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Poster-1

Sequential Multicolor Pulse-Chase Imaging of Protein Dynamics in Extracellular Matrix in Living Fibroblasts

Harumi Ito
RIKEN Center for Life Science Technologies (CLST)

Extracellular matrix (ECM) is a non-cellular three-dimensional macromolecular network that underlies structural and biomechanical properties of multicellular tissues. One of the core components of ECM is fibronectin. Fibronectin is synthesized in endoplasmic reticulum, secreted into the extracellular milieu, and then assembled into an insoluble fibrillar matrix. In this study, we visualized the organization process of the fibronectin matrix with sequential multicolor pulse-chase labeling method. We constructed a fibroblastic cell line expressing fibronectin fused with HaloTag, which can be covalently conjugated with a chloroalkane ligand bearing a small functional molecule such as a fluorescent dye. Fibronectin fused with HaloTag was labeled sequentially at fixed intervals with three ligands, which bear dyes with different fluorescence colors (Alexa Fluor 488, Alexa Fluor 660, and tetramethylrhodamine). The imaging analysis indicated that the secreted fibronectin initially forms a meshwork structure, which then grows to a thick fiber. Our results suggest that this dynamic remodeling process contributes to the complicated construction of the ECM network.

Poster-2

Mapping the ECM Specific to the Sites of Inter-tissue Interactions in the Hair Follicle

Ko Tsutsui
RIKEN Center for Developmental Biology (CDB)

Inter-tissue interactions are vital for the formation and function of all organs. Physiological functions and homeostasis of mammalian hair follicles are also maintained by the interactions between different types of epidermal stem cells and surrounding mesenchymal tissues. These compartmentalized stem cells are projected individually by sensory neuron, arrector pili muscles, and dermal papilla to establish functional units for tactile sensation, piloelection and hair growth, respectively. However, little is known about how these inter-tissue connections are molecularly regulated. To address this question, we screened for the extracellular matrix (ECM) proteins regionally deposited in these inter-tissue interfaces using RNA-seq transcriptome data and immunohistochemical examinations. Our observation suggested that ECM variety from the epidermal stem cells is the major determinant of the region-specific ECM compositions. As the signature ECMs for the upper bulge compartment, where sensory neurons innervate, we identified eight ECM genes including *Egfl6* and *Col4a4*. Deletion of *Egfl6* caused misaligned patterns of axonal endings and low touch response, demonstrating that epidermis-derived ECMs contribute inter-tissue connections and their functions. Signature ECM genes for other inter-tissue interaction sites were similarly identified. Our data provide both cellular origins and tissue localizations of the ECM, and suggest functional importance of ECMs for inter-tissue interactions.

Poster-3

Combined STED Nanoscopy and Enzymatic Labeling to Study the Fine Organization and Dynamics of Neuronal Organelles Across Distinct Neuronal Compartment

Giovanna Coceano
KTH Royal Institute of Technology/Science for Life Laboratory

Neurons are highly polarized cells composed of different compartments with distinct molecular composition and functions. The reasons behind organelles distribution, dynamics and interactions along axons and dendrites are not completely understood due to the lack of imaging methods operating at high spatio-temporal resolution. By using STimulated Emission Depletion Microscopy (STED) in combination with new cell-permeable dyes we investigate the fine morphology of mitochondria and endoplasmic reticulum in live hippocampal neurons. We further characterize their distribution and interaction across the actin cytoskeleton, which is involved in establishing neuronal polarity, transport of cargos and stabilization of synaptic structures. In the long processes of cultured hippocampal neurons, actin forms various structures, including bundles, patches and a recently reported periodic ring-like structure that can be uniquely explored with super resolution light microscopy thanks to the nanoscale spatial resolution. Using actin as our reference structure, we analyzed the precise localization of mitochondria and the endoplasmic reticulum in different cellular compartments, such as the periodic cytoskeleton in the axon and dendrites and small dendritic spines, aiming to increase our understanding of basic mechanism governing mitochondria-ER interactions.

Poster-4

Harnessing the CRISPR/Cas9 System in Mouse Genetic Engineering

Takaya Abe
RIKEN Center for Life Science Technologies (CLST)

The CRISPR/Cas9 system allows us to generate the genetically engineered mice by microinjection or electroporation in the zygotes, and a number of approaches have been reported recently. However, there is a room for improvement on the technology to insert a gene cassette knock-in. Here we report our current approaches for generation of designed-null alleles that are inserted a terminal codon and conditional (floxed) alleles with ssODN, as well as a knock-in alleles. For generating knock-in mice efficiently, we compared four different methods that have been reported: 1) a traditional homologous recombination-based method, 2) a method using a long single strand DNA as a donor plasmid, 3) the PITCh system employing microhomology-mediated end joining, 4) a homology-mediated end joining-based method. The ROSA26 locus was used as a pilot experiment for inserting a GFP expression cassette. We injected about 200 eggs each condition, and then GFP positive embryos were obtained from all of three methods. We would like to discuss about these pros and cons, and show examples of establishment of knock-in mice.

Poster-5

Translating the Fetal Heart Gene Expression Program to Adult Heart Failure-tissue by Spatial Transcriptomics

Michaela Asp

Science for Life Laboratory/KTH Royal Institute of Technology

Heart failure is a major medical complication linked to poor quality of life and high mortality rates. The adult heart has the ability to return to the fetal gene program during the pathogenesis linked to heart failure. Thus, tracking the activation of those fetal genes in the adult heart could potentially serve as useful biomarkers of heart failure progression[1]. In addition, complete gene profiles of the developing heart process could expand our knowledge on how mature cardiomyocytes develop, and provide new insights in the field of myocardial regeneration, with the potential outcome of alternative strategies for treating heart failure. Therefore, the intention of this study was to track all gene activity linked to human heart development, within a spatiotemporal context. To do this we used the spatial transcriptomics (ST) method[2], together with single-cell RNA sequencing to analyze fetal heart tissue. By combining ST and single-cell technologies, high-resolution functional clusters related to the architecture of the heart, as well as the spatial location of individual cells with key stem cell signatures, could be unraveled.

1. Asp, M, *et al.* Spatial detection of fetal marker genes expressed at low level in adult human heart tissue. *Sci. Rep.* **7,** 1–10 (2017).
2. Ståhl, P. L & Salmén F, *et al.* Visualization and analysis of gene expression in tissue sections by spatial transcriptomicsm Science **353**, 78-82 (2016).

Poster-6

Genetic Screen to Identify Factors that Give Resistance to Cancer, Obesity and Aging

Hiroshi Nishida
Yoo Physiological Genetics Laboratory, RIKEN Institute Laboratories

Organisms are constantly exposed to multiple stresses and respond to these stresses to maintain a homeostatic state. It has previously been shown that flies that live longer often have resistance to various stresses. This raises a possibility that there could be a correlation among resistances to multiple stresses. To address this question, we have been conducting EMS-based unbiased genetic screening by using *Drosophila melanogaster*. We established a library of approximately 1000 mutants. Each mutant has unique mutations generated randomly by EMS. Using this library, we are investigating if there is any correlation among resistances to multiple stresses. We focus on three stresses: oncogenic stress, high nutritional stress and aging stress. We already identified several interesting mutants in those examined. After completion of the screening, we will investigate if there is any correlation among resistances to different stresses. Our final goal is to identify genes that are responsible for the resistance to multiple stressors.

Poster-7

Nicotinic Cholinergic Regulation of Early Sensory Processing in Primary Auditory Cortex

Hideki D. Kawai
Soka University

The cholinergic system is involved in cognitive functions such as wakefulness, attention, learning and memory. Cholinergic neurons of the basal forebrain modulate cerebral cortex by releasing the neurotransmitter acetylcholine. Loss of cholinergic neurons during aging is associated with cognitive dysfunction in Alzheimer's diseases. While cholinergic deficits will affect higher cortical processing, it may do so by impairing lower cortical processing at the level of primary sensory cortex. We have shown previously that activation of nicotinic acetylcholine receptors enhanced auditory input-mediated intra-column activities while it suppressed inter-column activities in primary auditory cortex. Blocking the receptors prevented the nicotinic regulation of both intra- and inter-column activities. These data suggest that acetylcholine increases the signal-to-noise ratio of auditory information processing critical to cognitive functions. Consistent with this, nicotinic activation increased the number of tone-evoked responses with larger amplitudes, reducing the variability of responses in the thalamocortical input layer. The increased signal-to-noise ratio is also associated with an increased power of gamma frequency oscillation. These nicotinic cholinergic regulations may arise due to elevated excitability of pyramidal neurons with a combination of enhanced excitatory synaptic currents and reduced inhibitory currents. Taken together, cholinergic deficits likely affect cognitive functions by impairing lower cortical processing.

Poster-8

Intrinsic μ Opioid System in Medial Prefrontal Cortex Modulates Placebo Analgesia in Rats

Ying Zeng
RIKEN Center for Life Science Technologies (CLST)

Placebo analgesia is the beneficial effect that follows despite a pharmacologically inert treatment. Modern neuroimaging studies in humans have delineated the hierarchical brain regions involved in placebo analgesia. However, because of the lack of proper approaches to perform molecular and cellular manipulations, the detailed molecular processes behind it have not been clarified. To address this issue, we developed an animal model of placebo analgesia in rats and analyzed the placebo analgesia related brain activity using small-animal neuroimaging method. We show here that gabapentin-based Pavlovian conditioning successfully induced placebo analgesia in neuropathic pain model rats and hierarchical brain regions are involved in placebo analgesia in rats, including the medial prefrontal cortex (mPFC), rostral anterior cingulated cortex (rACC), ventrolateral periaqueductal gray matter (vlPAG), etc. The functional couplings in placebo responders among the mPFC, rACC, and vlPAG were interrupted by naloxone, an antagonist of μ opioid receptor. Moreover, both local chemical lesion and microinfusion of naloxone in the mPFC suppressed the placebo analgesia. These results suggest that the intrinsic μ opioid system in the mPFC causally contribute to placebo analgesia in rats, and the small-animal neuroimaging approach could provide important insights toward understanding the placebo effect in great detail.

Poster-9

Small-animal Neuroimaging Analysis of the Pain Matrix in Visceral Pain Model Rats

Tianliang Huang,
RIKEN Center for Life Science Technologies (CLST)

In recent decades, the inflammatory bowel disease (IBD) incidence is increasing rapidly around the world due to the lifestyle and dietary changes. Though, IBD is always accompanied by abnormal abdominal pain that seriously affects patient's quality of life, the central mechanism of the pain has remained unclear. Recently, we have developed a small-animal neuroimaging method combining 2-deoxy-2-[^{18}F] fluoro-$_{D}$-glucose ([^{18}F]FDG) PET imaging with statistical parametric mapping analysis to evaluate regional brain activity in the rodent brain. In order to investigate the" pain matrix" activated by IBD, we analyzed the regional brain activity in a IBD model rats in which the 2,4,6-trinitrobenzene sulfonic acid (TNBS), a chemical compound widely used to generate colitis, was applied to the rectum. FDG-PET imaging study demonstrated that the brain activity was increased in the Medial septal nucleus, accumbens nucleus, and decreased in the Gigantocelluar reticular nucleus, Medullary reticular nucleus in response to the spontaneous pain. In contrast, the brain activity was increased in the Secondary somatosensory cortex, Granular insular cortex, Lateral paragigantocellular nucleus, Raphe magnus nucleus, Lateral parabrachial nucleus, Ventromedial hypothalamic nucleus and decreased in the Primary somatosensory cortex, Secondary motor cortex after mechanical noxious stimulus, indicating different subsets of brain areas might be involved in spontaneous and evoked pain during IBD.

Poster-10

In vivo Imaging of Cancer Microenvironment Using Fiber-bundle Based Micro-endoscope

Sally Danno
RIKEN Center for Life Science Technologies (CLST)

Cancer tissue is composed of cancer cells, immune cells, and interstitial tissues such as blood vessels, lymph vessels, fibroblasts, and extracellular matrix, all with distinctive structures, different from normal tissues.
This "cancer microenvironment" is well-known cause of malignancy, which is shown by metastasis, infiltration, and drug resistance. Up to now, the cancer microenvironment has been mainly studied by powerful technique of two-photon microscopy.
It is, however, impossible to evaluate the "cancer microenvironment" of deep tissues in minimally invasive manner.
In our study, we developed microscopic endoscope system that can visualize structure of cancer tissues, from the surface to the deep areas, at the cell level spatial resolution by inserting an optical fiber bundle to the cancer tissue. The tip of our optical fiber bundle, with about 300mm diameter, polished in a bamboo spear shape, is minimally invasive.
Here we suggest that this system makes it possible to observe cancer microenvironment and the tissue structure at the cell level resolution continuously over a long period, and it could be a useful tool to evaluate mechanisms of action of the anticancer drugs.

Poster-11

Molecular Renovation Strategy: A Novel Synthetic Methodology for Expeditious Development of Molecular Probes

Takashi Niwa
RIKEN Center for Life Science Technologies (CLST)

Molecular probes bearing a functional moiety such as a fluorescence group and a positron emitting nuclide are useful tools for precise investigation of biological phenomena in life science researches. However, preparation of practical probes is not easy and often takes a long time.

To expedite the development of molecular probes, we recently proposed a straightforward synthetic methodology, namely the “molecular renovation strategy.” This approach consists of direct transformation of readily available original molecule-of-interest to a synthetic precursor of the probe compound and introduction of a functional moiety. We are trying to establish this concept by developing novel synthetic methods for organoboron compounds, which serves as versatile intermediates applicable to a wide spectrum of reliable derivatizations. With an aim to achieve straightforward synthesis of organoboron compounds from a wide variety of bioactive molecules, we have recently developed several new borylation reactions involving the cleavage of stable chemical bonds, such as C–F, C–S, and C–CO_2H. In this presentation is discussed the current status of our achievement regarding the renovation strategy, which includes an application of the method to expeditious preparation of molecular probes.

Poster-12

Palladium-Mediated Rapid ^{11}C-Cyanation of (Hetero)Arylborons and its Application for Syntheses of ^{11}C-Labeled PET Tracers

Zhouen Zhang
RIKEN Center for Life Science Technologies (CLST)

Positron emission tomography (PET) has become an important tool for disease diagnosis, drug R&D, and general biomedical researches. To increase the availability of PET tracers, much effect has been made to develop rapid labeling methods to introduce the short-lived positron-emission nuclei ^{11}C ($t_{1/2}$ = 20.4 min) into the functional molecules. Herein, we report a novel palladium(II)-mediated rapid ^{11}C-cyanation of (hetero)arylborons with [^{11}C]NH_4CN or [^{11}C]HCN for the synthesis of nitrile-containing PET tracers. This ^{11}C-cyanation reaction showed high radiochemical yield, and excellent tolerance to various functional-groups, including halogens, unprotected hydroxy or amino groups, several types of carbonyl groups such as esters or amides, cyano group, and heterocycles. By using this method, potential aromatase imaging tracers [*cyano*-^{11}C]cetrozole and [*cyano*-^{11}C]YM511 were smoothly prepared with sufficient radioactivity and specific activity for PET imaging studies. Furthermore, this rapid ^{11}C-cyanation of (hetero)arylborons was successfully applied to the synthesis of [^{11}C]5-aryltetrazoles and [^{11}C]2,4-diamino-6-aryl-1,3,5-triazines by subsequent cyclization of [*cyano*-^{11}C]cyanoarenes with trimethylsilyl azide or dicyandiamide, respectively. Since the highly functionalized arylborons are easily accessible by various borylation reactions, these ^{11}C-labeling methods utilizing arylborons as precursors enables facile preparation of a wide range of PET tracers, including [*cyano*-^{11}C]cyanoarenes, [^{11}C]5-aryltetrazoles, and [^{11}C]2,4-dimino-6-aryl-1,3,5-triazines.

Poster-13

PET Imaging Based Pharmacokinetic Analysis of 18F-Pitavastatin in Rats

Takayoshi Nakaoka
RIKEN Center for Life Science Technologies (CLST)

Abstract:
Positron emission tomography (PET) imaging is a powerful tool to visualize in vivo kinetics of molecules of interest non-invasively. PET imaging enables us to know the concentration of candidate drug in human and is expected to reduce the risk of drop-out of the candidate drug at clinical study phase. Interaction with other drugs sometimes inhibits the expected effect or enhances the risk of adverse effect. PET imaging enables us to investigate the drug-drug interaction at the safety dose because of high sensitivity and spatial resolution.
Statin is anti-hypercholesterolemia drug and is one of the most prescribed drug. Therefore, it is important to know the pharmacokinetics and drug-drug interaction. To achieve this, we have used 18F-labeled pitavastatin (18F-PTV) as a PET-tracer and conducted pharmacokinetic analysis.
In the present study we demonstrated that 18F-PTV was precisely measured by PET imaging and it was mainly distribute to the liver and excreted into the bile in rats. In addition we also demonstrated that rifampicin, an inhibitor for organic anion-transporting polypeptides, significantly reduced hepatobiliary transport of 18F-PTV.
PET study using 18F-PTV is expected to be useful to analyze pharmacokinetics of pitavastatin and to evaluate the contribution of potent interactor in the future clinical study.

Poster-14

PET Study on [^{11}C]Thiamine and [^{11}C]Fursultiamine for In Vivo Molecular Imaging of Vitamin B_1

Hiromichi Sugimoto
Takeda Consumer Healthcare Company Limited

Thiamine (vitamin B_1) was first reported in 1911 by Dr. Umetaro Suzuki (one of the founders of RIKEN) having been isolated from rice bran, which had been found to be an effective therapeutic agent against the disease, beriberi. Fursultiamine (thiamine tetrahydrofurfuryl disulfide) was developed as a vitamin B_1 prodrug and has been a commercially successful vitamin product available since the early 1960s.
With the aim of realizing in vivo molecular imaging of thiamine and fursultiamine in animal and human by positron emission tomography (PET), we have concentrated on synthesizing ^{11}C-labeled thiamine and fursultiamine by using Pd^0-mediated rapid *C*-[^{11}C]methylations under the collaboration of Takeda Consumer Healthcare Co., Ltd. and RIKEN Center for Life Science Technology. Each of ^{11}C-labeled thiamine and fursultiamine was intravenously injected into the tail vein of rats, and whole-body PET images were obtained. High accumulation of ^{11}C-labeled thiamine was observed in the liver, kidney, and urinary bladder. On the other hand, ^{11}C-labeled fursultiamine initially accumulated extensively in the heart, and finally in the urinary bladder. Additional animal PET studies using ^{11}C-labeled thiamine and fursultiamine are currently in progress, and a human clinical PET study is planned.

Poster-15

Solubility-Improved 10-*O*-Substituted SN-38 Derivatives with Antitumor Activity.

Tatsuya Kida
RIKEN Center for Life Science Technologies (CLST)

With the objective of improving the poor water solubility of the potent antitumor compound SN-38, 10-*O*-substituted SN-38 derivatives were developed by the introduction of fluoroalkyl, fluorobenzoyl, or bromobenzoyl groups. The 10-*O*-fluoropropyl-substituted SN-38 was found to be 17-fold more soluble than SN-38 in phosphate buffered saline, and it exhibited a level of biological activity ≈50% that of SN-38 in a cytotoxicity assay using the prostate cancer cell line PC-3. Five other derivatives did not show solubility improvement to the same extent, but their activities in cytotoxicity assays were almost the same as that of SN-38. In vivo study of 10-*O*-fluoropropyl-substituted SN-38 using PC-3 tumor-bearing mice revealed that it has higher antitumor activity than SN-38, even at lower dosage. These results will promote the medicinal chemistry application of the 10-*O*-modification of SN-38 and help reestablish the drug potential of SN-38. Furthermore, the inclusion of fluoro and bromo substituents means that the synthetic strategy developed here may be used to obtain ^{18}F- or ^{76}Br-labeled SN-38 derivatives for in vivo positron emission tomography studies.

Poster-16

Molecular Tools for Analysis of Drug Binding Characteristics

Rasel A Al-Amin
Uppsala University

Methods are needed to study selective binding and localization of candidate drugs and their target interactions in relevant clinical specimen during drug discovery and development. We describe a target engagement-mediated amplification (TEMA) technology, where target interactions by oligonucleotide-linked small molecules is visualized and measured with circularized oligonucleotide probe (padlock probe) via rolling-circle amplification (RCA). We established TEMA using kinase inhibitor precursor compounds and applied the assay for investigating target interaction at low nanomolar drug concentrations in a commercial array of 9000 recombinant human proteins and in pathology tissue section. The TEMA method proved useful to reveal the localization of drug binding in fixated cells and tissues. We also identified specific drug-target interaction and selectivity profiles among large collection of arrayed proteins. We conclude that TEMA is promising as a means to validate target binding by drugs during lead optimization, and for candidate selection during drug discovery.

Poster-17

Structural and Biochemical Studies on Inhibitors Against FAD-dependent Histone Lysine Demethylases

Hideaki Niwa
RIKEN Center for Life Science Technologies (CLST)

Histone lysine methylation regulates either transcription activation or repression of target genes depending on the sites of the lysine residues. Developing chemical probes for proteins that regulate lysine methylation, histone lysine methyltransferases and demethylases, will help to understand the role of each protein. In mammals, two histone lysine demethylases, lysine-specific demethylase 1 (LSD1/KDM1A) and lysine-specific demethylase 2 (LSD2/KDM1B), are known that catalyze the reaction using flavin adenine dinucleotide (FAD) as the cofactor. LSD1 is implicated in the regulation of intractable cancers, such as acute myeloid leukemia and glioma, and therefore inhibitors against LSD1 have been intensively developed, including those that covalently bind to FAD or those that non-covalently bind to the binding pocket. Molecules that inhibit LSD2 much more than LSD1, which will benefit the chemical-biological study of LSD2, have yet to be developed. To develop more potent inhibitors or chemical probes, we have been studying the structures of LSD1 and LSD2 in complex with 2-phenylcyclopropylamine (2-PCPA) derivatives. We report here our recent studies on new compounds, including structural and biochemical assessment, comparison of the binding pockets and inhibitor binding modes between LSD1 and LSD2, and prospect of further development of inhibitors or chemical probes for the histone lysine demethylases.

Poster-18

New Chemical Biology Tools for the Detection of Metabolic Cancer Biomarkers

Louis Conway
Uppsala University

Pancreatic cancer and colorectal cancer are two of the most common forms of cancer, collectively accounting for 24 % of all deaths from cancer in the Sweden (2011). Early-stage patients with these diseases frequently exhibit nonspecific or no symptoms. This fact, coupled with the highly invasive nature of current methods of diagnosis (colonoscopy, biopsy) results in late diagnoses and extremely poor five-year survival rates for patients with these cancers. Pancreatic cancer is particularly lethal, with a five year survival rate of only 2.8 % (1987 – 1999 in Sweden). The development of non-invasive methods for the early detection of cancer is therefore of critical importance.

The use of metabolomics to identify small-molecule markers of both pancreatic and colorectal cancer in plasma, serum, urine, or feces presents a tremendous opportunity for the non-invasive and routine screening of at-risk populations for signs of cancer, facilitating early diagnosis and greatly improved survival rates. To this end, we are applying new approaches to metabolomic analysis for the discovery of cancer biomarkers and the elucidation of the roles of oncogenes by developing new tools at the interface of chemistry and biology with a focus on the relationship between gut microbial metabolites and carcinogenesis.

Poster-19

Development of New Diagnostic Biomarkers for Chronic Fatigue Syndrome Using Metabolome Analysis

Emi Yamano
RIKEN Compass to Healthy Life Research Complex Program

Chronic fatigue syndrome (CFS) is a persistent and unexplained pathological state characterized by exertional and severely debilitating fatigue, with/without infectious or neuropsychiatric symptoms, lasting at least 6 consecutive months. Its pathogenesis was not fully understood. Because of incomplete understanding of aetiology and diagnostic uncertainty of CFS, there are no firmly established objective diagnosis or treatment recommendations.

In the present study, we performed comprehensive metabolomic analyses of plasma samples obtained from CFS patients and healthy controls to establish an objective diagnosis of CFS. CFS patients exhibited significant differences in intermediate metabolite concentrations in the tricarboxylic acid (TCA) and urea cycles. The combination of ornithine/citrulline and pyruvate/isocitrate ratios discriminated CFS patients from healthy controls yielding high area under the receiver operating characteristic curve values both in training and validation datasets. These findings provide compelling evidence that a clinical diagnostic tool could be developed for CFS based on the ratios of metabolites in plasma.

Poster-20

Host Proteome in HIV-1 Natural Control

Wang Zhang
Karolinska Institutet

Background: A small subset of HIV-1 positive individuals, the “Elite Controllers” (EC), is able to control viral replication without antiretroviral therapy. In this study, we aimed to explore plasma proteomics data in a well-defined Swedish cohort of HIV-1 positive ECs to identify the underlying mechanisms.

Method: Plasma samples were obtained from unbiased untreated HIV-1-positive EC (n=19), treatment naïve patients with viremia (VP, n=32) and HIV-1-negative persons (HC, n=23) and analyzed using PEA technology (Olink Bioscience AB, Sweden) targeting 92 soluble factors. *The* differential profile of the proteome was analysed using Qlucore Omics Explorer version 3.2.

Result: Among the 92 proteins, 82 were detectable in >50% of the samples and were further used for the analysis. Multi-group analysis by ANOVA identified clustering of 90% (29/32) of VPs together while EC and HC clustered separately from VP. Among the factors, 28 were statistically significant at a false discover rate (FDR) adjusted p (q) <0.001 using ANOVA. Interestingly, EC had lower levels of TNFSF6 (FasL) and TNFSF10 (TRAIL) compared to VP (significantly) and HC, TNFSF12 (TWEAK) was significantly decreased in VP and EC compared to HC, but no significant difference was seen between the two of them.

Conclusions: The plasma proteomics profile of the soluble factors analysis strongly indicated that cell surface receptor signaling pathway, programmed cell death, response to cytokine and cytokine-mediated signaling may synergistically play important role in virus replication control in EC.

Poster-21

Polysome-profiling in Small Tissue Samples

Shuo Liang
Karolinska Institutet/Science for Life Laboratory

Polysome-profiling is commonly used to study translatomes and applies laborious extraction of efficiently translated mRNA (associated with >3 ribosomes) from a large volume across many fractions. This property makes polysome-profiling inconvenient for larger experimental designs or samples with low RNA amounts. To address this, we optimized a non-linear sucrose gradient which reproducibly enriches for efficiently translated mRNA in only one or two fractions, thereby reducing sample handling 5-10 fold. The technique generates polysome-associated RNA with a quality reflecting the starting material and, when coupled with smart-seq2 single-cell RNA sequencing, translatomes in small tissues from biobanks can be obtained. Translatomes acquired using optimized non-linear gradients resemble those obtained with the standard approach employing linear gradients. Polysome-profiling using optimized non-linear gradients in serum starved HCT-116 cells with or without p53 showed that p53 status associates with changes in mRNA abundance and translational efficiency leading to changes in protein levels. Moreover, p53 status also induced translational buffering whereby changes in mRNA levels are buffered at the level of mRNA translation. Thus, here we present a polysome-profiling technique applicable to large study designs, primary cells and frozen tissue samples such as those collected in biobanks.

Poster-22

Membrane Composition as Risk Factor in Alzheimer's disease

Neha Sharma
Japan Advanced Institute of Science and Technology

Alzheimer's disease (AD) is an age-related brain disorder. Accumulation and aggregation of misfolded amyloid peptide causes this illness. It is known that exogenous amyloid can cause Endoplasmic Reticulum (ER) stress, after traversing inside the cells via endocytic transport. This transport is a membrane phenomenon, thus we were interested to observe the changes when membrane is altered and interacts with Aβ peptide. We have used oxysterols such as 25-hydoxycholesterol (25-OH) and 7-ketocholeserol (7-keto) to alter the composition of membrane of immune and neuronal cells.

In the presence of Cholera toxin B subunit and monosialotetrahexosylganglioside (GM1), internalization of Aβ aggregates was observed in 25-OH added cells. In our study, endocytic vesicular transport of Aβ-42 was clearly observed which indicates the importance of oxysterols and glycosyl chains for vesicular transport of the protein. Changes in the cell viability were also observed with the inclusion of oxysterol in the cells. Herein, we were interested in finding the pathway of internalized protein to reach ER.

Our results demonstrated that oxysterols and glycosyl chains are essential in the internalization of peptide, thus become a risk factor of AD. The findings will be beneficial to understand Aβ-induced cytotoxicity in neurological disorders.

Poster-23

Identification of RNA Polymerase I Inhibitors Among FDA-approved Drugs Using a Cell-based High-content Screening

Jaime A. Espinoza
Karolinska Institutet/Science for Life Laboratory

Ribosome biogenesis is a complex series of coordinated steps that occur in the nucleolus, where ribosomal genes are transcribed by RNA polymerase I (POL1) to produce ribosomes. There is evidence that inhibition of ribosome biogenesis (ribosomal stress) can result in selective growth inhibition of cancer cells, hence, there is a growing interest in identifying compounds capable of inducing ribosomal stress for in anti-cancer treatments
Our goal was to identify if some of ~1200 FDA-approved drugs, of known therapeutic function, may also have the ability to inhibit ribosome biogenesis, were we are specifically interested in POL1 as an additional target. To achieve this goal, we have developed a tool for the identification of POL1 inhibitors based on a cell line with a GFP-tagged RNA helicase able to translocate to the nucleolus upon genotoxic stress induction. In our model, the inhibition of POL1 activity prevents the translocation of the helicase toward the nucleolus, normally induced by genotoxic stress. We identified one compound (A) capable of trigger ribosomal stress pathway through ribosomal proteins RPL11 and RPL5, mediating subsequent p53 activation. Compound A induces degradation of catalytic subunit of POL1, leading to decreased synthesis of rRNA and alteration of nucleolar structure.

Poster-24

Oligomeric Ubiquitylation by the RNF52 Domain of BRCA1-associated Protein 2

Shisako Shoji
RIKEN Center for Life Science Technologies (CLST)

Post-translational modification of proteins by the addition of ubiquitin is known as ubiquitylation or ubiquitination. The modifier protein ubiquitin is structurally conserved in eukaryotes. Ubiquitin linkages are peptide bonds formed between the C-terminal glycine of donor ubiquitin and the N-terminal methionine or one of the seven lysine residues of acceptor ubiquitin. Thus, the proteins destined for ubiquitylation are modified by various types of ubiquitin chain linkages. The conventional model for ubiquitin-chain assembly indicates that ubiquitin-chain elongation occurs through the sequential addition of individual ubiquitin molecules. In contrast, we recently reported the mechanism of oligomeric ubiquitylation mediated by the ubiquitin ligase BRCA1-associated protein 2 (BRAP or BRAP2) [Biochem. J., 2017 Sep 08;474(18):3207-3226]. BRAP is also known as RING-finger protein 52 (RNF52). The central catalytic domain architecture of BRAP, comprising four subdomains (NBP–RING–ZfUBP–CC), is conserved in eukaryotic RNF52 orthologs from yeast to humans. Our study revealed the molecular characteristics of the RNF52 domain of BRAP. The RNF52 domain preferentially binds to ubiquitin chains (oligo-ubiquitin), but not to ubiquitin monomers, and it utilizes various ubiquitin chains for ubiquitylation. Here, we discuss an intracellular signaling pathway model associated with oligomeric ubiquitylation by the RNF52 domain.

Poster-25

The Activation Mechanism of a Circularly Permuted GTPase, RsgA in the Ribosome

Chie Takemoto
RIKEN Center for Life Science Technology (CLST)

RsgA has been found as a *r*ibosomal *s*mall subunit dependent *G*TPase and shown to play a crucial role in maturation of the ribosomal small subunit (30S subunit). For example, RsgA promotes dissociation of RbfA (ribosome binding factor A) from the 30S subunit. RsgA is composed of the N-terminal OB-fold domain, GTPase domain and the C-terminal zinc-binding domain. Interestingly, the GTPase motifs of RsgA are circularly permuted such that they follow a G4-G5-G1-G2-G3 order in the primary sequence instead of the canonical G1-G5 order. Several structures of RsgA alone and RsgA-30S complex have been reported. They revealed that RsgA binds in the vicinity of the decoding center and the OB-fold resembles that of translation initiation factor-1. However, since the functionally important switch-1 region is disordered in those structures, the activation mechanism of GTPase is unknown.

Here, we present results of directed hydroxyl radical probing of the 30S subunit upon binding of RsgA in the presence of GDPNP or GDP and cryo-EM single particle analysis of the RsgA-GDPNP-30S complex. The results show that RsgA destabilizes the 30S head structure, including late binding r-proteins. Moreover, the structural model of ribosome-bound RsgA in the GTP form reveals specific interactions with the 16S rRNA.

[3DEM DB: EMD-3661, EMD-3662, EMD-3663. PDB: 5NO2, 5NO3, 5NO4]

Poster-26

Crystal Structure of Eukaryotic Translation Initiation Factor 2B (eIF2B)

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Eukaryotic translation initiation factor 2B (eIF2B) is a heterodecamer composed of two copies each of five different subunits (the α-, β-, γ-, δ- and ε-subunits), and acts as the guanine nucleotide exchange factor specific for eIF2, a heterotrimeric G protein composed of the α-, β- and γ-subunits.
We developed a bacterial expression system for *Schizosaccharomyces pombe* eIF2B, and determined its structure at 3.0-Å resolution by X-ray crystallography. In the crystal structure, the $\alpha_2\beta_2\delta_2$ hexameric regulatory subcomplex is sandwiched between two γε dimeric catalytic subcomplexes. Based on this structure, we performed surface-scanning site-directed photo-cross-linking analyses, and discovered the interfaces for eIF2α and eIF2γ on the regulatory and catalytic subcomplexes, respectively. These interfaces are located far apart from each other, suggesting that the tight interaction between eIF2B and phosphorylated eIF2α keeps the eIF2-eIF2B complex in a 'nonproductive' state, which prevents nucleotide exchange on eIF2γ.

Poster-27

DNA Sequencing & Genome Informatics in RIKEN Kobe: How Technical Support and Original Research have Coexisted in a Lab

Shigehiro Kuraku
RIKEN Center for Life Science Technologies (CLST)

Modern life sciences largely rely on large-scale omics data production platforms. In the RIKEN Kobe branch, Phyloinformatics Unit of RIKEN Center for Life Science Technologies (http://www.clst.riken.jp/phylo/) provides genome, transcriptome, and epigenome data and opportunities of technical fostering, while it conducts original research projects focusing on vertebrate evolutionary genomics. In this presentation, the overview of the unit activity is introduced with a special emphasis on epigenetics and omics data production for non-model organisms. It will also cover current technical and organizational challenges for sustainable infrastructure management, which is expected to give rise to some seeds of possible continuous exchanges between the institutes.

Poster-28

Droplet Barcode Sequencing for Linked-read Haplotyping of Single HLA Alleles

David Redin
KTH Royal Institute of Technology/Science for Life Laboratory

Data produced with short-read sequencing technologies result in ambiguous haplotyping and a limited capacity to investigate the full repertoire of biologically relevant forms of genetic variation. The notion of haplotype resolved sequencing data has recently gained traction to reduce this unwanted ambiguity and enable exploration of other forms of genetic variation; beyond studies of just nucleotide polymorphisms, such as compound heterozygosity and structural variations. Here we describe Droplet Barcode Sequencing, a novel approach for creating linked-read sequencing libraries by uniquely barcoding the information within single DNA molecules in emulsion droplets, without the aid of specialty reagents or microfluidic devices. Barcode generation and template amplification is performed simultaneously in a single enzymatic reaction, greatly simplifying the workflow and minimizing assay costs compared to alternative approaches. The method has been applied to phase multiple loci targeting all exons of the highly variable HLA-A gene, with DNA from 8 individuals present in the same assay. Barcode-based clustering of sequencing reads confirmed analysis of over 2000 independently assayed template molecules, with an average of 753 reads in support of called polymorphisms. Our results show unequivocal characterization of all alleles present, validated by correspondence against confirmed HLA database entries and haplotyping results from previous studies.

Poster-29

Increased Switch Dynamics of *in vitro* Synthesized mRNA using Modified rNTP Nucleosides

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mRNA-based switches (e.g., RNP L7Ae switches, and miRNA switches) and the gene circuits that they build are gaining in popularity in various applications due to the advantages of using RNA-only delivery, and the dynamic responsiveness of post-transcriptional based-switches. However, the necessity of using modified rUTP and rCTP in the exogenous mRNAs to evade immune surveillance comes at the price of decreased translational output and dynamic range between OFF and ON states of the encoded switch. This is likely resulting from the altered interaction with RNA binding proteins, including those of the ribosomal complex, and altered hybridization kinetics between complementary RNA strands. The relative low dynamic range compared to pDNA or replicon encoded circuits, means alternative modifications are highly sought after. We found a single uridine substitute modification can restore protein translation to that of unmodified exogenous mRNA, further still the same modification outperformed pseudouridine in respects to immune surveillance evasion. Finally, we tested modified rNTPs combinations and found an optimal modification that could provide higher dynamic range equal to that of unmodified mRNA. Using miRNA switches to detect cell-type specific miRNA activity, this modification provides greater resolution in separating different cell types.

In future projects, we will further investigate the influence of various types of rNTP modifications on mRNA biogenesis using various ‘omic’ technologies, and RNA-targeted nuclease-dead CRISPR systems to detect and modify RNA modifications at single-base resolution. Using such systems will provide causative rather than correlative evidence of a particular type of RNA modification to RNA biogenesis and various biological processes.

Poster-30

A Two-step Mechanism for Recurrent Somatic Copy Number Alteration in APC Mutant Tumours

Taisaku Nogi
RIKEN Center for Life Science Technologies (CLST)

Chromosome aneuploidy, or somatic copy number alteration (SCNA), is a hallmark of cancer and has been posited to drive tumourigenesis1-3. Certain aberrations are associated with particular cancer types4,5, yet the molecular mechanisms underlying the selection of particular SCNAs and their functional consequences for malignancies remain largely unknown. Here, we identified a causal relationship between biochemical functions of the APC (adenomatous polyposis coli) tumour suppressor and signature SCNAs in APC-mutant colorectal cancers (CRCs), which can circumvent the adverse effects of APC inactivation on cell proliferation. Imaging-based quantitative cell phenotyping in culture revealed that APC inactivation induced growth retardation due to cell-cycle delay and frequent mitotic errors due to blockade of AURKA, mediated by elevated levels of β-catenin. These cells undergo chromosome alterations resembling CRC SCNAs, which restore active AURKA levels and stimulate proliferation. Aberrant AURKA activation was induced at an early-stage of tumourigenesis. Administration of AURKA inhibitor to APC-mutant mice decreased expression of proliferation markers in tumours. These results indicate that APC-mutant cells that acquired AURKA-activating SCNAs through chromosome instability become predominant as a result of selection. We propose a two-step model for SCNA formation, in which precursor cell properties drive selection of specific chromosomal regions to optimize growth potential.

Poster-31

RUNX1 Regulates Site Specificity of DNA Demethylation by Recruitment of DNA Demethylation Machineries in Hematopoietic Cells

Takahiro Suzuki
RIKEN Center for Life Science Technologies (CLST)

RUNX1 is an essential master transcription factor in hematopoietic development and plays important roles in immune functions. While the gene regulatory mechanism of RUNX1 has been characterized extensively, the epigenetic role of RUNX1 remains unclear. Here, we demonstrate that RUNX1 contributes DNA demethylation in a binding site-directed manner in human hematopoietic cells. Overexpression analysis of RUNX1 showed the RUNX1-binding site-directed DNA demethylation. The RUNX1-mediated DNA demethylation was also observed in DNA replication-arrested cells, suggesting an involvement of active demethylation mechanism. Coimmunoprecipitation in hematopoietic cells showed physical interactions between RUNX1 and DNA demethylation machinery enzymes TET2, TDG, and GADD45. Further chromatin immunoprecipitation-sequencing revealed colocalization of RUNX1 and TET2 in the same genomic regions, indicating recruitment of DNA demethylation machinery by RUNX1. Finally, methylome analysis revealed significant overrepresentation of RUNX1-binding sites at demethylated regions during hematopoietic development. Collectively, the present data provide evidences that RUNX1 contributes site-specificity of DNA demethylation by recruitment of TET and other demethylation related enzymes to its binding sites in hematopoietic cells.

Poster-32

Mapping Combinatorial Epigenetic Modifications at Single Nucleosome Resolution

Jen-Chien Chang

RIKEN Center for Life Science Technologies (CLST)

Post-translational modifications of histones, which mediate many nuclear processes, is one of the most well characterized epigenetic mechanisms. In recent years, it has become evident that specific combinations of histone modifications represent various chromatin states, and are associated with different regulatory functions. However, standard methods like chromatin immunoprecipitation probe one histone modification at a time; thus, the combinatorial patterns at each histone or nucleosome can be masked by heterogeneous population. Here, we apply single-molecule fluorescence imaging in analyzing the coexisting histone modifications at the single-nucleosome resolution. Initially, the method is demonstrated by using reconstituted epi-nucleosomes with known histone modifications. Next, we apply it to delineate the heterogeneous chromatin states during the Epithelial-Mesenchymal-Transition by comparing to time-course ChIP-seq data. Further development of this technology includes integrating with a single-molecule sequencer as well as improving sample preparation from less material. We expect this method to elucidate the functions of chromatin in different states, with the ultimate goal in providing a more complete picture of how epigenome is modulated in various cellular processes.

Poster-33

High-Resolution Characterization of Drug-Induced Cellular Response

Andrew T. Kwon
RIKEN Center for Life Science Technologies (CLST)

For drug mode of action analysis and repositioning, drug response expression profiling has emerged as a powerful technique as it allows us to characterize the cellular response to drug treatments at a molecular level. However, existing approaches and resources are limited by their low resolution at genomic, cell state, and cell type levels, as they rely on existing gene models and cannot adequately deal with cellular heterogeneity. With the single cell, high throughput transcriptomic approach, we can overcome these resolution limitations of both the genomic and cellular level. Not only can we examine both existing and novel promoters, enhancers, and lncRNA, but also resolve the minute differences in cellular make up and their internal states. Thus, by measuring gene expression levels using single cell sequencing methods in an unbiased manner, we can assess the effects of population heterogeneity on their response to drugs, and perform a more detailed mode of action analysis. In this study, we profile drug response in 3 different cell lines (fibroblasts, MCF7, and HepG2) to 2 types of histone deacetylase inhibitors using the newly developed C1 CAGE method, which can robustly detect the expression of individual promoters and enhancers at a genome-wide scale, in a single experiment. We identify and characterize the sub-populations of these cells based on their differential response to drug treatments.

Poster-34

Genetic Diagnosis for Influenza virus and CPT2 genotyping using SmartAmp method

Yuki Tanaka
RIKEN Center for Life Science Technologies (CLST)

Our group has been working on development of genetic diagnosis kits using the SmartAmp, an isothermal nucleic acid amplification method. Our technology can be used for two approaches in fighting with disease, one is pathogen detection for infectious diseases and the other is human genotyping for pharmacogenomics or prognosis prediction.
In this poster, rapid influenza detection and CPT (carnitine palmitoyl transferase) 2 genotyping kit are shown.
Influenza by itself is not fetal in most developed countries, however, some complications such as influenza associated encephalopathy cause severe cases. In mammals, CPT2 system is a pivotal component of ATP generation through mitochondrial fatty acid β-oxidation. It has been reported that a SNP [1055T>G/F352C] predominant in East sians in the CPT2 gene causes a thermolabile phenotype of CPT2 and suggested that it may be a principal genetic background of influenza-associated encephalopathy for infants. Since prompt and appropriate therapy improves prognosis for the patients with the SNP, rapid genotyping is important.
With the developed SmartAmp genotyping kit, the CPT2 gene were detected directly from a small amount of blood samples. 341 blood samples were tested and the SmartAmp results agreed totally with those of the sequencing method.

Poster-35

Functional Annotation of Long Non-coding RNAs in FANTOM6

Jordan Ramilowski
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The length of your abstract should be up to 200 words. Figures and photograph are not allowed. FANTOM (Functional ANnoTation Of the Mammalian genome) is an international research consortium aiming to comprehensively identify and annotate mammalian transcripts. Previous editions of FANTOM as well as ENCODE have provided evidence for pervasive transcription of the mammalian genome, generating vast numbers of long non-coding RNAs (lncRNAs). Specific lncRNAs have been shown to act as key regulators in a wide range of biological roles, and have been implicated in human disease in genome-wide association studies. Unlike proteins, for which a putative functional role can be assigned based on their similarity in amino acid sequence to previously annotated proteins, a functional annotation of lncRNAs based on their nucleotide sequence is not feasible due to the low sequence conservation between homologs and the paucity of functionally annotated lncRNAs. Most non-coding transcripts therefore currently do not have a functional annotation.

Here, we will present recent progress in the sixth edition of the FANTOM project (FANTOM6). FANTOM6 aims to systematically elucidate the function of lncRNAs in human using high-throughput strategies to perturb hundreds of lncRNAs in multiple cell types, followed by transcriptome profiling using CAGE to assess the molecular phenotype. The lncRNAs we selected for perturbation include published transcripts as well as novel transcripts discovered as part of FANTOM5. FANTOM6 data generated so far show the distinct response of the human transcriptome to the lncRNA perturbations, consistent with their functional role in cellular regulation.

Poster-36

Functional lncRNAs in Hepatocellular Carcinoma

Jonas Nørskov Søndergaard
Science for Life Laboratory

In recent years it has become apparent that long non-coding RNAs (lncRNAs) have a functional impact on the progression of hepatocellular carcinoma. In order for the lncRNAs to have a functional impact they must interact with protein partners (RNA binding proteins, RBPs). Therefore, this project aims to construct transcriptional networks governed by RBPs and to identify the involvement of lncRNAs in hepatocellular carcinoma using cutting-edge experimental and computational tools.
RNA from liver biopsies from paired tumor and healthy tissue from a hepatocellular carcinoma cohort were sequenced and compared to data from two independent cohorts (TCGA, FANTOM).
Differential gene expression analysis identified 109 significantly deregulated RBPs overlapping between the cohorts. The top 10 expressed RBPs were subsequently knocked down in Huh7 and HepG2 liver cancer cell lines, which affected the number of metabolically active cells, the viability,and the growth in 3D. RNA sequencing revealed deregulation of lncRNA transcripts affected by the RBP knockdown. The functional impact of impaired lncRNA transcription in controlling global gene expression during hepatocellular carcinoma is currently under investigation.

Poster-37

The FANTOM5 Integrated Expression Atlas of miRNAs and their Promoters

Michiel J. L. de Hoon
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MicroRNAs (miRNAs) are short non-coding RNAs that act as negative regulators by base-paring to complementary regions on targeted mRNAs and long non-coding RNAs. Dysregulation of miRNA expression has been implicated in a wide variety of diseases.
To understand the function and regulation of miRNAs in health and disease, a detailed characterization of miRNA expression patterns in different cell types and tissues, as well as of the genomic control regions regulating their expression, is essential. As part of FANTOM5 project, we performed deep sequencing of 492 short RNA libraries, each with a matching CAGE library, from 396 human and 47 mouse RNA samples. Using these data, we identified the promoter for 1,357 human and 804 mouse miRNAs, and found that the genome sequence in miRNA promoter regions is highly conserved between species. As the expression levels of primary and mature miRNAs were correlated, the primary miRNA expression measured by CAGE could be used as a proxy for the expression level of the mature miRNA, allowing us to extend to the miRNA expression atlas to the 1,829 human and 1,029 mouse CAGE libraries in the FANTOM5 collection. We thus provide the foundation for the detailed analysis of miRNA expression, regulation and function.

Poster-38

Development of in vivo Evaluation Systems for Novel non-coding RNA, SINEUP, to Enhance Translation Level of Target Genes as a Nucleic Acid Medicine

Kazuhiro R. Nitta

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Thanks to the development of deep transcriptome analysis, almost ten thousands of long non-coding RNAs (lncRNAs) have been identified in this decade. Among them, we discovered a novel class of lncRNAs (named SINEUPs) that can up-regulate the translation level of a target coding mRNA without any change in its mRNA expression level. SINEUPs consist of an antisense sequence to target mRNA at the 5′ end and an inverted SINEB2 sequence (Short Interspersed Nuclear Elements of B2 family, a type of transposable elements). It is known that the SINE B2s share their ancestral sequence and RNA secondary structure with tRNAs and are widely distributed in mouse genome. Though the mechanism of SINEUPs' activity is still under investigation, in theory, it can be applicable to any protein coding mRNA by means of target-specific binding domain (BD). We have reported several functional SINEUPs for neurodegeneration associated genes including PARK7/DJ-1 for Parkinson's disease (Zucchelli et al., 2015). In the paper, we demonstrated that the synthetic SINEUP-PARK7 specifically enhances the translation level of endogenous PARK7 mRNA in human neuroblastoma cells. These results encouraged us to lead the SINEUP technology to pharmacological targets.

To expand this technology as therapeutic applications, we have targeted one transcription factor, hepatocyte nuclear factor 4 alpha (Hnf4a). It is known to make an important role in liver development and its metabolisms. In addition, this gene is also known as a causal gene for maturity onset diabetes of the young 1 (MODY1). First, we have designed SINEUPs for Hnf4a to enhance its translation (SINEUP-Hnf4a). Those designed SINEUP-Hnf4as are tested in a mouse hepatocellular carcinoma cell line, and we have identified several functional SINEUPs. Those functional SINEUPs could up-regulated Hnf4a protein levels in the range of 1.7 to 2.4 folds. As a next step, we introduced the SINEUP-Hnf4a to mouse liver by using Adeno-associated virus. Introduced SINEUPs were expressed in the one-week after infected liver tissue, and the up-regulation of Hnf4a translation level was observed in the range of 1.7 to 2.2 folds. This result indicated that SINEUPs are functional also in mouse liver. Other effects in the tissue are under investigation. In parallel, we are trying to express SINEUPs in long-term (more than month) and to assess its long-term effect in animal liver. We will also discuss these our preliminary results.

Our results indicate that SINEUPs are potential molecules for the therapeutic application as the translation enhancer.

Poster-39

Whole Transcriptome Analysis of Thousands of Single Cells with nanoCAGE and CAGEscan

Stéphane Poulain
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The nanoCAGE/CAGEscan protocol is a powerful tool initially developed to perform whole transcriptome analysis in specimens for which low amount of input RNA is available. The method was recently updated to propose better transcriptome coverage (Arnaud et al., Biotechniques, 2016), improved multiplexing capabilities, and higher sequencing efficiency on Illumina sequencers (Poulain et al., Methods Mol Biol, 2017). By further miniaturizing the reverse transcription step with an acoustic liquid transfer system (Labcyte Echo), we analysed thousands of FACS-sorted single cells and sequenced the nanoCAGE libraries produced paired-end. The resulting dataset contains CAGEscan information at single molecule resolution for >8,000 single cells derived from cell lines either infected by the human papillomavirus of type 16 or 18. These data can be used for mapping TSSs, quantifying the expression of human and viral genes and comparing their profiles between the different cells analysed. Moreover, an update of the CAGEscan analysis pipeline enables to partially reconstitute the sequence of individual transcripts starting from their 5'-end, and thereby identify splicing events and virus integration points. We are now adapting our protocol to simultaneously examine the transcriptome of thousands of single cells in high throughput using custom microfluidic chips (Kim et al., Lab Chip, 2015).

Poster-40

Transcriptome Profiling of Liver Sinusoidal Endothelial Cells and Hepatocytes During Liver Regeneration by CAGE Analysis

Xian-Yang Qin
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Liver regeneration is a complicated but coordinated multistep process which requires spatially and temporally precise interactions between different populations of liver-composing cells, including liver sinusoidal endothelial cells (LSECs) and hepatocytes (HPCs). In this study, genome-wide transcriptional profiling of LSECs and HPCs isolated from partially hepatectomized mice was measured using the Cap Analysis of Gene Expression (CAGE) technology. Ingenuity Pathway Analysis showed enrichment in LSECs for genes encoding molecules regulating wound-healing pathways as early as 2 h after partial hepatectomy (PH), while in HPCs pathways related with cell cycle control were abundantly enriched at 30 h and 48 h after PH. A key upstream regulator of liver regeneration, orosomucoid (Orm1) whose expression was dramatically induced in HPCs after PH, was identified by the combination of statistical bioinformatics, including partial least squares-discriminant analysis, correlation analysis and Bayesian network analysis. Knockdown of Orm1 in mice and human hepatic cell cultures resulted in a decrease in hepatocyte growth accompanying suppressed signaling in controlling chromatin replication. This study revealed a potential interaction between early injury response in LSECs and later cell cycle regulation in HPCs. Orm1 is induced in response to hepatic injury and executes liver regeneration by activating cell cycle progression in HPCs.

Poster-41

Characterizing the Oscillating Transcriptome Over the Cell Cycle

Johan Bostrm
Science for Life Laboratory/Karolinska Institutet

The most well-known biological oscillation is the cell cycle, and while intensely scrutinized, many of its interactions with other cell pathways and systems remains to be investigated. With the development of fluorescent markers such as the FUCCI system for tracking live cell cycle oscillation, it is now possible to characterize e.g. shifts in mRNA expression over the cell cycle in unperturbed cells.

We sorted U2OS and HeLa Fucci cells using flow cytometry into three cell cycle phases and performed deep RNA sequencing on the fractions. We developed an algorithm (TriComp) to visualize, classify and analyse the pattern of expression over the three phases. The developed algorithm transforms three-group comparisons into three variables denoting baseline, relationship and intensity of relationship, and provides a tool to compare any three-group relationships between gene families, experiments, cell types etc. Our results provides a transcriptome-wide characterization of cell cycle oscillations and shines light on the complex dynamics of transcriptome control linked to the cell cycle. Apart from providing a resource, we focused further analysis on transcription factor regulation as well as the synchronicity of the circadian clock to the cell cycle in stable cell lines.

Poster-42

Analysis of Retrotranposition in Neurodegenerative Disorders

Giovanni Pascarella
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Nearly 50% of the human genome is composed by Transposable Elements (TEs), discrete units of DNA capable of moving within the genomic environment. DNA transposons duplicate though a cut-and-paste mechanism and have been inactivated during evolution, while retrotransposons duplicate through RNA intermediates that are reverse-transcribed by a self-encoded variant of RT and then inserted at new genomic locations. The human genome harbors at least three families of retrotransposons that are currently capable of transposition: LINE-1 (L1), Alu and SVA elements, all belonging to the subclass of non-LTR retrotransposons and accounting for approximately one-third of the human genome. Recent published data shows that the impact of active retrotransposons on the shape and the integrity of the human genome is far deeper than previously considered. With an estimated rate of one new insertion every 10-100 live births, active retrotransposons are able to influence genetic diversity and to cause diseases, as supported by several cases of de novo insertions involved in the outbreak of cancers and other genetic disorders. Yet, clinical studies of insertions involved in human diseases are usually focused on a very small and local genomic scale. Somatic retrotransposition has been shown to occur in the normal human brain at higher rates compared to other tissues, and de novo insertions can happen at different frequencies in different brain regions. We have developed an oligonucleotide based liquid-phase capture protocol in order to specifically capture and sequence young, active LINE-1 and AluY retrotransposons in the human genome. We have used this approach to study the brain retrotransposome in samples obtained from donors affected by idiopathic Alzheimer's disease (AD) and Parkinson's disease (PD). Our preliminary results show significant differential content of L1 and somatic retrotransposition in several brain regions of AD and PD patients versus controls.