting region (LIR), which is essential for recruitment of cytosolic LC3 and ER delivery to autolysosomes for clearance. In fact, ectopic expression of Sec62 elicits ER delivery to autolysosomes, whereas expression of a Sec62 variant displaying an inactive LIR prevents the delivery of specific ER markers to autolysosomes.
Here, we investigate how mammalian cells recover after the resolution of a transient ER stress activating autophagy like processes to re-establish a physiological ER size, content and function.

Molecular and Cellular Biosciences

DYNAMIC MEASUREMENT OF KINASE ACTIVITY IN LIVE SINGLE CELL

Selected for flash talk

Durandau, Eric

DURANDAU, Éric* (1)
(1) UniL / FBM, Département de Microbiologie Fondamentale

The phosphorylation of signaling cascade components is a key post-translational modification used in signal transduction. Although all kinases catalyze this biochemical reaction, each individual kinase possesses its specific pool of substrates. Moreover, the temporal activity of these enzymes varies from cell to cell depending on intra- or extracellular cues. Knowing that kinase mis-regulation is implicated in many diseases, such as cancer, it becomes crucial to quantify the heterogeneity of the dynamic kinase activity at the single cell level. Microscopy is an ideal technique for such investigation. However, fluorescent assays have to be established to quantify this enzymatic activity in living cells. Using a synthetic biology approach, we designed a fluorescent biosensor that undergoes nuclear-to-cytoplasmic relocation upon phosphorylation by the kinase of interest. Combination of time-lapse microscopy measurements and automated image analysis allows the quantification of the dynamics of kinase activity in hundreds of single cells. As proof of concept, we generated a Synthetic Kinase Activity Relocation Sensor (SKARS) for Mitogen Activated Protein Kinases (MAPK) of the mating pathway in S. cerevisiae. The kinetics of the MAPK activation exhibits a large heterogeneity between single cells due to an inhibition of signal transduction at specific stages of the cell-cycle. With our quantitative and dynamic assay at the single cell level, we revisit the cross-inhibition between the cell-cycle and the mating pathway activation.

Molecular and Cellular Biosciences

Roles of intussusception vs. sprouting angiogenesis in vascular plexus remodeling
Selected for talk – clarified today, 5th of Jan

Handrkova, Helena

Handrkova, Helena (1); Djonov, Valentin* (1)
(1) University of Bern, Institute of Anatomy

Intussusceptive angiogenesis (IA) or blood vessel splitting is mechanism of capillary network expansion and remodeling. The hallmarks of IA are thin intraluminal pillars which are hypothesized to form by contact of opposing capillary walls, subsequent perfusion of the contact zone and infiltration by collagen fibers and perivascular cells. Evidence for this alternative to sprouting angiogenesis was found in a variety of organs and species, but the molecular mechanism is unknown. IA is highly clinically relevant in tumor vasculature: previous studies of our group indicate that a switch from sprouting to IA causes resistance to anti-VEGF therapy.
Intussusceptive pillars have also been observed in caudal vein plexus of zebrafish embryos. Using transgenic zebrafish (Tg(Fli1:eGFP//Gata1:dsRed) to label endothelium and erythrocytes) and time-lapse confocal microscopy, we document for the first time formation and further fate of these intraluminal pillars. The ultrastructure is being analyzed using TEM or whole-mount staining. Our data suggest that there are several 'classes' of pillars that differ by mechanism of origin, ultrastructure, and their behavior.

Next, we'll investigate the molecular mechanism of pillar formation and we hope to identify novel targets for antiangiogenic therapy, complementary to VEGF pathway.

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Molecular and Cellular Biosciences

ESCRTing intralumenal vesicles formation in mammalian cells

Mercier, Vincent

Mercier, Vincent (1); Roux, Aurélien* (1); Gruenberg, Jean* (1)
(1) UNIGE, Biochemistry

The endocytic pathway is a regulator of the sensitivity of the cell to external stimuli. Once internalised at the plasma membrane, material merges with endosomal compartments where a second mechanism of internalisation occurs. This process is called intralumenal vesicle (ILV) formation and can serve to limit sustained receptor signalling by sending them for degradation in the lysosome. Biogenesis of ILVs is achieved by machinery called Endosomal Sorting Complex Required for Transport (ESCRT). This process is very well described in yeast while it is more obscure in mammals. The goal of this study is, by using both in-cellulo and in-vitro approaches, to better understand the role of mammalian ESCRTs in the formation of ILVs by determining the order of ESCRT action and analysing the link between physical membrane properties and ESCRT polymerisation on endosome.

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Molecular and Cellular Biosciences

The price to pay for 53BP1 nuclear bodies

Neelsen, Kai

Neelsen, Kai (1); Lukas, Jiri* (1)
(1) Copenhagen University, Novo Nordisk Foundation Center for Protein Research

Perturbed DNA replication often results in incomplete genome duplication before mitotic entry. The residual under-replicated DNA interferes with sister chromatid separation in mitosis and therefore necessitates specialized processing, as do segregation problems due to incomplete decatenation. A subset of these lesions is transferred to daughter cells, where they lead to formation of large chromatin compartments (nuclear bodies) occupied by 53BP1 and 53BP1-binding proteins. Consequently, the formation 53BP1 nuclear bodies in G1 cells is one of the hallmarks of replication stress. We have shown previously that during G1, 53BP1 bodies shield the inherited DNA lesions from further erosion but whether this is the only function of 53BP1 remains unknown. Evidence that these structures may indeed perform additional function(s) comes from our observation that 53BP1 bodies persist beyond G1 and are dissolved only in late S phase. We reason that elucidating the mechanism of 53BP1 nuclear body resolution might hold the key to better understand the function of these compartments. To this end, we combine Quantitative Image-Based Cytometry with confocal imaging, live imaging of single cells, and genetic silencing of DNA damage regulators to monitor 53BP1 body dynamics. Our data indicate that 53BP1 body dissolution is a replication-coupled process. We have identified PCNA and the RAD52 recombinase as determinants of 53BP1 body dissolution, implying homology-mediated DNA repair and replication mechanisms in the dissolution reaction. We speculate that the protection of DNA lesions by 53BP1 nuclear bodies comes at the cost of error-prone replication in the next cell cycle.

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Molecular and Cellular Biosciences

Maintenance of a robust neuromuscular system as a novel strategy to protect skeletal muscle from sarcopenia

PANNEREC, Alice

Pannérec, Alice (1); Feige, Jérôme* (1); Springer, Margherita (1); Chiffelle, Johanna (1)
(1) Nestlé Institute of Health Sciences, Muscle Aging

Declining muscle mass and function is a main driver of loss of independence in the elderly. Sarcopenia, the age-related loss of skeletal muscle mass and function, is associated with numerous cellular and endocrine perturbations, and it remains challenging to identify those changes that play a causal role and could serve as targets for therapeutic intervention. Using a rat model of natural aging, we have found a differential susceptibility of certain muscles to age-related decline as aging rats specifically lose muscle mass and function in the hindlimbs, but not in the forelimbs. By performing a comprehensive comparative analysis of these muscles, we demonstrate that regional susceptibility to sarcopenia is dependent on neuromuscular junction fragmentation, loss of motoneuron innervation, and reduced excitability. Remarkably, muscle loss in elderly humans also differs in vastus lateralis and tibialis anterior muscles in direct relation to neuromuscular dysfunction, suggesting that maintenance of the neuromuscular system is key for healthy muscle aging. We further found that circulating levels of the neurotrophic factor neuregulin-1 (NRG1) are decreasing with age in rats. In vitro analysis using a nerve/muscle co-culture assay revealed that NRG1 protects the neuromuscular system from damage, suggesting that maintaining proper circulating levels with advancing age would have beneficial effect on muscle. Indeed, when rats were treated with NRG1 at the onset of sarcopenia we observe a protective effect on muscle mass. Taken together, our results demonstrate that maintenance of the neuromuscular system is key to protect from sarcopenia and we provide evidence for the role of NRG1 in this process.

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Molecular and Cellular Biosciences

Characterization of the human protein THEM6, a potential thioesterase involved in cellular lipid metabolism.

Mary, Camille

Mary, Camille (1); Duek Roggli, Paula (2); Bairoch, Amos* (1); Lane, lydie* (2)
(1) University of Geneva, Department of Human Protein Sciences; (2) SIB-Swiss Institute of Bioinformatics, CALIPHO

In the human genome, there are around 2000 genes coding for proteins that lack functional characterization. For this project, we are interested in one of these uncharacterized proteins, named THEM6. This protein is overexpressed in a large variety of cancers suggesting that it may play a role in the apparition and/or the maintenance of cancer. Bioinformatics analysis revealed that THEM6 is able to adopt a 3D fold typical of the "hotdog" clan of proteins and we predicted that, like other members of this clan, it is an acyl-CoA thioesterase involved in the metabolism of lipids. Western blot analysis showed that THEM6 is well expressed in HEK293T, HeLa, PANC-1 and Caco2 and that the highest expression was observed in MCF7 cancerous breast cells.
Despite the prediction of a N-terminal signal peptide, the protein was not found in cell free media after overexpression. Immunofluorescence studies showed that THEM6 is mainly localized in the endoplasmic reticulum (ER) which is a key organelle for the metabolism of lipids.
Interestingly, we found that Seipin (BSCL2) co-immunoprecipitates with endogenous THEM6. Seipin is a regulator of lipid catabolism. It is localized in the ER and plays a tissue-autonomous role in controlling lipid storage in adipocytes and in preventing ectopic lipid droplet formation in non-adipose tissues. THEM6 localization and interaction corroborate its potential involvement in cellular lipid metabolism. This hypothesis is currently tested in CRISPR/Cas9 knockout HEK293T and MCF7 cell lines and by purifying recombinant THEM6 protein so as to validate its acyl-CoA thioesterase activity.

Molecular and Cellular Biosciences

Mechanical force induces mitochondrial fission via the canonical fission machinery

Selected for flash talk

Feng, Qian

Helle, Sebastian* (1); Feng, Qian* (1); Aebersold, Mathias (2); Hirt, Luca (2); Grüter, Raphael (2); Sirianni, Andrea (3); Mostowy, Serge (3); Snedeker, Jess (4); Zambelli, Tomaso (2); Kornmann, Benoît (1)
(1) Institute of Biochemistry, ETHZ; (2) Laboratory of Biosensors and Bioelectronics, Institute for Biomedical Engineering, ETHZ; (3) Section of Microbiology, MRC Centre for Molecular Bacteriology and Infection, Imperial College London; (4) Institute for Biomechanics, ETHZ

Eukaryotic cells are densely packed with macromolecular complexes and intertwining membranous organelles that continually change shape and engage in active trafficking. It is intriguing that organelles avoid clashing and entangling with each other during such dynamic movements in such limited space. Here we describe a mechanism that explains how mitochondria orderly cohabit with other organelles in the crowded space of the cytoplasm.

Mitochondria form extensive networks that are constantly remodeled by fission and fusion events. While the molecular machineries that execute mitochondrial fission and fusion processes are relatively well documented, little is known about what triggers these events and determines the fusion and fission sites. We show here that mechanical forces could trigger mitochondrial fission. Mechanical stimulation of mitochondria – via the encounter with motile intracellular pathogens, via external pressure applied by an atomic force microscope, or via cell migration across uneven microsurfaces – resulted in the recruitment of the canonical mitochondrial fission machinery and subsequent fission. The mitochondrial fission factor (MFF) preferentially accumulated at mitochondria of reduced diameter, implicating it as a membrane-bound force sensor that recruits the fission machinery to sites of mechanical strain. Thus, mitochondria may avoid entanglement with itself and other cellular structures by responding to biomechanical cues.
These results shed new light on mitochondrial dynamics, an important process that has been shown to influence cell migration, cardiovascular functions, neuronal plasticity, aging and more. That mechanical triggers can be coupled to biochemical responses in membrane dynamics also provides a new perspective in studying organelle communication in general.

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Molecular and Cellular Biosciences

Functional organelle remodeling during adipocyte differentiation

Freyre, Christophe

Freyre, Christophe (1); Klemm, Robin* (1)
(1) University of Zürich, Institute of Molecular Life Sciences

Cellular differentiation entails the functional remodeling of organelles including changes in shape, and molecular composition. Organelle shape is often important for their function, however, the mechanisms underlying shape generation as well as creating specific structural and spatial relationship between different organelles are not well understood. During fat cell differentiation, the nascent adipocytes generate a large lipid droplet (LD) that is unique in protein composition, size, and functional contacts with other organelles involved in fat metabolism. To meet the specific metabolic requirements this LD forms contact sites with other organelles, i.e. the endoplasmic reticulum, peroxisomes, or mitochondria, which are important for lipid metabolic pathways. To elucidate mechanisms that underlie the spatial and functional remodeling of adipocyte organelles we developed an image-based single cell approach to measure for the first time, the dynamics of various organelle features during differentiation in populations of fixed cells. Combining this approach with differential proteomics and RNAseq analyses, we show that, surprisingly, mitochondria increase drastically in abundance during early differentiation stages and discovered a membrane protein that likely forms functional contact sites between mitochondria and the LDs. These contact sites are likely important in mediating metabolic changes central in adipocyte differentiation.

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Molecular and Cellular Biosciences

How to pack ribosomal RNA, stick in ribosomal proteins and squeeze it through the nuclear pore complex to get a functional ribosome?

Gerhardy, Stefan

Gerhardy, Stefan (1); Gillet, Ludovic (1); van Nues, Rob (2); Moursy, Ahmed (1); Petkowski, Janusz (1); Allain, Frederic (1); Aebersold, Rudi (1); Grannemann, Sander (2); Panse, Vikram* (1)
(1) ETH Zürich, Department of Biology; (2) University of Edinburgh, Centre for Synthetic and Systems Biology

The eukaryotic ribosome is the central molecular machinery that translates the genetic information from DNA to protein. This ribozyme is composed of >79 ribosomal proteins and 4 different RNA species. Despite advancements in structural biology, little is known about assembly of this machinery in the cell. Ribosome synthesis requesters a vast majority of the cellular recourses and energy. Ribosomal RNA accounts for more than 80% of the total cellular RNA in a cell and is connected and shielded by ribosomal proteins in the mature ribosome. However during the assembly process of the ribosome, the RNA needs to be folded, modified and processed accurately and efficiently. Most of the early ribosome assembly factors are directly or indirectly contacting the RNA and help with the help of RNA helicases to ensure correct folding of the rRNA.
We show by a combination of mass spectrometry and cell based assays that regions of the ribosome that are rich in rRNA are sensible to temperature changes, which might result in rRNA misfolding. By using proteomic approaches, we could show that expression of rRNA stabilizing subcomplexes are upregulated at lower temperatures, where the rRNA might get trapped in energetic minima. One important region of the large subunit is the RNA rich subunit interface. We could show that a pumillio containing protein binds in this region and helps to fold the ribosomal RNA that aligns the EPA sites on the large subunit. Deletion or mutations within the protein fail to efficiently synthesize and export functional ribosomes. Additionally, these ribosomes are probably detected by the nuclear surveillance machinery and subsequently degraded. We further aim to gain molecular insight into the interaction and the folding process through and integrative structural approach on early pre-60S particles.

Next generation high capacity DNA damage detection assay for chemotherapy and genotoxic compound screening

Peter Sykora (1), Sandra Woodgate (2), Jay George (2), Robert W. Sobol (1), Maja Petkovic (3)

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Many chemotherapeutic agents act through repressing DNA repair or increasing DNA damage. However the measurement of DNA damage levels within cells has been notoriously difficult, and current methods to assess DNA damage potential of new chemotherapeutics have major technical flaws. The single cell gel electrophoresis (SCGE) assay is a long-standing method for measuring levels of DNA damage within a cell. The principle is that DNA damage can cause DNA strand breaks, causing the relaxation of the compact highly supercoiled DNA. Application of an electric field while the cells are embedded in agarose allows damaged DNA to migrate faster than intact DNA. The more breaks in the DNA, the further it travels, resulting in the formation of a "comet" tail, the size and length of which directly correlate to amounts of DNA damage. This method benefits from technical simplicity and high sensitivity; but major drawbacks are that the assay is extremely laborious, lacks appropriate controls and has poor reproducibility. We have overcome these drawbacks by developing a 96-well format, the "CometChip", which uses micro-pillar technology to create an agarose 96-well chip where each well has approximately 300 micro-wells to capture individual cells. Using this technology we can incorporate multiple treatments, controls and time points on a single CometChip which can be rapidly analyzed using fluorescence-based imaging. This new technology was tested using 75 different chemical compounds considered either genotoxic, non-genotoxic or unknown. Compounds were tested on two lymphocyte cell lines with different p53 status to compare accumulation and repair of DNA damage. The CometChip gives highly reproducible and accurate results without loss of sensitivity. In high throughput screening using multiple CometChips, we estimate the throughput of the assay to be approximately 10,000% greater than traditional comet analysis. This massive increase in processivity brings new opportunity for large-scale compound screening. Increased sensitivity coupled with large sample sizes will allow researchers to measure minor changes in DNA damage with unparalleled accuracy.

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Molecular&Cellular Biosciences

Lysobisphosphatidic acid and cholesterol storage in Niemann-Pick C

selected for flash talk

Vacca, Fabrizio

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Lysobisphosphatidic acid (LBPA) is an endosome-specific phospholipid, which accumulates in NPC cells and in other lysosomal storage disorders. It has been shown to play a crucial role in the dynamics of the organelle as well as in the traffic of cholesterol. Indeed, even if the lipid accumulates in NPC endosomes similarly to other phospholipids, it seems still to be limiting for cholesterol traffic. In fact, NPC phenotype can be reverted in cells by supplementing LBPA-containing liposomes.
We found, that cell treatment with HPCD, which is very effective in normalizing cholesterol levels in NPC cells, also causes a drastical reduction of LBPA levels (both in wt and in NPC cells). Strikingly, this reduction is accompanied by the release of LBPA and other phospholipids in the extracellular medium. Concomitantly, we also find other evidences persistent long-term increase of the fusion rate of endosomes/lysosomes with the PM upon HPCD treatment, including release of lysosomal enzymes to the medium and in the long term (24-48h) a partial depletion of endo/lysosomal luminal content. These findings reveal a possible mechanism for HPCD-induced normalization of cholesterol traffic in NPC cells, possibly by redistributing cholesterol to the PM.
We analyzed by mass spectrometry the LBPA acyl chain composition in cultured cells treated with HPCD and in livers from wt and NPC1 KO mouse. Our findings indicate the strong increase in LBPA in this lipid storage disease is accompanied by a drastic re-arrangement in the acyl chain composition with relative percentage decrease in polyunsaturated fatty acid (PUFA)-containing forms. Ether forms of the lipid are also strongly reduced. Liver analysis also indicates a very strong increase on the triple acylated form of the lipid (SLBPA) whose biological function is totally unknown and whose relevance in cholesterol traffic awaits further investigations.

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Molecular and Cellular Biosciences

Neutrophil Necroptosis is Triggered by Ligation of Adhesion Molecules following GM-CSF Priming

Wang, Xiaoliang

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Apoptosis is the most common form of neutrophil death under both physiological and inflammatory conditions. However, forms of non-apoptotic neutrophil death have also been observed. In the present study, we report that human neutrophils undergo necroptosis after exposure to GM-CSF followed by the ligation of adhesion receptors such as CD44, CD11b, CD18, or CD15. Using a pharmacological approach, we demonstrate the presence of a receptor interacting protein kinase-3 (RIPK3) - mixed lineage kinase-like (MLKL) signaling pathway in neutrophils which, following these treatments, first activates p38 mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3'-kinase (PI3K), the finally leads to the production of high levels of reactive oxygen species (ROS). All these steps are required for necroptosis to occur. Moreover, we show that MLKL undergoes phosphorylation in neutrophils in vivo under inflammatory conditions. This newly identified necrosis pathway in neutrophils would imply that targeting adhesion molecules could be beneficial for preventing exacerbation of disease in the neutrophilic inflammatory response.

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Nano Biotechnology

Second Harmonic Nanoprobes for Hematopoietic Stem Cell Labeling and Tracking

Selected for flash talk

Sonay, Ali Yasin

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Primary Stem cells are particularly sensitive to exogenous imaging probes, which can lead to significant adverse effects on their proliferation and differentiation potential. Nanoparticle-based single-molecule techniques that require very low labeling may obviate these adverse effects, but a better understanding of cellular uptake mechanisms and fate of nanoparticle probes in stem cells are required. Here, we report the synthesis and functionalization of second-harmonic generating (SHG) $BaTiO_3$ nanocrystals to image their cellular fate in different stages of murine hematopoietic stem cells (HSCs) using nonlinear imaging and electron microscopy. The high signal-to-noise ratio of intracellular SHG nanoprobes allows sensitive detection of single nanocrystals in live primary stem cells without bioadverse effects. Our results show that SHG nanoprobes enter HSCs mainly by clathrin-dependent endocytosis, are not exported by efflux pumps, and split between daughter cells upon cell division. Additionally, we have discovered the efficiency of uptake is highly dependent on the dormancy and proliferation of the stem cells state. The concentration of nanocrystals in proliferative multipotent progenitors is over 2.5-fold greater compared to dormant stem cells; this difference vanishes when HSCs enter a proliferative state while their potency remains unaffected. Our results provide a unique approach to stem cell labeling by taking advantage of their proliferative state while retaining their stem cell characteristics. Moreover, SHG nanoprobe labeling of stem cells holds great promise for understanding their engraftment and therapeutic potential using deep tissue imaging.

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Neuroscience

Prediction of seizure outcome improved by fast ripples detected in low-noise intraoperative corticogram

Fedele, Tommaso

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Objective: Fast ripples (FR, 250-500 Hz) in the intraoperative corticogram have recently been proposed as specific predictors of surgical outcome in epilepsy patients. However, online FR detection is restricted by their low signal-to-noise ratio. Here we propose the integration of low-noise EEG with unsupervised FR detection. Methods: Pre- and post-resection ECoG (N = 9 patients) was simultaneously recorded by a commercial device (CD) and by a custom-made low-noise amplifier (LNA). FR were analyzed by an automated detector previously validated on visual markings in a different dataset.
Results: Across all recordings, in the FR band the background noise was lower in LNA than in CD ($p < 0.001$). FR rates were higher in LNA than CD recordings (0.9 ± 1.4 vs 0.4 ± 0.9, $p < 0.001$). Comparison between FR rates in post-resectionECoG and surgery outcome resulted in positive predictive value PPV = 100% in CD and LNA, and negative predictive value NPV = 62% in CD and NPV = 80% for LNA. Prediction accuracy was 67% for CD and 89% for LNA.
Conclusions: Prediction of seizure outcome was improved by the optimal integration of low-noise EEG and unsupervised FR detection.
Significance: Accurate, automated and fast FR rating is essential for consideration of FR in the intraoperative setting.

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Neuroscience

Reconstruction and Simulation of Neocortical Microcircuitry

Ramaswamy, Srikanth

Ramaswamy, Srikanth (1); Muller, Eilif (1); Reimann, Michael (1); Markram, Henry* (1)
(1) EPFL, Blue Brain Project

The Blue Brain Project has established a unifying data-driven process for the digital reconstruction of neocortical tissue. The process unifies a vast body of anatomical and physiological data on ion channel kinetics and distributions, neuron morphologies, electrical types, and synaptic dynamics to yield a digital reconstruction of the cellular and synaptic organization of juvenile rat somatosensory cortex. We present a draft anatomical and physiological map of neocortical microcircuitry, which represents the first comprehensive integration of available data and knowledge in a quantitative in silico reconstruction of a part of the brain. The digital in silico reconstruction was achieved through a novel predictive strategy that was developed using sparse biological data on the cellular and synaptic organization of the somatosensory cortex of a two-week old rat. The reconstructed microcircuit is 0.29 mm3 in volume and contains about 31,000 neurons belonging to 55 morphological neuron types, and 207 morpho-electrical sub-types distributed across 6 layers. The resulting reconstruction is broadly consistent with current knowledge about neocortical microcircuitry and provides an array of predictions on its structure and function across neuronal, synaptic, and circuit levels. The experimental data and models in the reconstruction are available as a public resource for collaborative and iterative refinement, and in silico neuroscience.

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Neuroscience

An open-source framework for scalable analysis of brain imaging data

Luetcke, Henry

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Imaging techniques, such as two-photon microscopy, have become an important part of the neuroscience toolkit. However, analysis of the large amount of data produced by these techniques pose challenges for many labs because conventional approaches to data analysis scale poorly to datasets comprising hundreds of gigabytes. These problems can be addressed by novel Big Data computing frameworks, notably Spark, which promise scalable data analysis on commercial cloud computing platforms. While these systems are powerful, the technical know-how required for implementing workflows based on Spark is still beyond what is available in most labs. Moreover, the use of commercial cloud computing platforms may raise concerns regarding cost control and privacy. We have implemented a framework for scalable and easy-to-use analysis of imaging datasets, based on Spark, in an open-source cloud computing environment. The workflow is accessed with a web browser and encompasses import of raw data, a preprocessing pipeline, visualization and higher-level analytics. We apply our framework to the analysis of calcium imaging data acquired in mice performing a texture-discrimination task. Preprocessing of full frame movies is faster with our approach, compared to similar analysis on stand-alone machines, and scales well with the number of machines/cores in the cluster. Higher-level analyses, such as sliding-window correlation and regression, which are computationally too costly to run on a single machine, become possible with our distributed approach. The framework is freely available to the community and is expected to become a useful tool for the analysis of similar datasets acquired in different laboratories.

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Neuroscience
Studying axonal energy metabolism in compact white matter

Saab, Aiman

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Neuronal energy requirements depend to a large extent on electrical activity and the ATP demand of Na+/K+ ATPases. Astrocytes and oligodendrocytes are suggested to fuel neuronal ATP demands by providing lactate to neuronal compartments such as dendrites and axons. Axonal energy deficits are well known to cause white matter lesions in ischemic stroke, and may also underlie neurodegeneration in many chronic neurological diseases. However, little is known about the energy metabolism of myelinated axons. Here, we have combined electrophysiological recordings of compound action potentials and two-photon imaging of acutely isolated optic nerves to study activity-dependent metabolite dynamics in axons. Following intravitreal injections of AAVs and successful infection of retinal ganglion cells we achieved a robust expression of genetically encoded metabolite sensors for lactate or glucose in optic nerve axons. We additionally studied axonal ATP homeostasis by using a transgenic mouse line expressing an ATP-sensor specifically in neurons. Upon acute nerve stimulations axonal ATP levels dropped whereas lactate levels increased. The activity-induced rise in axonal lactate levels may result either from the associated increase in axonal glycolytic activity or from the lactate supply of surrounding glycolytic glial cells. When glucose was omitted from the perfusate axonal lactate levels declined first and were followed by an ATP level drop only when axonal lactate was almost diminished, suggesting that axonal steady-state ATP homeostasis critically relies on lactate availability. Our optic nerve model system will allow us to determine the cellular mechanisms that govern axonal energy metabolism under physiological and pathological conditions.

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Neuroscience

Diversity of microvascular endothelial cell markers in different regions of the human brain

Mbagwu, Smart

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Background
Microvascular endothelial cells in the brain have various important functions, including contribution to the blood-brain barrier and supply of nutrients and oxygen to the brain, as well coagulation control, inflammatory responses and angiogenesis. Endothelial cells have observable morphological and molecular differences in different tissues and organs. However little is known about differences of microvascular endothelial cells in the human brain. The aim of this study was to characterize qualitatively the expression of endothelial cell markers within different parts of the brain.
Methods
Frozen sections from different anatomical regions (precentral and postcentral gyrus, hippocampus, rhinal and visual cortex) of human formalin fixed brains (n=3) were stained immunohistochemically for endothelial cell markers, including CD31, claudin 5, occludin,von Willebrand Factor, ZO-1, as well as astrocyte (glial fibrillary acid protein) microglia (Iba1) markers.
Results
The expression patterns of the markers were heterogeneous in the different regions of the brain studied. CD-31 had the most positive expression among the different biomarkers especially in the precentral cortex. ZO-1 and and vWF had fairly positive expressions in the pre central and post central cortices compared to other regions.
Conclusion
The expression pattern of the different endothelial markers is heterogeneous in the microvasculature within the different anatomical regions of the cerebral cortex. These results indicate that the diversity in microvascular endothelial cells contributes to functional differences in the different brain regions.

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Neuroscience

Molecular determinants of transepithelial calcium transport in the endolymphatic sac of the murine inner ear

Bächinger, David

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Background: Calcium concentrations in the inner ear endolymph [Ca2+]e are actively maintained at an extremely low level (0.02 mM) in order to enable the proper transduction of acoustic and gravitational stimuli by the sensory receptor cells (auditory and vestibular hair cells). Previous studies suggested that the endolymphatic sac (ES) – a non-sensory epithelial portion of the inner ear membranous labyrinth – contributes critically to the maintenance of [Ca2+]e by so far unknown transport mechanisms. Here, we aimed to reveal the molecular determinants of a transepithelial calcium (Ca2+) transport system in the murine ES epithelium.
Methods: Diaminobenzidine (DAB) immunohistochemistry of Ca2+-transport proteins on paraffin-embedded sections of adult mouse ES. For each antigen, DAB-positive cell numbers were counted along the entire length of the ES epithelium (intraosseus and extraosseus portions). Immunofluorescence double-labelings were performed to analyze cell type-specific and subcellular localization patterns of Ca2+-transport proteins within the ES epithelium.
Results: Transient receptor potential cation channels TRPV5 and TRPV6, sodium/calcium exchanger NCX2, plasma membrane calcium ATPases PMCA1 and PMCA4, calcium-sensing receptor CaSR, and sarco/endoplasmic reticulum Ca2+-ATPases SERCA1 and SERCA2 were identified in distinct subcellular compartments of ES epithelial cell populations. Notably, almost all Ca2+ transport proteins exhibited a spatial expression gradient within in the ES epithelium, showing strongest expression in the extraosseus (distal) portion.
Conclusion: The extraosseus portion of the ES harbours a transepithelial Ca2+ transport system, which presumably contributes to endolymphatic Ca2+-homeostasis. Loss of this Ca2+-transport system is of potential significance in various pathophysiological states of disturbed endolymph homeostasis, such as in Meniere's disease.

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Neuroscience

Restoring cell surface expression of GABA (B) receptors: a potential strategy to limit neuronal death in cerebral ischemia

Selected for talk

Balakrishnan, Karthik

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Cerebral ischemia is a leading cause for long-term disability and mortality in adults due to prolonged massive neuronal death. One major mechanism behind ischemia-induced neuronal death is the excessive release of glutamate upon oxygen and glucose deprivation (OGD) occurring during ischemic stroke. Ischemic overexcitation of neurons downregulates GABA(B) receptors, which normally control excessive excitatory neurotransmission by mediating slow and prolonged neuronal inhibition. Sustained activation of glutamate receptors increases the intracellular Ca2+ concentration enhancing the activity of CaMKII, which leads to the phosphorylation of GABAB receptors at S867 in the C-terminal domain of the GABAB1 subunit. This sorts the constitutively endocytosed GABAB receptors to lysosomal degradation instead of recycling them back to the cell surface. In this study we tested the hypothesis that restoring cell surface expression of GABAB receptors may prevent neuronal death under ischemic conditions. We identified a short synthetic peptide that prevents the interaction of CaMKII with GABAB1 and consequently prevented the phosphorylation of GABAB1 (S867). This short-interfering peptide prevented glutamate-induced downregulation of GABA(B) receptors in cultured cortical neurons and limited glutamate and OGD-induced neuronal death. Therefore, we expect that the preserved cell surface GABAB receptors levels under ischemic conditions counteract the excessive neuronal excitation and thus limit neuronal death in-vivo.

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Neuroscience

Electrophysiological and behavioral characterization of a genetic mouse model of diminished synaptic inhibition in the spinal dorsal horn

Tudeau, Laetitia

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Diminished synaptic inhibition in the superficial dorsal horn is believed to underlie several chronic pain syndromes. Here, we describe electrophysiological and behavioral changes in a genetic mouse model of diminished spinal synaptic inhibition, i.e. in mice that lack a major GABAA receptor (GABAAR) alpha subunit (a2) from the spinal cord and the spinal terminals of peripheral sensory neurons (hoxb8-gabra2-/- mice). We first characterized changes in GABAergic inhibition in the superficial dorsal horn where a2GABAARs are abundantly expressed. These experiments were done on a vGAT::ChR2 transgenic background which allowed optogenetic activation of inhibitory neurons in spinal cord slices. In hoxb8-gabra2-/- mice, light-evoked GABAergic IPSC amplitudes were reduced by about half ($p = 0.017$) with no significant change in their decay kinetics. Despite the reduction in synaptic inhibition, hoxB8-gabra2-/- mice did not show behavioral sensitization to acute painful stimulation, suggesting the presence of a yet-to-be identified homeostatic plasticity mechanism. Hoxb8-gabra2-/- mice did not only show unchanged sensitivity to acute pain but also developed normal pain sensitization in the CCI model of neuropathic pain, indicating that altered expression and posttranslational modification of a2GABAARs are not essential for neuropathic hyperalgesia. However, the antihyperalgesic effects after systemic application of the GABAAR modulator HZ-166 were virtually abolished in hoxb8-gabra2-/- mice, and in line with this observation, modulation of GABAergic IPSCs by HZ-166 was strongly reduced. These results indicate that a2GABAARs are not required for the induction of neuropathic hyperalgesia but are essential for the antihyperalgesic actions of the novel benzodiazepine site agonist HZ-166.

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Neuroscience

A functional role for miR-709 in sleep homeostasis

Selected for talk

Kompotis, Konstantinos

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MicroRNAs are small non-coding RNAs with a pivotal role in the fine tuning of a plethora of biological processes, including sleep (Mongrain et al., 2010; Davis et al., 2012). We performed a microRNA-array analysis to determine which microRNAs are affected by sleep loss in the mouse and found miR-709 expression to be increased in the forebrain after 6h of sleep deprivation (SD). In situ analysis confirmed a robust upregulation in cortex and hippocampus suggesting a role for miR-709 in the sleep homeostatic process. The functional involvement of miR-709 in this process was essayed by in vivo down-regulation using miR-709 LNA inhibitors (Exiqon, Denmark). The EEG response to a 6h SD was measured in mice injected intracerebroventricularly (ICV) with either a) the miRNA-709 inhibitor, b) a non-functional "scrambled" control, or c) artificial cerebrospinal fluid (aCSF) as a vehicle control. Mice injected with the miR-709 inhibitor exhibited a higher increase in EEG spectral power, particularly in the slow delta frequency range (1.0-2.25 Hz), compared to the two control groups. Since EEG delta activity in NREM sleep is considered the most reliable proxy of the sleep homeostatic process, our data demonstrate that miR-709 is implicated in this aspect of the sleep homeostat. Moreover, because miR-709 is upregulated after stimulation of metabotropic glutamate receptors (mGluRs; Lusardi et al., 2012), which are directly involved in sleep homeostasis (Ahnaou et al., 2015), miR-709 might functionally link neuronal activation during wakefulness to the recovery process occurring during sleep.

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Neuroscience
Rhythmic sensory stimulation in mice and its effects on sleep

Kompotis, Konstantinos

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Slow rhythmic sensory stimulation (0.25Hz), broadly known as “rocking”, facilitates the transition to sleep and increases delta (1-4Hz) and spindle (11-15Hz) activity in the sleep EEG of healthy humans. In this project we investigated whether this effect is conserved in mice, to establish a mouse model to elucidate the mechanistic aspects of the phenomenon.
C57BL/6J mice implanted with EEG/EMG electrodes, were rocked in the horizontal plane at three different frequencies (0.25, 1, and 1.5Hz) during the light period. EEG/EMG signals were recorded continuously for two stationary days, one “rocking” day, and another stationary day, for each frequency. Shaking at 1Hz recapitulated the observations made in humans; NREM sleep was also promoted in the mouse, especially within the first three hours after the start of shaking. “Rocking” at 1Hz also shortened sleep onset latency after a 1h sleep deprivation. We hypothesized that the effects of “rocking” on sleep are mediated, at least in part, by the vestibular system. To test this hypothesis, we used the tilt mouse that has deficits in the vestibular system due to a point mutation in Otop1. Homozygous tilt mice did not show the beneficial effect of rocking on sleep quantity or sleep onset latency while the response in wild type littermates resembled that of C57BL/6J mice. We have established a mouse model allowing us to investigate the mechanisms by which rocking impinges on the sleep circuitry. The results confirm the involvement of the vestibular system and could help develop a non-pharmacological intervention to treat sleep disorders.

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Pharmacology

Accelerated ligand-induced nuclear transport of the AHR but not nucleocytoplasmic shuttling is strictly dependent on histidine 291

Tkachenko, Anna

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The aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor, which is activated by structurally diverse ligands. Due to its multiple functions in xenobiotic metabolism, cancer progression, inflammation and apoptosis, the AHR gained relevance not only in toxicology but also in physiology. During activation, ligands trigger two distinguishable effects, namely an accelerated nuclear transport and consequently rearrangements of nuclear receptor complexes that initiate transcription. So far, ligand promiscuity of the AHR and relevant residues within the ligand-binding domain (LBD) have mainly been analyzed in respect to target gene activation. However, very little is known on functional and structural requirements that affect nuclear transport and trafficking of receptor complexes.

Using online confocal microscopy and real-time quantitative PCR, we demonstrate here that biogenic and xenobiotic AHR ligands trigger maximum nuclear transition rates and induction of CYP1A1/1B1 at different concentrations. To specify distinct modes of action, the role of individual amino acids within the LBD was examined in ligand-dependent responses. Site-directed mutagenesis identified His291 of human AHR as a key residue that is essential for both steps of ligand-mediated AHR activation, but not for basal nuclear transport in the frame of nucleocytoplasmic shuttling. Moreover, previously reported residues Cys333 and Gln383 seem to play only marginal role in this process. The role of His291 in ligand binding was further investigated in silico using homology modeling and molecular dynamics simulations. Altogether, the presented data provide a probable model of the AHR-LBD and its ligand binding properties, suggesting His291 as a central player.

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Pharmacology

Investigation of Xanthine Oxidase Inhibition and Carbonic Anhydrase Properties of Metal Phthalocyanine Compounds Containing Chalcone

BARAN, ARİF

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(1) Institute of science, chemistry

In this study, three novel phthalonitriles (M = Zn, Co, Cd) were synthesized using the corresponding metal salts and (E)-4-(4-(3-(2,4,5-trimethoxyphenyl)acryloyl)phenoxy)phthalo-nitrile as chalcone ligand , which was prepared from the reaction of 4-nitrophthalonitrile with (E)-1-(4-hydroxyphenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one. These metallophthalocyanines showed quite solubility in organic solvents such as chloroform and dichloromethane. The novel compounds have been characterized using their elemental analysis data and UV–Vis, FT-IR, 1H-NMR, 13C-NMR and MALDI-TOF mass spectra. Then the xanthine oxidase inhibition and carbonic anhydrase properties of these molecules were investigated.

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Pharmacology

Intranasal administration of resveratrol successfully prevents lung cancer in A/J mice

Selected for talk

Monteillier, Aymeric

Monteillier, Aymeric (1); Furrer, Pascal (1); Allémann, Eric (1); Cuendet, Muriel* (1)
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Resveratrol, a phytoalexin found in various foods such as grapes, is one of the most studied natural products. It displayed several biological activities including cancer chemoprevention. However, the low bioavailability of resveratrol often limited the translation of the in vitro activities to in vivo studies. For example, oral administration of resveratrol effectively inhibited carcinogenesis in the digestive tract, but failed to protect mice from chemically-induced lung carcinogenesis. This failure was attributed in part to the metabolism that undergoes resveratrol when taken orally. Therefore, other non-invasive administration routes must be considered to bring sufficient doses to the lungs, and the pulmonary route seems the best one. In the present study, hydroxypropyl-betadex was used to solubilize resveratrol in saline solution (24 mg/ml) and intranasal administration proved to be a valid method to expose the lungs to a high amount (281 nmol/g tissue) of compound. This formulation was administered thrice a week for 25 weeks to A/J mice having 4-[methyl(nitroso)amino]-1-(3-pyridinyl)-1-butanone (NNK)-induced lung carcinogenesis. Resveratrol-treated mice showed a 27 % decrease in tumor multiplicity, with smaller tumors, resulting in 45 % decrease in tumor load. These results support the hypothesis that resveratrol low oral bioavailability may be responsible for the lack of activity observed in former studies. Further in vitro investigations revealed a dose dependent increase in NNK-induced-H2AX phosphorylation after resveratrol treatment, highlighting DNA repair modifications as a plausible mechanism of action. Overall, the present study supports the interest in intrapulmonary administration of resveratrol for further clinical development in lung cancer chemoprevention.

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Pharmacology

Repurposing tamoxifen for severe myopathies: from preclinical evaluation in animal models to clinical trials in patients

DORCHIES, Olivier

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Duchenne muscular dystrophy (DMD) is a severe X-linked disorder caused by the lack of dystrophin, a large protein normally expressed in muscle fibres. Boys affected by DMD develop progressive muscle wasting, cardiac and respiratory failure, and early death. Many signalling pathways and cellular processes are altered downstream of the missing dystrophin. This situation offers the opportunity to mitigate DMD symptoms via a variety of pharmacological targets and using diverse classes of drugs and nutraceuticals that are already approved for human use and are readily available and affordable.
We have shown that tamoxifen, a drug used for more than 30 years to treat breast cancer, efficaciously ameliorates muscle function and structure in dystrophic mice, an animal model of DMD. Based on our robust pre-clinical data, a phase 2a/2b multicentre, randomized, placebo-controlled clinical trial on 85-100 DMD patients, will start in 2017, led by Professor Dirk Fischer (UKBB, Children's Hospital, Basel). Compassionate use of tamoxifen in DMD boys for a year shows promising results.
Recently, we have evaluated tamoxifen in a mouse model of X-linked centronuclear (myotubular) myopathy (XLCNM/XLMTM), an extremely severe myopathy characterized by generalized hypotonia from birth and death before the age of 1. We found that tamoxifen dramatically prolongs the lifespan of XLCNM mice: from around 40 days in untreated mice, the median survival was extended several-fold, to >200 days using clinically relevant doses of tamoxifen.
Our work reveals previously unrecognized roles of estrogenic signalling as a disease modifier pathway in severe muscular diseases that have independent pathogenic mechanisms.

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Physiology

Sodium retention in nephrotic syndrome occurs independently of proteinuria

Theilig, Franziska

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Patients with nephrotic syndrome (NS) often present symptoms of volume retention, such as edema formation or hypertension. The primary dysregulation was localized to the renal cortical collecting duct and involves an inappropriate activation of the epithelial sodium channel, ENaC. Plasma proteases passing the leaky glomerular filter were made responsible; however clinical observations demonstrate volume retention before the onset of proteinuria. To elucidate its relationship and the underlying mechanisms, inducible podocinCre; Nphs2fl/fl developing FSGS with NS was used. Analysis of renal functional parameters from NS mice revealed sodium retention between day 4 – 7, hypertension and proteinuria starting at day 10 and 12 after FSGS induction. Morphological and biochemical analysis of NS mice kidneys demonstrated at 5 and 9 days increased full-length alpha- and gamma-ENaC expression and proteolytical cleavage of alpha-ENaC, and at 17 days increased full-length ENaC and cleaved subunit expression. Aldosterone and vasopressin levels remained unaltered. Urine analysis from NS mice revealed proteolytic activity starting at day 2 after FSGS induction which increased over the time. Urine from 2 – 9 of NS mice was separated by HPLC and proteases of proteolytic fractions were identified by mass spectrometry. From the identified proteases, cathepsin B and D cleaved alpha-ENaC in vitro in protease assays and in vivo of transiently transfected HEK cell.
We identified a new mechanism of volume retention in NS where proteolytical activation of alpha-ENaC led to sodium retention early in the development of NS which mainly depends on lysosomal proteases and less on an inappropriate filtration of plasma proteases.

78
Physiology
Role of arginase-II in regulation of aging

Xiong, Yuyan

Xiong, Yuyan (1); Yepuri, Gautham (1); Montani, Jean-Pierre (1); Yang, Zhihong (1); Ming, Xiu-Fen (1)
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Aging is the progressive functional decline of various organs, leading to increased risk of many aging-related diseases such as cancer, neurological degeneration, cardiovascular diseases, and glucose intolerance. Arginase-II (Arg-II) isoenzyme that metabolizes L-arginine to L-ornithine and urea has shown to promote atherosclerosis, vascular cell senescence, and obesity-associated type 2 diabetes. Here we further investigate whether targeting Arg-II would extend lifespan in mice. Wild type (WT) and Arg-II knockout (KO) offspring from hetero/hetero cross were interbred to obtain WT and Arg-II knockout mice, respectively. Mouse lifespan was analysed by using Kaplan-Meier survival curves log-rank test. A significant lifespan extension was observed in Arg-II knockout as compared to WT mice in females, but not in males. Combined data of both gender groups demonstrate a significant improved survival at early life stage. In female mice, the median life of Arg-II knockout mice was increased by 93 days from 666 to 759 days. However, ablation of Arg-II gene in males had no significant effect on median and maximum life span. Dampened p66shc and mTOR signaling pathways in heart not liver tissue of old female Arg-II knockout mice were observed. p16Ink4a (encoded by the INK4a/Arf locus) characterized as an important cellular senescence marker, was decreased in the heart and skin of aged female (but not male) Arg-II knockout mice as compared to age-matched WT animals. Our results demonstrate that disruption of Arg-II gene is capable of extending lifespan particularly in female mice related to suppression of p66shc expression, mTOR signaling and p16INK4a in certain tissues/organs such as heart and skin.

79
Physiology

Regulatory DNA elements modulating oxygen-regulated erythropoietin gene expression

Selected for talk

Orlando, Ilaria

Orlando, Ilaria (1); Wenger, Roland H.* (1)
(1) University of Zurich, Physiology

Circulating erythropoietin (Epo), the main hormone maintaining red blood cells homeostasis, is produced by the fetal liver and adult kidney. In response to anemia or inspiratory hypoxia, renal Epo synthesis is induced in peritubular interstitial cells, located at the juxtamedullary cortex. While Epo transcriptional regulation in the liver has been widely studied due to the availability of human hepatoma cell lines capable of hypoxic Epo induction (Hep3B and HepG2), the kidney-specific transcriptional regulatory elements remain to be clarified because no renal erythropoietin producing (REP) cell line is available. We previously identified a novel 5\' hypoxia response element (5\'-HRE) within the putative kidney inducible region upstream of the EPO gene and compared its function with the well-known downstream 3\'-HRE within the liver inducible region. Using Hep3B cells, we demonstrated that both the 5\' and 3\'-HREs are bound and trans-activated by hypoxia-inducible factor (HIF)-2. In order to further dissect the contribution of the 5\' and 3\'-HREs to endogenous EPO gene expression, we used CRISPR/Cas technology to disrupt the two loci in hepatoma and neuroblastoma (Kelly) cells. While the 3\'-HRE but not the 5\'-HRE is required for hypoxic Epo induction in hepatic cells, both the 5’ and 3’-HREs appear to contribute to neuronal Epo induction by hypoxia. These data suggest that Epo regulation in REP cells may have more in common with neuronal cells than with hepatic cells, a finding that is supported by the unexpected finding of neuronal markers on REP cells in vivo.

80
Physiology

Role of arginase-II in regulation of water balance

Selected for flash talk

Huang, Ji

Huang, Ji (1); Montani, Jean-Pierre (1); Verrey, François (2); Feraille, Eric (3); Ming, Xiu-Fen (1); Yang, Zhihong* (1)
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Aim
Arginase-II (Arg-II) is highly expressed in kidney with its most abundant expression in proximal tubule epithelial cells and also in collecting duct cells. However, the function of Arg-II in kidney remains largely unknown. In the present study, we aim to investigate the role of Arg-II in regulation of vasopressin-regulated water channel protein aquaporin 2 (AQP2) in collecting ducts and the impact on water balance.

Methods and results
In cultured mouse collecting duct cell line mCCDcl1, desamino-d-arginine vasopressin (dDAVP), a synthetic vasopressin receptor V2-agonist, stimulated expression and membrane translocation of AQP2 as expected and upregulated Arg-II levels as assessed by immunoblotting and/or immunofluorescence staining. Silencing Arg-II further enhanced AQP2 expression and membrane translocation in response to dDAVP. Conversely, overexpression of native or an inactive Arg-II mutant suppressed the effects of dDAVP. In agreement with these findings in vitro, total and membrane-associated AQP2 levels were significantly higher in Arg-II-deficient (Arg-II-/-) than wild-type (WT) mice, suggesting a negative regulation of AQP2 by Arg-II. Furthermore, the total and membrane-associated AQP2 levels in WT mice were increased by water deprivation paralleled with elevated Arg-II level in collecting duct cells, decreased urine volume and increased urine and plasma osmolality. Arg-II-/- mice showed more pronounced water preservation effect under the water deprivation condition.

Conclusion
Arg-II in collecting duct cells influences water balance through negative regulation of AQP2 expression and membrane translocation independently of its L-arginine:ureahydrolase activity.

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Physiology

Effect of Roux-en Y gastric bypass in ApoE*3Leiden.CETP mice

selected for talk

Tarasco, Erika

Tarasco, Erika (1); Neuner-Boyle, Christina (1); Whiting, Lynda (1); Pellegrini, Giovanni (2); Lutz, Thomas* (1)
(1) Institute of Veterinary Physiology, University of Zurich; (2) Institute of Veterinary Pathology, Laboratory for Animal Model Pathology, University of Zurich

The metabolic syndrome (MetS) is associated with obesity, insulin resistance, cardiovascular diseases and hypercholesterolemia. ApoE*3Leiden.CETP mice expressing the human genes ApoE (apolipoprotein E) and CETP (cholesterol ester transfer protein) present a humanized lipoprotein metabolism, thus they are a suitable model to describe MetS. Responder and non-responder mice have the same genetic background but non-responders (20% of the offspring) don't gain as much weight as responders when fed high fat diet + 0.25% cholesterol (HFDC). Roux-en-Y gastric bypass (RYGB) surgery allows to achieve sustained and long-term weight loss and to improve obesity-related comorbidities. The goal of these studies is to compare the metabolic status of ApoE*3Leiden.CETP responders versus non-responders and to assess the effect of RYGB on improving these metabolic parameters.
Glucose metabolism was investigated by performing oral glucose tolerance tests (OGTT; 2g/kg) and insulin tolerance tests (ITT; 0.5U/kg). Responders became glucose intolerant compared to non-responders after 1 month on HFDC. Parameters of lipid metabolism (total cholesterol, HDL, triglyceride, free fatty acids and beta-hydroxybutyrates) were analyzed at 25 weeks of age and were all increased in responders compared to non-responder mice. HFDC fed responders underwent RYGB surgery and showed sustained weight loss compared to sham ad libitum-fed mice. More importantly, glucose metabolism improved 2 weeks after surgery. Lipid metabolism improved in both RYGB and sham body weight matched mice compared to sham ad libitum-fed mice after 3 weeks after surgery. Our study underlines the importance of RYGB surgery not only to induce body weight loss but also to improve glucose metabolism.

82
Physiology

Functional characterization of Calsequestrin-1 mutation identified in patients affected by a vacuolar myopathy

Polverino, Valentina

Polverino, Valentina (1); Gamberucci, Alessandra (1); Saüc, Sophie (2); Frieden, Maud (2); Demaurex, Nicolas (2); Sorrentino, Vincenzo* (1)
(1) University of Siena, Molecular Medicine Section, Department of Molecular and Developmental Medicine; (2) University of Geneva, Department of Cell Physiology and Metabolism

Calsequestrin1 (CASQ1) is the most abundant high-capacity, moderate-affinity Ca2+-binding protein in the sarcoplasmic reticulum (SR) of striated muscle. The CASQ1 polymer is anchored to the SR membrane thanks to its interactions with junctin and triadin, modulating the Ca2+ release channel RYR1 (ryanodine receptor 1). A physical interaction between CASQ1 and Stromal Interaction Molecule 1 (STIM1) has been also reported; STIM1 through interactions with the Ca2+ permeable Orai1 channel on the plasma membrane activates the store-operated Ca2+entry (SOCE), mediating the Ca2+ influx after depletion of intracellular Ca2+stores. Recently we identified the first mutation in CASQ1 gene, in patients with a vacuolar myopathy characterized by the presence of inclusions due to the aggregation of CASQ1 and other SR proteins. The CASQ1 mutation (CASQ1D244G) affects a high affinity Ca2+-binding-site. To analyze the functional alteration of Ca2+ storage capacity of the ER and the modulation of SOCE between the CASQ1WT and CASQ1D244G, we transfected CASQ1WT or CASQ1D244G in Hela cells, and measured cytosolic Ca2+ changes by Fura-2. We applied thapsigargin (Tg) to deplete the stores followed by Ca2+ re-addition. The ER Ca2+ release induced by Tg was increased in cells overexpressing CASQ1WT compared to control cells, and to a lesser extend in cells overexpressing CASQ1D244G. In both CASQ1WT and CASQ1D244G expressing cells, the SOCE was decreased. We also measured the ER Ca2+ concentration with the ER targeted Ca2+ probe D1ER. Preliminary results provided support for the hypothesis that CASQ1D244G altered ER Ca2+ storage capacity compared to CASQ1WT.

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Physiology

Differential localization and role of STIM1 and STIM1L in skeletal muscle

Frieden, Maud

Saüc, Sophie (1); Bernheim, Laurent (2); Demaurex, Nicolas (3); Frieden, Maud* (3)
(1) University of Geneva, Cell Physiology and Matebolism; (2) University of Geneva, Basic Neurosciences; (3) University of Geneva, Cell Physiology and Metabolism

Store-Operated Ca2+ entry (SOCE) is a ubiquitous Ca2+ influx mechanism of particular importance for skeletal muscle and patients harboring mutations in SOCE genes suffer from muscular weakness and myopathies. SOCE is triggered by the Ca2+ depletion of the endoplasmic/sarcoplasmic reticulum (ER/SR), which initiates the oligomerization of the ER Ca2+ sensor STIM1 and its translocation to the plasma membrane (PM). At the PM, STIM1 opens Ca2+-selective channels of the Orai family allowing Ca2+ entry and ER/SR refilling. In human muscle two different isoforms of STIM1 are expressed: the classical protein (STIM1) and a longer isoform (STIM1L) resulting from an alternative splicing of the stim1 gene. To investigate the respective contribution of STIM1L vs STIM1 in muscle physiology, we first determined the localization of STIM1L in human adult tissue by immunostaining using an antibody directed specifically against STIM1L. Surprisingly, our results showed a preferential localization of STIM1L at the longitudinal part of the SR (not apposed to t-tubules) suggesting other role(s) of this isoform than gating PM Ca2+ channels. To characterize the specific role of STIM1 and STIM1L in Ca2+ fluxes between the different cellular compartments, we are working out a culture cellular system (Matrigel) to obtain mature human myotubes. After 10 days in culture, typical skeletal fibers striations were observed, confirming an advanced level of differentiation, while the t-tubules are still not formed at that stage. Further Ca2+ imaging experiments after invalidation of each isoforms will be performed using this culture system to assess the roles of both STIM1 isoforms.

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Physiology

STIM1 promotes migration, phagosomal maturation and antigen cross- presentation in dendritic cells.

Nunes-Hasler, Paula

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(1) University of Geneva, Department of Cell Physiology and Metabolism; (2) Université Paris Descartes, INSERM, Unité 1013; (3) University of Geneva, Department of Pathology and Immunology

Antigen cross-presentation by dendritic cells (DCs) is a central immune mechanism for the activation of cytotoxic T cells critical for the defence against viruses, intracellular pathogens and cancer. Antigens acquired through phagocytosis in DCs produce potent T cell responses, but the intracellular signalling and trafficking pathways regulating cross-presentation of phagocytically derived antigens are not well understood. In the present study, we show that in LysM-Cre;Stim1fl/fl mice lacking STIM1 in myeloid cells cross-presentation is impaired in vivo. This effect was reproduced in vitro using primary bone-marrow derived DCs, as well as a CD8+ murine DC cell line stably expressing a control or an shRNA directed against STIM1. DC migration was partially impaired in vivo whereas in vitro STIM1 ablation specifically reduced migration towards Ca2+-dependent chemoattractants. Although store-operated Ca2+ entry as well as periphagosomal Ca2+ hotspots were diminished, STIM1 was not required for the upregulation of differentiation marker CD11c, maturation markers CD40, CD80, CD86 and MHC-CII in response to microbial products LPS or CpG, nor for robust phagocytic ingestion. While phagosomal pH and ROS production remained unaffected by STIM1 ablation, a decrease in phagosomal proteolysis and phagolysosome fusion was observed that correlated with a reduced number of membrane contact sites between the ER and phagosomes. Together these data suggest that STIM1-dependent Ca2+ signalling enhances cross-presentation in DCs by facilitating fusion of endomembranes that deliver the required cohort of enzymes and transporters to phagosomes.

85
Physiology
Ion channel(s) gated by the new STIM1L isoform.

Dyrda, Agnieszka

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Depletion of the endoplasmic reticulum Ca2+ store leads to a Ca2+ entry called store-operated Ca2+ entry (SOCE), which is due to the activation of Orai1 channel gated by STIM1. We identified recently a new splice variant of STIM1, called STIM1L (long) that has an extra 106 aa in the C-term part. STIM1L is as efficient as STIM1 in eliciting SOCE, but so far, nothing is known about the channel(s) gated by STIM1L. In 50% of STIM1L/Orai1 expressing HEK cells a linear current developed upon store depletion. In the other 50% of recordings, we measured a current similar to ICRAC, but with a small amplitude. Removing external Na+ strongly reduced the inward STIM1L-induced linear current, demonstrating the non-selective nature of this current. We postulated that this linear current could be due either to STIM1L gating of other channels than Orai1 (i.e. endogenous TRPC), or the gating of Orai1 by STIM1L changes its ionic selectivity. To test this hypothesis we expressed the Orai1V102C mutant and tested if STIM1L is able to restore the Ca2+ selectivity of Orai1V102C, showing its ability to gate the channel. Indeed, we obtained a CRAC-like current with STIM1L, even if the Ca2+ selectivity was not as high as with STIM1. Overall, our data are in favor of a change of Orai1 Ca2+ selectivity upon STIM1L gating. The variation between Ca2+ selective and non-selective current might be due to the ratio of STIM1L and Orai1, which remains to be established.

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Plant Sciences

De novo assemblies of complex genomes based on long reads and chromatin interactions: continuity, completeness and accuracy

Selected for flash talk

Qi, Weihong

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Continuous, complete and accurate assemblies are the foundations for systematic investigation of genome functions and structures. For non-model organisms with complex and large genomes, recently generated genome drafts are soften based on short reads and tend to be incomplete and highly fragmented. In this study we explore long read only assemblies scaffolded with chromatin interaction data for complex genomes. We sequenced different cassava wild varieties using only long reads. In conjunction with the cassava reference genome we bench-marked the performance of different long read genome assembly methods, as well as the continuity and completeness of the assemblies. Consensus accuracies were measured using low error rate Illumina reads from the same variety. Variety specific chromatin interactions data were also generated. In combination with the high density genetic map and/or synteny with the reference genome, either hierarchically or simultaneously, we investigated the achieved chromosomal-level assemblies. Our results demonstrate that long sequencing reads and chromatin interaction data are invaluable in efficiently producing high quality assemblies for large and complex genomes. The case study also generate practice guidelines for similar research projects. Together with the decreasing sequencing cost on long read platforms, more investigators will be motivated to adopt these technologies.

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Plant Sciences

Genetic specificity of a plant-insect food web: Implications for linking genetic variation to network structure

Selected for flash talk

Barbour, Matthew

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Theory predicts that intraspecific genetic variation can increase the complexity of an ecological network. To date though, we are lacking empirical knowledge of the extent to which genetic variation determines the assembly of ecological networks, as well as how the gain or loss of genetic variation will affect network structure. To address this knowledge gap, we used a common garden experiment to quantify the extent to which heritable trait variation in a host plant determines the assembly of its associated insect food web (network of trophic interactions). We then used a resampling procedure to simulate the additive effects of genetic variation on overall food-web complexity. We found that trait variation among host-plant genotypes was associated with resistance to insect herbivores, which indirectly affected interactions between herbivores and their insect parasitoids. Direct and indirect genetic effects resulted in distinct compositions of trophic interactions associated with each host-plant genotype. Moreover, our simulations suggest that food-web complexity would increase by 20% over the range of genetic variation in the experimental population of host plants. Taken together, our results indicate that intraspecific genetic variation can play a key role in structuring ecological networks, which may in turn affect network persistence.

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Plant Sciences

Adaptive Reduction of Male Gamete Number in a Selfing Species

Kakui, Hiroyuki

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The number of male gametes produced is critically important for fitness, and is known to evolve in response to changes in the breeding-system in both plants and animals. In particular, a transition to self-fertilization is associated with a reduced production of male gametes. Through a genome-wide association study in the predominantly selfing model plant Arabidopsis thaliana, we identified a novel gene, REDUCED POLLEN NUMBER 1 (RDP1) as responsible for variation in pollen number. Analyses of rdp1-null mutants in standard and nonstandard accessions generated using the CRISPR-Cas9 system revealed that the natural variants affect cell proliferation in the male germline. Signatures of selective sweeps at RDP1 and other loci underlying pollen number variation suggest that selection for reduced male gamete production in recent evolutionary time.

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Plant Sciences

Flowering phenology and the environmental factors in the tropical tree genus Macaranga (Euphorbiaceae)

Yamasaki, Eri

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Environmental triggers for flowering have been a central question in Southeast Asian forests because of the lack of clear seasonality. Many scientists have especially focused on the characteristic irregular flowering event termed “general flowering”, in which diverse plant groups flower synchronously, and various environmental factors, such as drought, temperature drop and sunlight, have been advocated to be important as triggers for general flowering. However, while significant number of plant species do not join general flowering, little is known on which environmental factors do they use for synchronous flowering within a species. In this study, we focus on the flowering phenology of three pioneer tree species of the genus Macaranga (Euphorbiaceae) in Lambir Hills National Park, Borneo, Malaysia. Our 20 months phenology observation confirmed that these species exhibit different flowering phenology patterns depending on species: M. conifera did not show any flowers within this period, M. bancana flowered episodically 3 times synchronously within the population, and every M. winkleri individual was flowering almost continuously over the whole period. We examined the environmental triggers of the flowering of M. bancana using phenology observation and meteorological data. The analysis indicated that drought condition around the past one month is the most likely trigger for flowering of M. bancana. Previous studies have shown that relatively severe and prolonged drought is important as a trigger of general flowering in the study site. M. bancana may use more moderate drought as an environmental trigger of flowering than general-flowering species.

90 Selected for flash talk

Plant Sciences

Population genomic signature of environmental association in an ecologically divergent Hawaiian tree species

Izuno, Ayako

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Genome-wide markers enable us to study genetic differentiation within a species and the factors underlying it at a much higher resolution than before. In this study, the genomic differentiation in an ecologically divergent woody species, Metrosideros polymorpha, was investigated. This species occupies a wide range of ecological habitats across the Hawaiian Islands and shows remarkable phenotypic variation. A total of 1,659 single nucleotide polymorphism (SNP) markers annotated with the genome assembly was used for examining the population genetic structure of nine populations across five elevations and two ages of substrates on Mauna Loa, the island of Hawaii. The nine populations were differentiated into two genetic clusters distributed on the lower and higher elevations and were largely admixed on the middle elevation. Among the 1,659 SNPs, 34 SNPs (2.05%) were likely to be under divergent selection and the allele frequencies of 21 of them were associated with environmental changes among habitats, such as temperature and precipitation. Overall, this study demonstrated a genomic mosaic of M. polymorpha; among populations in diverse environments, most genomic polymorphisms were shared, but a small fraction of the genome was significantly differentiated and could be responsible for the dramatic adaptation to a wide range of environments.

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Plant Sciences

Environment-dependent homeolog expression in an allopolyploid Cardamine flexuosa in field

Akiyama, Reiko

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Whole genome duplication (polyploidy) is pervasive in plants. However, little is known about ecology and genomics of allopolyploids (polyploids originated from different parental species) in field. In particular, environmental-dependency in gene expression and expression ratio of homeologs (sets of genes derived from the different parental species) are yet to be explored. We found that an allopolyploid Cardamine flexuosa occurred along soil moisture gradient and coexisted with either of the parental species in dry or wet extremes in native areas in Switzerland. Total expression level and the ratio between homeologs of known water- and stomata-responsive genes differed between habitats. Furthermore, the pattern of expression varied at different time points. The results suggest that C. flexuosa utilises homeologs in response to environmental fluctuation. They also highlight that the genus Cardamine serves as a useful system to study the molecular basis of adaptive significance of allopolyploidy in wild plants.

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Plant Sciences

Genome assembly and annotation of Arabidopsis halleri, a model for heavy metal hyperaccumulation and evolutionary ecology

Briskine, Roman

Briskine, Roman V. (1); Paape, Timothy (1); Shimizu-Inatsugi, Rie (1); Nishiyama, Tomoaki (2); Akama, Satoru (3); Sese, Jun (3); Shimizu, Kentaro K.* (1)
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Arabidopsis halleri is a self-incompatible species closely related to the self-compatible model plant Arabidopsis thaliana. A. halleri is capable of heavy metal hyperaccumulation and has a broad European and Asian distribution, which make it a useful model for ecological genomics studies. We used long-insert mate-pair libraries to improve the genome assembly of the A. halleri ssp. gemmifera Tada mine genotype (W302) collected from a Japanese location highly contaminated by heavy metals. We performed five rounds of forced selfing, which reduced heterozygosity to 0.04% and facilitated subsequent genome assembly. The new assembly version now covers 196 Mb or 78% of the estimated genome size and has the scaffold N50 length of 712 kb. We validated the assembly and annotation based on the synteny between A. halleri Tada mine and a previously published high-quality reference assembly of another closely related species, Arabidopsis lyrata. Phylogenetic and syntenic analysis of the HEAVY METAL ATPASE4 (HMA4) and METAL TOLERANCE PROTEIN1 (MTP1) regions also suggest the high quality of the assembly. Three tandemly duplicated copies of HMA4, key gene involved in cadmium and zinc hyperaccumulation, were assembled on a single scaffold. The assembly will enhance the genome-wide studies of A. halleri as well as the allopolyploid Arabidopsis kamchatica derived from A. lyrata and A. halleri.

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Plant Sciences

Genetic diversity of two tropical trees (Dipterocarpaceae) following enrichment-plating strategy in Borneo: negative impact of planting in monocultures.

Ang, Cheng Choon

Ang, Cheng Choon (1); O'Brien, Michael J. (2); Ng, Kevin Kit Siong (3); Lee, Ping Chin (4); Hector, Andy (5); Schmid, Bernhard (1); Shimizu, Kentaro K.* (1)
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Tropical rainforests are well known for being the most species-rich of all the terrestrial ecosystems on earth. However, biodiversity in these forests is under threat due to logging and land-use conversion. Many restoration efforts focus on recovering species diversity and forest structure post-logging, but fewer have emphasized genetic diversity within species, which plays an important role in species adaptation to and persistence under novel climates and biological interactions. Therefore, we aimed to provide a detailed assessment of genetic diversity among seedlings used for enrichment planting for the restoration of selectively logged forests in Sabah, Malaysia, and to compare it with the levels in naturally regenerating seedlings. We sampled enrichment-planted seedlings from two dipterocarp species (Shorea leprosula and Parashorea malaanonan) within the Sabah Biodiversity Experiment (SBE) restoration project and compared their levels of genetic diversity with those natural seedlings from the surrounding forests. Our results showed that the genetic diversity estimates (heterozygosity and rarefied allelic richness) varied significantly between natural and enrichment-planted seedlings, particularly in S. leprosula. Interestingly, a reduction of genetic diversity was consistently observed in monocultures relative to the mixed-species plots in both species from the enrichment-planting site. This reduction of genetic diversity was likely caused by selective mortality of genotypes in monocultures over the last 13 years post-planting mortality, relative to that of the 16-species mixtures. The selective loss in monocultures may be the result of increased-density-dependent mortality, which would likely to remove genotypes that are poor competitors. Therefore, in the future, forest restoration of tropical tree species should adopt more species-rich planting schemes to prevent the loss of within-species genetic diversity associated with low species diversity.

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Plant Sciences
The power of flowers - Boosting monoterpenoid indole alkaloid production in Catharanthus roseus by transcriptional engineering
Cancelled
Schweizer, Fabian

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Plant Sciences

Genome wide evolution and heavy metal hyperaccumulation in an emerging model allopolyploid Arabidopsis species: Arabidopsis kamchatica

Paape, Tim

Paape, Timothy (1); Shimizu, Kentaro* (1); Briskine, Roman (1); Hatekayama, Masaomi (1); Shimizu-Inatsugi, Rie (1); Lischer, Heidi (1); Sese, Jun (2)
(1) University of Zurich, Dept. of Evolutionary Biology and Environmental Studies; (2) National Institute of Advanced Industrial Science and Technology (AIST), Artificial Intelligence Research Center

Allopolyploid hybrids are formed when two or more diploid species hybridize without genome reduction. In a single individual, the duplicated genome (homeologs) results in fixed heterozygosity of parentally derived polymorphisms. One possibility is that genome duplication would prevent beneficial mutations from being expressed due to masking of new mutations. However, population genetics theory predicts that most amino acid changing mutations would be neutral or deleterious rather than beneficial. Therefore, fixed heterozygosity may be advantageous. Genome wide polymorphism data in allopolyploids is required to study these questions empirically, but the inherent similarities of duplicated genome sequences complicates high-throughput sequencing. Using a new approach that uses two diploid parental genome assemblies to classify Illumina reads to their parental origins, we produced genome wide polymorphism data in the allotetreploid Arabidopsis kamchatica, a natural hybrid of the diploid species A. halleri and A. lyrata. We show that A. kamchatica inherited heavy metal hyperccumulation from the A. halleri parent and it is a constitutive phenotype in the species. This is supported by transcriptomics results indicating HEAVY METAL ATPASE4 (HMA4) AND METAL TOLERANCE PROTEIN 1 (MTP1) show significantly high halleri-derived expression. The halleri-origin HMA4 gene also shows a significant reduction in genetic diversity compared with surrounding regions as a result of hitch-hiking. Genome wide diversity analysis shows the decay in linkage disequilibrium (LD) is about 5-10 kb. We also show that the majority of the genome appears to be under purifying selection and gene expression levels predict that highly expressed genes show the strongest selection against deleterious mutations. Loss of function mutations are also at low frequency in both sub-genomes and very rarely found in both homeologs in the same individual as expected under genome wide sheltering of deleterious mutations.

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Plant Sciences

Molecular mechanism of adaptation to new environments in allotetraploid species

Selected for talk

Shimizu-Inatsugi, Rie

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Polyploidization is thought to promote evolution by doubling gene number as well as creating a new gene network. In particular, allopolyploidization may allow the adaptation to new environments by combining parental gene networks. In spite of its significance, little is known about the alteration of gene network control by polyploidization. In order to understand the molecular mechanism of adaptation to new environments, we chose two species of genus Cardamine of Brassicaceae living at either dry or wet habitat, and one allotetraploid species derived from them found at intermediate habitat, as a model case. We conducted microarray analysis and used the data for hierarchical clustering analysis, which revealed that the expression pattern of allotetraploid speies clustered to either of the diploid progenitors in wet and dry condition. Besides this similarity, the induction levels of many genes of the allopolyploid were lower than those in a diploid, suggesting a tradeoff of allopolyploidization. To further analyze the transcriptomics of polyploid species, distinguishing the regulation of homeologous genes is critical, even though the high sequence similarity between homeologous gene pairs has hampered the bioinformatics with high throughput sequencing. To obtain the both information about the total expression of homeologs and the ratio between them, we developed a pipeline named HomeoRoq using Arabidopsis kamchatica as a model polyploid. We use this pipeline to analyze the detailed transcriptome data in alloteteraploid species.

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Plant Sciences

Using and developing Genomics tools for molecular breeding of a tetraploid crop plant.

Aluri, Sirisha

Aluri, Sirisha (1); Patrignani, Andrea (1), Grüter, Simon (1), Schlapbach, Ralph (1), Shimizu-Inatsugi, Rie (2), Shimizu, K. Kentaro, (2), Karaba, N. Nataraja, (3), Ravikumar R. L., (4), Sreeman M. Sheshshayee (3)

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Many crop species are allopolyploids, resulting from interspecific hybridization and polyploidy. The genome wide analysis of polyploidy crops is lagging behind compared to diploid ones partly due to difficulties in assembling the polyploid genome resulting from the genomic complexities induced by combining two or more evolutionary divergent genomes.

We are taking advantage of currently available different long and short read sequencers and would like to apply hybrid assembly techniques to deal with proper assembly of a tetraploid crop plant. We have sequenced the genome using Illumina (with different sized shotgun and Matepair libraries) and PacBio. With the aid of BioNano sequencing in addition, we would like to complete the assembly of tetraploid crop plant. In house developed assembly pipeline in collaboration with Department of Evolutionary Biology and Environmental Studies, University of Zurich will be used for the tetraploid Genome assembly.

The assembled genome will then be used to identify SNPs in selected genotypes. These findings will form the basis for further development of focused molecular breeding program to enhance the crop yield and bioavailability of nutrients.

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Plant Sciences

Microscopy screening approach for identifying chromatin modifiers underlying plant cell transdifferentiation

Selected for flash talk

Rutowicz, Kinga

Rutowicz, Kinga* (1); Baroux, Célia* (1)
(1) University of Zurich, Institute of Plant and Microbial Biology, Plant Developmental Genetics

The regeneration of tissues occurring upon injury or loss of organ integrity is a fascinating developmental process. By contrast to the vast majority of animals, plants show a remarkable potential to regenerate from isolated cells and tissue fragments. This process that has been widely exploited in agriculture, horticulture and biotechnological applications. Yet, although described more than a century ago, the molecular basis of plant cellular plasticity enabling regeneration remains largely enigmatic. Transdifferentiation of isolated plant cells devoid of cell walls (protoplasts) followed by whole plant regeneration in vitro a robust experimental system amenable to genetic, molecular and cell biology analyses. Strikingly, protoplast transdifferentiation in Arabidopsis is accompanied by dramatic changes in chromatin composition and organization (1). We also show here that transdifferentiation is underlined by a rapid turnover of H1 linker histone variants. In order to identify regulators of H1 dynamics during transdifferentiation we establish a semi-automated microscopy-based phenotyping system by adapting an existing high-content screening platform and supervised-learning mediated image processing (2). We will present the design of our high-content genetic screen using a candidate gene approach in a pilot phase and progress in establishing a robust pipeline. Ultimately, the aim is to identify novel chromatin modifiers causally influencing protoplast transdifferentiation and plant regeneration. This work should shed light on the role of chromatin dynamics in plant pluripotency.

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Proteomics

Proteome-wide expression and turnover analysis quantify genetic impact in Down Syndrome

Selected for talk

Liu, Yansheng

Liu, Yansheng (1); Beyer, Andreas (2); Antonarakis, Stylianos* (3); Aebersold, Ruedi* (1)
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Introduction: Trisomy 21 (T21) is the cause of Down Syndrome. However, how T21 impact human functional proteome remains unclear. We hypothesize that the proteome-wide turnover analysis is crucial to understand the functional impact of T21.

Materials and Methods: We investigated the effect of the extra chr21 at the levels of transcript quantity, proteome quantity and protein turnover rate. We analyzed the primary fetal skin fibroblasts derived from a pair of monozygotic twins discordant for T21, which uniquely allowed us to characterize the proteome changes due to T21 without the noise of genomic variability. To validate, we also analyzed the fibroblasts from 11 unrelated T21 individuals and 11 controls. We applied the cutting-edge SWATH mass spectrometry to reproducibly measure the proteomes.

Results: We quantified 4056 unique proteins for expression and ~2200 proteins by pulsed SILAC experiment for turnover analysis in both normal and T21 fibroblasts from the twins. The proteome-wide T21/normal fold-change correlation was extremely low, indicating substantial post-transcriptional regulation and buffering effects in T21. Overall, the protein degradation was faster in trisomy cells than the controls. Remarkably, those Chr21 encoded proteins that are members of heteromeric protein complexes in particular seemed to be exempt from responding to copy number alternations, likely through accelerated protein degradation. Moreover, we found that both mitochondrial and cytosolic ribosomal proteomes were degraded heavily in T21, but different degree of translational regulation shaped their final, divergent expression levels.

Conclusion: Prevalent, organelle specific proteome remodeling was identified as the proteomic hallmark of T21 as compared to normal.

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Proteomics

Site-specific N-glycosylation in the endoplasmic reticulum and Golgi of mammalian cells

Mathew, Corina

Mathew, Corina (1); Losfeld, Marie Estelle (1); Lin, Chia-wei (1); Grant, Oliver (2); Aebi, Markus (1)
(1) ETH Zürich, D-BIOL; (2) University of Georgia, Biochemistry and Molecular Biology

Glycans from different glycosylation sites of the same protein show different degrees of glycan processing. This led to the hypothesis that the protein surface and its interaction with the covalently attached glycans is a major determinant of N-glycan processing.
To study the effect of the protein surface on glycan processing I am using in vivo and in vitro approaches.
For in vivo studies, a model protein where amino acids that are predicted to interact with the glycans are mutated, is expressed in CHO cells. To investigate the resulting glycan structures on individual sites we perform a comprehensive analysis by mass spectrometry developed in our lab.
For the in vitro approach, different glycan processing events are reconstituted thanks to ER and Golgi glycosyl-hydrolases and -transferases produced with the baculovirus system. Resulting glycoprofiles are analyzed with the same MS methods and compared to in vivo data.

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Proteomics

Differential Protein Expression Analysis by Mass Spectrometry as a Service

Nanni, Paolo

Nanni, Paolo* (1); Grossmann, Jonas* (1); Fortes, Claudia (1); Wolski, Witold (1); Kunz, Laura (1); Schlapbach, Ralph* (1); Roschitzki, Bernd (1); Panse, Christian (1)
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Protein expression information from proteomics high throughput shotgun (DDA) experiments is among the most popular proteomics services provided by the Functional Genomics Center Zurich (FGCZ). This quantitative protein expression data is used to test existing hypotheses and also to generate new scientific insights. To assure that generated hypotheses are statistically significant a formalized, semi automatized analysis process was established. We present the entire process including experimental design, quality control which stresses the inclusion of control samples, descriptive and differential data analysis methods. We opt for a robust solution, which can be used in most of projects and the suggested regulated target proteins should be relatively easy to validate by other methods such as western blotting.
The formal process starts with a mandatory pilot experiment where one biological sample is split into two aliquots and subjected to protein extraction. This helps to estimate the biochemical variance introduced by the customer preparing the protein extract. Sample processing is optimized to minimize technical variability and batch effects are avoided by randomization. We use optimal data transformations to ensure compatibility with statistical parametric tests and the biological research question.
This service should provide a robust and meaningful output. We provide a false discovery rate controlled list of identified and regulated proteins along with statistical measure for differential expression and fold changes. As an example, to illustrate our workflow, we show an experiment where yeast is grown at two different growth conditions.

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Proteomics

Proteomic interaction analysis of cancer mutations in the Dyrk2 kinase complex

Mehnert, Martin

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A central issue of current biology is the functional interpretation of the huge amount of genomic data derived from large scale DNA sequence analysis and their translation into disease phenotypes. The link between genotype and phenotype is mainly mediated by protein complexes that constitute the major functional modules of the cell. In this study we combine advanced proteomic and structural biology methods to investigate the functional and structural phenotype of cancer mutations in protein complexes. Due to its involvement in diseases and its clearly defined multisubunit organization we use the Dyrk2 kinase complex as prototypical protein module for our analysis. Dyrk2 belongs to the evolutionary conserved CMGC family of serine/threonine protein kinases and acts in central cellular processes such as cell cycle regulation, differentiation and apoptosis. The Dyrk2 kinase complex encompassing the ubiquitin ligase Ubr5 and the substrate receptor subunit DDB1-VprBP mediates the proteasomal degradation of various client proteins involved in mitosis and cell cycle progression. Thus, a malfunction of this complex is suggested to trigger tumorigenesis. We performed a proteomic interaction analysis of the Dyrk2 kinase network using AP-MS and BioID-MS and identified about 80 significant interactors, in particular factors of cell cycle regulation, apoptosis and nuclear transport. The integration of cancer associated point mutations into Dyrk2 affected the interaction network and caused a disassembly of the Dyrk2 kinase complex. Next steps will include a profiling of the cellular phosphoproteome followed by a topological analysis of the cancer perturbed kinase complex by XL-MS.

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Proteomics

Pathway monitoring identifies effectors of rescue from alpha-synuclein toxicity

Soste, Martin

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(1) Biochemistry, Biology

α-Synuclein (α-syn) is an aggregation-prone protein linked to Parkinson's disease. Approximately 100 modulators of α-syn toxicity have been identified in previous genetic screens done on S. cerevisiae expressing heterologous human α-syn. Some of these modulators are conserved and were shown to revert α-syn pathobiology in higher organisms indicating that yeast is a valuable model system for large-scale screening. While a small number of modulators have been validated, it is not known how this diverse set of genes exert their protective effects. To identify the effectors of rescue we used targeted mass spectrometry to screen pathway activation across a large set of modulators. Effector pathways suggest new points of intervention against α-syn toxicity.

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Proteomics

Systematic investigation of glucose metabolism in various cancers by in silico mining of PCT-SWATH data sets

Selected for flash talk

Hernandez-Alias, Xavier

Hernandez-Alias, Xavier (1); Zhong, Qing (2); Buljan, Marija (1); Guo, Tiannan (1); Wild, Peter (2); Aebersold, Ruedi* (3)
(1) ETH Zurich, Department of Biology, Institute of Molecular Systems Biology; (2) University Hospital Zürich, Department of Pathology and Molecular Pathology; (3) ETH Zurich and University of Zurich, Department of Biology (Institute of Molecular Systems Biology) and Faculty of Science

Higher rates of aerobic glycolysis have been widely reported in various cancers, known as the Warburg Effect. While cancer cells are highly heterogeneous, the variability of Warburg effect in various tumor samples remains elusive due to the lack of ability to quantify metabolic enzymes consistently across a large number of samples.
In this study, we in silico investigated the quantity of 102 enzymes involved in glycolysis in 195 tumor samples from 9 different tissue types. Each tumor sample was analyzed using pressure cycling technology (PCT) coupled with SWATH mass spectrometry in duplicates.
We built a 102-protein network of glucose metabolism based on literature. From our SWATH data containing three different datasets, we detected 53 protein participants in glycolysis. In the dataset of 105 prostate tumors, 10 were significantly upregulated (PCK2, GAPDH, FBP2, PDHB, ENO2, PGLS, LDHB, HK1, TALDO1, ENO1, LDHA) and 3 significantly downregulated (PYGM, LDHB, PGM5). Next, we found six proteins exhibited a significant correlation with cancer progression. Interestingly, glucose uptake in prostate cancer is increased by upregulation of HK1 rather than HK2, in contrast to what is generally supported in literature. We also hypothesize a putative central role of the rate-limiting step PFK/FBP in regulating the flux towards Pentose Phosphate Pathway. We further analyzed the PSA recurrence-free survival data for these proteins, and extended this analysis to other tumor types.
In conclusion, through in-silico mining several hundred tumor proteome, we uncovered dysregulated glycolysis proteins including HK1, LDHA/LDHB or FBP2 in prostate tumors, and identified their association with tumor progression.

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Proteomics

Quantitative proteomic analysis of human liver cells exposed to cyproconazole suggests complex mechanisms underlying the observed lipid accumulation

Stamou, Marianna

Stamou, Marianna (1); Hauser, Klara (1); Dietrich, Sabine (1); Wollscheid, Bernd (1); Sturla, Shana* (1)
(1) ETH Zurich, D-HEST

Cyproconazole, an azole fungicide used to protect plants from fungal disease, causes hepatomegaly in rodent studies. Cyproconazole-induced hepatocellular vacuolization is accompanied by changes in expression of genes involved in fatty acid metabolism, suggesting hepatic steatosis as an adverse outcome of relevance to cyproconazole exposure. In order to assess cyproconazole-induced steatosis in a human-relevant model, HepaRG cells were exposed to low levels (25, 50, 100 and 200 μM) of cyproconazole for 24 hours and 72 hours. As a phenotypic readout, lipid accumulation was assessed using Oil Red O dye. No changes in lipid content were observed following exposure to cyproconazole for 24 hours. In contrast, 72 hour exposure to 200μM cyproconazole resulted in significant accumulation of lipids in these cells. We investigated the proteotype of HepaRG cells in order to assess quantitative changes in protein abundance upon dose-dependent cyproconazole perturbation. 3553 proteins were identified and quantified in HepaRG cells by using a data-independent mass spectrometric data acquisition. The data show widespread protein abundance changes across all cyproconazole exposure conditions, indicating pleiotropic activities of cyproconazole. Cyproconazole-perturbed hepatic pathways include enzymes (hydrolases, oxidoreductases and transferases) and proteins involved in transcriptional regulation networks. Consistent with observed lipid accumulation, exposure to 200 μM cyproconazole resulted also in decreased levels of proteins involved in fatty acid metabolism. Together, the data obtained on the phenotype and proteotype level provide preliminary evidence that cyproconazole might induce hepatic steatosis by disrupting hepatocellular lipid metabolism.

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Proteomics

Digitalization of patient samples and multi-omics data in the context of personalized medicine

Selected for flash talk

Goetze, Sandra

Goetze, Sandra (1); Guo, Tiannan (2); Wild, Peter (3); Abersold, Ruedi (2); Wollscheid, Bernd (1)
(1) ETHZ, IMSB/DHEST; (2) ETHZ, IMSB; (3) USZ

Well-annotated and molecularly characterized clinical specimens are a critical resource for Personalized Medicine (PM). However, large amounts of molecular and clinical data are saved unstructured and are not accessible for metadata analysis. This is problematic because clinical samples are finite and cannot be reproduced. If at all in the context of personalized medicine, a more extensive (i.e. omics) type of molecular analysis is mainly limited to the genomic or transcriptomic level. However, neither genomic nor transcriptomic profiles correlate with or mechanistically explain particularly well disease phenotypes because disease phenotypes are determined on multiple levels in non-trivial ways.
In the context of ZurichCancerMaps (ZCM), we aim at the development of a more complete understanding of molecular events that lead to progression of cancer. By a coordinated effort we plan to integrated various types of omics and clinical data and make them publicly available in a form of a digital biobank. Recent technological advances also support the generation of accurate and reproducible profiles at the proteomic level. Therefore, in a pilot project specifically focusing on proteomics and digital biobanking, we aim at a comprehensive profiling of 500-1000 clinical specimens (primary tumor and metastasis). Standard sample processing and data evaluation pipelines have been developed for fresh-frozen and formalin-fixed, paraffin-embedded tissue (FFPE) fixed tissue biopsies and have been benchmarked in terms of IDs and reproducibility. In the future these data sets and assays will be made publicly available and transferred into the clinics with the goal of optimizing drug-discovery in a personalized fashion.

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Proteomics

Quantitative proteomic profiling of key members of TNFR1-associated complex I and II by SWATH-MS and XL-MS

Ciuffa, Rodolfo

Ciuffa, Rodolfo (1); Aebersold, Ruedi* (1)
(1) ETHZ, IMSB

The explosion of omics technologies in the last decades has uncovered an enormous amount of genomic variability and shown that the relationship between genotype and phenotype is complex, and cannot be explained by the simple paradigm of 'one-gene, one-function'. Instead, a new paradigm based on the idea that protein modules and networks are the functional units of their relationship has emerged. In my work, I apply a combination of advanced proteomic and structural biology methods to define these networks in the context of NF-kB signaling. In particular, I combine (1) structural mass spectrometry and cryo-EM with (2) quantitative affinity purification coupled to mass spectrometry and (3) quantitative proteome profiling. Cross-linking mass spectrometry (XL-MS) defines distance restraints in large protein assemblies and, if combined with additional structural methods, can reveal their organization and topology. SWATH-MS, an implementation of data independent acquisition mass spectrometry recently developed in the Aebersold group, is a promising method that combines the throughput of shotgun MS with the accuracy and precision of selected reaction monitoring (SRM). This method has been mainly applied to proteome profiling, and a proof of principle in the context of affinity purification has been recently produced. In my work, I apply this suite of methods to key players of the TNFR1-associated complex I and complex II and study their structural and topological reorganization over time upon system perturbation.

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Proteomics

Surfaceome analysis of skeletal muscle to elucidate molecular mechanism behind glucose uptake

Jaleh, Fateme

Jaleh, Fateme (1); Goetze, Sandra (2); Gilardoni, Paola (1); Soro Arnáiz, Ines (1); De Bock, Katrien (1); Wollscheid, Bernd (2)
(1) Institute of Human Movement Sciences and Sport, Dep. of Health Sciences and Technology; (2) Institute of Molecular Systems Biology, Dep. of Health Sciences and Technology

Skeletal muscle composes 40% of individual body mass and plays an essential role in locomotion as well as in regulating the whole-body carbohydrate metabolism. For instance, skeletal muscle controls glucose uptake in response to a wide range of stimuli such as insulin and contraction. It does so by promoting the translocation glucose transporter (GLUT4) containing vesicles, to the plasma membrane. However, whether insulin and/or contractions lead to increased membrane availability of other molecules, either or not involved in the control of glucose uptake, is not yet described.
In this project we will evaluate whether the surfaceome of skeletal muscle is modulated by insulin and exercise. To investigate this, we will use C2C12 mouse myotubes as a model system. Cells will be stimulated with insulin or with electric pulse stimulation which mimics the effects of contraction. To identify surfaceome of skeletal muscle, we will apply proteomics-based Cell Surface Capture (CSC) technology. In contrast to an antibody based technique, this chemoproteomic approach provides unbiased and quantitative analysis of all of the cell surface proteins. Our initial results show the identification of more than 400 proteins on C2C12 myotube surfaceome.
We hypothesize that elucidation of skeletal muscle surfaceome will increase our understanding on the molecular mechanisms through which insulin and/or contractions control glucose metabolism.

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Proteomics
Diversity and plasticity of the synaptic proteotype

van Oostrum, Marc

van Oostrum, Marc (1); Wollscheid, Bernd* (1)
(1) ETH Zürich, D-HEST

The dynamic organization of neuronal synapses provides the basis for formation and plasticity of complex neural circuits. The functional diversity of these circuits is reflected in the synaptic architecture, as neurons can utilize a plethora of protein combinations to build and diversify the synaptic microenvironment. Global analysis of the synaptic proteotype has proven challenging, mainly due to the lack of technologies to determine protein quantities and nanoscale organization in a comprehensive fashion. Chemoproteomic-technologies would open up new avenues to resolve the molecular architecture of synapses, and thus enable the investigation of synaptic diversity and plasticity. Therefore, we develop and apply cell surface labeling strategies to neuronal cultures and tissues to quantitatively map the neuronal surfaceome. Initially, we focused on mapping the entire surface proteome expressed in hippocampal cultures resulting in >1000 identified bona-fide cell surface proteins. Taking time-resolved snapshots of the neuronal surface now enables us to quantify dynamics during synapse formation and plasticity. Thus, we asked how the neuronal surfaceome adapts during chemical long-term potentiation and found a set of extracellular proteins to rapidly localize to the surface upon plasticity induction. Gradually increasing abundance during synapse development further pinpointed towards a potential role as synaptic organizer molecules. Next, we aim at deciphering their interaction partners in the context of a comprehensive extracellular interactome analysis and as first step towards functional characterization. Furthermore we employ proximity-based protein tagging to differentiate the proteotypes of distinct synapse subtypes. Together, we target the quantitative protein organization within synapses using chemoproteomic-technologies, a key step towards deciphering synaptic diversity and plasticity.

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Proteomics

Label-free PRM-based identification of quantitative differences in the ADP-ribosylome induced by mild to severe oxidative stress

Selected for flash talk

Bilan, Vera

Bilan, Vera (1); Leutert, Mario (1); Nielsen, Michael (2); Hottiger, Michael O.* (1)
(1) University of Zurich, Department of Molecular Mechanisms of Disease; (2) The Novo Nordisk Foundation Center for Protein Research, Department of Proteomics

Protein ADP-ribosylation is a reversible post-translational modification in which the ADP-ribose (ADPr) moiety of NAD+ is transferred onto specific amino acids of acceptor proteins. Under steady-state conditions, ADPr levels are generally low and hardly detectable. However, an increase in ADPr level occurs in response to stress conditions such as oxidative stress and ionizing radiation. We hypothesize that protein ADP-ribosylation undergoes quantitative changes with an increasing degree of oxidative stress and that specific ADP-ribosylated peptides can be used as quantitative sensors of oxidative stress. Thus, the aims of the presented work are to:

- Identify the cellular ADP-ribosylome upon different oxidative stress conditions (mild to severe) and to identify candidate proteins that can be used for quantitative mass spectrometric (MS) analysis.
- Develop a robust assay for the quantification of defined ADP-ribosylated peptides using a targeted MS approach.

By applying an enrichment strategy based on the ADP-ribose binding domain AF1521 before MS analysis, we have identified 146 ADP-ribosylated candidate peptides from HeLa cells treated with 1 mM H2O2 for the targeted analysis approach using shotgun label-free quantification. We have validated the candidate peptides and shown that the developed quantification method with prior enrichment is highly reproducible. Additionally, we have analyzed the oxidative stress-induced ADP-ribosylome of HeLa cells with the developed parallel reaction monitoring (PRM) method and succeeded to quantify ADP-ribosylated peptides even under very low oxidative stress conditions (under which the cellular ADP-ribosylation signal is undetectable by immunofluorescence analysis). Furthermore, application of the PRM method to a set of ovarian cancer cell lines has revealed quantitative differences in the level of ADP-ribosylated proteins under basal and H2O2-treated conditions.

The presented results provide evidence that ADP-ribosylated proteins can indeed be detected and quantified under various oxidative stress conditions.

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Proteomics

Two surfaceomes on the same cell - Polarized signal transduction in epithelial cells

Kötemann, Anika

Kötemann, Anika (1); Wollscheid, Bernd* (1)
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Apicobasal polarization is crucial for the function of epithelia as selective barriers for endogenous molecules and pathogens, but also as a gate for pharmaceutical drug delivery (e.g. in airways, endothelium or blood-brain-barrier). The epithelial cell surface, facing different physiological environments, is subdivided into two separate domains that are each able to orchestrate specific cellular functions. To this day it remains elusive how this is accomplished by the repertoire of extracellular accessible proteins (surfaceome) in each membrane domain. Chemoproteomic technologies and in vitro models of polarized epithelia provide now the opportunity to explore how the polarized surfaceome can fulfill distinct functions, i.e. perceive signaling molecules or transport cargoes differently.
We hypothesize that apicobasal functions can be mediated by the identity, quantity, as well as the post-translational modification state of cell surface proteins. Cell Surface Capture (CSC) technology and other protein labeling strategies enable the comprehensive characterization of the cellular surfaceome. Here we adapted these technologies to determine the quantitative distribution of proteins across the apicobasal surface, including their glycosylation state as a potential function modulator.
We found that proteins generally localize to both, the apical and basolateral side. However, we identified differences in protein abundances, indicating complex mechanisms of apicobasal protein trafficking. Moreover, a number of glycosylation-sites showed a preference for one or the other surface domain. Using our acquired map of the apicobasal surfaceome, we are now able to investigate the functional role of protein distribution and glycosylation patterns in the context of intracellular signaling and transport pathways.

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Proteomics

Investigation of the molecular mechanism of resistance in the wild relatives of pigeonpea against pod borer infestation

Roschitzki, Bernd

Roschitzki, Bernd (1); Nanni, Paolo (1); Sreevathsa, Rohini* (2); Kunz, Laura (3); Panse, Christian (1); Schlapbach, Ralph* (3)
(1) UZH, FGCZ; (2) Indian Council of Agricultural Research, NRCPB; (3) ETHZ, FGCZ

Pigeonpea is one of the most important sources of dietary protein in India. The pod borers, Helicoverpa armigera and Maruca vitrata are major insect pests of pigeonpea cultivar and that pest infestation is causing up to 80-100% crop loss. Interestingly some wild relatives of pigeonpea show resistance to the pod borer attacks. In this study we are investigating the molecular mechanism of pod borer resistance on gene and protein level. Using comparative genomics and proteomics approaches we are exploring the different expression levels of potential pest induced molecular signals to understand that complex mechanism of the plant-insect interactions.
As a first step towards the exploration of the underlying mechanisms of the pod borer resistance in the wild relatives of pigeonpea we have generated a comprehensive proteomic profile of the two genomics varies. We could identify over 6000 proteins for both pigeonpeas. In a second step we have studied the proteomic response of a pod borer infestation of the pigeonpea cultivar in comparison to the resistant wild-type. We generated a time resolved protein abundace map of pigeonpea up to 96 hours after pod borer infestation. During that 96 hour time course about 95% of all larvae's died that have been feed with the pod borer resistant pigeonpea wild-type. The deferentially regulated proteins and pathways maps are presented and discussed.

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Proteomics
Decoding ligand-receptor interactions

Wendt, Fabian

Wendt, Fabian (1); Sobotzki, Nadine (1); Rudnicka, Alina (2); Schafroth, Michael (3); Götze, Sandra (1); Carreira, Erick (3); Milani, Emanuela (1); Yamauchi, Yohei (2); Wollscheid, Bernd* (1)
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Proteins residing within the surfaceome are cellular gatekeepers enabling, but also restricting molecular information transfer into and out of the cell. Decoding extra- and intracellular interaction partners of cell surface receptors (CSRs) would provide unprecedented insights into mechanisms of signal transduction. Detailed molecular knowledge of the mechanisms of signal transduction is essential to understand and eventually modulate cell-cell signaling, drug action and host-pathogen interactions. However, due to a lack of suitable technologies the majority of cell surface receptors remain orphan receptors - receptors without known ligands and signal transduction network. We recently developed the TRICEPS-based Ligand-Receptor Capture (LRC) technology which in principle enables now the identification of receptors for orphan receptors/ligands. TRICEPS-LRC is a chemoproteomic technology which allows for the unbiased identification of ligand-receptor interactions on the surface of living cells under physiological conditions. We present now the next generation of the LRC technology which is based on a new tri-functional crosslinker molecule termed HATRIC. Using HATRIC we could extend the application space of the first generation LRC technology towards small molecules and sorted subpopulations of cells at physiological pH. We show applications of HATRIC LRC ranging from commercially available drugs to the decoding of more complex host-pathogen interactions in the context of Influenza entry. LRC technology enables now the systematic investigation of ligand-receptor interactions towards the elucidation of the extracellular interactome.

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Proteomics

Identification of the cellular and organ protein ADP-ribosylome by liquid chromatography - tandem mass spectrometry

Nowak, Kathrin

Nowak, Kathrin (1); Leutert, Mario (1); Bilan, Vera (1); Martello, Rita (2); Nielsen, Michael (2); Hottiger, Michael (1)
(1) University of Zurich, Department of Molecular Mechanisms of Disease; (2) The Novo Nordisk Foundation Center for Protein Research, Department of Proteomics

Protein ADP-ribosylation is a reversible, covalent post-translational modification (PTM) whereby ADP-ribose (ADPr) is transferred from NAD+ onto specific amino acid residues of target proteins. Moreover, mono-ADP-ribosylation (MARylation) can be extended to poly-ADP-ribosylation (PARylation) through attachment of further ADPr units. ADP-ribosylation alters the function and properties of modified proteins or generates a scaffold that promotes the recruitment of proteins that contain specific ADP-ribose binding domains.
To provide further insights into the biological functions of ADP-ribosylation, it is important to determine ADP-ribosylated proteins. However, the identification of the ADP-ribosylome by LC-MS/MS is challenging due to the low abundance and the rapid degradation of ADP-ribosylated proteins.
We have developed a shotgun proteomics approach for analyzing cell lysates or organ tissues. For this approach, the extracted proteins are first digested into peptides. Afterwards, the peptides are treated with poly-ADP-ribose glycohydrolase (PARG) to reduce the complexity of the poly-ADPr (PAR) chains down to mono-ADPr. Then, MARylated peptides are enriched with the AF1521 macrodomain, which has a high binding affinity to mono-ADPr. The peptides are identified by mass spectrometry.
The aim of my PhD is to further optimize the proteomic sample preparation techniques for high coverage identification of the ADP-ribosylome and to include quantitative analysis. We plan to analyze different cell types and organs under various defined biological conditions to understand how ADP-ribosylation regulates cellular processes. First results of analysed organ tissues and cell types will be discussed.

115
Proteomics

Singlet oxygen generators enable the identification of proximal proteins

Selected for flash talk

Müller, Maik

Müller, Maik (1); Wollscheid, Bernd* (1)
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Proteins are social. They interact with each other (and other molecules) in order to fulfill specific functions. It is therefore crucial to decode protein-protein interactions in order to understand the complex biological process of cellular signaling. Previously, mass spectrometry based proteomics has been applied within affinity purification strategies to identify protein-protein interactions.
However, the dynamic nature of often low affinity interactions requires the development of proximity-tagging technologies such as BioID and APEX.
Here we introduce a next generation chemoproteomic technology utilizing reactive singlet oxygen for the light-mediated tagging of dynamic protein-protein interactions.
We show that singlet oxygen generators (SOGs) can be employed to locally transform ambient oxygen to highly reactive singlet oxygen species, which in turn leads to the oxidation of proximal biomolecules within nanometer distance. Oxidized proteins carry unique amino acid modifications and can be chemically tagged and quantified using mass spectrometry based strategies. Excitation of ligand-guided SOGs using short bursts of monochromatic illumination therefore enables the decoding of ligand receptor interactions and associated nanoscale organization of receptors on the surface of living cells.
These molecular insights would not only deepen our understanding but also provide a basis for interventions to manipulate cellular signaling in health and disease.

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research data management

OpenBIS ELN-LIMS: an open resource for academic laboratories

Barillari, Caterina

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The Electronic Laboratory Notebook (ELN) and Laboratory Information Management System (LIMS) based on the open-source data management platform openBIS[1] is specifically tailored to academic life science laboratories. openBIS ELN-LIMS[2,3] combines a powerful data management platform with a digital lab notebook and a sample and protocol management system. This structure allows scientists to keep track of all the work performed in the lab and follow up analysis, from bench to publication, which is a requirement for reproducibility of published results. Full data history tracking is ensured by parent-child relationships between all entities in the database; biological and chemical samples can be tracked with the storage manager; integration with measuring instruments allows direct data transfer, annotation and storage in the database; integration with BLAST[4] allows sequence similarity searches within the database; integration with PlasMapper[5] allows on-the-fly generation of plasmid maps from fasta files. openBIS ELN-LIMS is an open, easy-to-use and flexible platform for managing the ever increasing amount of data generated in scientific labs.

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117
Single-molecule study

Single-Molecule Chemistry: Formation and Identification of Guanine Oxidation Products Studied by Using Zero-Mode Waveguide Technology

Sobek, Jens

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We are using a modified single-molecule DNA sequencer to investigate molecular interactions at single-molecule level. We studied as a model system the hybridisation kinetics of CY3 labeled short oligonucleotides to oligonucleotide probes immobilised in a zero-mode waveguide nanostructure. As a side reaction we identified the oxidation of nucleobases of the probe initiated by a photoinduced electron transfer to the excited dye. Oxidation products reveal by hybrid destabilisation leading to a faster dissociation step. Reaction products can be identified by single-molecule real-time sequencing and kinetic evaluation of polymerase activity.

118
Stem Cells

Modeling Neural Crest Stem Cell Maintenance and Lineage Specification using Pluripotent Stem Cells

Marzorati, Elisa

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(1) University of Zurich, Institute of Anatomy, Cell and Developmental Biology

Recent attention has been paid to the development of the neural crest (NC), a transient embryonic stem cell population that originates during the process of neurulation in the dorsal portion of the vertebrate neural tube. Notably, these cells undergo an epithelial-to-mesenchymal transition (EMT), delaminate from the closing neural tube, migrate widely throughout the embryo and colonize different niches where they differentiate into several cell types. In fact, NC cells can give rise to cells of mesenchymal, neuronal, secretory and pigmented identity.
There is a growing body of evidence suggesting that stem cells display unique metabolic and translational signatures and that shifts in these processes may act as leading causes for cell fate changes. Nevertheless, it remains largely unknown whether NC stem cells (NCSCs) also control their identity by showing distinct metabolic and translational profiles and whether switches in these levels underlie lineage specification. Based on these observations the main objectives of this research project are to investigate if metabolism and RNA to protein translation are implicated in NC stemness maintenance and during lineage specific differentiation. To achieve these goals, the main system employed will be human pluripotent stem cells (hPSCs)-derived NCSCs (induced NCSCs).

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Stem Cells

Quantitative imaging of transcription factor dynamics to study the emergence of early developmental asymmetry in vivo

Welling, Maaike

Welling, Maaike* (1); Pantazis, Periklis* (1)
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It remains debated how cells in the mammalian pre-implantation embryo acquire the first developmental differences that can predict lineage-patterning events. While recent developments in single-cell genetic and genomic techniques have provided much insight in the molecular mechanisms regulating development, these approaches offer limited information of biological dynamics in vivo. In this study, I aim to address the controversy about early lineage allocation in the embryo by systematically applying advanced quantitative imaging tools to mechanistically dissect earliest signs of the establishment of pluripotency in vivo. In particular, I focus on the emergence and origin of the difference in transcription factor (TF) kinetic behaviors at different stages of pre-implantation development. By combining CRISPR/Cas9 based genome editing to generate endogenous photoconvertible TF-Dendra2 reporter lines with confined primed conversion of Dendra2, a recently invented quantitative imaging modality by the host lab, I will be able to accurately track single proteins as well as trace individual cells in early embryos to provide a systems view of the establishment of pluripotency in vivo. The comprehensive analysis of the TF kinetic behaviors in living mouse embryos will provide unique insight into the events that are key for the establishment and plasticity of the first specialized embryo cells.

120
Stem Cells

Understanding single cell - level MAPK activation dynamics for manipulation of neuronal stem cell self - renewal and differentiation fates

Ender, Pascal

Ender, Pascal (1); Dobrzynski, Maciej (1); Song, Jiyoung (2); Jeon, Noo Li (2); Pertz, Olivier* (1)
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Stem cell differentiation fate is controlled in vivo via the secretion of signalling molecules. Providing stem cells in vitro with specific, developmentally – relevant ligands is thus a promising approach for the generation of differentiated cells of a desired type for further research or cell therapy. However, the efficiency of such protocols is often low, with many cells in the population escaping the desired fate. For example, adult neural stem cells from the mouse Dentate gyrus can be cultured as predominantly uncommitted, multipotent progenitor cells when EGF and FGF2 are provided in the medium or induced to differentiate into neurons and astrocytes when only low levels of FGF2 are provided. In both cases, however, the outcome is heterogeneous and ultimately determined at the single cell - level. Both EGF and FGF2 are prototypical activators of the Erk/MAPK – signaling pathway. We hypothesize that distinct temporal dynamics of Erk – pathway activation are responsible for the difference in cell fate. We use fluorescent Erk – activity biosensors, microfluidics technology and quantitative image analysis to precisely stimulate cultured mouse Dentate gyrus stem cells with dynamic growth factor inputs of varying amplitude and frequency and study the response of the Erk/MAPK – pathway at the single cell – level with high temporal resolution. This will give insight into how Erk – signalling induces either self - renewal or differentiation fate and might enable us to efficiently and homogeneously manipulate stem cell fate at the population level by applying specific growth factor input patterns.

121
Structural Biology

A novel proteomics approach to probe the structural landscape of alpha-Synuclein in cells and tissues

Selected for flash talk

Feng, Yuehan

Feng, Yuehan (1); Verbeke, Lynn (2); Malinowska, Liliana (1); Chen, Serene (3); Gerez, Juan (1); Bergqvist, Filip (1); Outeiro, Tiago (4); Lindquist, Susan (5); Dobson, Christopher (3); Cremades, Nunilo (3); Reiter, Lukas (2); Picotti, Paola* (1)
(1) ETH Zurich; (2) Biognosys AG; (3) University of Cambridge; (4) University of Göttingen; (5) Whitehead Institute

Alpha-synuclein is an abundant neuronal protein, which plays a central role in Parkinson's disease (PD). While its physiological function remains enigmatic, α-Syn's association to pathology has been established and α-Syn was found to constitute a major component of Lewy bodies (LB), a clinical hallmark of PD and other neurodegenerative diseases. In vitro studies in the last two decades have demonstrated the exceptional conformational plasticity of this protein, though little insights have been gained on the structure-function relationship in vivo, due to the lack of techniques, which can assess the structural features of α-Syn in their native environments. This is a substantial limitation for designing therapeutic strategies, as conformational states of α-Syn in cells and tissues may differ from those characterized in vitro, given the fact that the complex cellular milieu cannot be reproduced in the test tube.

To tackle this issue, we devised a new strategy relying on the combination of limited proteolysis (LiP) and selected reaction monitoring MS (SRM) to probe the structural features of α-Syn directly in cells and tissues. α-Syn-specific LiP-SRM assays were designed and employed to measure LiP patterns of α-Syn. These features were deployed in a mathematical framework to link well-characterized in vitro-conformational states of α-Syn to the ones present in biological systems. We systematically profiled the conformational landscape of α-Syn across a variety of cellular and tissue samples, including physiological and pathological states.

Generally speaking, our analyses conclude that the structural ensemble of α-Syn is often heterogeneous in biological systems in which the presence of cellular factors, most of them hard to reconstruct in vitro, may affect the structural properties of this protein to a great extent.

122
Structural Biology

The role of human DNA repair protein APE1/Ref-1 in transcriptional regulation of genes involved in cancer development.

Bazlekowa - Karaban, Milena

Bazlekowa - Karaban, Milena (1); Tudek, Barbara (1); Saparbaev, Murat (2); Prorok, Paulina (3); Taipakova, Sabira (2); Akishev, Zhiger (4); Kladova, Olga (5); Kuznetsov, Nikita (5); Ishchenko, Alexander (2); Le Cam, Eric (6)
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Apurinic/apyrimidinic endonuclease 1, also referred as redox effector factor 1 (APE1/Ref-1), is a DNA repair protein which removes single damaged bases in base excision repair (BER) and nucleotide incision repair (NIR) pathways. Additionally to its DNA repair role, APE1/Ref-1 stimulates binding of oxidized transcription factors (TFs) to the DNA, leading to promotion of angiogenesis, anaerobic metabolism, hypoxia, cell adhesion, cell growth and tumor resistance to anticancer therapies. TFs contain redox-sensitive cysteine residues at their DNA-binding sites. Therefore, thiol oxidation of TFs induced by oxygen radicals strongly inhibits their DNA binding activities and transcription of target genes. It is thought that APE1/Ref-1 could act through thiol-mediated redox reaction using its Cysteine-65 residue to attack the disulfide bond in oxidized protein. This reaction leads to formation of disulfide bond in APE1/Ref-1 between Cys65 and Cys93 and disruption of disulfide bond in target protein. However, this model was challenged by number of contradictory findings. At present, the molecular mechanism underlying the non-DNA repair functions of APE1/Ref-1 remains unclear. Our results demonstrate that APE1/Ref-1 stimulates oligomerization of TFs on the DNA via its N-terminal domain and does not act as a classical reducing molecule (e.g. GSH). APE1/Ref-1 fails to stimulate oligomerization of TFs on the DNA conformations with which it cannot interact. Our data obtained from biochemical studies and electron microscopy suggest that APE1 can assemble in multi-protein complexes on DNA duplex and induce changes in DNA conformation or topology to facilitate the assembly of DNA – binding factors.

123
Systems Biology

Cell-wide analysis of protein thermal unfolding across species reveals the determinants of proteome stability

Leuenberger, Pascal

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(1) Institute of Biochemistry ETH Zurich, D-BIOL

Temperature is crucially important to life. Small temperature changes can differentiate optimal and lethal growth conditions of living organisms. Due to the higher intracellular abundance and lower stability of proteins compared to other biological macromolecules, temperature-induced cell death is thought to be due to protein denaturation and loss of protein function. The determinants of thermal sensitivity of cellular proteomes remain however uncharacterized due to the lack of techniques to analyze the intracellular stability of proteomes. We have developed a strategy based on limited proteolysis and mass spectrometry to measure the stability of proteins on a proteome-wide scale, directly in a cellular matrix and with domain-level resolution. Application of our approach to the proteomes of Escherichia coli, the yeast Saccharomyces cerevisiae, the thermophilic bacterium Thermus thermophilus, and human cells yielded protein stability data for >8,000 proteins and ~20,000 domains. Our results indicate that temperature-induced cellular collapse is due to the loss of a subset of unstable proteins with key physiological functions. The data also reveal the molecular and biological bases of protein thermal stability, shed light on the evolutionary conservation of protein and domain stability and indicate that the fraction of intrinsically disordered proteins in a cell is far lower than initially predicted. Our results suggest that highly expressed proteins tend to be more stable because they are designed to tolerate translational errors that would lead to the accumulation of toxic misfolded species.

124
Systems Biology

Systems approach to understand the impact of genetics and diet on aging

selected for flash talk

WANG, Xu

Jha, Pooja (1); Auwerx, Johan* (1)
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Age is the greatest risk factor for nearly every major cause of mortality in developed nations. People are living longer but often suffering from multiple diseases or disabilities of aging. We aimed to find factors that can succesfully postpone, ameliorate or prevent the accumulations of morbidities during aging and promote healthy longivity. We have used a systems genetics approach to study the individual impact and combined interaction of genetics and diet on aging using BXD mouse Genetic Reference Population (GRP). Our results reveal that a healthy diet does lead to an average extension of the lifespan in BXD strains by ~95 days, however a high fat diet (western-type diet) favors the extension of lifespan in mice after 1000 days of age by extending their lifespan by ~100-125 days. We have identified Cox7a2l, a postulated mitochondrial supercomplex assembly factor, to increase the risk of death by 46% after 800 days of age. Additionally we validated Mapkapk3 (MAPK-Activated Protein Kinase 3) -known negative reulator of aging in C.elegans- to correlate negatively with aging in BXDs and in humans. Our ongoing omics analysis (transcriptomics, proteomics and metabolomics) will help us understand the genetic and dietary factors and networks favorable or detrimental for healthy aging.

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Systems Biology

Regulatory networks reconstruction using literature based knowledge

Dorier, Julien

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Regulatory networks are becoming widely accepted as a useful mathematical tool to build qualitative models of regulatory processes. Boolean networks, as opposed to more quantitative modeling frameworks, are particularly interesting since they can cope with relatively large systems, and do not suffer from the lack of data on the stoichiometry and kinetics of biochemical reaction. Boolean networks can be used to predict the behavior of the underlying biological system, by performing in-silico perturbations of any combination of genes and measuring the stable phenotypes reached by the network. Using Boolean networks to perform in-silico experiments is faster and cheaper than in-vivo/in-vitro experiments and, although it does not eliminate the need for in-vivo/in-vitro experiments, it can significantly reduce the number of experiments needed to find for example therapeutically interesting combination of treatments.
We developed a method to infer Boolean regulatory networks using as much orthogonally generated data as possible, ranging from prior knowledge network (PKN) obtained from the literature to training sets obtained from in-vivo/vitro experiments (gene-expression, FACS, ...). This poster presents the method and its application to macrophage differentiation and activation.

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Systems Biology

Systems analysis of the polyol pathway in tumorigenesis

Uzozie, Anuli

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Metabolic reprogramming is acknowledged as one of the prominent hallmarks of cancer. The earliest discovery of an altered metabolic phenotype in cells centered on glucose metabolism.The aim of this project is to investigate the functions of the polyol pathway in glucose metabolism and tumorigenesis using a systems biology approach.
Evidence of an altered polyol pathway would be investigated in different cancers representing some of the most common human cancer types. Global cellular perturbations related to tumorigenesis would be induced in cancer cell lines and cellular assays would be performed to examine the resulting effect of these perturbations on various tumor phenotypes and metabolic pathways. A high-throughput proteomic technique (SWATH-MS) would be used to investigate the central proteome of cancer cells in different perturbed states. Discovery and targeted metabolomic studies would also be performed to validate alterations in the cellular metabolome and proteome after perturbation of the polyol pathway. In addition to the well known cancer-related alterations in glucose metabolism, the polyol pathway, could be a contributing metabolic phenotype to carcinogenesis.

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Systems Biology

Rhea, an expert curated resource of biochemical reactions for enzyme annotation and genome-scale metabolic modeling

Lombardot, Thierry

Lombardot, Thierry (1); Morgat, Anne (1); Axelsen, Kristian (1); Aimo, Lucila (1); Niknejad, Anne (2); Hyka-Nouspikel, Nevila (1); Rosanoff, Steven (3); Onwubiko, Joseph (3); Coudert, Elisabeth (1); Redaschi, Nicole (1); Bougueleret, Lydie (1); Xenarios, Ioannis (1); Bridge, Alan* (1)
(1) SIB Swiss Institute of Bioinformatics, Swiss-Prot group; (2) SIB Swiss Institute of Bioinformatics, Vital-IT group; (3) EMBL-EBI, Protein function development team

Rhea (www.rhea-db.org) is a comprehensive and non-redundant resource of expert curated biochemical reactions designed for the functional annotation of enzymes and the description, analysis and reconciliation of genome-scale metabolic networks.
Rhea describes enzyme-catalyzed reactions (the IUBMB enzyme nomenclature list), transport reactions and spontaneously occurring reactions using species from the ChEBI (Chemical Entities of Biological Interest) ontology of small molecules. Rhea reactions are extensively curated with links to source literature and are mapped to other publicly available metabolic resources such as MetaCyc/EcoCyc, KEGG, Reactome and UniPathway.
Rhea reactions are used as a reference for the reconciliation of genome-scale metabolic networks in the MetaNetX resource (www.metanetx.org) and also serve as the basis for the computational generation of the library of lipid structures and analytes in SwissLipids (www.swisslipids.org). External resources that use Rhea include the EMBL-EBIs Enzyme Portal and MetaboLights resource as well as the Microscope platform for genome annotation developed by Genoscope.
Here we describe recent and forthcoming developments in Rhea, which include the development of a new website, substantial growth of Rhea through sustained literature curation, and the addition of parent-child reactions relationships to complement the IUBMB enzyme classification.
At the time of writing, Rhea (release 76, of November 2016) includes 9528 unique reactions involving 8296 unique reaction participants curated from 9373 unique PubMed identifiers, and already 679 parent-child relationships have been defined.

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Systems Biology

The SwissLipids knowledgebase for lipid biology

Bridge, Alan

Aimo, Lucila* (1); Liechti, Robin* (2); Hyka-Nouspikel, Nevila (1); Niknejad, Anne (2); Gleizes, Anne (2); Götz, Lou (2); Kuznetsov, Dmitry (2); David, Fabrice (3); van der Goot, Gisou (4); Riezman, Howard (5); Bougueleret, Lydie (1); Xenarios, Ioannis (6); Bridge, Alan* (1)

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Lipids are a large and diverse group of biological molecules involved in membrane formation, energy storage, and signaling. The lipid complement or lipidome of individual cells, tissues and organisms is tightly regulated in response to changes in cellular signaling and nutritional status. The frequent disruption of these regulatory mechanisms in diseases such as cancer, hypertension, allergy, diabetes and degenerative diseases highlights the growing importance of lipids as biomarkers and potential diagnostic tools.

A more complete understanding of the biological roles of lipids in health and disease will require the integration of lipidomic data with other types of biological data and prior knowledge. To facilitate this task we have developed SwissLipids, a knowledge resource for lipids and their biology. SwissLipids features over 300,000 known and putative lipid structures from human and a variety of model organisms and pathogens. These lipid structures are enriched with expert-curated data on thousands of enzymatic reactions, lipid-protein interactions, lipid functions, and lipid locations, as well as links to supporting evidence and source text from peer reviewed publications. SwissLipids is updated daily with new knowledge and is freely available to search, download and access programmatically at http://www.swisslipids.org/.

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Systems Biology

Integration of multilayered systems approaches optimizes the identification of metabolic genes

Li, Hao

Li, Hao (1); Wang, Xu (1); Sorrentino, Vincenzo (1); Rukina, Daria (2); Huang, Qingyao (3); Luan, Peiling (1); Komljenovic, Andrea (4); Williams, Evan (5); Robinson-Rechavi, Marc (4); Schoonjans, Kristina (3); Morgenthaler, Stephan (2); W. Williams, Robert (6); Auwerx, Johan* (1)
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Identifying the genetic and environmental factors that impact on complex traits and how they influence the development of common diseases is a challenge and priority in biomedical research. In this study, we developed and validated a series of systems approaches, including (expression-based) phenome-wide association, transcriptome-/proteome-wide association, mediation analysis, population-based gene set analysis. Applied to multi-omics datasets from a mouse genetic reference population, we were able to identify and validate many novel links between genes and clinical and molecular phenotypes, including links between Kdm5b and longevity, Rpl26 and body weight, Cpt1a and lipid metabolism, as well as C10orf107 and mitochondrial respiration. Importantly, unlike many links seen in classical loss-of-function mouse studies, the gene-phenotype links identified in population studies were robust and translated well across species. Our multilayered integrative approaches, have been implemented on the large multi-omic historic datasets available in the open-access web server (http://gn2.genenetwork.org), hence expediting and consolidating the systems dissection of gene function.

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Systems Biology

Inferring biological tasks using Pareto analysis of high-dimensional data.

Hausser, Jean

Hausser, Jean* (1); Hart, Yuval* (1); Sheftel, Hila* (1); Szekely, Pablo* (1); Bossel, Noa* (1); Korem, Yael (1); Tendler, Avichai (1); Mayo, Avi (1); Alon, Uri* (1)
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Approaches for analyzing high-dimensional data sets include dimensionality reduction techniques such as principal-component analysis (PCA), t-distributed stochastic neighbor embedding (t-SNE) and methods that split data points into groups, such as clustering and Gaussian mixture models (GMMs).

A recent advance in evolutionary theory suggests a complementary way to understand large biological data sets on the basis of Pareto optimality of biological systems with respect to multiple evolutionary tasks. Here we present the Pareto task inference method (ParTI) for inferring biological tasks from high-dimensional biological data. Data are described as a polygon (a multi-dimensional polygon), and features maximally enriched closest to the vertices (or archetypes) allow to identify the tasks the vertices represent.

We demonstrate that mouse tissues and human breast tumors are well described by tetrahedrons in gene expression space, with specific tumor types and biological functions enriched at each of the vertices, suggesting four key tasks and trade-offs between these tasks. In particular, the tasks of human breast tumors are shared with 9 other cancer types, suggesting common trade-offs across cancer types.

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Systems Biology

SourceData: a semantic platform to make data and figures discoverable

Liechti, Robin

Götz, Lou* (1); George, Nancy (2); El-Gebali, Sara (2); Crespo, Isaac (1); Chasapi, Anastasia (1); Xenarios, Ioannis (1); Lemberger, Thomas* (2); Liechti, Robin* (1)
(1) SIB Swiss Institute of Bioinformatics, Vital-IT; (2) EMBO, SourceData

In scientific publications, data are visually depicted in figures or tables. The original data behind the figures – the 'source data' – however are almost never available in a structured format that would make them findable and reusable. To address this issue, SourceData (http://sourcedata.embo.org) has built a suite of tools to capture the structure of published research data and to make published research papers discoverable based solely on their data content.
SourceData converts the narrative descriptions provided in figure legends into standardised, machine-readable metadata. Each biological component in a figure is consistently identified via links to reference databases and ontologies. The experimental design is, furthermore, captured in a structured format by classifying the role of each component. Computer-assisted manual identification and classification of biological entities is performed with a web-based curation tool. A separate interface allows authors to verify the accuracy of curated information. In a pilot project, the SourceData team has processed over 15,000 experiments from papers across 23 journals. The resulting network of connected data can be browsed through the SmartFigure application, which displays data in the context of related figures published in other papers and enables users to easily navigate between them. The SourceData search engine enables to directly retrieve data based on the design of an experiment.
SourceData is now integrated into the live editorial process of EMBO Press and inclusion of SmartFigures in an online publication environment is being demonstrated in the SmartFigures Labs (http://smartfigures.net) in collaboration with Wiley.

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Systems Biology

The transcription regulator BRD3 at the crossroads between lipid metabolism and membrane remodeling by oxidative stress.

Jiménez Rojo, Noemi

Jiménez-Rojo, Noemi (1); Gehin, Charlotte (2); Leonetti, Manuel (3); Riezman, Isabelle (1); Weissman, Jonathan (4); Riezman, Howard* (1)
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The control of membrane lipid homeostasis is an essential process that allows cells to maintain both their energetic balance and the structural integrity of their different membrane systems. However, despite the importance of this process, and although most of the enzymes involved in lipid metabolism have been already identified, little is known about the regulatory mechanisms.
In order to have a broader overview about how membrane lipid metabolism is orchestrated in cells, we present in this work a strategy that allows the monitoring of lipid changes in cells using a large-scale RNAi screening of human kinases combined with targeted lipidomic analysis by mass spectrometry. Statistical analysis of the screening highlights some genes whose knockdown induces changes in sphingolipid levels. Among them, cells lacking bromodomain-containing protein 3 (BDR3) have been found to have an increase in ceramide levels in different human cell lines, together with a decrease in glucosylceramide. Also, other changes in glycerophospholipids (i.e. ether-lipids, saturated lipids) can be detected, which shows that a remodeling of the membrane takes place upon BRD3 knockdown by siRNA or by CRISPRi technology. These changes are accompanied by an increase in oxidative stress levels and affect plasma membrane properties. We proposed a metabolic and/or functional crosstalk between ether-lipids and sphingolipids that may be important for the adaptation of cells against oxidative stress, showing the importance of maintaining membrane lipid homeostasis to preserve cell viability under stress conditions.

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Systems Biology

Deep learning approach for predicting CB2 affinities using recurrent convolutional neural networks

Mikołaj Mizera

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Cannabinoid analgesics were well tolerated in clinical trials with acceptable adverse event profiles. Their adjunctive addition to the pharmacological armamentarium for treatment of pain shows great promise. There was some evidence resulted not only from different animal models on the efficacy of analgesic effect of cannabinoids, but also provided from clinical trials which included e.g. patients with peripheral neuropathy in inflammatory bowel disease, multiple sclerosis and also in cancer pain.
The mechanism of activity of cannabinoids is connected with their affinities of selected receptors: CB1 and CB2. In presented study we applied deep learning approach for predicting affinities of molecules to CB2 receptor on basis of Quantative Structure-Affinity Relationship (QSAR). Data used for creation of QSAR model were 3500 compounds encoded in SMILES format with corresponding affinities expressed as pChembl value acquired from ChEMBL database. For each of molecules, 3D structures were generated using MMFF94 method. Parametrization Method 7 was applied in order to optimize structures and extract density matrices of each molecule in dataset. Extracted matrices were used as inputs to deep network constructed of consecutive layers involving convolutional operators, pooling operators, long-short time memory layers and multilayer perceptron.
Reported study showed that sufficiently deep models perform ability to infer the most optimal descriptors for given set of chemical structures, which makes them superior to classically used, empirically derived molecular descriptors.

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Systems Biology

Universal sample preparation for Omics–based applications in personalized medicine

Poveda, Lucy

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Combining "Omics" information from the analysis of diverse biomolecules, such as nucleic acids and proteins, opens up new opportunities in the field of personalized/precision medicine where patients can be monitored across multiple molecular readouts to improve disease diagnosis and treatment. Ideally, the data should be collected from the same sample using a universal sample preparation method, which enables the extraction of the different classes of biomolecules for sensitive analysis. Recently, the pressure cycling technology (PCT) has emerged as an efficient sample preparation strategy, enabling the extraction of proteins from small amounts of samples such as needle tissue biopsies (1).

Here, we performed the lysis of small amounts of porcine tissue biopsies using the PCT approach, enabling the downstream analysis of RNA and proteins. In total, more than 300 molecular readouts were generated from six different tissue types (pig liver, heart, spleen, lung, kidney brain) processed in four replicates and at four different tissue weights (0.2, 0.5, 1 and 3 mg) each. For RNA, the PCT workflow was combined with standard DNA/RNA isolation kits enabling the purification of total RNA from the samples. Sufficient RNA amounts were isolated to enable next generation sequencing (NGS) experiments. The integrity and quality of RNA on all tissue weights and organs reflected low degradation of the biomolecules during sample processing. For protein, estimated amounts resulting from the PCT workflow were directly proportional to the tissue weights and demonstrated high reproducibility between replicates and organs. After isolation, all tested samples were further processed for transcriptome and proteome profiling using NGS analysis and mass spectrometry, respectively. As expected, a high correlation was identified between transcript and protein abundances for the same organ, but a lower correlation was measured across organs. Pathway enrichment analysis revealed several activated molecular pathways that were found to be expressed strictly organ and tissue-specific. The presented robust and sensitive method for the isolation of multiple molecular species from minimal samples provides the basis for an integration of complex data sets from proteomics and genomics for a comprehensive view on cells or organs under defined physiological states, a prerequisite in system biology and personalized medicine.

1. Guo et al., Rapid mass spectrometric conversion of tissue biopsy samples into permanent quantitative digital proteome maps. Nat Med. 2015 Apr;21(4):407-13. doi: 10.1038/nm.3807.

Systems Biology

Analysis of Translational Pausing by Disome Profiling

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During translation, ribosomes traverse along the linear mRNA template at non-uniform speeds. Conceivably, at sufficiently low speeds of translation, newly arriving ribosomes can be stacked behind the pausing ribosome increasing the local density of ribosomes. Such transient pausing of ribosomes potentially represents an important regulatory mechanism as it could affect a variety of processes, such as folding of the nascent polypeptide, efficiency of protein biosynthesis and ribosomal frameshifting. A number of factors can influence ribosomal pausing: transcript structure, codon usage, tRNA abundance and nascent peptide sequence. However, there are limited numbers of techniques providing genome-wide high resolution data on ribosome pausing and stacking, making it challenging to understand their causes and regulatory effects.
Here, we report on disome profiling, a variant of ribosome profiling, as a new approach to study translational pausing. A disome is formed by two adjacent ribosomes on an mRNA that sterically exclude nucleases and hence protect approximately a 60 nt stretch of the transcript. By transcriptome-wide sequencing of disome footprints, we were able to map exact locations of stacked ribosomes. We validated our approach by demonstrating that disome footprint densities correlated with the presence of signal peptides that are recognized by the signal recognition particle (SRP), binding of which has been known to induce an 'elongation arrest'. We applied established techniques from information theory and machine learning to different features of disome footprints, such as their size and location, to identify specific regulatory factors of translational pauses. The latest results on the application of disome profiling in understanding the kinetics of translation will be presented.

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Systems Biology

An integrative proteogenomics strategy to identify the entire protein coding potential of prokaryotic genomes

Selected for flash talk

Varadarajan, Adithi

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Proteogenomics, a research area at the interface of proteomics and genomics, has gained much popularity recently. Searching mass spectrometry (MS) data against complete genome sequences generated from NGS technologies has enabled researchers to re-annotate genomes and to identify alternate proteoforms, novel protein coding genes (CDSs), and to pin-point genomic variations, all of which can improve systems biology based studies. Several software solutions and tools have been developed to match MS data against genomic information. However, they are relying on one or multiple genome annotation resources, which have been shown to differ widely. A comprehensive and integrated analysis is thus still lacking. We present here a general proteogenomics approach that integrates CDSs from multiple reference genome annotations, gene prediction algorithms and in silico ORFs in a minimally redundant yet highly informative, integrated proteogenomics database (iPtgxDB), which covers the entire protein-coding potential of a prokaryotic genome. Searching high quality, condition-specific proteomics data against an iPtgxDB applying a stringent FDR uncovered evidence for novel small ORFs (sORFs), alternative start sites and expressed pseudogenes. We demonstrate the general applicability of the approach for E.coli, B. diazoefficiens and B. henselae. Importantly, applying this strategy on a de novo assembled genome of a lab strain provided evidence for single amino acid changes compared to the NCBI reference genome, i.e. information particularly relevant for the field of infection biology and personalized medicine. The use of iPtgxDBs is a promising approach to iteratively improve genome annotations and is expected to be useful in the field of system biology.

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Systems Biology

Elucidation of dynamic cell surface protein-protein interactions by using chemoproteomic technologies

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Dynamic protein-protein interactions enable, but also limit cellular signaling and communication. Although a wealth of knowledge was generated by the biomedical community for intracellular protein-protein interactions our current knowledge about extracellular interactions remains limited, mainly due to technological limitations. We developed a new methodology using a proximity-based protein tagging strategy to study the nanoscale organization of the surfaceome and their dynamic interactions. We used the tyramide-based signal amplification strategy (TSA) in combination with state of the art quantitative proteomic workflows. TSA is based on the transformation of the substrate biotin-phenol into highly reactive phenoxyl radicals by horseradish peroxidase (HRP) in the presence of H2O2. HRP can be targeted to specific surfaceome residing proteins by coupling to an affinity binder or ligand. The generated biotin-phenoxy radicals react either with themselves or with primarily tyrosine residues of proteins in their vicinity ultimately leading to a biotinylation. Affinity-purified biotinylated proteins/peptides can subsequently be analyzed and quantified by mass spectrometry. We demonstrate that the TSA method allows for the detection of a) heterodimer partners (Erbb2/Erbb3), b) large scale organizational changes at the cell surface (lipid rafts) and c) the micorenvironment of receptor tyrosine kinases (EGFR). Finally, the TSA reaction was performed targeting ten different cell surface receptors in EGF stimulated cells. The identified proteins were quantified in order to build a cell surface nanoscale matrix, which provide new insight into functionally relevant nanodomains around EGFR. Proximity-dependent chemoproteomic technologies enable the identification of dynamic protein-protein interactions and reveal the nanoscale organization of the surfaceome.

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Systems Biology

Assessing proteome organization by native SEC coupled to SWATH-MS

Moritz, Heusel

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Introduction

Living systems are organized in modular networks of interacting molecules. Therein, most cellular functions are carried out by macromolecular complexes, frequently protein-protein complexes, representing physically linked functional modules in the cellular network. The identification and quantification of these functional units is required for understanding cellular systems. Multiple techniques to characterize protein complexes have been established. Often protein network modules are inferred from binary interaction networks generated by techniques such as yeast2hybrid (Y2H) and affinity purification coupled to mass spectrometry (AP-MS). These techniques are limited by the need for genetic engineering, low throughput and low accuracy of the resulting modules representing actual protein complexes. Alternative approaches better suited to systematically analyze modular proteome organization in cell systems are dearly sought after.

Methods

SEC-SWATH-MS generates quantitative size exclusion chromatography (SEC) elution profiles of a proteome under native conditions aimed at maintaining the integrity of macromolecular complexes. First, protein complexes were liberated from cycling HEK293 cells using a mild lysis protocol commonly applied in AP-MS. Protein complexes were then concentrated and fractionated by high resolution SEC into 80 fractions spanning a mass range of ~ 2 MDa to the small molecule range. Tryptic peptides of the SEC fractions were quantified by SWATH-MS, drawing a detailed map of the HEK293 protein mass partitioning into diverse complex formation states. For data acquisition and interpretation, concepts from peptide analysis in targeted proteomics were extended.

Preliminary data

With the goal to develop the SEC-SWATH-MS technique for the characterization of protein network modules, the native SEC elution profiles of the HEK293 cell line were queried for the presence of previously characterized complexes as annotated in the CORUM database. Computational methods were devised to i) detect protein elution profiles in the SEC dimension and ii) detect protein complex formation as indicated by co-eluting subunit proteins. Based on manual annotation of complex features represented in the dataset, methods were benchmarked and optimized, reaching sensitivities for the recovery of true positive features of up to 71 % with false positive assignments at below 10

percent, demonstrating the technique's readiness for testing further external hypotheses on protein network modules.

With the goal to estimate the degree of higher order organization of the HEK293 proteome, specifically the phenomenon of protein moonlighting in different complex formation states, protein elution features in the SEC dimension were analyzed in detail. Extending the targeted proteomics concept, protein elution was evidenced by co-elution of peptide features in the SEC dimension. Protein mass could be tracked to an average of 3.4 different elution profiles per protein, suggesting a high degree of moonlighting. Relation of the observed SEC retention times to those expected for the respective monomers, over 70 % of the protein mass was detected in a complex-bound or higher order organizational state, confirming a large degree of higher order organization to be a hallmark feature of higher eukaryotic cell systems.

Novel aspect
SEC-SWATH-MS depicts the higher order organization of a human cell system at unprecedented resolution, coverage and accuracy.

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Virology

Antigen-loaded oxidation sensitive polymersomes enhance B cell immunity towards a weakly immunogenic viral surface antigen

Clara, Galan Navarro

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Neutralizing antibodies are decisive during vaccine-mediated protection against viral infections. However, several highly pathogenic emerging viruses only elicit weak or delayed protective antibody responses potentially leading to disease. Development of safe and efficient subunit vaccines capable of generating robust protective antibodies titers is a major challenge, frequently cuased by the inherently low immunogenicity of viral attachment proteins, which represent most significant targets for protective antibodies. Here, we evaluated a newly developed polymersome (PSs) nanocarrier vaccine platform for its ability to enhance humoral immunity against poorly immunogenic virus surface antigens. The PSs platform drives the loaded antigen to lymphoid tissues, enhances DCs intake and increases antigen presentation. As a relevant example, we used the envelope glycoprotein (GP)-1 of the highly human pathogenic Lassa virus (LASV) currently threatening thousands of lives in western Africa. Based on recent structural information, we designed a suitable LASV GP1-derived immunogen that was encapsulated into the aqueous core of PSs and evaluated the immune response in a mouse model. In contrast to the free antigen, the PSs vaccine formulations induced robust titers of virus-binding antibodies capable to target LASV GP1 in its native conformation at the virus surface. Screening with peptide arrays revealed that antigen delivery by PSs significantly increased the epitope-range of anti-LASV GP1 antibodies, unveiling relevant epitopes, which were previously identified and showed implication in neutralization. Our research shows PSs ability to markedly improve the quality of the antibody response towards weakly immunogenic viral surface proteins, with major implications for the design of subunit vaccines.

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Virology

Phosphoproteomic-based kinase profiling of influenza A virus infected cells reveals GRK2 as a crucial host-factor for viral entry

Selected for flash talk

Yángüez, Emilio

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Influenza A viruses (IAVs) harness the cellular endocytic machinery to enter the cell and traffic through the cytoplasm to reach the replication site in the nucleus. Coordinated early activation of particular signalling pathways has been shown to be crucial for the entry process and the outcome of viral infection. However, the complexity of these signalling cascades, tightly connected with each other, requires a broader analysis to identify the main routes, key mediators and direct effectors involved in the early steps of viral replication. In order to obtain a comprehensive view of the signalling events induced during IAV entry, we have conducted a proteome-wide SILAC-based quantitative phosphoproteomic screen of A549 cells within minutes post-infection. We have quantified around 3000 phosphorylation sites from 1300 different proteins and identified infection-induced changes in the phosphorylation of an important subset. Bioinformatic analysis has revealed that MAPK signalling is the main pathway activated early during IAV infection and it is accompanied by significant changes in the phosphorylation of key proteins of the cytoskeleton and the vesicle-mediated transport. Using an algorithm based on the differential phosphorylation of putative targets, we have identified the G protein-coupled receptor kinase 2 (GRK2) as one of the key kinases activated during IAV entry. Both silencing or chemical inhibition of GRK2 leads to drastic reduction in viral replication, which is related with a defect in the virus trafficking through the endosomal pathway. Altogether, our results indicate that targeting GRK2 kinase activity could be a promising strategy for future antiviral interventions.

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Virology

Investigation of the IFITM-mediated restriction of influenza A virus

Lanz, Caroline

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Interferon-inducible transmembrane proteins (IFITMs) are potent antiviral factors active against various viruses, including influenza A virus (IAV). Localizing to either the plasma membrane or endosomes, IFITMs block the entry of incoming viral particles. Recently, it has been found that IFITMs also get incorporated into the membrane of HIV virions upon budding, thereby decreasing their infectivity.
Here, we report our findings on IFITM incorporation into IAV particles. Making use of IAV VLPs having the M1 protein fused to beta lactamase we show that IAV VLPs incorporated IFITM3 and were reduced in their entry capacity when IFITM3 was present in the VLP producing cell. Interestingly, this effect was alleviated in a dose-dependent manner when increasing amounts of HA were co-transfected into the VLP producing cells. While we also observed incorporation of IFITM3 into wildtype IAV particles via Western Blotting and EM studies of purified virus, we did not detect a reduction in virus infectivity associated with incorporation of IFITM3. However, preliminary studies suggest that IAV grown in the presence of IFITM3 shows increased sensitivity to neutralization by hemagglutinin-directed antibodies, potentially compromising the virus in an in vivo situation. In summary, we established a role for IFITM3 in late stages of IAV infection.

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Virology
Characterization of bat influenza virus entry

Karakus, Umut

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Two novel influenza A-like virus sequences have recently been isolated from two South American bat species. Initial studies revealed that these bat influenza viruses, classified as H17N10 and H18N11, are highly divergent from known influenza A virus (IAV) strains. Unlike conventional IAVs, hemagglutinins (HAs) of the bat derived influenza strains do not bind the canonical IAV receptor sialic acid. Moreover, their neuraminidases (NAs) seem to lack any neuraminidase activity. Little is known about target cells and the entry characteristics of the bat influenza viruses. In order to identify cell lines susceptible to bat influenza virus, we have established a flow cytometry-based system to detect binding of recombinant H17 and H18 proteins on a panel of different bat and mammalian cell lines including those derived from Sturnira lilium, the bat species from which the H17N10 sequences originate. Furthermore, we have developed reporter assays to investigate entry of virus-like particles (VLPs) pseudotyped with the bat influenza virus glycoproteins H17N10 or H118N11 on different cell lines. Aiming at the identification of the cellular receptor of H18N11, we performed a comparative transcriptome analysis of two different MDCKII clones differing in susceptibility to H18N11 entry. Thus, we can test potential receptor candidates by silencing or ectopic expression experiments. Altogether, we want to study bat influenza virus entry and uncover their cellular receptor to get a deeper insight into the biology of the newly discovered IAV subtypes.

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Virology

Hijacking of the class IA phosphatidylinositol 3-kinase pathway by a viral protein

Selected for flash talk

Tsolakos, Nikolaos

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Influenza A virus (IAV) manipulates several host factors to ensure efficient replication and spread one of which is the class IA phosphatidylinositol-3 kinase (PI3K), a key enzyme in the regulation of cellular growth, metabolism and proliferation. Class IA PI3K is a dimer of a regulatory (p85α, p50α, p55α, p85β or p55γ) and a catalytic (p110α, p110β or p110δ) isoform. PI3K is activated by IAV via the direct and selective interaction of the viral non-structural protein 1 (NS1) with the p85β regulatory isoform. However, little is known about the catalytic isoforms involved in this process and the functional consequences of this interaction for the virus and the host. Here, we show that NS1 binding to p85β during IAV infection leads to the selective activation of the p110α catalytic isoform. NS1 achieves this by releasing the inhibitory regulation of p85β over p110α and causing p110α translocation to receptor tyrosine kinases on the plasma membrane. Interestingly, p85β-p110β dimers are instead targeted by NS1 into endosome-like structures with unknown function. An oncogenic activating mutation in p85β led to similar phenotypes suggesting that NS1 mimics a generic mechanism of PI3K hyper-activation. Using a phospho-proteomic approach, we have established that IAV activates the canonical PI3K/Akt signalling pathways involved in protein synthesis, cell survival and glucose metabolism but further affects phosphorylation of proteins involved in processes like stress granule formation, autophagy and ribonucleoprotein complex assembly. Understanding the role of these alterations for viral growth and/or cellular homeostasis will be the aim of future research efforts.

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Virology

Influenza virus employs ubiquitin and deubiquitinating enzymes to enter the host cell

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Due to their minimal genomes, viruses rely on a multitude of host factors that support their life cycle. Endocytosis is a common entry strategy for many clinically relevant viruses. However, the mechanism by which these viruses escape the endosomes and gain access to the cytosol is not well characterized. Influenza A virus (IAV), a highly relevant human pathogen, usurps ubiquitination in order to establish infection. We previously showed that histone deacetylase 6 (HDAC6) – a cytoplasmic deacetylase with ubiquitin-binding activity - supports IAV capsid disassembly during cell entry. In this study, we hypothesised that deubiquitinating enzymes (DUBs), which are proteases that remove and edit ubiquitination events, may also play a role in IAV entry. Using small-molecule inhibitors and RNA interference in human airway cells, we identified ubiquitin specific protease 7 (USP7) as a pro-viral factor for IAV. We further employed quantitative IAV entry assays based on confocal microscopy, high-throughput immunofluorescence microscopy and FACS, and concluded that USP7 was required at a post-fusion, pre-nuclear step of entry. IAV uncoating is supported by actin and microtubules. Since USP7 has been described as a regulator of actin assembly at endosomes, it may be involved in IAV uncoating. This project is relevant for novel therapeutic interventions aimed at targeting cellular rather than viral proteins. Moreover, it will help us attain a better picture of how ubiquitination events regulate virus uncoating and cell entry.

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Virology

Assessment of the Antiviral Activity of MxA against Influenza A Virus

Steiner, Fiona

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Human MxA protein belongs to the family of dynamin-like large GTPases and exerts antiviral activity primarily against negative-stranded RNA viruses, including influenza A (IAV). MxA is able to form higher order oligomeric structures. However, the mode of action of MxA remains unknown. There is increasing evidence that MxA targets the IAV nucleoprotein (NP) in conjunction with UAP56, a cellular DEAD-box RNA helicase required for efficient IAV replication. In order to better understand the molecular mechanism of action of MxA we analyzed in detail the interplay between MxA, UAP56 and the viral target NP.
To assess the potential interactions of MxA, NP and UAP56 we employed co-immunoprecipitation (Co-IP) as well as a tripartite split-GFP system. In particular, we investigated the effect of various NP mutants on binding and sensitivity to MxA. Our data clearly showed that only the dimeric variant of MxA (R640A) is able to form stable complexes with NP and UAP56. In addition binding of UAP56 to MxA is greatly enhanced in the presence of NP. Moreover, we observed that the dimeric form of MxA is able to sequester UAP56 from a UAP56-NP complex, while the catalytically inactive form of MxA (T103A) fails to do so. Mutations in NP reducing its nucleo-cytoplasmic shuttling activity strongly increased binding to MxA, while mutations abrogating oligomerization of NP did not affect binding. These data demonstrate that the dimeric form of MxA plays a critical role for its antiviral function. Hence, the oligomeric MxA (presumed storage form) has first to disassemble into dimers in order to sequester UAP56 from the UAP56-NP complex.

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Virology

Identification of novel ISGs involved in the antiviral response against influenza A virus

Müller, Eva

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(1) Institute of Medical Virology

Interferons (IFNs) are cytokines that have strong antiviral effects and play a central role in the immune defense of the host cell against virus infections. Interferon stimulated genes (ISGs) are the mediators of IFN's antiviral activity and hundreds of different ISGs are known to be upregulated upon virus infection. However, only a few have been found to be antivirally active against influenza A virus (IAV). Our hypothesis is that the currently known factors alone cannot explain the strong effect of IFN against IAV, and we aim to find novel ISGs which have yet to be described. For this purpose, an siRNA library of 152 different human ISGs was created, and these siRNAs are currently being screened to identify those that increase the replication of a reporter human IAV which expresses Renilla luciferase. Our assay was been tested with siRNAs targeting different control ISGs, including IFITM3 and MxA (well known inhibitors of IAV infection), and has proved to be robust and applicable for high-throughput approaches. We are also applying our new reporter virus system to other strains of IAV, including those that infect birds and pigs, with the future aim of identifying specific ISGs that may act as host-range restriction factors. Overall, we have established an efficient reporter virus system which can be easily adapted to different kinds of infection experiments, and which is currently being used for our ongoing screen of the human ISG siRNA library.

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Virology

Characterising the function of SUMO E3 ligase TRIM28 during influenza A virus infection

Schmidt, Nora

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In order to successfully establish an infection and replicate within the host cell, a virus has to reprogram cellular pathways and overcome host defence barriers. During influenza virus infection, a massive retargeting of the small ubiquitin-like modifier (SUMO) to a subset of host proteins was observed, which is involved in the regulation of many nuclear functions. In a previous mass spectrometry approach, our group identified cellular SUMO target proteins that change their SUMOylation status during infection. In this follow-up study, we are focusing on the SUMO E3 ligase TRIM28 that is known to have anti-retroviral activity as well as to be involved in regulation of latency of DNA viruses.
TRIM28 strongly decreases in SUMOylation during both influenza A and B virus infections. Interestingly, this does not coincide with phosphorylation on Ser824 of TRIM28 which occurs in DNA damage response and HCMV infections. Additionally, the localisation of TRIM28 did not change upon deSUMOylation. Depleting TRIM28 by siRNA transfection in influenza A virus growth experiments revealed a potential pro-viral function of TRIM28. In order to investigate the role of TRIM28 during influenza virus infection in more detail, we generated a TRIM28 knockout cell line using the CRISPR-Cas9 system in A549 cells. Having established this cell line, we are planning to re-introduce mutant forms of TRIM28 that lack SUMO E3 ligase activity and/or cannot be SUMOylated. This will help elucidating whether the SUMO E3 ligase activity is required for the pro-viral function of TRIM28 and whether this is regulated by its SUMOylation.

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Virology

SUMOylation in the Regulation of Antiviral Host Innate Immunity

Patzina, Corinna

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Upon detection of invading viruses, cells can rapidly mount an innate immune response by activating specific antiviral signalling pathways, such as the type-I/III interferon and NF-kB responses. This results in a potent protection of infected and surrounding cells. To avoid exacerbated or insufficient innate immune activation, these signalling pathways are tightly controlled, amongst others by posttranslational modifications like SUMOylation. SUMO has just recently been associated with innate immunity and can profoundly impact its antiviral action.
To get a more detailed view on which cellular functions are altered during an innate immune response, we performed mass spectrometry to characterise overall changes in the cellular SUMO proteome after infection with an influenza virus with an impaired ability to counteract cellular defence systems. We focused on factors that show a strong and reproducible increase in SUMOylation under these conditions, but not during wildtype virus infection, selecting for proteins involved in the innate immune response. Amongst the top hits, we identified a subunit of NF-kB and members of the cavin protein family. We further investigate whether the SUMOylation of these factors changes their function and/or impacts innate immune signalling, for example by examining their impact on antiviral gene expression as well as on virus replication. In this context, we will also determine (novel) SUMOylation sites in the proteins as well as the SUMO machinery involved.
These data will present novel insights into how SUMO contributes to the tight regulatory network controlling innate immunity that is often deregulated in inflammatory diseases or severe virus infections.

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Virology

Nanomaterials with virucidal activity against HSPGs dependent viruses in vitro, ex vivo and in vivo

Selected for talk

Talk only, no poster

Tapparel Vu, Caroline

Cagno, Valeria (1); Galloux, Marie (2); Jones, Samuel (3); Kaiser, Laurent (4); Stellacci, Francesco (3); Tapparel, Caroline (1)
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Viral infections kill millions of people every year and despite this burden, there is currently no antiviral nor vaccine available for most of them. In addition, existing drugs are virus-specific and rapidly induce emergence of resistant variants. An ideal strategy to fight viral infections would be to develop broad spectrum antivirals that irreversibly inhibit infectivity. We designed sulfonic acid decorated nanomaterials that mimic heparan sulfate proteoglycans, a cell attachment-receptor used by many distinct viruses. We tested this material in cells, in relevant tissue culture models and in mice. This nanomaterial induced an irreversible loss in viral infectivity and proved active against important human pathogens such as Zika virus, Respiratory Syncytial virus, Papillomavirus and Herpes virus. We also demonstrated an absence of toxicity and an effectiveness in nanomolar concentrations in vitro, ex vivo and in vivo. These broad-spectrum virucidal drugs may thus represent an ideal tool to fight both endemic and emerging viruses.

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Virology

Identification of a novel supersite of neutralization vulnerability within the HIV-1 membrane proximal external region

Selected for flash talk

Friedrich, Nikolas

Stiegeler, Emanuel* (1); Friedrich, Nikolas* (1); Reinberg, Thomas (2); Morin, Mylène (3); Hansen, Simon (2); Eroglu, Mustafa (1); Schaefer, Jonas V. (2); Zerbe, Katja (3); Marrero Nodarse, Aniebrys (3); Robinson, John (3); Plückthun, Andreas (2); Trkola, Alexandra (1)
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The highly conserved membrane proximal external region (MPER) in the gp41 envelope glycoprotein of HIV-1 is the target of various broadly neutralizing antibodies (bnAbs) and hence an attractive vaccine target. However, despite intensive efforts all MPER vaccination strategies thus far have failed to elicit neutralizing responses, in part due to autoreactivity of MPER reactive B cells leading to clonal deletion. Alternative MPER vaccination strategies are thus urgently needed. Here we explored if additional epitopes within the MPER domain exist that would allow for broad neutralization. Using a structurally arrested MPER peptide mimetic as target we employed Designed Ankyrin Repeat Proteins (DARPins), small synthetic binding proteins, to select HIV-1 entry inhibitors. Two MPER-specific DARPins with nanomolar affinities for the MPER domain were generated and proved to have exceptional breadth, neutralizing 100% (n=67) of a multi-subtype virus panel. While the monovalent MPER-specific broadly neutralizing DARPins (BNDs) exceeded the known MPER bnAbs in breadth, their potency was lower (5 to 30 fold) but in a relatively close range. Intriguingly, the BNDs proved to target a structural epitope composed of highly conserved residues that form a contiguous hydrophobic patch. HIV-1 proved only to partially escape the MPER BND neutralization pressure in vitro by inducing mutations outside of MPER. Uncovering this novel site of neutralization vulnerability that allows maximal breadth coverage highlights further the potential of the MPER domain and gives new perspectives for designing MPER vaccines and therapeutics.

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Virology

The interferon-induced MxB protein is a novel herpes virus restriction factor

Crameri, Michel

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The human myxovirus resistance proteins MxA and MxB are expressed upon stimulation with type I and type III interferons (IFNs) in response to viral infection. While MxA is well-known to elicit potent antiviral activity against many viruses, MxB has long been unrecognized to serve any antiviral function. Recently, a potent antiviral effect of MxB against human immunodeficiency virus type 1 (HIV-1) and other primate lentiviruses has been discovered. Evolutionary analyses then indicated that MxB is likely to exert inhibitory activities against additional viruses.
To test this hypothesis, we selected herpes simplex virus type 1 (HSV-1) as a representative of nuclear-replicating large DNA viruses. We first utilized T98G cells as a model due to their high endogenous MxB expression. Then, we tested MxB in absence of other IFN-induced genes by generating A549 cells stably overexpressing MxB (A549-MxB).
We observed that silencing of MxB expression in IFN-treated T98G cells partially rescued HSV-1 replication. Moreover, HSV-1 replication was strongly inhibited in A549-MxB cells as compared to control cells. In line with this, HSV-1 replication in A549-MxB cells recovered to a large extent after siRNA-mediated knock-down of MxB. Similar results were obtained using HSV-2 and Kaposi's sarcoma-associated herpesvirus (KSHV). Further experiments then revealed that MxB acts early upon infection, i.e. after particle uncoating and cytoplasmic trafficking of capsids but prior to viral immediate-early gene expression.
In conclusion, out data indicate that MxB interferes with nuclear entry of herpesviral DNA, suggesting a gatekeeper function of MxB at the nuclear pore.

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ViralZone: recent updates to the virus knowledge resource

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ViralZone (viralzone.expasy.org) is a web resource that links sequence data with virus biology knowledge. The information is organized into viral fact sheets for each viral genus that describe virion shape, genome, molecular biology and epidemiology with links to the corresponding sequences in GenBank and UniProtKB/Swiss-Prot. ViralZone provides a detailed view of viral molecular biology through a 200 pages dictionary of controlled vocabulary that describes the common steps of viral replication cycles and host-virus interactions. Users can navigate back and forth from genus fact sheet to relevant molecular vocabulary.
Based on the controlled vocabulary assembled in ViralZone, we have created ontology in Gene Ontology (GO), and UniProtKB/Swiss-Prot. Ontologies provide a formal representation of knowledge that is amenable to computational as well as human analysis. This viral molecular ontology facilitates annotation of virus genes in sequence databases and helps large-scale analysis. More than 80,000 protein annotations have already been implemented in databases using this ontology.

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MorphoSphere: A deep learning framework to score cancer cell proliferation and oncolytic virus efficacy in 3D tumor models

Selected for flash talk

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Cancer is among the most prevalent causes of death in the world, and increasing in aging societies. It entails uncontrolled cell proliferation and tumor formation in a complex environment with multifactorial causalities. Mimicries of tumor tissues are developed to untangle the complexity of tumor biology. Cancer cell spheroids are suitable organotypic models to study the formation and proliferation of tumors. They exhibit more biological complexity than monolayer cell cultures, are readily accessible, controllable from the environment and allow in-depth mechanistic studies of both normal and malignant growth and cell proliferation. We have developed cancer cell spheroids to explore the mechanisms by which oncolytic viruses kill tumors. Here, we present a platform for high-throughput screening of scaffold-free spheroids inoculated with different viruses. We employ high-throughput live cell imaging and automated image analysis, in conjunction with a newly developed automated deep learning image quantification framework, called MorphoSphere. MorphoSphere monitors spheroid dynamics based on morphological and textural features and classifies spheroidicity and viability of cell aggregates using a convolutional neural network based on a custom-designed training set. We showcase the anti-tumor potential of viruses in cancer cell spheroids by combining transmission light analyses with quasi-tomographic light-sheet microscopy. This allows us to compare the impact of a panel of replication-competent and -incompetent viruses, and for the first time correlate the macroscopic tumor killing with virus spreading between cells. We find that the extend of oncolytic spheroid killing tightly correlates with the speed and efficacy with which viruses spread deeply into the tumor model tissue.