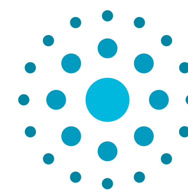

The multiple facets of RNA in development and disease

February 7-9 2018

Grand Château, Parc Valrose, Nice

Invited speakers

Victor Ambros (UMASS, USA)
Edouard Bertrand (IGMM, France)
Laurence Colleaux (Imagine Institute, France)
Andrzej Dziembowski (iBB, Varsaw, Poland)
Reini Fernandez de Luco (IGH, France)
Aimee Jackson (Miragen therapeutics, USA)
Markus Stoffel (ETH, Switzerland)
Marvin Tanenbaum (Hubrecht Institute, Netherlands)
Gian Gaetano Tartaglia (CRG, Spain)
Jernej Ule (Crick Institute, UK)
Hervé Vaucheret (IJBP, France)

http://univ-cotedazur.fr/events/rna2018

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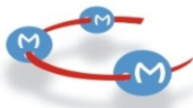

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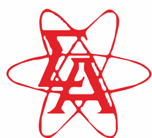

SIGMA-ALDRICH

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PROGRAMME

WEDNESDAY FEBRUARY 7th 2018

2:15 – 2:30 pm – **WELCOME REMARKS** - Barbara Bardoni Florence Besse

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2:30 – 3:30 pm – **OPENING Lecture**: Introduction and Chairperson: Bernard MARI

VICTOR AMBROS (UMASS, USA) - *Perspectives on the functions of MicroRNAs in animal development*

Session 1: Non coding RNAs: from basic research to therapy

(Chairpersons: Pierre Abad; Thomas Maurin)

3:30 – 4:00 pm - **Aimee Jackson (Miragen Therapeutics, USA)** - *Development of microRNA-based Therapeutics*

4:00 – 4:15 pm - **David Gilot (Université de Rennes, France)** - *A noncoding function of TYRP1 mRNA promotes melanoma growth*

4:15-4:45 pm Coffee break

4:45 – 5:15 pm - **Reini Fernandez de Luco (IGH, Montpellier, France) –** *Beyond the DNA: the role of chromatin and lncRNAs in the regulation of alternative splicing*

5:15 – 5:30 pm - **Maria Duca (ICN, Nice, France)** - *Interference with oncogenic microRNAs network using synthetic small molecules: scope and applications*

5:30 – 5:45 pm - **Valérie Grandjean (C3M, Nice, France)** - *RNA-mediated epigenetic inheritance of environmentally-induced pathologic variations*

5:45 – 6:15 pm - **Hervé Vaucheret (IJBP, Paris, France) -** *Regulation of gene expression by small RNAs in plants*

6:15 – 6:30 pm - **Stéphanie Jaubert-Possamai (INRA, Valbonne, France)** - *Characterization of small RNAs involved in the plant response to parasitic nematodes, genus Meloidogyne*.

THURSDAY FEBRUARY 8th 2018

Session 2: RNA Metabolism in Cancer

(Chairpersons: Enzo Lalli; Sophie Tartare-Deckert)

9:00 – 9:30 am - **Andrzej Dziembowski (iBB, Warsaw, Poland)** - *The role of FAM46C, a novel non-canonical poly(A) polymerase, in the pathogenesis of multiple myeloma*

9:30 – 9:45 am - **Patrick Brest (IRCAN, Nice, France)** - *Unexpected miRNA dynamics in tumor progression*.

9:45 – 10:00 am - **Martin Teichmann (Université de Bordeaux, France)** - *Regulation of RNA polymerase III transcription during transformation of human IMR90 fibroblasts with defined genetic elements*

10:00 – 10:15 am – **Felix Kröner (Dynamic Biosensors GmbH, Munich, Germany)** - *Biophysical characterization of Cas9 – DNA Interactions with Electro-Switchable Biosurfaces - switchSENSE*

10:15-10:45 am Coffee break

Session 3: RNA metabolism in Disease and Development

(Chairpersons: Patrick Brest ; Jean-Louis Nahon)

10:45 – 11:15 am - **Markus Stoffel (ETH, Switzerland)** - *RNA regulatory networks in metabolism*

11:15 – 11:30 am - **Elias Bechara (CRG, Barcelona, Spain)** - *When STARs influence pluripotency*

11:30 – 11:45 am - **Grégoire Savary (University of Lille 2, France)** - *Targeting non-coding RNA networks in fibrogenic diseases*

11:45 – 12:00 am - **Thomas Maurin (IPMC, Valbonne, France)** - *Fragile X Syndrome : from CLIP-assay a clue for effective therapies.*

12:00 – 12:15 pm - **Amélie Piton (IGBMC, Illkirch, France)** - *Novel genes encoding RNA binding proteins involved in post-transcriptional regulation of gene expression are mutated in intellectual disability*

12:15 – 12:45 pm - **Laurence Colleaux (Imagine Institute, Paris, France)** - *Role of miR-146a in differentiation and neural lineage determination of neural stem cell: relevance for neurodevelopmental disorders*

12:45-2:30 pm- Lunch

Session 4: Methodological advances

(Chairpersons: Florence Besse; Arnaud Hubstenberger)

2:30 – 2:45 pm - **François Berdou (Thermo Fisher) -** *Robust RNA biomarkers selection made easy with Clariom arrays*

2:45 – 3:00 pm - **Laure Emmanuelle Zaragosi (IPMC, Valbonne, France)** - *Single-cell transcriptomics unravels cell trajectories during normal and pathological regeneration of the airway epithelium*

3:00 – 3:15 pm - **Mohamed Fareh (Delft University of Technology, the Netherlands)** - *Single-molecule fluorescence reveals how Dicer-TRBP recognizes precursor microRNA in crowded cellular environment*

3:15 – 3:30 pm - **Marion Peter (IGMM, Montpellier, France)** - *A dual protein/RNA localization screen reveals locally translated mRNAs in human cells*

3:30 – 3:45 pm – **Sergio Afonso (Advanced Cell Diagnostics, Newark, USA)** - *Visualization of gene expression and of their in-situ modulations: applications of in-situ RNAscope® and BaseScope hybridization technologies*

Session 5: Spatio-temporal regulation of gene expression

(Chairpersons: Barbara Bardoni; Gaël Cristofari)

3:45 – 4:15 pm - **Edouard Bertrand (IGMM, Montpellier, France)** - *Visualization of gene expression in single living cells*

4:15 – 4:30 pm - **Danielle Adekunle (MIT, Cambridge, USA)** - *Transcriptome-wide discovery of subcellular RNA and protein localization in liver*

04:30-05:00 pm Coffee break

5:00 – 5:15 pm - **Florence Besse (iBV, Nice, France)** - *Regulating neuronal granules in space and time*

5:15 – 5:30 pm - **Jeshlee Vijayakumar (iBV, Nice, France)** - *Role of the Prion-like domain of Imp in RNP granules*

5:30 – 6:00 pm - **Marvin Tanenbaum (Hubrecht Institute, the Netherlands)** - *Live cell single molecule imaging reveals translation dynamics and control mechanisms*

Session 6: Poster session

6:30-8:30pm Cheese & Wine – Poster Session

FRIDAY FEBRUARY 9th 2018

Session 7: RNA regulatory networks

(Chairpersons: Pascal Barbry; Michele Trabucchi)

9:15 – 9:45 am - **Gian Gaetano Tartaglia (CRG, Barcelona, Spain)** - *Discovery of Protein-RNA Networks*

9:45 – 10:00 am - **Silvia Bottini (C3M, Nice, France)** - *A Nucleoplasmic Post-transcriptional Control of microRNA-Mediated Silencing*

10:00 – 10:15 am - **Diogo Ribeiro (TAGC Inserm U1080, Marseille, France)** - *Long non-coding RNAs predicted to scaffold human protein complexes*

10:15-10:45 am Coffee break

10:45 – 11:00 am - **Arnaud Hubstenberger (iBV, Nice, France)** - *P-body purification reveals how repressed mRNA regulons condense*

11:00 – 11:30 am - **Jernej Ule (Crick Institute, UK) –** *Dual evolutionary consequences of LINE-dependent RNP assembly shaped the mammalian transcriptomes*

11:30 – 11:45 am - **Gaël Cristofari (IRCAN, Nice, France)** - *Developing new genomic approaches to study LINE-1 activation in human cells*

11:45 – 12:00 am - **CONCLUSION Remarks** (Bernard Mari, Florence Besse)

12:00-13:00 pm Lunch

ORAL PRESENTATIONS

– OPENING LECTURE –

Perspectives on the functions of microRNAs in animal development

Victor Ambros

University of Massachusetts Medical School, Worcester MA USA

MicroRNAs are small non-coding RNAs that can base-pair to specific mRNAs, and thereby function, in association with an Argonaute partner protein, as post-transcriptional regulators of target gene expression. Animal genomes encode hundreds of distinct microRNAs, and since a given microRNA can base pair to scores of distinct mRNAs, this class of regulators extensively impacts the regulation of diverse biological processes, including growth, cell division, cell fate determination, and buffering developmental processes against stress. Genetic analysis of microRNA gene function in experimental animals such as C. elegans has provided an understanding of how microRNAs are integrated into broader gene regulatory networks to control and coordinate developmental processes. Broadly speaking, microRNAs can be grouped into two functional classes: One class control developmental switches, the simplest form of which consists of a single microRNA regulating expression of a single major target gene; loss-of-function mutation of such a switch-like microRNA will cause visible phenotypes owing to overexpression of the major target. A second class of microRNAs would be those that exert redundant and/or conditional functions in the context of conferring developmental or physiological robustness; loss-of-function mutations of this class of microRNA do not display visible phenotypes except in the context of particular stresses, and/or in particular genetic backgrounds. Analysis of the functions of evolutionarily conserved microRNAs in distinct experimental organisms provides insight into the roles of microRNAs in the evolution of animal form and function.

MRG-106, an Oligonucleotide Inhibitor of miR-155, Coordinately Regulates Multiple Survival Pathways to Reduce Cellular Proliferation and Survival in Cutaneous T-Cell Lymphoma

Anita G. Seto, Xuan Beatty, Joshua M. Lynch, Melanie Hermreck, and Aimee L. Jackson

Miragen Therapeutics, Inc., Boulder, USA

miR-155, a microRNA associated with poor prognosis in lymphoma and leukemia, has been implicated in the progression of cutaneous T-cell lymphoma (CTCL), in particular the mycosis fungoides (MF) subtype. We developed MRG-106, an oligonucleotide inhibitor of miR-155. Inhibition of miR-155 with MRG-106 in CTCL cell lines resulted in decreased proliferation, increased apoptosis, and transcriptional changes consistent with the function of miR-155 in survival signaling. Using clinical samples from MF patients, we demonstrate that the expression of these pharmacodynamic biomarkers is dysregulated in MF. These data demonstrate that miR-155 regulates multiple parallel survival pathways that are associated with the pathology of MF, and that inhibition of miR-155 has the potential for therapeutic benefit by modulating signaling through these multiple pathways.

A noncoding function of TYRP1 mRNA promotes melanoma growth

D. Gilot, M. Migault and MD Galibert

IGDR, Rennes, France

Competition among RNAs to bind miRNA is proposed to influence biological systems. However, the role of this competition in disease onset is unclear. Here, we report that TYRP1 mRNA, in addition to encoding tyrosinase-related protein 1 (TYRP1), indirectly promotes cell proliferation by sequestering miR-16 on non-canonical miRNA response elements (MREs). Consequently, the sequestered miR-16 is no longer able to repress its mRNA targets, such as RAB17, which is involved in melanoma cell proliferation and tumor growth. Restoration of miR-16 tumor suppressor function can be achieved in vitro by silencing TYRP1 or increasing miR-16 expression. Importantly, TYRP1-dependent miR-16 sequestration can also be overcome in vivo by using small oligonucleotides that mask miR-16 binding sites on TYRP1 mRNA. Together, our findings assign a pathogenic noncoding function to TYRP1 mRNA and highlight miRNA displacement as a promising targeted therapeutic approach in melanoma.

Beyond the DNA: the role of chromatin and lncRNAs in the regulation of alternative splicing

Reini Fernandez de Luco

IGH-CNRS, Montpellier, France

Classically, epigenetics and chromatin conformation have been involved in the regulation of purely DNA-related processes, such as DNA transcription, replication or repair. However, it has become more and more evident that regulation of gene expression is a much more complex and multifactorial process in which chromatin is intimately implicated at multiple levels, from the DNA to the RNA. One of such processes is alternative splicing, which has been involved in cell definition and cell faith. Alternative splicing is one of the most general and important biological processes in the eukaryotic cell. It affects more than 90% of human genes, it is essential for protein diversity and any misregulation of the highly cell-specific alternative splicing programs can lead to disease, such as cancer. However the mechanisms of cell-specific alternative splicing regulation are still largely unknown. I will first present the existing evidence for a role of chromatin, and in particular histone modifications, in the regulation of cell-specific alternative splicing. I will then extent those evidences by showing the combinatorial role of these histone marks in splicing regulation and the importance of antisense long non-coding RNAs in the establishment of the chromatin signatures necessary for inclusion of cell-specific splicing isoforms, adding a new layer in the regulation of alternative splicing.

Interference with oncogenic microRNAs network using synthetic small molecules: scope and applications

Cathy Staedel[1], Duc Duy Vo[2], Anh Tran[2], Fabien Darfeuille[1], Audrey Di Giorgio[2], Maria Duca[2]

1- Laboratoire ARNA, INSERM U1212 CNRS UMR5320, Université de Bordeaux, Bordeaux, France
2- Université Côte d'Azur, Institut de Chimie de Nice, UMR7272, Parc Valrose 06100 Nice, France

MicroRNAs are key factors in the regulation of gene expression and their deregulation has been directly linked to various pathologies such as cancer. The use of small molecules to tackle the overexpression of oncogenic miRNAs has proved its efficacy and holds the promise for therapeutic applications. Our work aims at the development of small-molecule drugs targeting oncogenic miRNAs production in a selective manner. Toward this aim, we employ two different but complementary approaches: (i) the design and synthesis of multimodal RNA ligands and (ii) the screening of chemical libraries for the identification of new and unexpected RNA binding compounds. Both in vitro biochemical and intracellular studies are performed in order to elucidate the molecular mechanism of action of the identified hits leading to the description of structure-activity relationships for the newly discovered RNA ligands. Thanks to these works, we demonstrated that it is possible to inhibit selectively miRNAs production using synthetic small molecules. Various applications will be described, such as the induction of the differentiation of cancer stem cells based on the involvement of miRNAs in cancer heterogeneity. These RNA ligands could thus be applied in future anticancer therapies and could also find important applications as chemical biology tools for the improvement of our understanding of miRNAs biological pathways.

RNA-mediated epigenetic inheritance of environmentally-induced patologic variations

Valérie Grandjean

Inserm, Nice, France

It is now clearly established that it is not just genes that are inherited. Our environment (i.e., dietary habits, chemical and/or stress exposure) can positively or negatively impact our lives and the ones of our progeny. This process has been defined as epigenetic inheritance. Recently, we and others extended the concept of "epigenetic inheritance" to RNA-mediated paternal heredity. Briefly, we microinjected naive embryos with sperm RNA of mice fed with high-fat (HFD) or chow diet (CD). We found that mice derived from embryos microinjected with HFD-sperm RNA, developed metabolic pathologies, such as obesity and diabetes, even when they have been fed with chow diet. We then performed a small RNA high-throughput sequencing from HFD- and control-testis RNA, uncovering the deregulation of several small-RNAs, including microRNA, piRNA and tRNA-derived small RNAs. Interestingly, those deregulated small RNA were sufficient to mediate the paternal diet-induced heredity, as demonstrated by microinjecting them into naive one-cell embryos. Altogether, these data demonstrated that sperm small RNAs represent a type of paternal epigenetic vector involved in intergenerational inheritance of diet-induced metabolic disorders.

Regulation of gene expression by small RNAs in plants

Hervé Vaucheret

Institut Jean-Pierre Bourgin, UMR1318 INRA, AgroParisTech, CNRS, Université Paris-Saclay, Centre INRA de Versailles, RD10, 78000 Versailles, France

Our group deciphers the molecular mechanisms that plant cells use to inactivate at the transcriptional or post-transcriptional level invasive sequences of endogenous (transposons, duplications) or exogenous origins (pathogens, transgenes). We are particularly interested in the interplay between these silencing mechanisms and other processes, including DNA repair, chromatin assembly, transcription, RNA maturation and export, and RNA quality control.

Characterization of small RNAs involved in the plant response to parasitic nematodes, genus Meloidogyne.

S. Jaubert-Possamai, C. Medina, M. Da Rocha, P. Abad and B. Favery

ISA, Sophia Antipolis, France

Root-knot nematodes (RKN), genus Meloidogyne, are plant parasitic worms that have the striking ability to transform plant cell fate from root vascular cylinder into hypertrophied, multinucleate and metabolically highly active feeding cells. Redifferentiation into feeding cells is the result of a massive transcriptional reprogramming of root cells targeted by RKN with a large repression of plant gene expression. Since RKN are able to induce similar feeding cells in roots of thousands of plant species, these worms are thought to manipulate essential and conserved plant molecular pathways. Our work aims to investigate the role of plant small non-coding RNAs, the microRNAs and the siRNAs, in the control of the massive transcriptional reprogramming observed during redifferentiation of root cells into feeding cells. Firstly, function of microRNA and siRNA and DNA methylation machinery in plant response to RKN was investigated by analyzing infection rate of various Arabidopsis thaliana mutants. Then small RNAs of roots infected with the RKN Meloidogyne incognita from the model plant species A. thaliana and from the tomato S. lycopersicum were sequenced by high throughput sequencing technologies. A catalog of microRNAs expressed in uninfected and infected roots was established. In Arabidopsis, we identified 24 Arabidopsis microRNAs that are differentially expressed in infected roots and we showed a role for the miR159 family in the regulation of a plant MYB transcription factor in response to M. incognita. Beyond analysis of miRNAs, we used Shortstack algorithm to analyse siRNA populations from infected and uninfected roots. We identified siRNA producing clusters that are differentially expressed in infected roots and evidenced an over-representation of the 23-24nt siRNAs in infected roots. This size corresponds to the canonical size of heterochromatic siRNAs (hcsiRNAs) which are known to regulate expression of transposons and genes at the transcriptional level, mainly by inducing DNA methylation. Comparison of expression of siRNA clusters with previous transcriptomic data identified a list of protein coding genes differentially expressed in galls that may be repressed either directly by siRNA induced post transcriptional gene silencing or indirectly via transcriptional gene silencing of neighbouring transposable elements. By comparing the results we obtained with A. thaliana and tomato we will investigate conservation of these regulations.

– SESSION 2: RNA METABOLISM in CANCER –

Regulation of Gene Expression by non-canonical poly(A) and poly(U) polymerases

Andrzej Dziembowski

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

In eukaryotes, almost all RNA molecules are processed at their 3' ends and most mRNAs are polyadenylated in the nucleus by canonical poly(A) polymerases (PAPs). Recently, several non-canonical poly(A) and poly(U) polymerases have been discovered that have more specific regulatory roles. In contrast to canonical ones, their functions are more diverse; some induce RNA decay while others, especially cytoplasmic ncPAPs, activate translationally dormant deadenylated mRNAs. In this talk, I will summarize our recent studies in which we have discovered novel unexpended functions of poly(A) and poly(U) polymerases in humans.

Unexpected miRNA dynamics in tumor progression.

Joséphine Zangari, Marius Ilie, Florian Rouaud, Laurie Signetti, Mickael Ohanna, Robin Didier, Barnabé Roméo, Dana Goldoni, Nicolas Nottet, Cathy Staedel, Jocelyn Gal, Bernard Mari, Baharia Mograbi, Paul Hofman, Patrick D Brest

IRCAN, Nice, France

MiRNAs are an important component of gene regulation, eliciting either decay or translational repression of target mRNAs. With the potential to regulate more that 30% of human genes, miRNAs are critical in biology and particularly in every step of tumor development from initiation, progression, to metastasis, behaving either as tumor suppressors or oncogenic miRNAs. Control of these master regulators thus represents a fundamental aspect of gene regulation. While mechanisms of miRNA upregulation and processing have been well documented, little is known about how miRNAs are downregulated, a necessary facet of dynamic expression. It has been postulated but not yet proven that extracellular-miRNA (ex-miRNA) once engulfed into surrounding cells, are able to promote persistent pro-tumoral activities. Nevertheless, this concept, i.e. stability of ex-miRNA, does not explain the dynamic cell plasticity observed during development and in human malignancies. To better define the function of ex-miRNA we decided to focus our attention on invasion, a spatio-temporal process in which cancer cells undergo epithelial-mesenchymal transition (EMT) and its reversal, mesenchymal-epithelial transition (MET).

Regulation of RNA polymerase III transcription during transformation of human IMR90 fibroblasts with defined genetic elements.

Stéphanie Durrieu-Gaillard[1], Helene Dumay-Odelot[1], Frédéric Chibon[2], Robert G. Roeder[3], Dominique Joubert[4], Stéphan Vagner[5] and Martin Teichmann[1*]

1- Université de Bordeaux, INSERM, U1212 – CNRS UMR 5320, F-33076 Bordeaux, France
2- Génétique et Biologie des Sarcomes - INSERM U1218, F- 33000 Bordeaux, France
3- The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA
4- Institut de Génomique Fonctionnelle, UMR 5203 CNRS, F-34000 Montpellier, France
5- Institut Curie, CNRS UMR 3348, 26 rue d'Ulm, 75248 Paris, France
* corresponding author

RNA polymerase (Pol) III transcribes small untranslated RNAs that are essential for cellular homeostasis and growth. Its activity is regulated by inactivation of tumor suppressor proteins and overexpression of the oncogene c-MYC, but the concerted action of these tumor-promoting factors on Pol III transcription has not yet been assessed. In order to comprehensively analyse the regulation of Pol III transcription during tumorigenesis we employ a model system that relies on the expression of five genetic elements to achieve cellular transformation. Expression of these elements in six distinct transformation intermediate cell lines leads to the inactivation of TP53, RB1, and protein phosphatase 2A, as well as the activation of RAS and the protection of telomeres by TERT, thereby conducting to full tumoral transformation of IMR90 fibroblasts. Transformation is accompanied by moderately enhanced levels of a subset of Pol III-transcribed RNAs (7SK; MRP; H1). In addition, mRNA and/or protein levels of several Pol III subunits and transcription factors are upregulated, including increased protein levels of TFIIIB and TFIIIC subunits, of SNAPC1 and of Pol III subunits. Strikingly, the expression of POLR3G and of SNAPC1 is strongly enhanced during transformation in this cellular transformation model. Collectively, our data indicate that increased expression of several components of the Pol III transcription system accompanied by a 2-fold increase in steady state levels of a subset of Pol III RNAs is sufficient for sustaining tumor formation.

Biophysical Characterization of Cas9 – DNA Interactions with Electro- Switchable Biosurfaces - switchSENSE®

Félix Kröner

Dynamic Biosensors GmbH, Münich, Germany

From recent studies it is seen that the CRISPR/Cas9 system has the potential to become a versatile tool to revolutionize medicine by facilitating researchers to edit DNA targets. Although the system shows promising applications in the field of gene therapy to improve its utility its off-target activity still must be greatly suppressed. Here we present the findings of switchSENSE technology to study the binding kinetics of CRISPR/Cas9 system which binds and cuts DNA using guide RNAs.

switchSENSE® is an automated biosensor chip based technology that employs electrically actuated DNA nanolevers for the real-time measurement of binding kinetics, affinities, and enzymatic activities. switchSENSE demonstrates biomolecular interactions involving proteins, nucleic acids, and small molecules with exceptional sensitivity, i.e. the quantification of dissociation constants (K_D) in the femto-molar concentration regime and the real-time measurement of association and dissociation rate constants (k_{on}, k_{off}). High-affinity and complex interactions are characterized by employing variable capture molecule densities and DNA nanostructures.

Apart from presenting the binding kinetics of the Cas9 system we also elucidate a standardized workflow to study sequence variations by hybridizing unlabeled target DNAs of interest to a labeled anchor-DNA tethered to the gold surface. We also compare the influence of mismatches in sequences on the association and dissociation rates of Cas9 using a simplified workflow.

Furthermore, we assessed the influence of Heparin on specific and unspecific binding of Cas9 to its target sequence at different concentrations of Heparin and Cas9. We found that while Heparin suppresses unspecific binding of Cas9, at a concentration of 30 µg/ml, the specific binding is further suppressed to less than 1%.

– SESSION 3: RNA METABOLISM in DISEASE and DEVELOPMENT –

RNA regulatory networks in metabolism

Markus Stoffel

ETH, Zürich, Switzerland

When STARs influence pluripotency

Alessandro Dasti, Maria Carla Antonelli, Elias Bechara and Gian Gaetano Tartaglia

Centre for Genomic Regulation, Barcelona, Spain

The molecular basis underlying embryonic stem cell (ESCs) pluripotency and their differentiation in all types of somatic cells is subject of intensive research. Recently, the potential involvement of RNA Binding Proteins (RBPs) in these processes started to gain high interest. The Signal Transduction and Activation of RNA (STAR) is a family of RBPs that consists of 5 members, Sam68, Quaking, Slm1, Slm2 and SF1. They are highly conserved and share a common KH domain allowing them to bind RNA targets. They are involved in regulating RNA processing, localization, stability and translation. Making use of knock down and Knock out experiments using shRNAs and CRISPR-Cas9 respectively, we have found that Sam68 depletion affects cell proliferation and colony formation rather than the exit from pluripotency. On the other hand, RNA-seq data show that Sam68 is involved in mESCs differentiation, leading to a higher expression of neuroectodermal markers and a reduced expression of mesodermal markers. These results were confirmed by real time PCR. Moreover, Sam68 depletion leads to a defect in the generation of the mesodermal layer in teratoma assay. iCLIP and ribosome profiling experiments will help us to decipher the molecular mechanisms through which Sam68 is acting to regulate embryonic stem cells differentiation. Ongoing analysis of the other members of the family will unravel the involvement of each protein in cell fate during embryonic development.

Targeting non-coding RNA networks in fibrogenic diseases.

Savary G, Buscot M, Dewaeles E, Nottet N, Fassy J,Van der Hauwaert C, K Lebrigand, Paquet A, Crestani B, Glowacki F, Bellusci S, M Perrais, Barbry P, Marquette CH, Cauffiez C, Mari B, Pottier N

IPMC, Valbonne, France

Given the paucity of effective treatments for fibrotic disorders, new insights into the deleterious mechanisms controlling fibroblast activation, the key cell type driving the fibrogenic process, are essential to develop new therapeutic strategies. We identified the long non-coding RNA DNM3OS as a critical downstream effector of TGF-Î_-induced myofibroblast activation. Mechanistically, DNM3OS regulates this process in trans by giving rise to 3 distinct profibrotic mature miRNAs (i.e. miR-199a-5p/3p and miR-214-3p), which influence both SMAD and non-SMAD components of TGF-Î_ signaling in a multifaceted way, through two modes of action consisting of either signal amplification or mediation. Finally, we provide preclinical evidence that interfering with DNM3OS function using distinct strategies not only prevents fibrosis but also improves established fibrosis, providing thus a novel paradigm for the treatment of refractory fibrotic diseases.

Fragile X Syndrome: from CLIP-assay a clue for effective therapies.

Thomas Maurin, Kevin Lebrigand, Sara Castagnola, Agnes Paquet, Marielle Jarjat, Mauro Grossi, Florence Rage, Barbara Bardoni

IPMC, Valbonne, France

Fragile X syndrome (FXS), the most common form of inherited intellectual disability, is due to the functional deficiency of the Fragile X Mental Retardation Protein (FMRP), an RNA-binding protein. To date, no specific treatment for FXS is available. We searched for FMRP targets by HITS-CLIP in multiple mouse brain regions at a developmental time when FMRP is most highly expressed and synaptogenesis reaches a peak. Our regional approach allowed us to identify the largest set of FMRP targets in the brain to date. Newly identified mRNA partners are expressed in the hippocampus and the cerebellum and point to specific roles of FMRP in these tissues, further highlighting the need of a regionalized analysis. Indeed, we classified FMRP targets in the different tissues according to their gene ontology annotations and could identify tissue specific regulated pathways such as ceramide signaling in CB and ephrin a signaling in the HC. We next questioned the cellular origins of FMRP brain targets. Our results show that FMRP binds primarily to neuronal RNA both in pyramidal cells and interneurons, with a few notable exceptions in astrocytes, microglial cells, oligodendrocytes and ependymal cells. Investigating the determinants of FMRP binding to RNA, we validated several new canonical FMRP binding sites i.e. G4 forming structures. We then identified five motifs underpinning FMRP/mRNA interaction in brain. We investigated RNA secondary structure in vivo to refine the binding modalities of FMRP to RNA. Our results show that FMRP binds to stem-loop folded regions and that a quaternary structure is required for FMRP interaction. Last, we analyzed the functional consequences of FMRP binding by performing reporter assays. We show that the translation of the vast majority of reporters is upregulated in the absence of FMRP further confirming a direct role of FMRP in repressing the translation.

Novel genes encoding RNA binding proteins involved in post-transcriptional regulation of gene expression are mutated in intellectual disability

Amélie Piton

IGBMC, Strasbourg, France

Intellectual disability (ID) is a group of neurodevelopmental disorders characterized by an extreme genetic heterogeneity, with more than five hundred genes now implicated in mendelian forms of ID. These genes encodes synaptic proteins, proteins involved in cystokeletton modulation, or protein involved in the regulation of gene expression. These latter include in one hand transcription factors and chromatin remodellers regulating transcription, and in the other hand RNA binding proteins (RNP) regulating different posttranscriptional steps which lead to protein translation (mRNA splicing, transport, degradation or translation). Twenty five years ago, the FMR1 gene, encoding the RNP FRMP, was identified as responsible for one of the most frequent monogenic form of ID, the Fragile-X syndrome. The advent of new sequencing technologies (high throughput sequencing, HTS) has revolutionized human genetics, and its use both in research and diagnostic laboratories have led to the identification of numerous novel genes responsible for ID in the last past years. Here we describe mutations identified by HTS in novel ID genes encoding RNP involved in regulation of splicing (NOVA2), mRNA degradation or initiation of translation (CNOT3, etc). We also review all the RNP recently identified as involved in ID or other neurodevelopmental disorders.

Role of miR-146a in neural stem cell differentiation and neural lineage determination: relevance for neurodevelopmental disorders

Lam Son Nguyen, Julien FREGEAC, Christine Bole-Feysot, Patrick Nitschke, Anand Iyer, Jasper Anink, Eleonora Aronica, Olivier Alibeu, Nicolas Cagnard, Laurence Colleaux

Imagine Institut, Paris, France

MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression at the post-transcriptional level. miRNAs have emerged as important modulators of brain development and neuronal function and are implicated in several neurological diseases. Previous studies found miR-146a upregulation is the most common miRNA deregulation event in neurodevelopmental disorders such as autism spectrum disorders (ASD), epilepsy and intellectual disability (ID). Now, we elucidated its role in early brain development using in vitro and in vivo techniques. We first showed that miR-146a is upregulated in the temporal lobe of autistic children. Using H9-derived human neural stem cells, we found that miR-146a overexpression enhances neurite outgrowth and branching and favors differentiation into neuronal like cells. Expression analyses revealed that ten percent of the transcriptome was deregulated and organized into two modules critical for cell cycle control and neuronal differentiation. We also found deregulated neuronal lineage markers. Transcriptomic analyses of mouse brain samples at E14, P30 and P60 indicated spatial- and temporal-specific effects of miR-146a inactivation. We observed that loss of miR-146a mainly impacts neuron development. Pyramidal and interneurons are the most severely affected cell types with deregulated pathways reflecting different stages of neuronal differentiation and synaptic maturation. Further, in silico analysis suggests the SHH signaling pathway is a master mediator of miR-146a deregulation effects. Taken together, our results suggest that the pro-neuronal effects of miRNA overexpression is caused by an impaired balance between neural progenitor cell renewal and neuronal differentiation and that miR-146a also plays a key role in neural lineage determination. Our results provide new insight into the molecular events that link miR146a overexpression to impaired neurodevelopment and may yield new therapeutic targets and strategies.

– **SESSION 4: METHODOLOGICAL ADVANCES –**

Robust RNA biomarkers selection made easy with Clariom arrays

François Berdou, ThermoFisher

Existing gene expression solutions either lack the depth coverage to identify rare transcripts, or are resource-intensive for large-scale biomarker investigations.
The new Clariom arrays offer the most comprehensive coverage of the human, mouse, and rat transcriptomes commercially available with content sourced from 15 public data sources, including RefSeq, NONCODE, Ensembl, Vega, lncRNAWiki, and RNAcentral, among others. That ensure that the discoveries are real and biomarkers are not missed.
You can extract data from as little as 100 pg of total RNA from a wide range of common and challenging sample types, including formalin-fixed, paraffin-embedded (FFPE) tissues and whole blood, without the need for globin mRNA reduction or rRNA removal.
Analysis the datas with the free Transcriptome Analysis Console software, you can independently go from sample to analyzed results in 3 days.

Two transcriptome-level solutions

- Clariom D (deep) Quickly find key biomarkers with transcriptome-level assays that detect coding and long non-codinggenes, exons, and alternative splicing events, including rare transcripts.
- Clariom S (shallow) Get answers with gene-level assays that rapidly measure changes in well-annotated genes and pathways.

When you have precious samples

- Unravel expression profiles from as little as 100 pg of total RNA or as few as 10 cells.
- Analyze RNA from a wide variety of sample types including LCM cells, whole blood, and fresh/fresh frozen or formalin-fixed paraffin-embedded (FFPE) tissues.

Preserve sample integrity and reduce variability with no need for globin or ribosomal RNA removal.

Single-cell transcriptomics unravels cell trajectories during normal and pathological regeneration of the airway epithelium

Sandra Ruiz Garcia, Marie Deprez, Kevin Lebrigand, AgnÃ¨s Paquet, Marie-Jeanne Arguel, AmÃ©lie Cavard, Brice Marcet, Pascal Barbry, Laure-Emmanuelle Zaragosi

IPMC, Valbonne, France

The upper airway epithelium is mainly composed of 3 cell types: multiciliated (MCCs), goblet, and basal cells. After respiratory injuries, this tissue regenerates through proliferation and differentiation of progenitor cells in order to restore a proper mucociliary function. In chronic airway diseases, such as chronic obstructive pulmonary disease or asthma, the injured epithelium frequently displays defective repair leading to tissue remodeling, characterized by a loss of MCCs and mucus hyper-secretion. As the accurate events governing the differentiation of the airway epithelium have not yet been characterized, we have used single cell transcriptomics to characterize the sequence of cellular and molecular processes taking place during airway mucociliary epithelium regeneration in healthy or pathological conditions such as asthma. This single-cell approach has made possible the accurate characterization of airway subpopulations with high resolution, and lineage tracing algorithms have unraveled cell trajectories from basal cells to differentiated cells, identifying novel intermediate progenitor cell populations. Finally, we have applied this technique to the analysis of pathological tissue remodeling and have reported a drastic modification of cell populations with redistribution within the basal cell compartment, decrease of MCCs and increase in the goblet cell populations. Our study demonstrates the power of single-cell transcriptomics to elucidate cell lineages during differentiation processes.

Single-molecule fluorescence reveals how Dicer-TRBP recognizes precursor microRNA in crowded cellular environment

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The RNA binding protein TRBP is a central component of the Dicer complex. Despite a decade of biochemical and structural studies, the essential functionality of TRBP in microRNA (miRNA) biogenesis remains poorly understood. Here we show that TRBP is an integral cofactor for time-efficient Dicer processing in crowded cellular environments. We competed for Dicer processing of pre-miRNA with a large amount of cellular RNA species and found that Dicer-TRBP, but not Dicer alone, remained resilient (1). To apprehend the mechanism of this substrate selectivity, we employed single-molecule fluorescence technique (1,2). The real-time observation revealed that TRBP acts as a gatekeeper, precluding Dicer from engaging with pre-miRNA-like substrates. During the initial collision, double-stranded RNA (dsRNA) binding domains of TRBP randomly trap dsRNA molecules and orient them toward Dicer's PAZ domain to probe the authenticity. This dual recognition mechanism allows Dicer-TRBP to rapidly reject pre-miRNA-like molecules and engage a productive binding with canonical pre-miRNA. Our recent single-molecule data also show that LoqsPD, a Drosophila homolog of TRBP, assists Dicer-2 in the recognition and processing of viral dsRNA (3). This cooperation between Dicer proteins and their dsRBP cofactors accomplishes an efficient way of discarding non-canonical substrates. Our finding fundamentally changes the perception of the role of cofactors in microRNA and viral RNAs processing and suggests that other RNA processing enzymes might use similar dual mechanism for time-efficient substrate recognition.

(1) Fareh et al, Nature Communications, 2016
(2) Fareh et al, Methods, 2016
(3) Fareh et al, Nucleic Acids Research, (accepted)

A dual protein/RNA localization screen reveals locally translated mRNAs in human cells

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While most mRNAs are randomly distributed in the cytoplasm, some are localised in particular sub-cellular areas. This can be involved in mRNA storage/processing, or it can lead to local translation, a process involved in many biological functions, such as the regulation of cell polarisation, asymmetric cell division, cell migration, and neuronal functions. In order to obtain a broad view of mRNA localisation in human cells, we performed a screen in which we simultaneously analysed localisation of mRNAs and their encoded protein. This was achieved by performing single mRNA detection (smFISH) with anti-GFP oligonucleotide probes, on a collection of HeLa cell lines expressing GFP-tagged genes from stably transfected bacterial artificial chromosomes (BACs). The analysis of more than 500 mRNAs showed that around 8% of them displayed non-random localisation patterns, with several novel patterns and subcellular structures found in both interphase and mitotic cells. Interestingly, only a fraction of the localised mRNAs colocalised with their corresponding proteins, suggesting that mRNA localisation can be involved in other functions than localising the mature protein. To better understand these cases, we used an improved SunTag system to directly label translation sites. In one case where the mRNA was localised in cytoplasmic aggregates from which the mature protein was absent, we could show that the mRNA aggregates function as specialised translation factories. Our study highlights the diversity of functions of mRNA localisation in human cells.

Visualization of gene expression and of their in-situ modulations: applications of in-situ RNAscope® and BaseScope hybridization technologies

Sergio Afonso

Advanced Cell Diagnostics, Newark, USA

– SESSION 5: SPATIO-TEMPORAL REGULATION of GENE EXPRESSION –

Visualization of gene expression in single living cells

Edouard Bertrand

IGMM, Montpellier, France

Transcriptome-wide discovery of subcellular RNA and protein localization in liver

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2- Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL, USA

While a modest number of RNA transcripts have been shown to require specific subcellular localization patterns for their cellular functions, the majority of transcripts exhibit specific RNA localization patterns. General organizing principles of RNA localization remain elusive, and a first step towards better understanding these processes is mapping of RNA localization patterns in cells. We generated subcellular profiles of RNAs by resolving mouse liver homogenate through density gradient centrifugation. Proteomic analysis of the gradient confirmed separation of distinct subcellular compartments. We analyzed transcriptomes of each gradient fraction and clustered transcripts into subgroups by their expression profiles across the gradient. Strikingly, RNAs exhibiting similar profiles encode proteins associated with similar cellular compartments, protein complexes, and/or biological function according to Gene Ontology categories. For many RNA clusters, we observed enrichment of specific cis-elements within 3' UTRs, suggesting that specific trans-factors mediate the localization patterns of groups of transcripts. Similar to our observations with gene expression, preliminary analyses revealed distinct RNA isoform composition across the entirety of the gradient. In summary, high resolution subcellular fractionation on a transcriptome-wide scale can provide important insights into RNA localization regulation, by revealing groups of RNAs that co-segregate within the cell and identifying cis-elements and trans-factors that mediate these patterns.

Regulating neuronal RNP granules in space and time

Florence Besse

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In neurons, high order ribonucleoprotein (RNP) assemblies termed neuronal RNP granules have been implicated in the long-distance transport of mRNAs to axons or dendrites, and in their local translation in response to external cues. Although it has become clear that the properties of these complexes are modulated in response to developmental and environmental cues as well as aging, how such changes are achieved at the molecular and cellular levels is currently poorly understood.
Using the fruitfly as a model organism, we have identified the conserved RNA binding protein Imp as a component of neuronal RNP granules, and have shown that its transport to axons is tightly regulated during brain maturation. Furthermore, Imp function is essential for the developmental remodeling of axonal branches. Our current work aims at understanding how neuronal RNP granules are assembled, and how their properties and transport are regulated during development. Assembling and maintaining such large complexes represents a challenge for cells, as RNP granule components must first undergo a demixing reaction that segregates them from the soluble cytoplasmic fraction, and then establish extremely dynamic interactions with each others. While defective demixing will prevent the formation of RNA granules, alterations in RNA granule dynamics will generate static pathological inclusions. To identify factors controlling RNA granule assembly and turnover, we are combining different approaches including structure/function analyses, purification of RNP granules, and high throughput microscopy-based RNAi screen.

Role of the Prion-like domain of Imp in RNP granules

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Eukaryotic mRNAs are bound by RNA Binding Proteins (RBP) and packaged into diverse range of macromolecular assemblies named RNP granules. In neurons, transport RNP granules are implicated in the transport of specific mRNAs to axons or dendrites, and in their local translation in response to external cues. Although little is known about the assembly and regulation of these granules *in vivo*, growing evidence indicates that the presence of Prion Like domains (PLD) within RBPs favours multivalent protein-protein and protein-RNA interactions, promoting the transition of soluble complexes into RNP granules. Our group has recently uncovered the conserved RBP Imp as a core component of RNP particles that are actively transported to axons upon neuronal remodelling in *Drosophila* (Medioni et al., 2014). Furthermore, Imp function is required for axonal remodelling during *Drosophila* nervous system maturation. Here, we have explored the function of a PLD located at the C-terminus of Imp. In cultured cells, we observed that Imp granules formed in the absence of the PLD, yet their number and size were increased. Proteins with scrambled PLD sequence accumulated in granules of normal size and number, implying that the degree of disorder of this domain, and not its sequence, is essential for granule homeostasis. Moreover, FRAP experiments revealed that Imp PLD is important to maintain the turnover of these granules. *In vivo*, this domain is both necessary and sufficient for efficient transport of Imp granules to axons. Furthermore, mutant forms lacking the PLD do not rescue the axon remodelling defects observed upon imp loss of function. Finally, a swapping experiment in which we moved Imp PLD from the C-terminus to the N-terminus of the protein revealed that the functions of Imp PLD in granule transport and homeostasis are uncoupled, and that PLD-dependent modulation of Imp granule properties is dispensable *in vivo*. Together, our results show that Imp PLD of is not required for the assembly of RNP granules, but rather regulates granule number and dynamics. Furthermore, we uncovered an unexpected *in vivo* function for a PLD in axonal transport and remodelling during nervous system maturation.

Paradoxical role for ribosomes in Ago2-mediated mRNA cleavage

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Guided by a small RNA, Argonaute 2 (Ago2) interacts with its target mRNA, resulting in mRNA cleavage and decay. In vitro measurements have provided important insights in the binding and cleavage kinetics of Ago2, but much less is known about the behavior of Ago2 in living cells. Here, we describe a method based on our previously developed SunTag translation imaging system, which allows us to observe cleavage of single mRNAs by Ago2 in living cells. We find that Ago2 frequently cleaves its target mRNA within minutes after nuclear export, at a time that precisely coincides with the arrival of the first translating ribosome at the Ago2 binding site. Using translation drugs and different mRNA reporters, we show that ribosomes can stimulate mRNA decay by Ago2, by promoting the release of cleaved mRNA fragments from Ago2. However, the role of ribosomes in modulating Ago2 activity is paradoxical, as ribosomes also inhibit mRNA cleavage by Ago2, by displacing Ago2 molecules from the mRNA before cleavage can occur. Whether ribosomes promote or inhibit cleavage depends on the sequence of the small RNA associated with Ago2, as Ago2 showed distinct cleavage kinetics when associated with different small RNAs. In summary, through live-cell single molecule imaging, we found that ribosomes profoundly alter Ago2â€™s mRNA cleavage activity by modulating distinct rate constants of the Ago2 cleavage cycle.

– SESSION 6: RNA REGULATORY NETWORKS –

Discovery of Protein-RNA Networks

Gian Gaetano Tartaglia

CRG, Barcelona, Spain

A Nucleoplasmic Mechanism to Control microRNA-Mediated Silencing

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There is a growing body of evidence about the presence and the activity of the miRISC in the nucleus of mammalian cells. Here, we showed by quantitative proteomic analysis that Ago2 interacts with nucleoplasmic Sfpq in a RNA-dependent fashion. By HITS-CLIP and transcriptomic analyses in P19 stem cells, we demonstrated that Sfpq directly controls the miRNA targeting of a subset of miRNA-target mRNAs when it binds locally (for both ectopic let-7a and endogenously expressed miRNAs). These data were validated in both mouse P19 and human NTERA-2 stem cells. Although Sfpq interacts with Ago2/miRNAs only in the nucleoplasm, it modulates miRNA targeting in both nucleoplasm and cytoplasm, indicating a nucleoplasmic imprinting of Sfpq-target mRNAs. Mechanistically, Sfpq binds to a set of long 3'UTR recognizing two specific binding motifs with a core composed by UGU sequence, as we determined by de novo motif analysis from Sfpq HITS-CLIP analysis. Sfpq uses these two binding motifs to form long aggregates to optimize miRNA position/recruitment to selected binding sites, as we showed by HITS-CLIP analysis and on Lin28A 3'UTR using gene reporter assay and atomic force microscope. Finally, Sfpq regulates the let-7-dependent gene expression program to ultimately elicit the exit from stem cell status and to promote differentiation towards a neuron-like phenotype. In conclusion, these data extend the miRNA-mediated post-transcriptional gene silencing into the nucleoplasm and indicate that a unique Sfpq-dependent post-transcriptional strategy takes place in cells for controlling miRNA-targeting activity on mRNA containing long 3'UTRs.

Long non-coding RNAs predicted to scaffold human protein complexes

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The human transcriptome contains thousands of long non-coding RNAs (lncRNAs). Characterizing their molecular and functional properties is a current challenge. An emerging concept is that lncRNAs serve as protein scaffolds, forming ribonucleoproteins and bringing proteins in proximity. However, only a few scaffolding lncRNAs have been characterized and the prevalence of this function is yet unknown. Here, we propose the first computational approach aimed at predicting scaffolding lncRNAs at a large scale. We produced the largest predicted human lncRNA-protein interaction network to date using the catRAPID omics algorithm. In combination with tissue expression and statistical approaches, we identified 847 lncRNAs (~5% of the long non-coding transcriptome) predicted to scaffold half of the known protein complexes and network modules. We show that our lncRNA scaffolding candidates contain a significant number of known functional lncRNAs such as lncRNAs affecting cell growth, harboring mammal-conserved structural features or disease-associated lncRNAs. Lastly, we show that the association of certain lncRNAs to disease may involve their scaffolding ability.Overall, our results suggest for the first time that RNA-mediated scaffolding of protein complexes and modules is a common mechanism in human cells.

P-body purification reveals how repressed mRNA regulons condense

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Within cells, soluble RNPs can switch states to co-assemble and condense into liquid droplets, semi-liquid hydrogels, or solid aggregates. We previously showed how these phase transitions are tightly controlled during development and identified regulatory pathways (1,2). Although phase transitions have been reconstituted, for endogenous bodies the diversity of the components, the specificity of the interaction network, and the function of the co-assembly remain to be identified. By developing a Fluorescent Activated Particle Sorting method to purify cytosolic P-bodies from human cells (3), we identified hundreds of proteins and thousands of mRNAs that structure a uniquely dense network of specific interactions, separating P-bodies from other RNPs. mRNAs segregating to P-bodies are translationally repressed but not decayed, which explains part of poor genome-wide correlation between RNA and protein abundance. Condensation itself strengthens the repression of thousands of mRNAs, adding a layer of coordination. Last, P-body mRNAs strikingly encode regulatory processes. Thus, we uncovered unique mRNA regulons, where RNP condensation segregates co-repressed mRNAs that can be mobilized for adaptive and coordinated expression.

(1) Hubstenberger, A., Noble, S.L., Cameron, C., and Evans, T.C. (2013). Translation Repressors, an RNA Helicase, and Developmental Cues Control RNP Phase Transitions during Early Development. Dev Cell 27, 161-173.
(2) Hubstenberger, A.*, Cameron, C., Noble, S.L., Keenan S., and Evans, T.C (2015). Modifiers of solid RNP granules control normal RNP dynamics and mRNA activity in early development. J Cell Biol 211(3), 703-16.
(3) Hubstenberger, A., Courel, M., BÃ©nard, M., SouquÃ¨re, S., Ernoult-Lange, M., Chouaib, R., Yi, Z., Morlot, J.B., Munier, A., Fradet, M., Daunesse, M., Bertrand, E., Pierron, G., Mozziconacci, J., Kress, M., Weil, D. (2017). P-body purification reveals the condensation of repressed mRNA regulons. Mol Cell 68(1),144-157.

Dual evolutionary consequences of LINE-dependent RNP assembly shaped the mammalian transcriptomes

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It is challenging for RNA processing machineries to correctly select exons within long intronic regions. We find that LINE repeat sequences (LINEs) contribute to this selection by recruiting dozens of RNA-binding proteins (RBPs). This includes MATR3, which promotes binding of PTBP1 to LINEs. Both RBPs repress splicing and 3â€™ end processing within and around LINEs, as demonstrated in RNAseq data from cultured human cells and mouse brain. Notably, dozens of repressive RBPs preferentially bind to evolutionarily young LINEs, which are confined to deep intronic regions. These RBPs insulate both LINEs and surrounding regions from RNA processing. Upon evolutionary divergence, gradual loss of insulation diversifies the roles of LINEs. Older LINEs are located closer to exons, are a common source of tissue-specific exons, and increasingly bind to RBPs that enhance RNA processing. Thus, LINEs are hubs for assembly of repressive RBPs, and contribute to evolution of new, lineage-specific transcripts in mammals.

Developing new genomic approaches to study LINE-1 activation in human cells

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The impact of transposable elements in human health and disease, and particularly LINE-1 (L1), has received considerable attention these last years. Despite their extraordinary abundance within the human genome, and their consequences on genome stability, we are only starting to understand the biological processes underlying their reactivation. L1 retrotransposons are transcriptionally silent in many somatic tissues, but are expressed and mobilized in the early embryo, in the brain, and in nearly half of epithelial cancers. Of note, among the hundreds of thousands L1 insertions present in the human genome, only the youngest and human-specific subset, named L1HS, is able to jump in modern humans and to generate disease-causing mutations, even if older families can be transcribed. Despite the diversity of L1 subfamilies, most studies consider L1 elements as a whole, and never at single copy resolution. We have tackled a simple but fundamental question to understand L1 mobilization: Is the high L1 activity observed in particular cell-types derived from a few deregulated L1HS instances or does it represent a bulk activation of most copies? Approaching this question has so far presented a formidable technical challenge, due to the highly repetitive and dispersed nature of L1HS elements and their extreme degree of insertional polymorphism between individuals. We will present how novel genomics approaches have allowed us to answer this question.

POSTER PRESENTATIONS

Characterization of long non coding RNAs (LncRNA) implicated in T cell acute lymphoblastic leukemia (T-ALL)

Jose David Abad Flores[1] Eve-Lyne Mathieu[1], Mohamed Belhocine[1], Marc-Antoine Garibal[4], Nicolas Fernandez[3], Beatrice Loriod[3], Agata Cieslak[2], Denis Puthier[1], Vahid Asnafi[2] and Salvatore Spicuglia[1]

1- Technical advances for Clinics and Genomics Laboratory, Marseille
2- Necker-Enfants-Malades Institute, Paris
3- Transcriptomic and Genomic Marseille-Luminy, Marseille
4- Cancéropole PACA, Marseille

Transcription of essentially the entire eukaryotic genome generates a myriad of long non-coding RNA (lncRNA) species that show complex overlapping patterns of expression and regulation. Although the function of most lncRNAs remains unknown, many of them have been suggested to play important roles in the regulation of gene expression during normal development and diseases, including cancers. T cell acute lymohoblastic leukemias (T-ALLs) are aggressive hematologic tumors resulting from the malignant transformation of T cell progenitors. We used RNA-Seq to identify and characterize the expressed lncRNAs in human T-cell precursors, as well as, in a subset of T-cell acute lymphoblastic leukemia (T-ALL) samples. These different datasets led to the identification of potentially deregulated lncRNAs in T-ALLs. It is expected that some of the lncRNAs identified by this project will have critical functions during T-cell development and/or being implicated in leukemogenesis. Thus, they could provide potential new targets for the development of drugs or at least, they could be used as biomarkers for the detection and prognostic of T-ALL. Since the function of LncRNAs cannot be predicted based on the genomic sequences as for the coding genes, we are using different approaches in order to prioritize the LncRNAs most likely to be involved in T-ALL leukemogenesis. The prioritization approaches are based on 1) epigenomic profiles; 2) a high throughput screening of functional LncRNAs (CRISPRi) and 3) a candidate approach based on the expression variance. Notably, we are showing here the proof of concept that epigenetic marks could be used as a prioritization strategy, based on bio-informatics and biological analysis.

Differential expression of P2RX7 and its splice variants in human lung cancer

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2- Department of Pulmonary Medicine and Thoracic Oncology, Pasteur Hospital, FHU Oncoage, Nice, France
3- Laboratory of Clinical and Experimental Pathology and Biobank, Pasteur Hospital, FHU Oncoage, Nice, France.

Scientific context: Lung cancer is a frequent and severe disease. Its prognosis is bad when tumour is diagnosed at a late stage. Therefore, this pathology represents a health public problem. Despite recent progresses in the care of those patients (targeted therapies and imunotherapies), such tumors are often untreatable and new approaches are needed. We believe that the purinergic P2RX7 receptor could represent a future therapeutic target since it has been previously shown in our laboratory that this protein behaves as a tumor suppressor. P2RX7 is an ATP gated cation channel. Further, stimulation with high concentration of ATP causes the opening of a non selective large pore which requires an intact C-terminal tail and has been associated to P2RX7 cytotoxicity. Functional P2RX7 is made of three monomers. SNPs and alternative splicing were described for P2RX7.These genetic modifications affect the tridimensional structure of P2RX7, with a loss of function effect leading to the loss of the antitumoral function of P2RX7. In this study, we focused on splice variants expression in healthy and pathologic human lung tissues.Results: Among the ten splice variants identified in the literature, we have designed specific primers to identify human alternative splicing. We particularly focused on the expression of three variants, B corresponding to a C-terminal tail truncated receptor which is associated with a loss of large pore formation function, H as a N-term tail truncated receptor which is associated with a loss of ATP-binding capacity, and J as a C-term and extracellular loop truncated receptor which correspond to a negative dominant. We quantified these splice variants in two types of human lung tissue: healthy and non small cell lung cancer. We observed an over-expression of splice variants in non tumour cells, but an overexpression in immune cells of each cells.
Conclusion : The aim of this project is to determine the expression of P2RX7 and its splice variants in human lung cancer and their regulation in immune and non immune cell compartments.
Perspective: Several splice variants can be expressed in one cell, implying a potential heterotrimeric structure. It is important to determine if an heterotrimeric association of P2RX7 lead to an activated receptor or not. We are in the process of investigating this point. Potential therapeutic use of P2RX7 in cancer requires elucidation of its biological characterization.

FGF8 controls COUP-TFI translation through the action of microRNA-21 in neocortical progenitor cells

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The morphogen FGF8 plays a pivotal role in neocortical area patterning through its inhibitory effect on rostral expression of the nuclear receptor COUP-TFI; however, its mechanisms of inhibition is poorly understood. In this study, we have first established an in vitro model of mouse embryonic stem cell corticogenesis, in which COUP-TFI protein is inhibited by the activation of FGF8 signaling. Then, we have over-expressed the COUP-TFI 3'UTR and showed that it significantly reduces the inhibitory effect of FGF8 on COUP-TFI translation, suggesting that this region might contain micro-RNAs responding to FGF signaling. Among the few miRNAs that target the COUP-TFI 3'UTR in silico, we found that miR-21 can efficiently counteract the FGF8 inhibitory effect in vitro and COUP-TFI protein expression in vivo. Accordingly, miR-21 expression is complementary to COUP-TFI protein distribution during early corticogenesis. These data contribute to our understanding on how regionalized expression of area mapping genes is established during neocortical arealization.

Fragile X Mental Retardation Protein regulates calcium signaling via Cacna1a

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Fragile X syndrome (FXS) is the most common form of intellectual disability and primary cause of autism. It originates from the lack of Fragile X Mental Retardation Protein (FMRP), which is an RNA-binding protein encoded by the Fmr1 gene. FMRP is involved in different steps of RNA metabolism, ranging from RNA transport (from the nucleus to the cytoplasm, but also along neuronal prolongments) to translational control of mRNAs at soma and at synapses. In the last 20 years researchers have found a large number of FMRP targets, but it is still not clear which are those playing a critical role in the syndrome. We performed Cross-Linking ImmunoPrecipitation (CLIP) experiments and found many putative targets of FMRP (around 4000). One of the most enriched targets was Cacna1a, which gene encodes for the pore-forming subunit of the Cav2.1 Voltage-Gated Calcium Channel (VGCC). This channel is particularly expressed in neurons, both in the axon terminals and in the somatodendritic compartments, and it allows the entry of calcium in the cytosol of neurons upon arrival of an action potential. This Ca2+ influx promotes many intracellular changes, like neurotransmitter release and calcium-dependent gene transcription. There are many studies in the recent literature correlating FXS with defects in ion channels (also called channelopathies), ranging from K channels, to h-channels, and of course Ca2+ channels, but Cacna1a was never studied in relation to FXS. Our goal is to analyze the correlation between the lack of FMRP and the expression of Cacna1a in primary neuronal cultures, in order to define the exact role of Cacna1a in the pathophisiology of FXS. For this purpose, we carried out calcium-imaging experiments on cultured Fmr1-null cortical/hippocampal neurons and we observed that these neurons display a weaker and slower Ca2+ response to KCl (which mimics the arrival of a depolarizing action potential) than wild type neurons. This abnormality might be due to an increased activity/expression of Cacna1a, with FMRP being the regulator of Cacna1a mRNA translation. We also showed that Cacna1a has a reduced activity/expression on the cell membrane of Fmr1-KO neurons, suggesting a possible membrane trafficking impairment.

TARBP2 a novel regulator of autophagy

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TARBP2 (Trans-Activation-Responsive RNA binding protein 2) is a double-stranded RNA binding protein and is known for its role in miRNA biogenesis. Besides binding to miRNA precursors, TARBP2 can also interact with TAR (Trans-Activation Responsive RNA) RNA and sRSE (structural RNA stability elements) loop structures of mRNAs and regulate their expression through translation or mRNA stability. Here, we identified ATG2A (Autophagy related 2A) as novel interacting transcripts of TARBP2. Using gain- and loss-of-function experiments, we showed that TARBP2 suppressed ATG2A expression. Furthermore, TARBP2-mediated suppression of ATG2A also reduced LC3-II level with and without bafilomycin AI treatment, suggesting its suppressive effect on autophagy. Importantly, we further demonstrated that TARBP2 regulation of ATG2A and autophagy is independent of Dicer or miRNA function. In conclusion, our study uncovers a novel function of TARBP2 for autophagy regulation.

***Drosophila* Imp granules, a paradigm to identify factors that regulate the assembly, stability and distribution of RNP granules**

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In vivo, RNAs and proteins are frequently packaged into dynamic macromolecular structures named mRNP granules. These include P-bodies, stress granules and neuronal transport granules. Defects in their assembly, localization and/or dynamics have been associated with several human pathologies such as the fragile-X syndrome or amyotropic lateral sclerosis. However, to date, our knowledge of the mechanisms underlying mRNP granule assembly and dismantling relies essentially on the study of P bodies and stress granules. Our laboratory is interested in the highly conserved RNA binding protein Imp whose mammalian counterparts overexpression correlates with poor prognosis in several cancers. In vivo, the laboratory has shown that Imp, involved in the axonal remodeling of mushroom body gamma neurons, is present in RNP granules visible both in neuronal cell bodies and axons. These Imp-containing RNP granules are distinct from P-bodies but contain other conserved RNA binding proteins. They are also detected in Drosophila S2 or S2R+ cultured cells. Taking advantage of this cellular model, we have undertaken a genome-wide RNAi-based visual screen to identify factors that regulate the assembly, the stability and/or the distribution of Imp-containing granules. This implies combining high throughput microscopy with the development of a computational pipeline for automatic image analysis. This pipeline first segments nuclei, discriminates healthy from dead nuclei and stores this information in an interactive SQLite database that enables experimental quality control. Then, the cytoplasm of healthy cells is segmented by filtering operations, thresholding, mathematical morphology, then refinement by active contour. Finally, GFP-Imp granules are detected within each selected cytoplasm using the SPADE algorithm, and their characteristics extracted and stored in an interactive SQLite database. Data from the pilot screen we have performed to validate the experimental design and develop our pipeline for data mining will be presented.

Identification and characterization of new molecular determinants of cisplatin resistance in lung adenocarcinoma.

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Lung cancer is a leading cause of cancer related death worldwide. Non-small cell lung cancer (NSCLC) is a common (80% of lung cancer) and aggressive subtype responsible for more than one million deaths annually. Platinum-based therapy is the first line treatment for the majority of patients with advanced stage of NSCLC. However, efficacy of cisplatin is limited by chemoresistance and side effects including nephrotoxicity. While significant progress has been made in the understanding of NSCLC drug resistance, new approaches that can reverse cancer cell resistance phenotype, potentiate cancer cells response to cisplatin warrant investigations. As recent studies have demonstrated that microRNA dysregulation can modulate resistance to anti-cancers drugs, we performed a functional screening of 1000 mimic miRNAs to comprehensively identify those significantly involved in cisplatin resistance using A549 cell line. This screening approach uncovered 6 miRNAs whose increased expression was sufficient to induce resistance of A549 cells to cisplatin. In this study, we focused on the best miRNA candidate, and further showed a significant association with survival of patients with early stage of lung adenocarcinoma. Mechanistically, we demonstrated that this miRNA targets genes involved in apoptosis and vitamin B6 metabolism, two major components of cisplatin cytotoxic effects. We are currently assessing the use of miRNA inhibitors on tumor sensitivity to cisplatin using xenograft mouse model of lung adenocarcinoma. In conclusion, this study may lead to the identification of a new therapeutic target which may potentiate cancer cell response to cisplatin treatment.

The pro-fibrotic miR-143/145 cluster regulates an extracellular matrix (ECM) remodeling program during adaptive and acquired resistance of melanoma cells to targeted

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Melanoma cells are known for their plasticity and ability to phenotype switch toward an invasive de-differentiated mesenchymal state leading to an aggressive clinical behavior. The emergence of this mesenchymal phenotype has been shown during primary, adaptive and acquired resistance to MAPK-targeted therapies in BRAF mutant melanoma cells. The mesenchymal acquired resistance is achieved by overexpression of receptor tyrosine kinases and we recently evidenced that this cell state is associated with expression of markers observed in fibrotic diseases and acquisition of cancer-associated fibroblasts (CAFs)-like ECM remodeling activities to generate a drug-tolerant microenvironment. However, epigenetic or posttranscriptional signaling networks that regulate this poorly understood phenotype remains to be defined. In this perspective, our study focused on the analysis and characterization of a pool of microRNAs (miRNAs) involved in fibrotic diseases, acting as pro-fibrotic or anti-fibrotic regulators, called "FibromiR". Here, we identify the pro-fibrotic miR-143~145 cluster as a good candidate for the ECM program activation in drug-tolerant melanoma cells through the regulation of ECM deposition and remodeling and its potential contribution in acquisition of invasiveness. Moreover, we analyzed its biological role and regulation in response to BRAF and MEK inhibitors treatment. The two miRNAs are found strongly overexpressed in mesenchymal resistant cells versus sensitive cells, and induced upon TGF-Î² or PDGF stimulation as well as in response to MAPK signaling pathway inhibition in mutant BRAFV600E melanoma cell lines. In addition, inhibition of TGF-Î² or PDGF signaling pathways in drug resistant cells leads to a decrease in cluster expression, meaning that constitutive activation of these pathways, typical of mesenchymal resistant state, might be responsible for activation of pro-fibrotic miR-143~145 cluster expression. Ectopic expression of either miRNA triggers activation of the ECM signature and induction of mesenchymal traits related to activation of STAT3, whereas inhibition of miR-143 or miR-145 impairs the up-regulation of ECM components and remodeling proteins induced by MAPK pathway inhibition. Overall, our data indicate that the miR-143~145 cluster contributes to phenotypic cell plasticity and acquisition of CAFs-like ECM remodeling activities that occur during the rewiring of melanoma cells signaling in response to MAPK-targeting therapies.

CaMKII : a novel regulator of *Drosophila* Imp neuronal RNA granules

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Ribonucleoprotein (RNP) granules are dynamic supramolecular structures composed of RNAs and proteins. Neuronal granules store and transport dormant mRNAs to axons and dendrites where they switch from packed to more accessible conformations, thus allowing local translation in response to specific signals such as developmental cues or neuronal activity. In mature neurons, RNP granule remodeling and local protein synthesis can be triggered at activated synapses upon neurotransmitter release, a process that was shown to contribute to synaptic plasticity. To date, little is known about the in vivo regulation and dynamics of neuronal granules. To tackle this question, we use as a paradigm Drosophila neuronal RNP granules containing the conserved RNA Binding Protein Imp and target mRNAs. In adults, Imp granules localize to the processes of Mushroom Body (MBs) neurons, which are involved in learning and memory and are functionally related to hippocampal neurons. Interestingly, we found that forced neuronal depolarization induced by constitutive expression of the NaChBac sodium channel, or by feeding with Acetylcholine, leads to a dramatic change in Imp granule properties in vivo. To find regulatory components of Imp neuronal RNP granules, we immunoprecipitated Imp from adult fly brains and identified associated proteins by Mass Spectrometry. As expected, a significant enrichment in RNA Binding Proteins was observed. Furthermore, we identified the activity-dependent Calcium (Ca2+) and Calmodulin (CaM)-dependent serine/threonine kinase II (CaMKII), known for its role in synaptic plasticity, learning and memory, as an Imp partner. The interaction between CaMKII and Imp is RNA-independent and relies on CaMKII activity, suggesting a model in which activity-dependent remodeling of Imp RNP granules might be mediated by CamkII activation. To study the impact of CaMKII activation/inactivation on Imp RNP regulation, we are using a combination of genetic gain and loss-of-function in vivo approaches and cultured cell-based assays. So far, our data indicate that CaMKII regulates the size, turnover and motility of Imp granules in response to Ca2+ influx, suggesting its involvement in Ca2+-dependent, and possibly activity-dependent regulation of Imp RNPs

The nuclear hypoxia-regulated NLUCAT1 long non-coding RNA variant is endowed of protumoral activity in lung adenocarcinoma

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Lung cancer is the leading cause of cancer death worldwide, with poor prognosis and a high rate of recurrence despite early surgical removal. It is therefore essential to identify new prognostic markers and new therapeutic targets. We are interested in gene regulation related to hypoxia, a factor associated with relapse of lung adenocarcinomas (LUAD). While long non-coding RNAs (lncRNA) are increasingly recognized as major gene expression regulators through various molecular mechanisms, their roles in cancer development and hypoxic response are still largely unexplored. Combining profiling studies on early stages LUAD biopsies and on A549 LUAD cell line cultured in normoxic or hypoxic conditions, we identified a subset of 8 lncRNAs that are both correlated to the hypoxic status of tumors and regulated by hypoxia in vitro. We focused on a new nuclear transcript, named NLUCAT1 that is strongly up-regulated by hypoxia in vitro and correlated to hypoxic markers and bad prognosis in LUAD samples. Full molecular characterization of NLUCAT1 showed that it is a large 9807 nt nuclear transcript variant of LUCAT1 composed of 6 exons and mainly regulated by NFÄ¸B and NRF2 (NFE2L2) transcription factors. Targeted deletion of NLUCAT1 using a CRISPR/CAS9 strategy in the A549 LUAD cell line, revealed a decrease in proliferative and invasive properties, an increase in oxidative stress and a higher sensitivity to cisplatin-induced apoptosis. Interestingly, the analysis of NLUCAT1-deficient cells transcriptome revealed repressed gene networks controled by NRF2, HIF and NFÎºB transcription factors, suggesting that this transcript could exert a positive feedback on these pathways. We identified 4 genes of the NRF2-regulated anti-oxidant response that were downregulated in NLUCAT1 knockout cells, GPX2, GLRX, ALDH3A1 and SOD2 and we demonstrated that their concomitant RNA interference significantly increased the ROS-dependent caspase activation of LUAD cells, thus partially mimicking the consequences of NLUCAT1 inactivation. Overall, our data strongly demonstrate that NLUCAT1 exerts pro-tumoral activities in early-stages hypoxic lung cancers and suggest it could represent a new potential therapeutic target in LUAD.

Non-canonical post-transcriptional regulation of SQSTM1 in tumour programming

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Population densification and ageing pose a new risk for cancer development: Exposure to concentrated air pollution. Previous studies have shown that air pollution is strongly associated with mortality from lung cancer. In France, the prevalence lung cancers has reached epidemic proportions. Although critical, there are little, if any, therapeutic options to alter cancer progression. Air pollution and particularly the particulate matter are known carcinogens. However, the causal pollutant components and physiologic mechanisms underlying inflammation and carcinogenesis are not understood. In response to these environmental pollutants, our cells exert autophagy which is an immediate adaptive cellular process that activates cells repair mechanisms and eliminates dysfunctional organelles or damaged macromolecules. Impaired autophagy does not allow anymore to maintain cell homeostasis and thus can lead to cancer. The autophagic gene SQSTM1 is particularly interesting because impaired autophagy is associated with a large list of chronic age-related diseases and especially lung cancer characterized by un accumulation of SQSTM1. Recently it has been established a non-exhaustive list of potential targets of DROSHA whose SQSTM1. DROSHA is a double-stranded RNA-specific RNase III enzyme which initiates microRNA maturation. DROSHA has also the capacity to cleave messenger RNA, known as its non-canonical activity but it remains unclear to what extent DROSHA functions outside the miRNA pathway. In order to define the non-canonical activity of DROSHA and its impact on the regulation of SQSTM1, our strategy is to characterize the cellular phenotype induced by DROSHA invalidation or overexpression. Furthermore, we are going to investigate whether pollutants, which affect autophagy, could deregulate DROSHA and contribute to tumour programming.

Steroidogenic Factor-1 is a Goldilocks transcription factor regulating key genes implicated in adrenocortical tumourigenesis

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The transcription factor Steroidogenic Factor-1 (SF-1; NR5A1) regulates tissue-specific gene expression in steroidogenic cells and has an essential role in the development of adrenal glands and gonads. Furthermore, it is implicated in the pathogenesis of adrenocortical tumours and its overexpression correlates with a severe prognosis in adrenocortical carcinoma (ACC). We have previously shown that SF-1 regulates distinct set of genes in ACC cells according to its dosage. Those genes are involved in defining several aspects of the malignant phenotype in ACC cells. We have performed detailed kinetic analyses of the expression of genes regulated by SF-1 in ACC H295R cells. Our results reveal that different thresholds of gene activation or repression exist according to the precise levels of SF-1 expression. We could also ascertain that SF-1 autoregulates the expression of its own transcript, a property that can be relevant to modulate its levels in ACC. The scenario then emerges of SF-1 working as a Goldilocks transcription factor, each dosage of the protein optimally regulating a specific set of target genes. These properties are likely to have an important role for transcriptional regulation of gene expression by SF-1 in steroidogenic tissue development, physiology and disease. Further studies are in progress with the aim to correlate SF-1 target gene expression to local chromatin modifications and to perform single-cell gene expression analysis in conditions of basal and increased SF-1 dosage in tissue culture cells and in a new mouse model of Sf-1 overexpression in the same tissues expressing its endogenous transcript.

Root knot nematode target plant host RNA splicing machinery allowing cellular transcriptional reprogramming and feeding cells ontogenesis.

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Plant parasitic nematodes are microscopic worms. The most damaging species have adopted a sedentary lifestyle within their hosts. These obligate endoparasites are biotrophs that induce the differentiation of root cells into hypertrophied, multinucleate feeding cells. Effector proteins synthesized in the nematode esophageal glands are injected into the plant cells via a syringe-like stylet and are required to modulate many aspects of plant cell morphogenesis and physiology leading to the establishment of the feeding giant cells. Genomics and transcriptomics approaches allowed identification of a large panel of putative effectors. We identified a gene (MiEFF18) specifically expressed in the esophageal glands of parasitic juveniles that encode a protein carrying both a nucleolar and a nuclear localization signal. Using a yeast-two hybrid approach, we identified a nuclear host target of the effector MiEFF18 which encode for a plant component of the spliceosome modulating alternative splicing. We validated this interaction in planta. Silencing or knock-out of this target impacted the fitness of the nematode in different plants. We aim at deciphering the role of MiEFF18 effector in modulation of alternative splicing to allow transcriptional reprogramming of vascular root cells and giant cells formation.

MicroRNA Analysis Paired with a Novel Live Cell Viability Assay

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The requirements for nucleic acid purification for use in RNA profiling have expanded with the growing interest in the role of microRNAs (miRNAs) and other small non-coding RNAs in cancer cell growth and metastasis.This evolution of expression analysis highlights the need for more sophisticated tools for total RNA isolation beyond traditional mRNAs. Here we describe tools developed to isolate total RNA, for mRNA and miRNA analyses, in the context of an epigenetic workflow with cancer cell lines. Using this workflow with two breast cancer cell lines, with distinct responses to histone deacetylase (HDAC) inhibition, we can measure cell health, cytotoxicity, HDAC activity, and mRNA plus miRNA expression profiling in a single experiment. The manual total RNA isolation method we describe can also be used with 3D cell cultures.

Single cell RNAseq at the UCAGenomiX platform

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Introduction: It is now possible to analyze the genome of a single cell, compare it to the one from its neighbors, and detect the expression of all genes specifically expressed therein, thus establishing a molecular identity card for each cell in a specific state. Such a project, which was still unreachable a few years ago, is now achievable thanks to advances made in single cell manipulation and nucleic acid sequencing. Results: The UCAGenomiX platform has been providing single cell RNAseq technologies since the end of 2014. Our service starts after tissue dissociation into single cell. We provide commercial solutions for single cell isolation and library preparation (Fluidigm C1 since 2014, 10X Genomics Chromium System since December 2016), but also library preparation after custom single cell isolation into 96 well plates via FACS sorting, pipeting or laser capture, using Clontech Smarter V4 kits. All our in-house library preparation protocols include RNA molecules tagging with Unique Molecular Identifiers (UMI) to eliminate PCR bias (Arguel, 2016). Library sequencing is performed on our Illumina NextSeq500. Since the acquisition of our chromium system, we have run 40 samples, from 5 research projects. The system has shown high robustness, with only 2 sample failures. Our bioinformatics analyses includes read mapping and UMI counting, unsupervised clustering analysis for cell types identification and tSNE visualization of the identified clusters, then differential expression analysis between clusters for marker gene detection. Our bioinformatics team can further collaborate on additional analyses, such as cell cycle determination, trajectory or RNA velocity inference that may be relevant to your project. Conclusion: The UCAGenomiX team has been providing single cell RNAseq technologies since 2014. We are available to discuss the best wetlab and bioinformatics approaches for your research projects. Information on our current offerings and costs can be found on our website: http://www.genomique.info/

Novel Insights Into Neuronal RNP Granules Upon Aging

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Tight spatio-temporal regulation of gene expression is achieved at least partly by packaging the nascent transcripts with regulatory proteins into ribonucleoprotein (RNP) granules. In neurons, neuronal RNP granules transport mRNAs to axons or dendrites, and control their local translation in response to external stimuli. While the post-transcriptional regulatory functions of these supramolecular assemblies have been extensively studied, how neuronal RNP granule assembly and dynamics are regulated in vivo in response to developmental signals or aging remains elusive to date. Yet, proper regulation of their dynamics and size is necessary, as the formation of toxic RNP aggregates has been proposed to disrupt cell ribostasis, leading to neurodegenerative disorders such as Amyotrophic Lateral Sclerosis (ALS). In the laboratory, we study in Drosophila neuronal RNP granules that are characterized by the presence of IMP, the Drosophila ortholog of ZBP1. These granules localize in the cell body of CNS neurons and are transported selectively to the axons of Mushroom Body (MB) gamma neurons. To study the impact of aging on neuronal RNP granules, we have analyzed IMP granules in flies of increasing age. Strikingly, a progressive increase in the size and number of IMP granules was observed with increasing age of analyzed flies. Such an increase did not result from variations in Imp expression levels. Furthermore, the large IMP granules observed in aged flies contained *profilin* mRNA, and did not colocalize with Ubiquitin or aggregation markers such as p62, suggesting that they do not correspond to protein aggregates. Age-dependent increase in Imp granule size is also not induced by neuronal activity, as it persisted after inactivation of MB gamma neurons. Finally, no similar change in granule properties was observed for FMRP, Syncrip, PABP granules in aged MB neurons, suggesting a certain degree of specificity. To unravel the molecular mechanisms regulating IMP granule assembly and dynamics during aging, we have started testing the role of cellular pathways altered in aging, including ROS signaling, autophagy, proteasomal pathway and post-translational modifications. Preliminary results suggesting that IMP granules respond to the MAPK phosphorylation pathway, and physically interact with the Drosophila MAP Rolled will be presented.

Unusual cytoplasmic roles of the histone lysine methytransferase SETDB1

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SETDB1 is a lysine methyltransferase belonging to the SUV39 subfamily, which methylates histone H3 on lysine 9 (H3K9), mainly involved in gene repression. Beyond histones, SETDB1 methylates many non-histone substrates, such as UBF, p53 and Tat. Setdb1 is essential in mouse embryonic stem cells (ESCs) pluripotency and self-renewal; its knockout is lethal at the peri-implantation stage at 3.5 dpc. It is also essential for the differentiation of many progenitor cell types (spermatogenesis, neurogenesis, skeletal muscle differentiation). Moreover, SETDB1 is involved in several pathologies; SETDB1 gene is amplified in melanoma and overexpressed in lung cancer, Huntington's disease and schizophrenia. SETDB1 shows distinct subcellular and subnuclear distribution in several cell types. Our lab recently showed a SETDB1 relocalization in the cytoplasm during muscle terminal differentiation, the meaning of which remains elusive. Thus, we aimed at uncovering the cytoplasmic roles of SETDB1 and the mechanisms regulating its shuttling. For this purpose, we are trying to identify SETDB1 non-histone substrates, its partners and post-translational modifications in different sub-cellular compartments. In order to identify SETDB1-dependent proteome-wide lysine methylome we have used both the PTMScan assay coupled to SILAC (Stable Isotope Labeling by Amino acids in Cell culture) and a MBT pull-down assay (based on the enrichment of methylated proteins using L3MBTL1 protein coupled to MS). Doing this, possible SETDB1 nuclear and cytoplasmic non-histone substrates involved in the regulation of the cell cycle, transcription and translation processes were identified. We are trying now to confirm these data by in vitro methylation tests combined to functional studies. To determine SETDB1 partners, we have performed both TAP-tag coupled to mass spectrometry (MS) and a yeast two-hybrid (Y2H) screening assays. Our preliminary data confirmed that SETDB1 interacts with proteins involved in RNA processing and protein translation. In collaboration with Dr B Cosson, through the SunSET assay, we showed a statistically significant decrease in the amount of synthetized proteins in the absence of SETDB1 in mESCs, a phenotype rescued by SETDB1 WT re-expression in SETDB1 KO. In conclusion, our current data unravel unusual functions of a firstly supposed histone lysine methyltransferase and provide new insights.

UV crosslinked mRNA-binding proteins captured from leaf mesophyll protoplasts

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The complexity of RNA regulation is one of the current frontiers in animal and plant molecular biology research. RNA-binding proteins (RBPs) are characteristically involved in post-transcriptional gene regulation through interaction with RNA. Recently, the mRNA-bound proteome of mammalian cell lines has been successfully cataloged using a new method called interactome capture. This method relies on UV crosslinking of proteins to RNA, purifying the mRNA using complementary oligo-dT beads and identifying the crosslinked proteins using mass spectrometry. We describe here an optimized system of mRNA interactome capture for Arabidopsis thaliana leaf mesophyll protoplasts, a cell type often used in functional cellular assays. We established the conditions for optimal protein yield, namely the amount of starting tissue, the duration of UV irradiation and the effect of UV intensity. We demonstrated high efficiency mRNA-protein pull-down by oligo-d(T)25 bead capture. Proteins annotated to have RNA-binding capacity were overrepresented in the obtained medium scale mRNA-bound proteome, indicating the specificity of the method and providing in vivo UV crosslinking experimental evidence for several candidate RBPs from leaf mesophyll protoplasts. The described method, applied to plant cells, allows identifying proteins as having the capacity to bind mRNA directly. The method can now be scaled and applied to other plant cell types and species to contribute to the comprehensive description of the RBP proteome of plants.

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