RNA Biology in Cancer and other Diseases

Barcelona

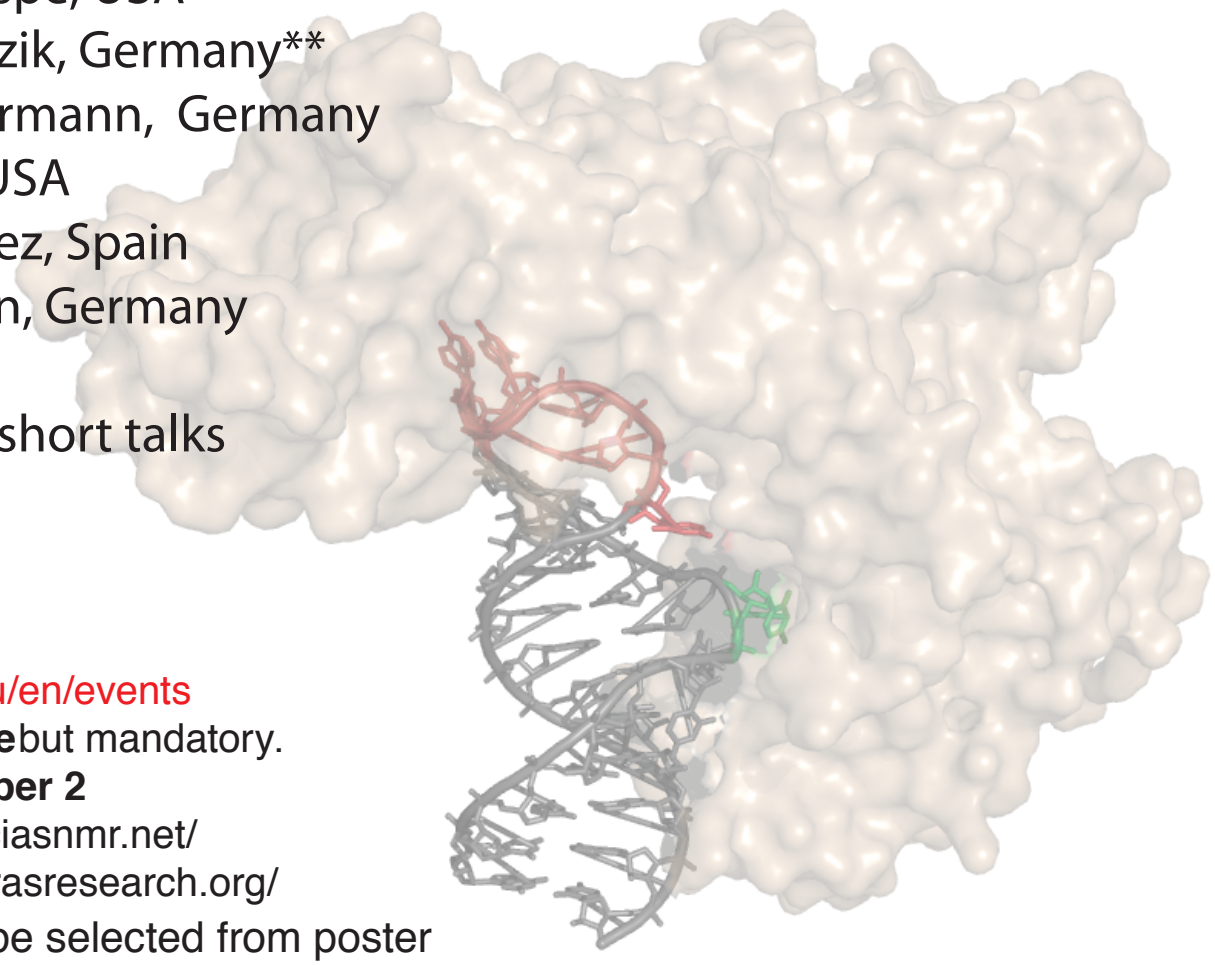
November 24-26 2015

2nd congress

Organized by

Consolider RNAREG Consortium and Josep Carreras Leukaemia Research Institute (IJC)

Claudia Bagni, Belgium
Victoria Cowling, UK*
Myriam Gorospe, USA
Andreas Kulozik, Germany**
Reinhard Luhrmann, Germany
Joel Richter, USA
Mayka Sanchez, Spain
Britta Skawran, Germany



+17 selected short talks

** EMBO lecture
*EMBO YIP Lecture

Registration at:
http://www.crg.eu/en/events
Registration is **free** but mandatory.
Deadline **November 2**
http://rnareg.maciasnmr.net/
http://www.carrerasresearch.org/
Short talks will be selected from poster abstracts.

Organized by:
Mayka Sanchez
Juan Valcarcel

JOSEP CARRERAS LEUKAEMIA RESEARCH INSTITUTE
For a future without leukaemia

RNAREG

CRG Centre for Genomic Regulation

Generalitat de Catalunya

GOBIERNO DE ESPAÑA
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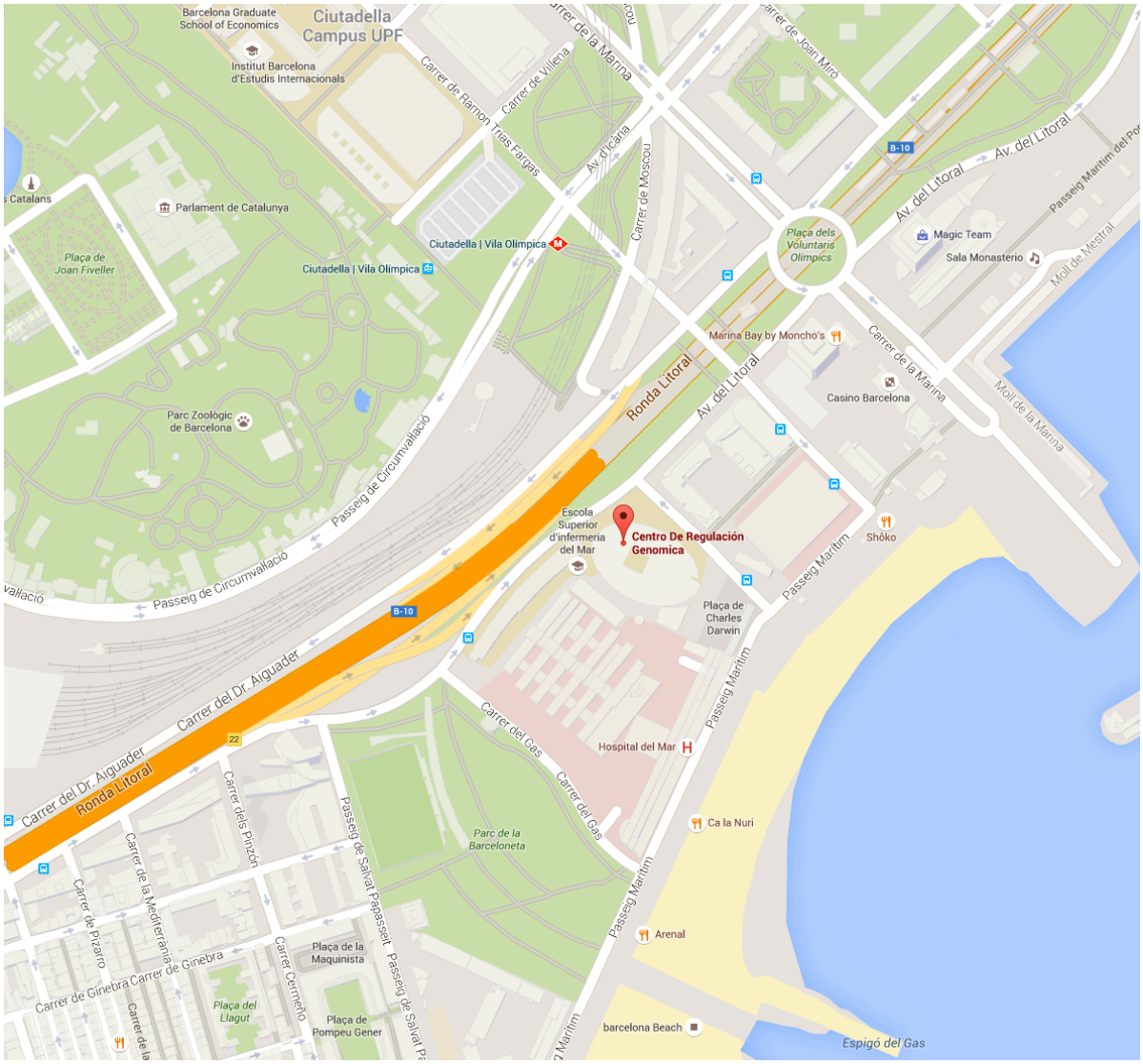
Consolider

EMBO excellence in life sciences

Transportation:

The Parc de Recerca Biomedica de Barcelona, PRBB (Barcelona Biomedical Research Park) hosting the Centre for Genomic Regulation (CRG) is located at numer 88 of Dr. Aiguader St. In the courtyard, at first floor level, is the auditorium, where the RNAREG meeting will take place. The PRBB is very close to the Mapfre Tower and Hotel Arts, at the Olympic Village. There are several different ways to get to the CRG. For further information visit the website of Transports Metropolitans de Barcelona, TMB (Barcelona Metropolitan Transports).



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Second International Meeting
"RNA Biology in Cancer and other Diseases"
Co-organized by RNAREG consortium and Josep Carreras Leukaemia Research Institute (IJC)

24-26 November 2015

Venue: Auditorium of the Barcelona BiomedicalResearch Park (PRBB), Barcelona, Spain
Organizers: Mayka Sánchez,Juan Valcárcel
Scientific Committee: Fátima Gebauer, Pablo Menéndez, Mayka Sánchez, Marisol Soengas, Francesc Solé, Juan Valcárcel

Scientific Program

Tuesday, 24th November 2015

14:00-15:00 Registration and poster hanging
15.00-15:15 Presentation RNAREG Consolider project - Juan Valcárcel, RNAREG Coordinator
15.15-15:30 Presentation of Josep Carreras Leukaemia Research Institute (IJC)- Evarist Feliu, IJC Director

Session 1
Chairperson: Francesc Solé (IJC)

15.30-16.20 Prof. Myriam Gorospe. National Institute on Aging-NIH, Baltimore, USA
Cytoplasmic RNA regulation in cancer and aging

16.20-16:35 Laurence Wurth, CRG, Barcelona, Spain
UNR/CSDE1 drives a post-transcriptional program to promote melanoma invasion and metastasis
16:35-16:50 Marina Barriocanal, CIMA, Pamplona, Spain
HCV infection upregulates EGOT, a PKR-induced long noncoding RNA that favours viral replication by affecting the antiviral response
16:50-17:05 Francesco Marchese, CIMA, Pamplona, Spain
A long non-coding RNA involved in DNA replication and sister chromatid cohesion

17:05-17:30 Coffee break

17.30-18.20 Prof. Claudia Bagni. VIB center for the Biology of Disease, Leuven, Belgium
FMRP-based control of neuronal function

18:20-18:35 Davide Cirillo, CRG, Barcelona, Spain
Discovery of ribonucleoprotein networks
18:35-18:50 Metehan Cifdaloz, CNIO, Madrid, Spain
New oncogenic networks controlled by the RNA binding factor CUGBP1 in melanoma identified by an integrated transcriptomic and proteomic analysis
18:50-19:05 Rosario Francisco-Velilla , CBMSO, Madrid, Spain
Analysis of the composition of the Gemin5 interactome

19:05-20:30- Poster Session I

Wednesday, 25th November 2015

Session 2

Chairperson: Fátima Gebauer (RNAREG, CRG)

09.00-09:50 Prof. Roderic Guigó. Centre for Genomic Regulation, Barcelona, Spain
The GTEx project: The human transcriptome across tissues and individuals

09.50-10:05 Babita Singh, UPF, Barcelona, Spain
Alternative Splicing as Driver of Cancer
10:05-10:20 Julián CerónIDIBELL, Barcelona, Spain
C. elegans as system to study the Retinitis Pigmentosa subtype caused by mutations in core spliceosome components
10:20-10:35 Panagiotis Papasaikas, CRG, Barcelona, Spain
Definition of the regulatory modules underlying tissue-specific splicing

10:35-11:00 Coffee break

11:00-11:50 THE EMBO LECTURE. Prof. Andreas Kulozik. Molecular Medicine Partnership Unit EMBL and University Heidelberg, Germany
Regulated 3' end Processing- implications for the control of Blood coagulation, Innate Immunity and Tumor spread

11:50-12:05 Alfonsina Ballester-López, UAB, Barcelona, Spain
A novel method of genetic diagnosis in McArdle disease: taking advantage of peripheral blood mononuclear cells transcription
12:05-12:20 Luisa Vigevani, CRG, Barcelona, Spain
Molecular basis for the differential sensitivity of 3' splice sites to antitumor drugs targeting U2 snRNP

12:20-13:15 Lunch

13:15-15:30 Poster Session II

Session 3

Chairperson: Pablo Menéndez (IJC)

15.30-16:20 Prof. Joel Richter. University of Massachusetts, Worcester, USA
Role of CEBP proteins in neurodegeneration

16.20-16:35 Jordina Guillén-Boixet, IRB, Barcelona, Spain
CPEB4 activity and meiotic progression are controlled through multiple phosphorylations in CPEB4 intrinsically disordered domain and the assembly of higher order structures
16:35-16:50 Rosa Pascual, IRB, Barcelona, Spain
Sequential CPEB functions regulate local translation at the mitotic spindle to coordinate metaphase to anaphase transition and cytokinesis
16:50-17:05 Olga Coll, CRG, Barcelona, Spain
Dicer-2 is involved in mRNA activation through cytoplasmic polyadenylation

17:05-17:30 Coffee break

17:30-18:20 Dr. Mayka Sánchez. Josep Carreras Leukaemia Research Institute, Barcelona, Spain
Post-transcriptional regulation by the Iron regulatory proteins and iron-response element system: new players

18:20-18:35 Carlos Maíllo, IRB, Barcelona, Spain
Translation control of hepatic metabolism by the CPEB4
18:35-18:50 Francesco Albano, University of Cantazaro, Italy
Modulation of gene expression by IBTK, a novel E3-Ubiquitin-Ligase
18:50-19:05 Gloria Lozano, CBMSO, Madrid, Spain
Fingerprinting the junctions of RNA structure by an openpaddlewheel diruthenium compound

19:05-20:30 Poster Session III

Thursday, 26th November 2015

Session 4

Chairperson: Marisol Soengas (RNAREG)

09.00-09:50 Prof. Britta Skawran. Hannover Medical School, Germany
Regulation of microRNA genes by histone acetylation in hepatocellular carcinomas

09.50-10:05 Juan L. Trincado, UPF, Barcelona, Spain
The prognostic potential of alternative transcript isoforms across human tumors
10:05-10:20 Rory Johnson, CRG, Barcelona, Spain
Detection of long non-coding RNA driver genes across 1104 tumour genomes with ExInAtor
10:20-10:35 Jordi Hernández, CRG, Barcelona, Spain
Mechanisms of NUMB alternative splicing regulation in lung cancer cells by RBM10 and SF1/BBP

10:35-11:00 Coffee break

11:00-11:50 **YIP EMBO Lecture. Dr. Victoria Cowling. School of Life Sciences, University Dundee, United Kingdom**
mRNA cap regulation in stem cell pluripotency and differentiation

11:50-12:05 Jennifer Jungfleisch, UPF, Barcelona, Spain
The DEAD-BOX helicase DHH1 promotes translation of highly structured mRNAs
12:05-12:20 Hila Gingold, Weizmann Institute of Science, Rehovot, Israel
A dual program for translation regulation in cellular proliferation and differentiation
12:20-12:35 Maria S. Soengas, CNIO, Madrid, Spain
Lineage-specific roles of CPEB4 in the control of oncogenic drivers in melanoma

12:35-12:45 Closing remarks and end of the meeting

ABSTRACTS
PLATFORM PRESENTATIONS
(in the order of the program)

MYRIAM GOROSPE

National Institute on Aging-NIH, Baltimore, USA

Cytoplasmic RNA regulation in cancer and aging

Abstract: Senescent cells accumulate in aging tissues, and their metabolic and gene expression profiles are linked to many age-related pathologies, including cancer. I will discuss our recent studies on the expression patterns and functions of senescenceassociated noncoding RNAs and the RNA-binding proteins (RBPs) with which they interact. Senescence-associated RBPs bound to long noncoding RNAs (lncRNAs) can alter protein expression patterns in the cell by suppressing the translation of select mRNAs (e.g., *LincRNA-p21* [1]), by modulating ubiquitin-mediated proteolysis of select proteins (e.g., *HOTAIR* [2]), and by inhibiting p53 production (e.g., *7SL* [3]). Other senescence-associated RBPs can bind to microRNAs; I will discuss our recent studies on the interaction of RBP AUF1 with let-7, a microRNA involved in senescence and cancer [4].

[1] Yoon et al., *Mol Cell* (2012)
[2] Yoon et al., *Nat Commun* (2013)
[3] Abdelmohsen et al., *Nuc Acids Res* (2014)
[4] Yoon et al., *Genes Dev.* (2015)

Laurence Wurth

Centre for Genomic Regulation (CRG), The Barcelona Institute of Technology, Barcelona, Spain

Universitat Pompeu Fabra (UPF), Barcelona, Spain

UNR/CSDE1 drives a post-transcriptional program to promote melanoma invasion and metastasis

Abstract: RNA binding proteins (RBPs) are gaining great attention in cancer research for their potential to regulate nearly all steps of tumor development. Yet, the molecular mechanisms that underlie these capacities are unclear, particularly in aggressive cancers such as melanoma, where RBPs remain largely uncharacterized. Here we show that the conserved RBP UNR/CSDE1 is over-expressed in melanoma tumors and selectively favors melanoma metastasis formation. iCLIP-Seq, RNA-Seq and ribosome profiling combined with in silico studies unveiled an unanticipated network of UNR targets, many of which had not been previously linked to melanoma. Mechanistically, UNR was found to control the stability and translation (remarkably at the elongation phase) of coherent sets of key pro-metastatic factors previously unknown to be regulated at the posttranscriptional level and revealing functional RNA regulons. Among these targets, Vimentin was found to restore the loss of oncogenic potential of UNR depleted cells. These results identify UNR as an oncogenic modulator of melanoma progression, unravel the underlying molecular mechanisms, and provide a resource of novel potential targets for this therapeutically challenging malignancy.

Contributions: Panagiotis Papasaikas[1,2], Guadalupe T. Calvo[3], Santiago Guerrero[1,2], Nadine Bley[4], Javier Martinez-Useros[5], María García-Fernández[3], Stefan Hüttelmaier[4], Maria S. Soengas[3] and Fátima Gebauer[1,2]*

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(2) Universitat Pompeu Fabra (UPF), Barcelona, Spain
(3) Spanish National Cancer Research Centre (CNIO), Madrid, Spain
(4) Institute of Molecular Medicine (IMM), Martin-Luther-University (MLU), Halle, Germany
(5) Health Research Institute - University Hospital "Fundación Jimenez Díaz", Madrid, Spain

Marina Barriocanal

Center for Applied Medical Research (CIMA)

HCV infection upregulates EGOT, a PKR-induced long noncoding RNA that favours viral replication by affecting the antiviral response

Abstract: We have identified hepatitis C virus (HCV)-Stimulated long noncoding RNAs (lncRNAs) (CSRs) by RNASeq and microarray analysis. Interestingly, several oncogenic lncRNAs such as PVT1 or UCA1 are induced to high levels after HCV infection, providing a novel link between HCV infection and development of liver tumors. Expression of some CSRs seems induced directly by HCV while others are upregulated by the antiviral response against the virus. In fact, sensing of the virus by the RNA-activated kinase PKR induces the expression of several CSRs. Therefore, PKR, a well-known inhibitor of translation initiation, leads to the production of molecules that function in the absence of translation. PKR senses HCV genome and activates NF-KB, which transcribes for CSR32/EGOT. Genome-wide guilt-by-association studies show that EGOT may function as a negative regulator of the interferon pathway. Accordingly, depletion of EGOT increases the expression of several interferon-stimulated genes and leads to decreased HCV replication. Therefore, after HCV infection PKR inhibits translation of cellular proteins and allows expression of lncRNAs that function to regulate the antiviral response.

Contributions: C.Prior, E.Carnero, V.Segura, E.Guruceaga and P.Fortes

CIMA/UNAV/IDISNA

Francesco P. Marchese

Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain

A long non-coding RNA involved in DNA replication and sister chromatid cohesion

Abstract: Faithful DNA replication and proper sister chromatid cohesion ensure the correct propagation of the genetic material to daughter cells during cell division. A large number of factors involved in these processes have been identified and characterized, as well as their alterations associated to genome instability and eventually tumorigenesis. However, the involvement of the non-coding components of the genome in DNA replication and cohesion remains still uncovered. Here we describe a novel human long non-coding RNA (lncRNA), DDI-1 involved in DNA replication and sister chromatid cohesion. Our results indicate that DDI-1 is cell cycle-regulated, and its depletion by siRNA or CRISPR-Cas9 affects DNA replication and cell cycle progression, leading to decreased cell proliferation and increased cell death. Moreover, cells depleted of DDI-1 show severe defects in sister chromatid cohesion, suggesting an essential role for DDI-1 in cohesion establishment and/or maintenance during cell division. Our data indicate that DDI-1 physically interacts with DDX11 helicase, which is known to be involved in DNA replication and sister chromatid cohesion. Mutations in DDX11 have been in fact associated to a rare pathological condition known as Warsaw breakage syndrome; a syndrome characterized at cellular level by sister chromatid cohesion defects. Results suggest that the binding of DDI-1 to DDX11 may be necessary for the proper function of the DNA helicase during cell division.

Finally, our results indicate that DDI-1 may act as an oncogene. It is transcriptionally controlled by c-MYC and presents higher levels in tumour cells with inactive p53. DDI-1 expression is significantly increased in a number of tumour types, suggesting a contribution of the lncRNA to the development and maintenance of the tumour.

Contributions: Alejandro Athie, Elena Grossi, Oskar Marín-Béjar, Jovanna González, Alicia Amadoz and Maite Huarte

Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain

Claudia Bagni

VIB/KULeuven, Center for the Biology of Disease, Leuven, Belgium

KULeuven, Center for Human Genetics and Leuven Research Institute for Neuroscience and Disease (LIND), Leuven, Belgium

Department of Biomedicine and Prevention, University of Rome Tor Vergata, Rome, Italy

The multi-tasking protein FMRP wires mammalian brain

Abstract: Deficiencies in the fragile X mental retardation protein (FMRP) lead to the most frequent form of inherited intellectual disability and Autism Spectrum Disorders (ASDs), the fragile X syndrome (FXS), with symptoms manifesting during infancy and early childhood. Because FMRP is expressed at very early stages of embryonic development, we hypothesize that FXS is the result of complex regulatory mechanisms occurring prenatally and at early postnatal stages, when synaptogenesis occurs.

We show here that FMRP regulates the positioning of neurons in the cortical plate during embryonic development, affecting their multipolar-to-bipolar transition (MBT). We identified a few FMRP-regulated targets crucial for MBT in embryonic brain, among them N-cadherin. Spontaneous network activity and high-resolution brain imaging at earlier postnatal stages revealed embryonic defects in the establishment of excitatory and inhibitory neuronal networks.

Furthermore, we show that the b-amyloid precursor protein (APP), involved in Alzheimer's disease, plays a role postnatally in synapse formation, and is upregulated in FXS and other intellectual disabilities. In FXS, APP signals through the metabotropic receptor that, activating the MAP kinase pathway, leads to synaptic and behavioral deficits. Proper control of APP processing is crucial for healthy spine formation and function(s).

We propose that the affected brain wiring in FXS is in part the result of dysregulated mRNA metabolism that starts during the first weeks of life and persists with remnants into adulthood.

Davide Cirillo

Centre for Genomic Regulation (CRG), Spain

Discovery of ribonucleoprotein networks

Abstract: RNA-binding proteins regulate a number of cellular processes, including synthesis, folding, translocation, assembly and clearance of RNAs [1,2]. Recent studies have reported that an unexpectedly large number of proteins are able to interact with RNA, but the partners of many RNA-binding proteins are still uncharacterized.
Using a theoretical approach [3,4], we are studying ribonucleoprotein interactions linked to inherited intellectual disability, amyotrophic lateral sclerosis, Creutzfeuld-Jakob, Alzheimer's, and Parkinson's diseases. We previously investigated RNA interactions with fragile X mental retardation protein FMRP, self-regulatory associations between proteins and their own transcripts as well as formation of ribonucleoprotein granules [5,6,7]. Our results are in striking agreement with previous experimental evidence and provide new insights that we are currently testing in our wet lab.
We recently found that co-expressed protein and RNA molecules have a high propensity to interact, which allows us to screen ribonucleoprotein networks and select candidates amenable for experimental validation. The integration of in silico and ex vivo data unraveled two major types of protein-RNA interactions, with positively correlated patterns related to cell cycle control and negatively correlated patterns related to survival, growth and differentiation [8].

References

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2. Cirillo, D., Livi, C. M., Agostini, F. & Tartaglia, G. G. Discovery of protein-RNA networks. Mol Biosyst 10, 1632–1642 (2014).
3. Bellucci, M., Agostini, F., Masin, M. & Tartaglia, G. G. Predicting protein associations with long noncoding RNAs. Nat. Methods 8, 444–445 (2011).
4. Agostini, F. et al. catRAPID omics: a web server for large-scale prediction of protein-RNA interactions. Bioinformatics 29, 2928–2930 (2013).
5. Agostini F., Cirillo D., Bolognesi B., Tartaglia GG. X-inactivation: quantitative predictions of protein interactions in the Xist network. Nucleic Acids Res. 2013 Jan 7;41(1):e31.
6. Zanzoni A., Marchese D., Agostini F., Bolognesi B., Cirillo D., Botta-Orfila M., Livi CM., Rodriguez-Mulero S., Tartaglia GG. Principles of self-organization in biological pathways: a hypothesis on the autogenous association of alpha-synuclein. Nucleic Acids Res. 2013 Dec;41(22):9987-98.
7. Cirillo D., Agostini F., Klus P., Marchese D., Rodriguez S., Bolognesi B., Tartaglia GG. Neurodegenerative diseases: quantitative predictions of protein-RNA interactions. RNA. 2013 Feb;19(2):129-40.
8. Cirillo D., Marchese D., Agostini F., Livi CM., Botta-Orfila T., Tartaglia GG. Constitutive patterns of gene expression regulated by RNA-binding proteins. Genome Biol. 2014 Jan 2;15(1):R13.

Contributions: Domenica Marchese, Riccardo Delli Ponti and Gian Gaetano Tartaglia

Centre for Genomic Regulation (CRG), Spain

Metehan Cifdaloz

Spanish National Cancer Research Center (CNIO)

Melanoma Laboratory, Molecular Oncology Programme

New oncogenic networks controlled by the RNA binding factor CUGBP1 in melanoma identified by an integrated transcriptomic and proteomic analysis

Abstract: Despite great progress in identifying genetic and epigenetic defects accumulated during melanoma progression, the molecular bases underlying the aggressive behavior of this tumor type are not completely understood. For example, malignant melanocytic cells accumulate a plethora of changes in the transcriptome and the proteome. Consequently, distinguishing drivers from inconsequential passenger events has been a main challenge in this disease. Intriguingly, although it is recognized that oncogenes and tumor suppressors can be regulated by alternative splicing of their mRNAs, the expression and regulation of spliceosome proteins remains a virtually unexplored area of research in melanoma. Here we focused on CUGBP1, a RNA binding protein that can influence RNA splicing, RNA transcription and RNA decay. Intriguingly, the limited information on CUGBP1 in cancer cells suggests a minimal overlap in the bound/regulated transcripts, a factor we considered of interest to test in melanoma. Histologically, CUGBP1 was found overexpressed in human melanoma cells and tissue specimens. Targeted gene depletion demonstrated that CUGBP1 is required to sustain melanoma cell proliferation. Splicing-sensitive mRNA arrays, RNA immunoprecipitation (RIP)-followed by mRNA sequencing and iTRAQ-MS/MS proteomics were then performed to further assess the mechanism of action of CUGBP1. Surprisingly, this combined strategy revealed minor changes in alternative splicing after CUGBP1 downregulation. Instead, Gene Set Enrichment Analysis revealed an unanticipated large network of tumor-associated factors with key roles in the control of cell cycle and chromatin metabolism acting downstream of CUGPB1. Intriguingly, while the overlap with other systems was again negligible, we identified the oncogene DEK as a common target of CUGBP1 in melanoma and different tumor cell types. Mechanistically, CUGBP1 was found to bind to the 3'UTR of DEK stabilizing its mRNA levels and ultimately allowing for an efficient cell proliferation. These results illustrate the power of comprehensive analyses of RNA regulators in the identification of novel malignant features of cancer cells.

Contributions: Lisa Osterloh[1], Erica Riverio-Falkenbach[1], Tonantzin G. Calvo[1], Belén M. Gomez[2], Pilar X.-Embún[3], Javier M. Peralta[3], Osvaldo G. Castro[4], Gonzalo G. Gómez[4], Juan Valcárcel[2] and María S. Soengas[1]

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[2]Regulation of Alternative pre-mRNA Splicing Laboratory, Gene Regulation, Stem Cells and Cancer Programme, Center for Genomic Regulation (CRG), Barcelona, Spain;

[3]Proteomics Core Unit, CNIO, Madrid, Spain;

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Rosario Francisco-Velilla

Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Nicolás Cabrera 1, 28049-Madrid, Spain

Analysis of the composition of the Gemin5 interactome

Abstract: RNA-binding proteins play a key role in every step of gene expression regulation. Gemin5 is a predominantly cytoplasmic protein with at least two distinct RNA-binding domains. The Nterminal domain binds specifically to snRNAs, and targets them for assembly into spliceosomal snRNPs. In addition, this protein harbors a non-canonical RNA binding site at the C-terminal domain that determines its interaction with IRES elements, and represses internal initiation of translation. Here we report the network of Gemin5 partners using subcellular fractionation, TAP methodologies, and polysome profiles. Interestingly, this global analysis identified a set of riboproteome components, consisting of a large set of proteins with a strong enrichment for RNA-binding proteins. Using a panel of polypeptides corresponding to N-terminal and Cterminal domains of Gemin5, we obtained a comparative characterization of the cellular partners interacting with each of these domains of the protein. We found that Gemin5 partners mainly consist of RNA-binding proteins and ribosomal proteins, with a bias towards the Nterminal domain of the protein. Importantly, RNA bridges interconnect the vast majority of Gemin5 partners, since exhaustive RNase A treatment during the process of affinity purification strongly decreased the number of proteins interacting with the N-terminal region of Gemin5.

Contributions: Javier Fernandez-Chamorro, Jorge Ramajo, and Encarnación Martinez-Salas*

Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Nicolás Cabrera 1, 28049-Madrid, Spain

Roderic Guigó

Center for Genomic Regulation, Universitat Pompeu Fabra

The GTEx project: the human transcriptome across tissues and individuals

Abstract: The pilot phase of the Genotype-Tissue Expression (GTEx) project has produced RNASeq from 1,641 samples originated from up to 43 tissues from 175 postmortem donors, and constitutes a unique resource to investigate the human transcriptome across tissues and individuals. Clustering of samples based on gene expression recapitulates tissue types, separating solid from not solid tissues, while clustering based on splicing separates neural from non-neural tissues, suggesting that post-transcriptional regulation plays a comparatively important role in the definition of neural tissues .About 47 % of the variation in gene expression can be explained by variation of across tissues, while only 4% by variation across individuals. We find that the relative contribution of individual variation is similar for lncRNAs and for protein coding genes. However, we find that genes that vary with ethnicity are enriched in lncRNAs, whereas genes that vary with age are mostly protein coding. Among genes that vary with gender, we find novel candidates both to participate and to escape X-inactivation. In addition, by merging information on GWAS we are able to identify specific candidate genes that may explain differences in susceptibility to cardiovascular diseases between males and females and different ethnic groups. We find that genes that decrease with age are involved in neurodegenerative diseases such as Parkinson and Alzheimer and identify novel candidates that could be involved in these diseases. In contrast to gene expression, splicing varies similarly among tissues and individuals, and exhibits a larger proportion of residual unexplained variance. This may reflect that that stochastic, non-functional fluctuations of the relative abundances of splice isoforms may be more common than random fluctuations of gene expression. By comparing the variation of the abundance of individual isoforms across all GTEx samples, we find that a large fraction of this variation between tissues (84%) can be simply explained by variation in bulk gene expression, with splicing variation contributing comparatively little. This strongly suggests that regulation at the primary transcription level is the main driver of tissue specificity. Although blood is the most transcriptionally distinct of the surveyed tissues, RNA levels monitored in blood may retain clinically relevant information that can be used to help assess medical or biological conditions.

Babita Singh[*]

Universitat Pompeu Fabra, Dr. Aiguader 88, E08003 Barcelona, Spain

Alternative Splicing as Driver of Cancer

Abstract: Change in alternative splicing is known to play crucial role in many diseases including cancer. Many hallmarks of cancer such as cell proliferation, angiogenesis and metastasis involve aberrant splicing patterns. However role of alternative splicing in tumor development and factors that regulate these splicing has not been studied yet in an exhaustive way. To understand splicing in cancer, we analyzed RNA and DNA sequencing data provided by The Cancer Genome Atlas (TCGA) for 11 solid tumor types from more than 4000 patients. We also studied 1348 RNA-binding proteins to understand the mechanisms behind these changes. We found several differential splicing patterns in genes between normal and corresponding tumor samples. We found novel mutations and copy number variations on RNA-binding proteins associated with splicing changes in cancer drivers and oncogenic pathways. We observed differential splicing of NUMA1, a cancer driver gene involved in mitotic process, in breast and kidney tumors. This was mainly controlled by MBNL1, a splicing factor known to be involved in cell differentiation. Knock down experiments of MBNL1 or using antisense oligonucleotides to modify NUMA1 splicing pattern lead to enhanced cell proliferation, mimicking tumor-like behavior in a normal breast epithelial cell model. Our study therefore unveils novel splicing patterns in multiple tumors and propose detailed splicing regulatory network that potentially contribute to tumor development and progression.

Contributions: Endre Sebestyén[1,*], Belén Miñana[1,2], Amadís Pagès[1], Francesca Mateo[3], Miguel Angel Pujana[3], Juan Valcárcel[1,2,4], Eduardo Eyras[1,4,5]

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Julián Cerón

Cancer and Human Molecular Genetics, Bellvitge Biomedical Research Institute-IDIBELL, Hospitalet de Llobregat, Barcelona 08908, Spain.

C. elegans as system to study the Retinitis Pigmentosa subtype caused by mutations in core spliceosome components

Abstract: Some genes identified as responsible for the autosomal-dominant form of Retinitis Pigmentosa (adRP) are ubiquitous spliceosome components whose impairment causes disease only in the retina. The fact that these proteins are essential in all organisms has hampered genetic and genomic studies in animals. We performed transcriptomic analyses of worms where s-adRP genes were partially inactivated by RNAi. Such inactivation causes mild intron retention in developing animals but not in adults, suggesting a positive correlation between intron retention and transcriptional activity in these RNA-treated animals. Interestingly, RNAi of s--- adRP genes produces an increase in the expression of *atl---1* (homolog of human ATR), which is normally activated in response to replicative stress and certain DNA---damaging agents. The upregulation of *atl-1* correlates with the ectopic expression of the pro-apoptotic gene *egl-1* and apoptosis in hypodermal cells, which produce the cuticle, but not in other cell types. We are investigating a novel mechanism based on genomic instability to explain the retina-specific cell death in s-adRP patients. The parallelisms between adRP and the phenotypes observed in our model encourage us to introduce adRP mutations in worms by CRISPR to explore potential therapies.

Contributions: Karinna Rubio-Peña, Laura Fontrodona, David Aristizábal-Corrales, Silvia Torres, Eric Cornes, Francisco Javier García-Rodríguez, Xènia Serrat, Montserrat Porta-De-La-Riva

Cancer and Human Molecular Genetics, Bellvitge Biomedical Research Institute-IDIBELL, Hospitalet de Llobregat, Barcelona 08908, Spain

Panagiotis Papasaikas

Centre for Genomic Regulation (CRG), Barcelona, Spain

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DEFINITION OF THE REGULATORY MODULES UNDERLYING TISSUE-SPECIFIC SPLICING

Abstract: The shifting of pre-mRNA intronic boundaries via the process of Alternative Splicing (AS) has a key role in establishing the differential expression programs that underlie cell differentiation and tissue morphogenesis in metazoa. Specification of AS patterns in the different cell contexts largely relies on the varying activity of a swarm of auxiliary RNA-binding splicing factors that modulate the interaction between the pre-mRNA and the spliceosome, the multipartite machinery tasked with delineating intron boundaries and catalyzing their removal. Previous work in the lab of Juan Valcarcel identified a network of functional interactions among spliceosome components and auxiliary splicing factors based on their effects on the regulation of a small number of alternative splicing events (1). Here we take advantage of the copious data generated by the GTEx consortium, including wholetranscriptome quantifications for multiple samples across over 50 tissues and subtissues, to characterize the molecular circuitry of tissue-specific splicing. Our strategy derives composite regulatory modules and the functional relationships among their components using a stepwise network reconstruction process: First modules of high-confidence tissue-specific exons along with sets of candidate regulators are inferred on the basis of their across-tissue covariance. Next, we derive refined heterogeneous networks that simultaneously capture co-regulated groups of exons as well as their putative cognate regulators utilizing information on within-tissue expression variance. Finally, we infer functional interactions between splicing regulators by modeling the predicted effects of their concentration gradation on exon inclusion. Our work offers a comprehensive view of the tissuespecific splicing regulatory circuitry and has the potential to highlight and assign function to previously uncharacterized components of the system and to pinpoint novel regulatory relationships.

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From Blood Clots to cancer, stress and regulated 3' end mRNA processing: what diseases tell us about mechanisms of gene regulation

Abstract: On New Year's Eve 1866, Armand Trousseau came to the awful realization that he would soon be dead. A celebrated French physician, he had noted that cancer patients often suffered from a greatly increased tendency to develop blood clots. These clots were associated with cancers, particularly the stomach. But on that fateful night, a mere two years after he had made his discovery, Trousseau himself developed a thrombosis in his left arm. The following day, he confided in a colleague that his symptoms left no doubt that he was suffering from gastric cancer. Sadly, he was right. The association between thrombosis and cancer has been referred to Trousseau syndrome the causes of which have remained unknown for more than 150 years. Thrombin is a key protease involved in blood coagulation, complement activation, angiogenesis, responses to inflammation and tumor invasion. Although induced in many (patho-) physiological conditions, the underlying mechanisms controlling prothrombin expression remained enigmatic. Previously, we discovered that prothrombin expression is physiologically inefficient but can be upregulated by a post-transcriptional regulatory mechanism of 3' end mRNA processing responding to stress and inflammation. This mechanism is triggered by external stimuli that activate p38 MAPK. In turn, p38 MAPK up-modulates components of the canonical 3'end processing apparatus and phosphorylates the RNA-binding proteins FBP2 and FBP3 that inhibit 3'end processing of mRNAs such as prothrombin mRNA that bear a defined upstream sequence element (USE) in their 3'UTRs. Upon phosphorylation, FBP2 and FBP3 dissociate from the USE making it accessible to proteins that stimulate 3'end processing. This mechanism induces inflammatory hypercoagulation and tumor invasion. Building on these data, we recently explored the effect of cellular stress on mRNA polyadenylation and investigated the implications of regulated polyadenylation site usage on gene expression. We combined high-confidence polyadenylation site mapping with global pre-mRNA and mRNA expression profiling and show that stress induces an accumulation of mRNAs with alternative polyadenylation (APA) sites, which affects the length of 3'UTRs, impacts on open reading frames, and quantitatively modulates the expression of distinct mRNA isoforms. This study highlights the impact of posttranscriptional mechanisms on stress-dependent gene regulation and reveals the differential expression of alternatively polyadenylated transcripts as a common stress-induced mechanism. The analysis of the molecular mechanisms predisposing to thrombosis have thus revealed regulated 3'end processing as a key mechanism of gene regulation with broad medical implications and, more generally, highlights the potential of an in-depth analysis of human disease to provide important biological insight into the complex molecular network that governs gene regulation.

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A novel method of genetic diagnosis in McArdle disease: taking advantage of peripheral blood mononuclear cells transcription

Abstract: Purpose: McArdle disease is a metabolic disorder caused by pathogenic mutations in the gene (PYGM) encoding myophosphorylase. Timely diagnosis is often difficult with direct genomic analysis, thereby requiring studies in cDNA from muscle transcripts. Yet the 'nonsense mediated mRNA decay' (NMD) eliminates tissue-specific aberrant transcripts and is highly prevalent in this disease. However, there is some residual transcription of tissue-specific genes in virtually all cells, e.g., peripheral blood mononuclear cells (PBMCs). Methods: We studied a subset of the main types of PYGM mutations (missense, nonsense, deletion, silent, or splicing mutations) in cDNA from easily accessible cells (PBMCs) in 12 McArdle patients. Results: Those mutations that might not be easily found with direct genomic DNA analyses (large deletions, silent mutations, splicing mutations) were detected in PBMCs. Because the NMD mechanism does not seem to operate in non-specific cells, PBMCs were more suitable for detecting some PYGM mutations than muscle biopsies, notably the a priori predicted synonymous silent mutation c.645G>A (p.K215), whose pathogenic effect in the splicing of intron 6 was previously unnoticed in muscle transcriptomic studies. Conclusion: We propose to use PBMCs to study mutations causing this and possibly other neuromuscular disorders to improve diagnostic protocols and gain insight into 'pathogenomics'.

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MOLECULAR BASIS FOR THE DIFFERENTIAL SENSITIVITY OF 3' SPLICE SITES TO ANTI-TUMOR DRUGS TARGETING U2 snRNP

Abstract: Three families of natural compounds with anti---tumor properties (FR901463---5, GEX1 and Pladienolides) and their synthetic derivatives (e.g. Spliceostatin A, Meayamycin and Sudemycins) share a common pharmacophore and target U2 snRNP components, notably SF3B1. We have combined genome---wide transcriptome and bioinformatic analyses with minigene assays in cancer cells in culture and biochemical experiments in nuclear extracts to study sequences and mechanisms that mediate the effects of some of these drugs. Under conditions in which the drugs exert cytostatic effects, different 3' splice sites display differential drug sensitivities, resulting in alternative splicing. Remarkably, drugs with very similar structures display distinct profiles of splicing alterations, with examples of introns whose splicing is inhibited by one drug and enhanced by another. Drug sensitivity correlates with the presence of various sequence motifs 5' of the branch point region that can be transferred to a different 3' splice site. Motifs that confer drug sensitivity include sequences that resemble branch point decoys, consistent with previous results, while additional functional branch points and polypyrimidine tracts confer drug resistance. These results show that drug response can be exquisitely sensitive to sequence context ---even to single nucleotide differences--- in the region 5' of the branch site, depending on the functional consequences of drug---induced promiscuous base pairing of U2 snRNA in this region. Collectively, our data argue for a key function of SF3B1 in 3' splice site selection, which can be modulated by anti---tumor drugs.

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Translational Homeostasis and the Fragile X Syndrome

Abstract: Translational regulation is an essential regulatory process in the brain where it mediates higher cognitive and other neural functions. When translation goes awry, cognitive impairment may result such as in the case of some autism spectrum disorders, particular the Fragile X Syndrome (FXS). FXS afflicts 1 in ~4000 children and is characterized by mental insufficiency, developmental and speech delays, avoidance of social interactions, epileptic-like seizures, and other maladies. FXS is causes by a triplet repeat expansion in the Fmr1 gene, which leads to transcriptional silencing. FMRP, the protein product of Fmr1, represses translation; when it is absent, protein synthesis in the brain is excessive and this results in the syndrome. Restoration of translational homeostasis in the brain rescues FXS in mice; experiments determining how this rescue occurs will be discussed.

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CPEB4 activity and meiotic progression are controlled through multiple phosphorylations in CPEB4 intrinsically disordered domain and the assembly of higher order structures

Abstract: Cytoplasmic polyadenylation element binding proteins (CPEBs) are a family of RNA-binding proteins essential for the translational regulation of mRNAs in various biological contexts. CPEBs recognize CPE elements in the 3' untranslated region of target mRNAs and regulate their translational fate through cytoplasmic polyadenylation. This process is especially important during meiosis, since its progression relies on the translational activation of stored maternal mRNAs. CPEB1 and CPEB4 are the two members of the family required for meiotic progression. While CPEB1 mediates the translational activation of mRNAs until metaphase I (MI), CPEB4 activates mRNAs from interkinesis to metaphase II (MII). CPEBs share a conserved RNA-binding domain and regulate overlapping mRNA subpopulations. Hence, the requirement of two distinct CPEBs to complete meiosis leans on their differential posttranslational regulation. In fact, the N-terminal domain of the CPEBs is highly variable and harbours different regulatory motifs. While CPEB1 is activated by Aurora A kinase and is targeted for degradation by Cdc2 and Plk1-mediated phosphorylation, CPEB3 is controlled by monoubiquitination and SUMOylation, which regulate the transition from an inactive monomeric CPEB3 form to an active beta-amyloid-like aggregate. How the other CPEBs are posttranslationally regulated is unknown. Nevertheless, unveiling how the different CPEBs are differentially regulated is crucial for understanding how they respond to different stimuli and how they are interconnected in particular scenarios of co-existence. We found that CPEB4 activity is regulated by hyperphosphorylation during the meiotic cell cycle. Specifically, CPEB4 is phosphorylated in twelve residues by two different kinases and in a phase-specific manner. All phosphorylated residues are located in the intrinsically disordered N-terminal half of CPEB4 and are required for cytoplasmic polyadenylation of target mRNAs. Accordingly, these twelve phosphorylation sites are essential for meiotic progression. Furthermore, we have shown that hyperphosphorylation of CPEB4 disordered domain modulates its aggregation properties. Hence, non-phosphorylated CPEB4 forms non-amyloid aggregates that specifically recruit and repress CPE-containing mRNAs, whereas hyperphosphorylated CPEB4 remains monomeric and is active in cytoplasmic polyadenylation. Importantly, CPEB4 aggregates are dynamic and reversible upon phosphorylation on the identified phosphosites. These results contribute greatly to the understanding of how the CPEBs are differentially regulated and how they would differentially respond in a given cellular environment.

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Sequential CPEB functions regulate local translation at the mitotic spindle to coordinate metaphase to anaphase transition and cytokinesis

Abstract: The CPEB family of RNA-binding proteins regulate, temporally and spatially, translational activation of maternal mRNAs to drive unidirectional phase-transitions during meiotic progressions. Although all CPEB-members bind the same Cytoplasmic Polyadenylation Element in the 3' UTR of target mRNAs their activation is differentially regulated by cell cycle regulated kinases, resulting in phase specific translational activation by distinct family members in the transcriptionally silent oocyte. In this work we have studied whether mitotic cells also utilize similar mechanisms. We found that CPEB1 localizes repressed CPE-mRNAs to mitotic spindles where they are activated after CPEB1 is phosphorylated by AurKA. This activation generates a translational gradient from the spindle poles to the metaphasic plate and, if disrupted, generates aneuploidies. In turn, one of the mRNAs regulated by CPEB1 in metaphase encodes for CPEB4, which is locally synthetized in the spindle and activated by Cdk1, probably generating a reverse gradient. Replacement of CPEB1 by CPEB4 is further directed by the CdK1-mediated destruction of CPEB1 providing a molecular basis for metaphase to anaphase transition irreversibility. Disruption of CPEB4 results in chromosomal fusions and fragmentation and cytokinesis defects. Genome wide identification of spindle-localized mRNAs bound by either CPEB1 or CPEB4 indicates that these proteins have sequential functions in the spindle and that both are required for proper mitotic completion. A temporal and spatial model of gene expression regulation in the mitotic spindle will be presented.

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Dicer-2 is involved in mRNA activation through cytoplasmic polyadenylation

Abstract: Cytoplasmic poly(A) tail elongation is a widespread mechanism to regulate mRNA translation. The biochemistry of cytoplasmic polyadenylation has been elucidated in vertebrates, where it requires two sequence elements in the 3' UTR of substrate mRNAs: the U---rich cytoplasmic polyadenylation element (CPE) and the AAUAAA hexanucleotide (Hex). These elements are recognized by the canonical polyadenylation factors CPEB and CPSF, respectively. In Drosophila early embryogenesis, cytoplasmic polyadenylation occurs in the absence of the CPEB homolog Orb. We have previously found that a non-canonical cytoplasmic polyadenylation machinery operates at this time of development (1). To identify the components of this machinery, we have identified relevant polyadenylation elements in the substrate mRNA Toll. RNA affinity chromatography using these elements uncovered the siRNA processing factor Dicer---2 as a candidate for non--- canonical polyadenylation. Depletion and co---immunoprecipitation experiments indicate that Dicer-2 interacts with the cytoplasmic poly(A) polymerase Wispy and is necessary for polyadenylation and translation of Toll and r2d2 reporters in vitro. Furthermore, embryos derived from Dicer-2 mutant mothers show defects in r2d2 polyadenylation, while general cellular polyadenylation is unaffected. These results uncover a novel function of Dicer-2 in activation of mRNA translation through cytoplasmic polyadenylation.

(1) Coll et al. A novel, non-canonical mechanism of cytoplasmic polyadenylation operates in Drosophila embryogenesis. Genes Dev. 2010, 24: 129-134.

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Post-transcriptional regulation by the Iron regulatory proteins and iron-response element system: new players

Abstract: Cellular iron homeostasis is controlled post-transcriptionally by the IRP/IRE regulatory system. The iron-regulatory proteins (IRP1 and IRP2) can recognize cis-regulatory mRNA motifs called iron-responsive elements (IREs), a conserved RNA element located in the untranslated regions (UTR) of mRNAs that encode proteins involved in iron metabolism. A canonical IRE structure is composed of a 6-nucleotide apical loop (5'-CAGWGN-3') on a stem of five paired nucleotides, a small asymmetrical bulge with an unpaired cytosine on the 5' strand of the stem, and an additional lower stem of variable length. IRP/IRE interactions regulate the expression of the mRNAs encoding proteins for iron acquisition (transferrin receptor 1, TFR1; divalent metal transporter 1, SLC11A2), iron storage (ferritin H, FTH1; ferritin L, FTL), iron utilization (erythroid 5-aminolevulinic acid synthase, ALAS2; mitochondrial aconitase, ACO2; Drosophila succinate dehydrogenase, Sdh), and iron export (ferroportin, FPN) and oxygen sensing (HIF2α). Both IRPs inhibit translation initiation when bound to 5'UTR IREs, whereas their association with the 3'UTR IREs of the TFR1 mRNA decreases its turnover by preventing mRNA degradation via an as yet unidentified endoribonuclease. Disruption of both copies of the IRP1 and the IRP2 genes in mice is embryonic lethal, indicating that the IRP/IRE regulatory network is essential. Interestingly, IRP1-deficient mice display polycythemia and pulmonary hypertension in mice through translational de-repression of HIF2α and IRP2 knock-out mice develop mild microcytic hypochromic anaemia with altered body iron distribution. Overall, the regulation of the IRE-binding activities of IRP1 and IRP2 assures the appropriate expression of IRP target mRNAs and cellular iron balance. Given the central role of the IRP/IRE regulatory system in iron homeostasis, together with the observed differences in the IRP1 and IRP2 knock-out mouse phenotypes, it has been postulated the existence of extra common and also specific IRP1/2-target mRNAs with yet undiscovered function in iron metabolism. Using a high-throughput genome-wide approach we have identified the whole repertoire of mRNAs regulated by both IRPs (CDC14A, HIF2α and others) and a cohort of IRP1 and IRP2-specific binding mRNAs. These findings open new questions about the role of the novel identified IRP-target mRNAs in iron physiology and their regulation by IRP, which will enhance our knowledge in iron related diseases and enable the development of new treatment strategies. An appropriate equilibrium in iron metabolism is essential for human health. Mutations in the IRE of Ferritin L and Ferritin H have been reported in the human disease Hereditary Hyperferritinemia with cataract Syndrome (OMIM 600886) and in an autosomal dominant hyperferritimemia with iron overload syndrome (OMIM 615517). Here we will discuss about new players controlled by the IRPs, as well as new mutations affecting IRE motifs in human diseases.

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Translation control of hepatic metabolism by the CPEB4

Abstract: Cytoplasmic polyadenylation element binding proteins (CPEBs) are sequence-specific RNA-binding proteins (RBPs) that control the translation of specific target messenger RNAs through the modulation of their poly(A) tail length. CPEB1 mediated translation in mouse liver is a key process that regulates hepatic metabolism through the control of insulin signaling. However, little is known about the role of the rest of the CPEBs in the regulation of hepatic metabolism, particularly of CPEB4, the most highly expressed CPEB in hepatocytes. To address this issue, we have generated ubiquitous and liver specific CPEB4- knockout mouse models and we have discovered that the absence of CPEB4 leads to the development of severe obesity and hepatic steatosis upon high fat diet feeding. Mechanistic studies show that these metabolic alterations are caused by impaired lipoprotein secretion and reduced mitochondrial β-oxidation in CPEB4-null livers. In hepatocytes, CPEB4 localizes to the endoplasmic reticulum (ER) where it regulates the translation of several mRNAs encoding for ER and mitochondrial proteins. Our results show that CPEB4 mediated translational control in liver is essential to maintain hepatic energy homeostasis in conditions of metabolic stress.

(1) Coll et al. A novel, non-canonical mechanism of cytoplasmic polyadenylation operates in Drosophila embryogenesis. Genes Dev. 2010, 24: 129-134.

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Modulation of Gene Expression by IBTK, a novel E3-Ubiquitin-Ligase

Abstract: The protein IBtkα is characterized by BTB-Ankyrin domains that are found prevalently in proteins of very diverse function, such as transcription regulators, ion transporters and signal transduction proteins. Recently, we demonstrated that IBTK is a component of Cul3-dependent E3 Ligase and associates with the tumor suppressor Pdcd4, a negative regulator of translation of specific mRNAs, promoting its ubiquitylation coupled to proteasomal degradation. In order to understand whether IBTKα could modulate the gene expression, like other BTB protein, we analyzed the transcriptome of silenced for IBTKα or control cells, by Deep RNA-Sequencing. In HeLa cells we found 184 (0.3%) out of 63128 mapped genes were differentially expressed in IBTK-shRNA as compared to control-shRNA, with 105 genes (57.1%) being up-regulated, and 79 genes (42.9%) down-regulated. In K562 cells, we found that 491 (0.78%) out of 63128 mapped genes were differentially expressed in IBTK-shRNA as compared to control-shRNA, being 219 (44.6%) upregulated genes and 272 (55.4%) downregulated. Only a small set of 7 genes was deregulated by IBTK depletion in both HeLa and K562, suggesting that IBTK differently affects the gene expression profile depending on the cellular context. By gene ontology classification we observed that differentially expressed genes by IBTK are involved in many biological process such as metabolism, immune system response, development, cell communications. Taken together, these data show that IBTKα significantly affects the transcription depending on the cellular context, providing additional novel information on IBTKα functions.

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Fingerprinting the junctions of RNA structure by an openpaddlewheel diruthenium compound

Abstract: RNA function is determined by its structural organization. The RNA structure consists of the combination of distinct secondary structure motifs connected by junctions, that play an essential role in RNA folding. Selective 2´-hydroxyl acylation analyzed by primer extension (SHAPE) probing is an established methodology to analyze the secondary structure of long RNA molecules in solution, which provides accurate data about unpaired nucleotides. However, the residues located at the junctions of RNA structure usually remain undetected. Here we report an RNA probing method based on the use of a novel open-paddlewheel diruthenium (OPW-Ru) compound [Ru2Cl2(μ-DPhF)3(DMSO)] (DPhF = N,N'-diphenylformamidinate). This compound has four potential coordination sites in a singular disposition to establish covalent bonds with substrates. As a proof of concept, we have analyzed the reactivity of OPW-Ru towards RNA using two viral internal ribosome entry site (IRES) elements whose function depends on the structural organization of the molecule. Our study suggests that the compound OPW-Ru preferentially attacks positions located one or two nucleotides away from junctions or bulges of the RNA structure. The OPW-Ru fingerprinting data differ from that obtained by other chemical reagents and provides new information about RNA structure features.

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Regulation of microRNA genes by histone acetylation in hepatocellular carcinoma

Abstract: Histone deacetylation regulates chromatin remodelling and transcriptional down-regulation of specific genomic regions, and is altered in many types of cancer cells, in particular hepatocellular carcinoma (HCC). We have shown that histone deacetylases (HDAC1-3) are consistently upregulated in HCC. To improve the generally very poor prognosis of HCC patients (www.dep.iarc.fr), treatment options targeting cell migration are urgently needed. Recently, it has become clear that not only protein-coding genes, but also miRNA genes are strongly altered by histone modifications. To understand the functional role and to test the potential as a drug target of the epigenetically regulated miRs, we aim to identify target genes and cellular pathways deregulated by distinct miRs during hepatocarcinogenesis. Using miR expression profiling we identified miRs that were reactivated by HDAC inhibitor- induced histone acetylation. Among them, miR-449a and miR-129-5p showed the strongest upregulation. MiR-449a is coded in a cluster together with its seed-sharing family members miR-449b and miR-449c. We comparatively analyzed the role of miR-449a, miR-449b, and miR-449c regarding their regulated target genes and functional effects in HCC. We found that miR-449a, miR-449b, and miR-449c are together induced by histone acetylation. Interestingly, we could show that epigenetic silencing of the tumor suppressive miR-449 family and miR-129-5p contributes to tumorigenesis of HCC by aberrant activation of several pathways including MAPK-, TGF-ß- and Wnt/ß-catenin pathways, leading to increased cell survival, growth and migration. In conclusion, the tumor suppressive miR-449 family and the tumor suppressive miR-129-5p are reactivated by histone acetylation and therefore contribute to the anti-cancer effects of HDAC inhibitors. In future, miR-449 family members or miR-129-5p may be considered for miRNA replacement therapy especially for HCCs with deregulated MAPK-, TGF-ß- or Wnt/ß-catenin signaling.

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The prognostic potential of alternative transcript isoforms across human tumors

Abstract: Molecular signatures can improve tumor stage identification, which is essential for therapy selection and patient prognosis. These signatures are often based on gene expression changes that occur during the activation of aggressive phenotypes. However, it is not yet known whether specific patterns of relative abundances of transcript isoforms are informative for clinical stage and survival. Here we integrate RNA sequencing data with clinical annotation from 12 tumor types from The Cancer Genome Atlas project to build and test predictive models of staging and clinical outcome. Transcript isoforms accurately separate early from late stage and metastatic from non-metastatic tumors, and are predictive of survival for patients with undetermined stage. We describe transcript isoform differences between breast tumors according to estrogen receptor status, and between melanoma tumors according to an invasive or proliferative phenotype, and derive accurate predictive models of tumor stage and survival for each subtype. Our analyses reveal new signatures that characterize tumor phenotypes and their progression independently of gene expression, which can contribute towards current strategies of precision cancer medicine. Here we report the identification of changes in the relative abundance of transcript isoforms that are predictive of clinical stage and survival in 12 different tumor types types, as well as in breast and melanoma tumor subtypes with different phenotypic properties. These analyses provides evidence for a role of RNA processing alterations during tumor progression and metastasis, and provides a rich resource of molecular information that can complement current strategies of precision cancer medicine.

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Detection of long non-coding RNA driver genes across 1104 tumour genomes with ExInAtor

Abstract: Large-scale cancer genome sequencing makes possible the systematic discovery of mutated non-coding genomic elements that drive tumour progression. Long noncoding RNAs (lncRNAs) are a recentlydiscovered and numerous class of genes that represents a large reservoir of undiscovered cancer drivers. Here we present ExInAtor, a tool for identifying cancer driver lncRNAs using tumour genome mutations. The approach assumes that driver lncRNAs will accumulate oncogenic mutations in exons, while introns will experience the local neutral mutation rate. The program takes as input gene annotations in GTF format and somatic mutations in BED format, and returns predicted drivers with adjusted statistical significance. Analysis of 1104 tumour genomes from 23 cancer types yielded a total of 103 candidate lncRNAs at a false discovery rate of 0.01. In control analyses, ExInAtor retrieves known coding and noncoding drivers with high precision, including the master tumour suppressor TP53 and proto-oncogene BRAF, as well as the oncogenic lncRNA, MALAT1. Both protein-coding and lncRNA drivers are more likely to be expressed in their tumour of origin than expected by chance. Putative driver lncRNAs display intriguing mutation patterns, including mutations in multiple different exons, or mutations across multiple cancer types. Thus ExInAtor reveals the landscape of lncRNA genes driving cancer, and will be a useful discovery tool in future tumour genome sequencing projects.

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MECHANISMS OF NUMB ALTERNATIVE SPLICING REGULATION IN LUNG CANCER CELLS BY RBM10 AND SF1/BBP

Abstract: Alternative splicing of NUMB exon 9 generates mRNAs encoding proteins with antagonistic functions in the NOTCH pathway and in cancer cell proliferation. Increased exon 9 inclusion is among the most frequent splicing alterations in lung cancer (1) and mutations in the exon 9- promoting skipping factor RBM10 are among the most frequent genetic lesions in lung adenocarcinomas (3,4). We have systematically mapped regulatory sequence elements in exon 9 using modified antisense oligonucleotides and identified regulatory factors using a spliceosome-wide RNAi screen. These analyses identified two potent splicing enhancers whose effects are mediated, respectively, by SRSF6/SRSF1/hnRNP K and – surprisingly- by the Branchpoint Binding Protein (BBP/SF1). Biochemical, ex vivo and in vivo results indicate that a branchpoint-mimic sequence acts as an exonic enhancer that can mediate the effects of SF1/BBP, revealing a novel function for this protein in splicing regulation. Our results using lung cancer cells in culture and in mouse xenografts indicate that RBM10 acts as a tumour suppressor, while mutant variants found in lung cancer promote cell proliferation and tumour growth. We have used a battery of mutant forms of RBM10 found in lung cancer to carry out a structure/function analysis of domains and residues important for its activity in promoting NUMB exon 9 skipping. The results reveal the requirement for both of the RRMs, the second Zn-finger and OCRE domains. In addition our data indicate that while mutation of valine 354 to glutamic acid in the second RRM disrupts regulation without affecting RNA binding, a natural RBM10 variant lacking valine is equally active in promoting NUMB exon 9 skipping, suggesting that V354E is a gain of function mutant that binds to the polypyrimidine tract preceding exon 9 but fails to assemble a repressive complex. Work in progress aims to identify protein partners of RBM10 whose interaction is affected by this mutation.

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Regulation of mRNA capping in stem cell pluripotency and differentiation

Abstract: The mRNA cap structure recruits factors essential for transcript processing and translation initiation. We report that regulated mRNA cap methylation is required for embryonic stem cell (ESC) differentiation and fibroblast reprogramming. Expression of the mRNA cap methyltransferase activating subunit, RAM, is elevated in ESCs resulting in high levels of mRNA cap methylation and translation of the ESC gene regulatory network. During differentiation, RAM is suppressed leading to loss of pluripotency and associated translational changes. An established requirement of differentiation is increased ERK1/2 activity, which suppresses pluripotency-associated genes. During differentiation ERK1/2 phosphorylates RAM Serine-36 triggering its ubiquitination and proteosomal degradation, ultimately resulting in changes in mRNA translation associated with loss of pluripotency. Restoration of RAM expression is ratelimiting for achieving pluripotency during fibroblast reprogramming. Thus, the mRNA cap emerges as a dynamic epigenetic mark that instructs change in the translational landscape during differentiation and reprogramming.

Victoria Cowling is a group leader in the Centre for Gene Regulation and Expression at the University of Dundee. Her group investigates how oncogenes and cellular signalling pathways influence the mRNA cap structure resulting in changes in gene expression and cellular physiology. The major contribution of Vicky's group is to uncover that the mRNA cap is dynamically regulated in the cell, integrating diverse signalling pathways to drive changes in protein synthesis and cell proliferation. Vicky's group are exploring the mRNA cap as a therapeutic target with which to inhibit cancer cell and parasite growth and proliferation.

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THE DEAD-BOX HELICASE DHH1 PROMOTES TRANSLATION OF HIGHLY STRUCTURED mRNAS

Abstract: Genome-wide studies of mRNA structure coupled with ribosome profiling experiments have uncovered the great influence of RNA structures in translation. As mRNA unfolding in vivo is mainly ATP-dependent, RNA helicases are predicted to play a major role in translation control which impact is still emerging. The yeast DEAD-box helicase Dhh1 and its highly conserved human ortholog DDX6 promote translation repression of mRNAs that are then fed into decay or storage. In contrast, we here demonstrate that Dhh1 activates in an ATPase-dependent manner translation initiation of positive-strand RNA viral genomes. This function is linked to highly structured regions located in the UTRs and the ORF of the viral RNA. Moreover, genome-wide ribosome profiling analyses and further validations demonstrate that Dhh1 activates translation of a specific set of functionally related cellular mRNAs, a set of them related to malignant phenotypes. These mRNAs present higher structured 5´UTR and ORF regions than those translationally repressed or not affected by Dhh1, strengthening the link between Dhh1 function and mRNA structure properties. Under stress, Dhh1 is described to change its cytosolic availability and phosphorylation status. Indeed, stress conditions reverse the translation status of Dhh1 mRNA targets. As deregulation of translation is tightly linked to malignant phenotypes and DDX6 is overexpressed in several types of tumors, Dhh1-mediated translational control may play an important role in tumor development..

Victoria Cowling is a group leader in the Centre for Gene Regulation and Expression at the University of Dundee. Her group investigates how oncogenes and cellular signalling pathways influence the mRNA cap structure resulting in changes in gene expression and cellular physiology. The major contribution of Vicky's group is to uncover that the mRNA cap is dynamically regulated in the cell, integrating diverse signalling pathways to drive changes in protein synthesis and cell proliferation. Vicky's group are exploring the mRNA cap as a therapeutic target with which to inhibit cancer cell and parasite growth and proliferation.

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A dual program for translation regulation in cellular proliferation and differentiation

Abstract: A central choice for metazoan cells is between proliferation and differentiation. Measuring tRNA pools in various cell-types, we found two distinct subsets, one that is induced in proliferating cells, and repressed otherwise, and another with the opposite signature. Correspondingly, we found that genes serving cell-autonomous functions and genes involved in multi-cellularity obey distinct codon-usage. Proliferation-induced and differentiation-induced tRNAs often carry anti-codons that correspond to the codons enriched among the cell-autonomous and the multi-cellularity genes, respectively. Since mRNAs of cell-autonomous genes are induced in proliferation and cancer in particular, the concomitant induction of their codon-enriched tRNAs suggests coordination between transcription and translation. Histone modifications indeed change similarly in the vicinity of cell-autonomous genes and their corresponding tRNAs, and in multi-cellularity genes and their tRNAs, suggesting the existence of transcriptional programs coordinating tRNA supply and demand. Hence, we describe the existence of two distinct translation programs that operate during proliferation and differentiation.

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Lineage-specific roles of CPEB4 in the control of oncogenic drivers in melanoma

Abstract: Cytoplasmic polyadenylation element binding proteins (CPEBs) are a family of RNA-binding proteins essential for the translational regulation of mRNAs in various biological contexts. CPEBs recognize CPE elements in the 3' untranslated region of target mRNAs and regulate their translational fate through cytoplasmic polyadenylation. This process is especially important during meiosis, since its progression relies on the translational activation of stored maternal mRNAs. CPEB1 and CPEB4 are the two members of the family required for meiotic progression. While CPEB1 mediates the translational activation of mRNAs until metaphase I (MI), CPEB4 activates mRNAs from interkinesis to metaphase II (MII). CPEBs share a conserved RNA-binding domain and regulate overlapping mRNA subpopulations. Hence, the requirement of two distinct CPEBs to complete meiosis leans on their differential posttranslational regulation. In fact, the N-terminal domain of the CPEBs is highly variable and harbours different regulatory motifs. While CPEB1 is activated by Aurora A kinase and is targeted for degradation by Cdc2 and Plk1-mediated phosphorylation, CPEB3 is controlled by monoubiquitination and SUMOylation, which regulate the transition from an inactive monomeric CPEB3 form to an active beta-amyloid-like aggregate. How the other CPEBs are posttranslationally regulated is unknown. Nevertheless, unveiling how the different CPEBs are differentially regulated is crucial for understanding how they respond to different stimuli and how they are interconnected in particular scenarios of co-existence. We found that CPEB4 activity is regulated by hyperphosphorylation during the meiotic cell cycle. Specifically, CPEB4 is phosphorylated in twelve residues by two different kinases and in a phase-specific manner. All phosphorylated residues are located in the intrinsically disordered N-terminal half of CPEB4 and are required for cytoplasmic polyadenylation of target mRNAs. Accordingly, these twelve phosphorylation sites are essential for meiotic progression. Furthermore, we have shown that hyperphosphorylation of CPEB4 disordered domain modulates its aggregation properties. Hence, non-phosphorylated CPEB4 forms non-amyloid aggregates that specifically recruit and repress CPE-containing mRNAs, whereas hyperphosphorylated CPEB4 remains monomeric and is active in cytoplasmic polyadenylation. Importantly, CPEB4 aggregates are dynamic and reversible upon phosphorylation on the identified phosphosites. These results contribute greatly to the understanding of how the CPEBs are differentially regulated and how they would differentially respond in a given cellular environment.

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POSTERS
(alphabetical order by first author)

Carme Arnan **Poster 1**

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Single-oligo, dual-CRISPR deletion of genomic elements including long non-coding RNAs

Background: CRISPR genome-editing technology makes it possible to quickly and cheaply delete non-protein-coding regulatory elements. We present a vector system adapted for this purpose called DECKO (Double Excision CRISPR Knockout), which applies a simple two-step cloning to generate lentiviral vectors expressing two guide RNAs (gRNAs) simultaneously. The key feature of DECKO is its use of a single 165 bp starting oligonucleotide carrying the variable sequences of both gRNAs, making it fully scalable from single-locus studies to complex library cloning.

Results: We apply DECKO to deleting the promoters of one protein-coding gene and two oncogenic lncRNAs, UCA1 and the highly-expressed MALAT1, focus of many previous studies employing RNA interference approaches. DECKO successfully deleted genomic fragments ranging in size from 100 to 3000 bp in four human cell lines. Using a clone-derivation workflow lasting approximately 20 days, we obtained 9 homozygous and 17 heterozygous promoter knockouts in three human cell lines. Frequent target region inversions were observed. These clones have reductions in steady-state MALAT1 RNA levels of up to 98 % and display reduced proliferation rates.

Conclusions: We present a dual CRISPR tool, DECKO, which is cloned using a single starting oligonucleotide, thereby affording simplicity and scalability to CRISPR knockout studies of non-coding genomic elements, including long non-coding RNAs.

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Contributions: Estel Aparicio-Prat, Ilaria Sala, Núria Bosch, Roderic Guigó, Rory Johnson

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Marina Barriocanal **Poster 2**

Center for Applied Medical Research (CIMA)

HCV infection upregulates EGOT, a PKR-induced long noncoding RNA that favours viral replication by affecting the antiviral response

Abstract: We have identified hepatitis C virus (HCV)-Stimulated long noncoding RNAs (lncRNAs) (CSRs) by RNASeq and microarray analysis. Interestingly, several oncogenic lncRNAs such as PVT1 or UCA1 are induced to high levels after HCV infection, providing a novel link between HCV infection and development of liver tumors. Expression of some CSRs seems induced directly by HCV while others are upregulated by the antiviral response against the virus. In fact, sensing of the virus by the RNA-activated kinase PKR induces the expression of several CSRs. Therefore, PKR, a well-known inhibitor of translation initiation, leads to the production of molecules that function in the absence of translation. PKR senses HCV genome and activates NF-KB, which transcribes for CSR32/EGOT. Genome-wide guilt-by-association studies show that EGOT may function as a negative regulator of the interferon pathway. Accordingly, depletion of EGOT increases the expression of several interferon-stimulated genes and leads to decreased HCV replication. Therefore, after HCV infection PKR inhibits translation of cellular proteins and allows expression of lncRNAs that function to regulate the antiviral response.

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mRNA SPLICING DEFECT AS CAUSE OF CONGENITAL HYPOTRANSFERRINEMIA

Abstract: Congenital hypotransferrinemia or atransferrinemia (OMIM#209300; ORPHA1195) is a rare autosomal recessive hematologic disease first described in 1961 (Heilmeyer et al, 1961) and caused by mutations in the TF gene that lead to a deficiency of transferrin, a serum glycoprotein responsible for the proper transport of iron in blood. Disease onset occurs in early childhood and is characterised by severe hypochromic microcytic anaemia and iron overload in non-hematopoietic tissues, such as liver and heart. To date, only 16 cases of 14 families have been reported worldwide. Here, we describe two new patients from a non-consanguineous Spanish family who is the first known case characterized at the molecular level in the Spanish population and the 9th case worldwide. We report two previously non-described variants found in the TF gene, including the first splicing mutation ever reported for this disease (c.[2062+20T>G]). The c.[1253C>A] variant causes a missense mutation (p.Ala418Glu) that changes the electrostatic environment around the iron binding site of transferrin towards more hydrophobic, most probably affecting protein stability. Studies in vitro reveal that the intronic variant c.[2062+20T>G] leads to the creation of a new donor splice site generating an aberrant TF mRNA.

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Shirley Brenner **Poster 5**

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Dual Regulatory Role of Polyamines in Adipogenesis

Abstract: Adipogenesis is a complex process, accompanied by a chain of interdependent events. Disruption of key events in this cascade may interfere with the correct formation of adipose tissue. Polyamines are ubiquitous polycations that were demonstrated essential for fundamental cellular processes, including cellular proliferation and differentiation. Polyamines form electrostatic interactions with various cellular components such as DNA, RNA, membranes, receptors and channels. We examined the effect of polyamine depletion on the differentiation of 3T3-L1 preadipocytes. Our results demonstrate that polyamines are required early in the adipogenic process. Polyamine depletion inhibited the second division of the mitotic clonal expansion (MCE), and inhibited the expression of PPARγ and C/EBPα, the master regulators of adipogenesis. However, it did not affect the expression of their transcriptional activator, C/EBPβ. Additionally, polyamine depletion resulted in elevation of mRNA and protein levels of the stress induced C/EBP homologous protein (CHOP), whose dominant negative function is known to inhibit C/EBPβ DNA binding activity. Conditional knockdown of CHOP in polyamine depleted preadipocytes restored PPARγ and C/EBPα expression, but failed to recover MCE and differentiation. Thus, our results suggest that the need for MCE in the adipogenic process is independent from the requirement for PPARγ and C/EBPα expression. We conclude that de-novo synthesis of polyamines during adipogenesis is required for down regulation of CHOP mRNA, to allow C/EBPβ activation, and for promoting MCE.

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Metehan Cifdaloz **Poster 6**

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New oncogenic networks controlled by the RNA binding factor CUGBP1 in melanoma identified by an integrated transcriptomic and proteomic analysis

Abstract: Despite great progress in identifying genetic and epigenetic defects accumulated during melanoma progression, the molecular bases underlying the aggressive behavior of this tumor type are not completely understood. For example, malignant melanocytic cells accumulate a plethora of changes in the transcriptome and the proteome. Consequently, distinguishing drivers from inconsequential passenger events has been a main challenge in this disease. Intriguingly, although it is recognized that oncogenes and tumor suppressors can be regulated by alternative splicing of their mRNAs, the expression and regulation of spliceosome proteins remains a virtually unexplored area of research in melanoma. Here we focused on CUGBP1, a RNA binding protein that can influence RNA splicing, RNA transcription and RNA decay. Intriguingly, the limited information on CUGBP1 in cancer cells suggests a minimal overlap in the bound/regulated transcripts, a factor we considered of interest to test in melanoma. Histologically, CUGBP1 was found overexpressed in human melanoma cells and tissue specimens. Targeted gene depletion demonstrated that CUGBP1 is required to sustain melanoma cell proliferation. Splicing-sensitive mRNA arrays, RNA immunoprecipitation (RIP)-followed by mRNA sequencing and iTRAQ-MS/MS proteomics were then performed to further assess the mechanism of action of CUGBP1. Surprisingly, this combined strategy revealed minor changes in alternative splicing after CUGBP1 downregulation. Instead, Gene Set Enrichment Analysis revealed an unanticipated large network of tumor-associated factors with key roles in the control of cell cycle and chromatin metabolism acting downstream of CUGPB1. Intriguingly, while the overlap with other systems was again negligible, we identified the oncogene DEK as a common target of CUGBP1 in melanoma and different tumor cell types. Mechanistically, CUGBP1 was found to bind to the 3'UTR of DEK stabilizing its mRNA levels and ultimately allowing for an efficient cell proliferation. These results illustrate the power of comprehensive analyses of RNA regulators in the identification of novel malignant features of cancer cells.

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Davide Cirillo **Poster 7**

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Fine-grained model of the sensorimotor control underlying larval chemotaxis

Abstract: RNA-binding proteins regulate a number of cellular processes, including synthesis, folding, translocation, assembly and clearance of RNAs[1,2]. Recent studies have reported that an unexpectedly large number of proteins are able to interact with RNA, but the partners of many RNA-binding proteins are still uncharacterized.
Using a theoretical approach[3,4], we are studying ribonucleoprotein interactions linked to inherited intellectual disability, amyotrophic lateral sclerosis, Creutzfeuld-Jakob, Alzheimer's, and Parkinson's diseases. We previously investigated RNA interactions with fragile X mental retardation protein FMRP, self-regulatory associations between proteins and their own transcripts as well as formation of ribonucleoprotein granules[5,6,7]. Our results are in striking agreement with previous experimental evidence and provide new insights that we are currently testing in our wet lab.
We recently found that co-expressed protein and RNA molecules have a high propensity to interact, which allows us to screen ribonucleoprotein networks and select candidates amenable for experimental validation. The integration of in silico and ex vivo data unraveled two major types of protein–RNA interactions, with positively correlated patterns related to cell cycle control and negatively correlated patterns related to survival, growth and differentiation8. of turns, we are developing a high-resolution larval tracker. Apart from high spatio-temporal resolution, this tool would provide optogenetic targeting of specific dorsal organs in a freely moving larvae. To achieve high-speed dorsal organ detection and tracking an algorithm initially developed offline FGA is being implemented.

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PROFILIN-2, A NEW PLAYER IN IRON METABOLISM

Abstract: The IRPs/IRE regulatory network plays a central role in the control of cellular iron homeostasis. Using a high throughput approach, we have previously identified novel IRP1 and IRP2 interacting mRNAs. Among the identified mRNAs, we studied more in depth Profilin2 (Pfn2), a protein involved in endocytosis and neurotransmitters release. The aim of this work is to characterize Pfn2 as a novel IRPs target mRNA and study its role in iron homeostasis. Mouse and human Pfn2 mRNAs were tested by non-radioactive competitive electrophoretic mobility shift assays (EMSA) for the binding to IRP1 and IRP2. To test the responsiveness of Pfn2 to IRP activity, Pfn2 mRNA levels were analyzed in mice with intestinal IRP1 and IRP2 deficiency. The labile iron pool (LIP) was measured in HeLa and Hepa1-6 cell lines with transient or stable overexpression of Pfn2. Tissues derived from Pfn2 knock-out mice were analyzed for iron content, measured by atomic absorption or colorimetric assay, and for mRNA and protein levels of iron-related genes. Combination of EMSA experiments and bioinformatic analyses allowed the identification of a novel and conserved 3'UTR iron responsive element in Pfn2 mRNA with an atypical hexanucleotide apical loop (AAGUGG). Pfn2 mRNA levels were significantly reduced (~20-25%) in duodenal samples from mice with IRP1 and IRP2 intestinal specific ablation, suggesting that IRPs exert a positive effect on Pfn2 mRNA expression in vivo. Overexpression of Pfn2 cDNA in HeLa and Hepa1-6 cells reduces LIP levels compared to control cells. Finally, analysis of Pfn2 KO mice showed iron accumulation in discrete areas of the brain (olfactory bulb, hippocampus and midbrain) together with an hepatic iron deficiency with ferritin reduction. Our results indicate that Pfn2 is controlled by the IRP regulatory system in vivo and that Pfn2 is a new iron gene that modulates iron homeostasis in cell lines and mice. Funding: Work supported by grant SAF2012-40106 from Spanish Secretary of Research, Development and Innovation (MINECO) and grant CIVP16A1857 "Ayudas a proyectos de Investigación en Ciéncias de la Vida - Fundación Ramón Areces", 2014 SGR225 (GRE) Generalitat de Catalunya and economical support from Fundació Internacional Josep Carreras i de la Obra Social "la Caixa" Spain to M.S. M.S. held a research contract under the Ramón y Cajal program from the Spanish Ministry of Science and Innovation (RYC-2008-02352).

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Elisabeth Daguenet **Poster 9**

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Searching for lncRNAs as potential regulators of Alternative Splicing

Abstract: The discovery of large RNA transcripts that do not code for proteins – termed long non- coding RNAs (lncRNAs) – provides a new perspective to understand the central role of RNA in gene regulation. A few lncRNAs have been functionally characterized in mammalian systems, and those have been associated with cellular processes such X-chromosome inactivation, maintenance of pluripotency, lineage commitment and apoptosis. LncRNAs form ribonucleic acid-protein complexes that carry out a variety of functions, including modulation of chromatin-modifying complexes and transcription. Recently, a role for the lncRNAs MALAT1 and Gomafu in modulating the activity of pre-mRNA alternative splicing (AS) factors was found. The mechanisms by which lncRNAs can regulate AS remain largely unknown. To unravel general functions of lncRNAs in AS, we took advantage of publicly available CLIP (Cross-Linking Immunoprecipitation)-sequencing data for splicing factors and searched for lncRNAs harboring high density of binding for particular splicing regulatory factors, including PTB, hnRNP C and TIA1/TIAR proteins. To further test the relevance of these interactions on AS, we evaluated the impact of downregulating these lncRNAs on a subset of AS events that are known to be strongly regulated by these particular splicing regulatory factors. Interestingly, we found one lncRNA whose knockdown affects the splicing pattern of events controlled by TIA1/TIAR proteins. We are currently dissecting the mechanistic aspects of this regulation using engineered minigenes and also extending our study at the genome-wide level using RNA-sequencing. This work could provide novel insights into the physiological role of lncRNAs and allow us to decipher novel molecular mechanisms of AS regulation, with implications in fundamental biological and pathological processes, including cell differentiation and cancer.

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Eduardo Eyras **Poster 10**

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Alternative splicing drivers of cancer

Abstract: Alternative splicing is a molecular mechanism regulated by RNA-binding proteins and affecting most eukaryotic genes. We have systematically analysed genomic and transcriptomic data in multiple tumor types to study the splicing alterations in cancer. We uncover novel splicing networks that may contribute to cancer development and progression, and find specific splicing alterations are predictive of tumor stage and survival. A subset of these splicing alterations eliminate or add protein domains that are frequently mutated in tumors, and some potentially impact protein-protein alterations. Additionally, some of these splicing alterations are mutually exclusive with mutations in tumor drivers. Our study reveals a catalogue of alternative splicing changes that potentially confer a selective advantage to tumors and provide novel prognostic signatures and targets of therapy.

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Sequential functions of CPEB1 and CPEB4 regulate VEGF expression during pathological angiogenesis

Abstract: Abnormal angiogenesis driven by VEGF is a major hallmark in pathological states like chronic liver disease (CLD), but the mechanisms underlying pathological VEGF overproduction and neovascularization remain unclear. Here, by analyzing human samples, in vivo animal models, genetically modified mice and in vitro angiogenesis assays, we elucidated a mechanism required for VEGF overexpression and pathological angiogenesis, but dispensable for physiological VEGF production, through sequential and non-redundant functions of cytoplasmic polyadenylation element-binding (CPEB) proteins 1 and 4. CPEB1 promotes alternative processing of VEGF and CPEB4 premRNAs, shortening their 3'-untranslated regions (3'UTRs) and excluding translationinhibition elements from their mature transcripts. As a result, CPEB4 is overexpressed and polyadenylates VEGF mRNA, further increasing its translation and angiogenesis. The regulation of CPEB4 by CPEB1 and the CPEB4 autoamplification loop ensues a coordinated switch-like induction of pathological angiogenesis. Hence, CPEBs may be potential targets to treat CLDs and other angiogenesis-dependent diseases.

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TRANSCRIPTOME ANALYSIS OF SPERMATOGENESIS-DEFICIENT Zrsr1 MUTANT MICE REVEALS EXTENSIVE IMPACT ON U12 INTRONS

Abstract: ZRSR1/2, also known as U2AF35-related proteins (Urps) have been implicated in 3' splice site recognition of both U2 and U12 introns. Recent sequencing studies revealed that somatic mutations of ZRSR2 are frequent in hematological malignancies including myelodysplastic syndromes (MDS). To study the function of ZRSR1 in vivo, we generated Zrsr1 mutant mice containing nonsense mutations in the RNA-recognition motif, thus leading to the predicted expression of truncated proteins containing one of the zinc finger (CCCH) domains, which could potentially exert a dominant negative effect on ZRSR1 function. Homozygous mice mutants exhibited severe defects in erythrocyte maturation and spermatogenesis, with germ cell sloughing and apoptosis ultimately leading to azoospermia and male sterility. To investigate the molecular basis for the spermatogenesis defects, RNA-Seq analyses of testis at day 15 (immediately after the highest expression of Zrsr1 in developing testis) were carried out. The results revealed changes in expression of 736 genes, enriched in gene ontology pathways related to meiosis, gamete generation and male fertility. Increased intron retention was the most frequent splicing alteration (44%, 300 introns). Thirty-two percent of these events correspond to U12 introns (a significant enrichment over the overall proportion of U12 introns in the mouse transcriptome -0.5%-) and 17% actually correspond to U2 introns in U12-containing genes, with the U2-affected intron typically positioned adjacent to a U12 intron, suggesting an effect of U12 splicing on neighboring U2 introns. Further analyses and validations, including RNAi and rescue experiments in cells in culture, revealed that ZRSR1 is required for splicing of most of the U12 introns examined and that the truncated protein cannot compensate for the absence of ZRSR1/2. These results underscore a specific role for ZRSR1 in U12 intron splicing and suggest that the loss of function of the truncated protein can be at the basis of the splicing defects observed in homozygous mice**.**

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Role of UNR/ CSDE1 in histone mRNA metabolism

Abstract: Upstream of N-ras (UNR/ CSDE1) is an RNA-binding protein that regulates mRNA translation and stability. We have recently found that UNR promotes melanoma development by coordinating RNA regulons important for invasion and metastasis. A group of mRNAs that are regulated by UNR en mass are the histone mRNAs. iCLIP experiments revealed that UNR binds to ~80% of the histone mRNAs expressed in melanoma cells. RNA-Seq data indicate that depletion of UNR results in histone mRNA down-regulation. Intriguingly, UNR binds close to a regulatory stem-loop structure in the 3' UTR necessary for efficient translation and stability. We are currently investigating the consequences of UNR binding to histone mRNAs using reporter constructs, and the possible interplay of UNR with the histone mRNA degradation machinery.

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Francesco P. Marchese **Poster 14**

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A long non-coding RNA involved in DNA replication and sister chromatid cohesion

Abstract: Faithful DNA replication and proper sister chromatid cohesion ensure the correct propagation of the genetic material to daughter cells during cell division. A large number of factors involved in these processes have been identified and characterized, as well as their alterations associated to genome instability and eventually tumorigenesis. However, the involvement of the non-coding components of the genome in DNA replication and cohesion remains still uncovered. Here we describe a novel human long non-coding RNA (lncRNA), DDI-1 involved in DNA replication and sister chromatid cohesion. Our results indicate that DDI-1 is cell cycle-regulated, and its depletion by siRNA or CRISPR-Cas9 affects DNA replication and cell cycle progression, leading to decreased cell proliferation and increased cell death. Moreover, cells depleted of DDI-1 show severe defects in sister chromatid cohesion, suggesting an essential role for DDI-1 in cohesion establishment and/or maintenance during cell division. Our data indicate that DDI-1 physically interacts with DDX11 helicase, which is known to be involved in DNA replication and sister chromatid cohesion. Mutations in DDX11 have been in fact associated to a rare pathological condition known as Warsaw breakage syndrome; a syndrome characterized at cellular level by sister chromatid cohesion defects. Results suggest that the binding of DDI-1 to DDX11 may be necessary for the proper function of the DNA helicase during cell division.

Finally, our results indicate that DDI-1 may act as an oncogene. It is transcriptionally controlled by c-MYC and presents higher levels in tumour cells with inactive p53. DDI-1 expression is significantly increased in a number of tumour types, suggesting a contribution of the lncRNA to the development and maintenance of the tumour.

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Elena Martín **Poster 15**

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Alternative splicing regulation by the SPF45-SR140-CHERP complex

Abstract: The splicing factor SPF45 is important for alternative 3' splice site recognition and interacts with other splicing factors to regulate alternative splicing (AS). SPF45 overexpression is present in numerous tumours and results in multidrug resistance. A functional splicing network developed in our lab links SPF45 with the splicing factors SR140 and CHERP, as their knockdowns have very similar effects on a series of AS events involved in cell proliferation and apoptosis. Interestingly, SPF45, SR140 and CHERP are all U2 snRNP-related factors. Our aim is to decipher the functional links between SPF45, SR140 and CHERP and the mechanisms of action to understand their coordinated effects on AS regulation. We have shown that the protein levels of all three proteins are decreased upon the individual knockdowns of each of them and that they can co-immunoprecipitate in HEK293 and HeLa cells. These results suggest that the three factors form a functional complex. We are now focusing on the interacting domains and their importance in AS regulation. Furthermore, SPF45, SR140 and CHERP depleted cells show decreased cell proliferation and cell cycle arrest. On going experiments are studying the effects of the depletion and overexpression of the three factors in cell migration, invasion and multidrug resistance. To identify the genome-wide targets of SPF45, SR140 and CHERP we are now performing RNA-seq analysis of depleted cells. Future studies will focus on candidate AS events involved in cell proliferation, cell cycle progression, migration or invasion as well as in multidrug resistance that can explain these phenotypes.

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Raigul Niyazova **Poster 16**

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Properties of miRNA Binding Sites in mRNA Genes Involved in the Development of Various Diseases

Abstract: We searched miRNA binding sites using the MirTarget program. The objectives of this work was to identify the target genes of miRNAs, which may contribute to the development of various diseases. One miRNA that has multiple binding sites is miR-466-3p, which has more than 300 target genes. The targets genes of miR-466-3p serve various functions. Many are transcription factors and kinases, some are involved in the cell cycle, apoptosis, and other processes. miR-3960 has 2,563 mRNA binding sites with high affinity for 375 human genes. miR-3960 has 565 binding sites in 5'UTRs and 515 sites in mRNA coding sequences (CDS). The nucleotide sequences of the binding sites located in CDSs encode polyalanine or polyproline. Hsa-miR-1322 has more than 2,000 binding sites in the mRNAs of 1,058 genes. This includes 1,889 binding sites in CDSs, 215 binding sites in 5'UTRs, and 160 binding sites in 3'UTRs. We identified 266 target human genes for miR-574-5p and six target genes for miR-574-3p. A significant substantial part portion of the target genes of miR-574-5p are transcription factors and kinases, which are involved in apoptosis and cell cycle. Changes in the expression of miR-574-5p and miR-574-3p are correlated with changes in the expression of their target genes, at which are associated with the development of many pathologies, including cardiovascular diseases and cancer.

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Lisa Osterloh **Poster 17**

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Essential role of the RNA helicase DDX46 in melanoma growth and metastasis

Abstract: Nearly every oncogene and tumor suppressor identified to date, can suffer alternative splicing in its mRNA. Moreover, the spliceasome has been recently reported as a potential therapeutic target in different tumor types. A malignant disease where alternative splicing is likely of be of particular relevance is cutaneous melanoma. Melanoma is the most aggressive form of skin cancer and a paradigm of histopathologically complex tumors that progress with a plethora of changes in the transcriptome. However, to date, the expression and regulation of spliceosome proteins remains virtually unknown in melanoma. In a screening for splicing factors and RNAbinding proteins altered in melanoma, we found the RNA helicase DDX46 (an essential component of the U2 subunit of the spliceosome) to be overexpressed in multiple melanoma cell lines. We pursued the mechanistic analysis of DDX46 as the impact of this protein in cancer is largely unknown. Targeted genetic inactivation and whole-genome exon arrays revealed new roles (and targets) of DDX46 in melanoma cell proliferation, adhesion and motility. In particular, we identified unexpected roles of DDX46 in the control of the invasive behavior of melanoma cells via the regulation of the Plexin B1 pathway. These data show how changes in splicing regulators can contribute to melanoma progression and support the feasibility of pharmacological targeting of spliceosomal proteins in melanoma cells.

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Evaluation of expression changes of selected long non-coding RNAs during the development of colorectal cancer

Introduction: Colorectal cancer (CRC) is one of the most common malignant diseases and a significant burden especially in the Czech Republic. Till this day there are still not enough (in number and validity) molecular markers that are able to describe tumour status, development and progression - particularly in metastatic settings. In this project we focus on a novel group of possible marker - long non-coding RNA (lncRNA), which are non-protein coding RNA molecules that play an important role in gene regulation. In addition to tracking changes in gene expression between normal and cancer tissue, selected candidates will be investigated regarding their potential functions in cell biology.

Aim: Our goal is to measure relative expression of a selected set of lncRNAs to find expression differences that correlate with clinical data and therefore with overall survival (OS). Co-expression of lncRNA and their correlation with clinical data might also be valuable and give a more detailed insight in tumour progression and metastasis probability.

Methods: Total RNA from tumour tissue after resection and from healthy mucosa was isolated using TriReagent extraction. cDNA was prepared in reverse transcription reaction with random hexamer primers and relative expression was assessed by quantitative real time PCR using TaqMan probes. Statistical analysis was performed using Statistica 11 software and R.

Results: Currently, paired samples from 62 patients were analysed. Out of the followed lncRNAs we were able to show significant correlation of CCAT-1 (colon cancer associated transcript 1) and GAS5 (growth arrest specific-5) with lymph node involvement (p=0,0167 and p=0,0102, respectively).

Conclusion: On the studied set of patients, we were able to track expression changes of selected lncRNAs to the clinical status of the tumour. We are now extending the study group to evaluate obtained data and to pinpoint most valuable candidates for functional testing. Results could be promising regarding the deepening of our knowledge about the development of colorectal cancer and links between the gene expression and clinical behaviour of the tumour, with the possible role as prognostic factors.

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Analysis of the composition of the Gemin5 interactome

Abstract: RNA-binding proteins play a key role in every step of gene expression regulation. Gemin5 is a predominantly cytoplasmic protein with at least two distinct RNA-binding domains. The Nterminal domain binds specifically to snRNAs, and targets them for assembly into spliceosomal snRNPs. In addition, this protein harbors a non-canonical RNA binding site at the C-terminal domain that determines its interaction with IRES elements, and represses internal initiation of translation. Here we report the network of Gemin5 partners using subcellular fractionation, TAP methodologies, and polysome profiles. Interestingly, this global analysis identified a set of riboproteome components, consisting of a large set of proteins with a strong enrichment for RNA-binding proteins. Using a panel of polypeptides corresponding to N-terminal and Cterminal domains of Gemin5, we obtained a comparative characterization of the cellular partners interacting with each of these domains of the protein. We found that Gemin5 partners mainly consist of RNA-binding proteins and ribosomal proteins, with a bias towards the Nterminal domain of the protein. Importantly, RNA bridges interconnect the vast majority of Gemin5 partners, since exhaustive RNase A treatment during the process of affinity purification strongly decreased the number of proteins interacting with the N-terminal region of Gemin5.

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CIMA/UNAV/IDISNA

Several long non-coding RNAs induced in hepatocellular carcinoma are upregulated in liver cirrhosis

Abstract: Several long non-coding RNAs (lncRNAs) are key regulators of processes leading to cancer. Therefore, they are of particular interest in tumors where therapeutic targeting of protein-coding drivers has proven unsuccessful, with hepatocellular carcinoma (HCC) being one of them. We have identified lncRNAs upregulated in HCC by bioinformatic analysis of TCGA transcriptomes. The best candidates are expressed in several HCC cell lines, where most are preferentially enriched in the nucleus. This suggests that they could function as regulators of gene expression. In fact, the expression of several candidates correlates with the expression of neighboring genes involved in cell proliferation. Given that most HCCs develop in cirrhotic livers, we have evaluated the expression of these lncRNAs in healthy livers, cirrhotic livers and HCC samples from cirrhotic patients. Fifteen lncRNAs showed very low expression in healthy livers and high expression in most of the HCCs tested. Surprisingly, the expression of nine of them was also high in non-tumoral cirrhotic livers. Further studies are ongoing to evaluate the oncogenic potential of these lncRNAs.

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Luisa Vigevani **Poster 21**

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MOLECULAR BASIS FOR THE DIFFERENTIAL SENSITIVITY OF 3' SPLICE SITES TO ANTI-TUMOR DRUGS TARGETING U2 snRNP

Abstract: Three families of natural compounds with anti---tumor properties (FR901463---5, GEX1 and Pladienolides) and their synthetic derivatives (e.g. Spliceostatin A, Meayamycin and Sudemycins) share a common pharmacophore and target U2 snRNP components, notably SF3B1. We have combined genome---wide transcriptome and bioinformatic analyses with minigene assays in cancer cells in culture and biochemical experiments in nuclear extracts to study sequences and mechanisms that mediate the effects of some of these drugs. Under conditions in which the drugs exert cytostatic effects, different 3' splice sites display differential drug sensitivities, resulting in alternative splicing. Remarkably, drugs with very similar structures display distinct profiles of splicing alterations, with examples of introns whose splicing is inhibited by one drug and enhanced by another. Drug sensitivity correlates with the presence of various sequence motifs 5' of the branch point region that can be transferred to a different 3' splice site. Motifs that confer drug sensitivity include sequences that resemble branch point decoys, consistent with previous results, while additional functional branch points and polypyrimidine tracts confer drug resistance. These results show that drug response can be exquisitely sensitive to sequence context ---even to single nucleotide differences--- in the region 5' of the branch site, depending on the functional consequences of drug---induced promiscuous base pairing of U2 snRNA in this region. Collectively, our data argue for a key function of SF3B1 in 3' splice site selection, which can be modulated by anti---tumor drugs.

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Claudia Vivori **Poster 21**

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Alternative splicing regulation in pluripotent stem cells

Abstract: Alternative splicing is a crucial mechanism of post-transcriptional regulation affecting virtually every biological process in higher eukaryotes. Embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are pluripotent cells, capable of self-renewal and differentiation into the three germ layers, both in vitro and in vivo. While transcriptional regulation and epigenetic modifications have been the primary focus of research in the field, outstanding progress has been made in the last years in revealing how post-transcriptional mechanisms also play a fundamental role in controlling stem cell fate.

Reprogramming of a somatic cell into an iPS by transient expression of OSKM factors is a highly inefficient process which can be divided into a stochastic and a deterministic phase. Transient expression of C/EBPα transcription factor during a 18h pulse prior to OSKM induction poises mouse B cells for rapid and efficient reprogramming into iPS [Di Stefano et al., Nature 2014]. Although the effects of C/EBPα are largely assumed to be due to the opening of chromatin in pluripotency genes promoters, involving DNA demethylation by Tet2, recent evidence showed that many splicing regulators are specifically interacting with C/EBPα [Grebien et al., Nat Chem Biol 2015]. Therefore, to investigate whether C/EBPα pulse could also affect alternative splicing patterns, additionally contributing to poise B cells for reprogramming, we analysed RNA sequencing data (unpublished data, Di Stefano and Graf) examining alternative splicing events and following their changes during early steps of reprogramming after C/EBPα pulse. K means clustering analysis allowed us not only to separate classes of alternative splicing events that behave differently during this process, but also to follow potential regulators featuring similar patterns in gene expression. Following appropriate validation, modulation of these selected events through antisense oligonucleotides will be carried out to determine whether inducing specific changes can contribute to reprogramming. The final aim will be to understand how C/EBPα could be affecting, directly or indirectly, alternative splicing and whether this phenomenon is independent of its chromatin-opening function, to potentially exploit alternative splicing modulation to reduce stochasticity during the process of reprogramming..

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M. Barriocanal **Poster 22**

CIMA/UNAV/IDISNA

Several long noncoding RNAs induced by IFN regulate the antiviral response

Abstract: Few studies have analyzed the antiviral role of long noncoding RNAs (lncRNAs). We have identified several "ISRs", Interferon (IFN) Stimulated long noncoding RNAs (lncRNAs) by transcriptome analysis of cells treated or not with IFN. Interestingly, some ISRs are located in the genome close to IFN stimulated genes (ISGs). lncBST2/BISPR, ISR12 and ISR8 locate close to BST2/tetherin, IL6 and IRF1 respectively. Downregulation of BISPR causes a decrease in the levels of BST2, indicating that BISPR is a positive regulator of BST2 expression. Instead, ISR12 is a negative regulator of the expression of distant ISGs, as depletion of ISR12 leads to increased levels of the ISG GBP1. Preliminary experiments suggest that ISR12 could induce GBP1 silencing. Finally, clones with altered ISR8 expression obtained using the CRISPR-Cas system do not stablish a successful IFN response. They fail to induce expression of several ISGs regulated by IRF1. In summary, our results show that lncRNAs induced after IFN treatment function as positive or negative regulators of the IFN response. The molecular mechanisms that allow gene expression regulation are under evaluation.

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New layer of regulation to the combinatorial code of CPE-mediated translational control

Abstract: The translational reactivation of maternal mRNAs encoding the drivers of vertebrate meiotic progression is accomplished mainly by the cytoplasmic elongation of their poly(A) tails. The Cytoplasmic Polyadenylation Elements (CPEs) present in the 3' UTR of these maternal transcripts, together with their cognate CPE-binding proteins (CPEBs), define a combinatorial code that determines the timing and extent of translational activation upon re-initiation of meiosis. An additional layer of regulation is provided by RNA-binding proteins (RBPs) that modulate CPEB activity or by CPE-independent mechanism(s) of cytoplasmic polyadenylation. The RBP Musashi1 (Msi1) regulates the polyadenylation of CPE-containing mRNAs, such as c-mos mRNA, by an as yet undefined CPEB-dependent or -independent mechanism. Here we show that Msi1 alone does not support cytoplasmic polyadenylation. However, the binding of Msi1 triggers the remodeling of RNA structure, thereby exposing adjacent CPEs and stimulating polyadenylation. Thus, Msi1 directs the preferential use of specific CPEs, which in turn affects the timing and extent of cytoplasmic polyadenylation during meiotic progression. Genome-wide analysis of CPEB1- and Msi-associated mRNAs identified 491 transcripts coregulated by Msi1 and CPEB1, thus revealing a new layer of regulation to the combinatorial code of CPE-mediated translational control..

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