



Institut
d'Estudis
Catalans



Societat Catalana
de **BIOLOGIA**

X Jornada de Cromatina i Epigenètica

***Organitzada per la Secció de Cromatina i Epigenètica
de la
Societat Catalana de Biologia (SCB)***

amb el Barcelona Chromatin Club (BCC)



Resums de les comunicacions

X Annual Chromatin and Epigenetics symposium

*Organized by the Chromatin and Epigenetics section of the
Catalan Society of Biology (SCB)
—Albert Jordan—*

*and the Barcelona Chromatin Club (BCC)
—Sonia Forcales—*

March 6, 2020

**IEC: carrer del Carme, 47, Barcelona
Prat de La Riba hall**

Sponsored by:

Institut d'Estudis Catalans

Active Motif

Covalab

Gene

BioNova

PROGRAM

9.00-9:30 Registration and documentation pickup

9:35 Opening

Session I. Chair: *Sonia Forcales*

9:40-10:00

15min +5

Bernhard Payer (CRG)

Structural remodeling of the mouse X Chromosome during reprogramming to pluripotency

10:00-10:20

15min +5

Marcus Buschbeck (IJC)

The histone variant macroH2A regulates chromatin architecture and the inflammatory response of cancer cells

10:20-10:40

15min +5

Beatrice Borsari (CRG)

Dynamics of gene expression and chromatin marking in a model of human trans-differentiation

10:40-10:50 Sponsor' talk

7min +3

Sarantis Chlamydas (Active Motif)

Epigenetics in the era of -omics, new paths and novel technologies

10:50-11:00

10 x 1min

Speed poster presentations

11:00-11:40 **Coffee break and poster session**

Session II. Chair: *Marcus Buschbeck*

11:40-12:00

15min +5

Luciano di Croce (CRG)

Chromatin regulators in cell differentiation and cancer processes

12:00-12:20

15min +5

Marian Martínez Balbas (IBMB-CSIC)

H3K9 dynamic methylation/demethylation is essential to maintain genome stability in early neurogenesis

12:20-12:40

15min +5

Maribel Parra (IJC)

HDAC7 in B cell development and leukemia

12:40-13:00

15min +5

Laia de Nadal (IRB)

Regulation of gene expression in response to stress

13:00-13:20

15min +5

Alejandro Vaquero (IJC)

Epigenetic regulation of stress response and genome stability by Sirtuins

13:20-14:40 **Lunch and poster session**

Session III-BCC10.

Chair: Marian Martínez Balbas

14:40-15:05

20min +5

María Gómez (CBM)

Reductions in histone H1 content causes non-coding transcript retention on chromatin and replicative stress

15:05-15:25

15min +5

Tanya Vavouri (IJC)

Effects of new transposon insertions on gene expression in mouse

15:25-15:45

15min +5

Gregoire Stik (CRG)

Role of CTCF in dynamic cellular processes

15:45-16:05

15min +5

Alfred Cortés (CRESIB)

Adaptation of malaria parasites to changes in their environment: epigenetics in motion?

16:05-16:40 **Coffee break and poster session**

Session IV. *Chair: Alejandro Vaquero*

16:40 Short Talk 1

7min +3

Julia Questa (CRAG)

Building and maintaining epigenetic memory in plants

16:50 Short Talk 2

7min +3

Clara Penas (UAB)

BET protein inhibition on macrophages to enhance axonal regeneration after peripheral nerve injury

17:00 Short Talk 3

7min +3

Jordi Moreno (CRAG)

A role for the Arabidopsis chromatin remodeler LHP1 in modulating the response to plant proximity

17:10 Short Talk 4

7min +3

Octavio Romero (IJC)

Histone demethylase inhibitors promote an epigenetic short-circuit in smarca4-deficient tumors

17:20 Short Talk 5

7min +3

Eudald Pascual (UB)

ATAC-seq and RNA-seq analysis reveal new elements of planarian posterior organizer

17:30 Short Talk 5

7min +3

Iva Guberovic (IJC)

A conserved role of macroH2A1.1 histone variant in regulating nuclear NAD⁺ metabolism from amoebas to humans

17:45 *Meet together for a beer*

Secretaries of SCB:

Mariàngels Gallego and Maite Sánchez

Societat Catalana de Biologia

C/ Maria Aurèlia Capmany, 14-16, 08001 Barcelona.

Tel. 933 248 584; E-mail: scb@iec.cat

Organized by:

Albert Jordan Vallès

Coordinator of the Chromatin and Epigenetics section of the SCB

Dept. Molecular Genomics, Institut de Biologia Molecular de Barcelona (IBMB-CSIC), Parc Científic de Barcelona

A/e: albert.jordan@ibmb.csic.es

Coorganized by:

Sònia Forcales

Coordinator of the Barcelona Chromatin Club

UB

POSTERS

1. Alejandro Valdivieso (ICM-CSIC)

Family-dependent variation in the multigenerational effects on sex ratios in zebrafish exposed to elevated temperature: changes in the testicular epigenome of unexposed offspring.

2. María José Barrero (CNIO)

Therapeutic potential of CREBBP/EP300 bromodomain inhibitors.

3. Julen Mendieta (CNAG-CRG)

3D genome structure reconstruction from sparse 3C-based datasets.

4. Lejuan Shi (UB)

Contribution of macrosatellite NBL2 and its associated lncRNA to neuroblastoma; promising new biomarkers and targets for therapy.

5. Núria Serna (IBMB-CSIC)

Histone H1 depletion in cancer cells promotes changes on genome architecture related to gene expression deregulation.

6. Pablo Coronel (UB)

Genome wide approaches reveals a putative *wnt1* enhance with a *foxG* motif as a key element to specify posterior fate in planarians.

7. Paula Escudero (IBMB-CSIC)

Centromeric control of nuclear envelope dynamics.

8. Christian Griñán (UB)

Transgenerational epigenetic memory imprinted by early life toxic stress in *C. elegans*.

ABSTRACTS SHORT TALKS

Epigenetics in the era of omics: success and challenges

Sarantis Chlamydas, Activemotif

It is clear that a new era of epigenetics study is now possible, thanks to modern high-throughput techniques like Reduced Representation Bisulfite sequencing (RRBS), Hi-C for chromatin conformation studies, ChIP-seq in clinical samples (Biopsies, FFPE), ChIP-seq followed by Mass Spec, Mass Spec analysis of Histone Modifications (Mod Spec) and ATAC seq.

Recently we have introduced to our pipeline the single cell analysis of the chromatin accessibility in order to classify single cell populations in tissue samples (sc-ATACseq).

These new techniques are mainly dependent upon high quality, proven reagents and high scientific expertise, suggesting that research reagents like specific validated antibodies, high performance High throughput sonicator (PIXUL) are still as relevant and necessary in this field today as they have ever been.

In this technical talk we will highlight our recent advances in the field of chromatin biology and gene regulation. We will present an overview of our novel tools, epigenetic reagents and discuss a series of data generated by our RnD and Epigenetics Services laboratories in the area of drug discovery and human diseases.

Building and maintaining epigenetic memory in plants

Julia Questa (CRAG)

Both in plants and animals, phase transitions rely on stable epigenetic silencing of key developmental genes, mostly achieved by the action of Polycomb Repressive Complexes (PRC1 and PRC2). Whereas successful germination is achieved following the inactivation of the embryonic program, the reproductive transition in *Arabidopsis thaliana* depends on epigenetic silencing of the floral repressor *FLOWERING LOCUS C (FLC)*. Prolonged cold of winter induces the assembly of a specialized PHD-PRC2 complex at *FLC* chromatin. On return to spring warm temperatures, PHD-PRC2 spreads across the locus delivering H3K27me3 to maintain long-term silencing, thus triggering the floral transition. *Arabidopsis* accessions have adapted to grow in a wide range of climates and the dynamics of *FLC* silencing vary in accessions collected from different locations. In a Northern Swedish accession, Lov-1, *FLC* silencing requires many more weeks of cold exposure and this was found to be the result of *cis* polymorphisms (SNPs) that quantitatively modulate the silencing. Our analysis of Lov-1 *FLC* behaviour revealed further distinct phases in the sequence of events in Polycomb silencing, with the SNPs causing instability in epigenetic memory. These findings not only further our understanding of Polycomb mechanisms but also may have implications for flowering time across plants with a broad range of reproductive strategies.

BET protein inhibition on macrophages to enhance axonal regeneration after peripheral nerve injury

Georgina Palomés, Xavier Navarro, Clara Penas

Institut de Neurociències, Departament de Biologia Cel·lular, Fisiologia i Immunologia,
Facultat de Medicina, Universitat Autònoma de Barcelona

Background: Peripheral nerve injuries (PNI) result in partial or total loss of motor, sensory and autonomic functions in the denervated segments of the body, and produce a decreased quality of life of patients. PN axons have a capacity to regenerate, but usually regeneration is not complete, leading to the mentioned chronic functional deficits. Presently, there are no available treatments that yield significant functional restoration. The bromodomain and extra terminal domain (BET) protein family comprises BRD2, BRD3, BRD4 and BRDT, and regulates gene expression by recruiting transcriptional regulatory complexes to poly-acetylated chromatin. We have previously observed that BET proteins have an important role in inflammation and are therapeutic targets after spinal cord injury (Sanchez-Ventura et al., 2018). However, if BET protein inhibition affects axonal regeneration is not known. Materials and methods: Female C57BL/6 mice underwent PNI by sciatic nerve crush, and were treated with the BET inhibitor JQ1, or vehicle, to determine the effect on nerve regeneration. Dorsal root ganglia (DRG) explants were cultured ex vivo and were treated with JQ1, or vehicle to ascertain the effect of BET inhibition on neurons. DRG explants were also treated with conditioned media from BET-inhibited or vehicle-treated macrophages and neurite outgrowth was measured to ascertain an indirect effect of inflammatory cells on neurons. Results: Electrophysiological and histological results of the in vivo study demonstrated that BET inhibition has a limited capacity to promote axonal regeneration after PNI in mice. In fact, a direct inhibition of BET proteins ex vivo, produced decreased neurite outgrowth of the DRG neurons. However, conditioned media from BET-inhibited macrophages enhanced neurite outgrowth ex vivo. Therefore, BET-inhibited macrophages secrete growth promoting factors that affect neurite outgrowth. Our preliminary results indicate that these factors are anti-inflammatory cytokines. Conclusions: The present study reveals that nerve regeneration may be enhanced by inhibiting BET proteins specifically on macrophages. Thus, this project may bring valuable insight on future targets and novel therapies for PNI patients

A role for the *Arabidopsis* chromatin remodeler LHP1 in modulating the response to plant proximity

Jordi Moreno-Romero¹, Renze Jacobi¹, Karel Vives^{1,2} Hugo Somoza¹ and Jaime F. Martínez-García^{1,3}

¹Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Cerdanyola del Vallès, Campus UAB, 08193-Barcelona, Spain.

²Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Barrio Universitario, Concepción, Región del Biobío 4030000, Chile.

³Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluís Companys 23, 08010-Barcelona, Spain.

In both natural and agronomic environments, competition for light might be intense between closely growing neighboring plants. In order to survive vegetation shade, plants have evolved a set of growth altering responses called the shade avoidance syndrome (SAS). In the model plant *Arabidopsis thaliana*, one of the more evident changes of the SAS is the promotion of hypocotyl elongation. To implement this response, changes in gene expression mediated by phytochrome photoreceptors are taking place rapidly after perception of plant shade. However, little is known about the epigenetic regulation of this developmental response, that is, how chromatin reorganization modulates gene expression to drive the elongation response. Genetic analyses have involved the chromatin remodeler LIKE HETEROCHROMATIN PROTEIN1 (LHP1) in the shade-induced elongation of hypocotyl in *A. thaliana* seedlings. We have observed that this chromatin remodeler impacts the rapid changes in both gene expression and auxin levels induced by shade perception. At the chromatin level, LHP1 is known to bind the histone mark H3K27me3. Using previous genome-wide data, we have found that LHP1 is present in a significant amount of shade-induced genes, which are enriched on the H3K27me3 mark. Further analyses showed that this mark is removed after the plant exposure to shade in a subset of these genes. We will present our latest data on the role of LHP1 on the epigenetic control of SAS.

Histone demethylase inhibitors promote an epigenetic short-circuit in smarca4-deficient tumors

Octavio Romero (IJC)

Background:

The standard treatment of non-small cell lung cancer (NSCLCs) is surgery. New therapeutics, such as tyrosine kinase inhibitors or immunotherapy may improve survival, but these treatments are only effective in small cohorts of patients. Thus, hopes of improving survival of lung cancer patients are related to the advent of novel therapeutic strategies. The classic epigenetic research focuses in the reversion of gene promoter DNA hyper methylation or histone code modifications, using a battery of unspecific drugs addressed to modify the global epigenetic code in cancer cells. In our previous work, we identified frequent inactivating mutations in the epigenetic gene BRG1 (about 20% of NSCLC), which were mutually exclusive with amplifications in the MYC oncogenic family. Unfortunately, BRG1-mutant cancer cells were also not able to respond to certain epigenetic therapies whereas cancer cells carrying MYC amplification, which are BRG1 proficient, appear to be highly sensitive to these combinations of treatments. These results show that MYC amplification could be used as a **prognostic biomarker for a specific personalized therapy**. In this project we observed that the mutational status of BRG1 directly correlates with the expression levels of several Lysine methyltransferases (KMTs) and Lysine demethylases (KDMs) in cancer cells. We also found that inactivating mutations in KDMs and KMTs tend to be mutually exclusive with inactivating mutations in BRG1, mutations in other SWI/SNF members and mutations in MYC oncogenic genes. Thus, we focused on targeting histone-modifying enzymes, in BRG1 proficient or deficient lung cancers cell lines.

Methods:

For this propose we integrate state of the art technology like genome-wide chromatin modification and transcriptome analysis, using human cell lines and preclinical models for lung cancer, including in vivo models of mice such as xenografts, subjected at different concentrations of histone deacetylase inhibitor (HDACi), lysine demethylase inhibitor (KDMi) and EZH2 inhibitor (EZH2i) to **design a personalized epigenetic treatment with high efficacy and low toxicity**.

Results:

Our results showed that BRG1 deficient cells are not sensitive to HDACi, displaying an unexpected increase in H3K27me3 after treatment that correlates with a global enrichment of H3K27me3 and EZH2 occupancy at gene promoter in BRG1-mutant cells. Otherwise, BRG1 directly regulates KDMs expression in lung cancer and demonstrates that inactivating mutations in BRG1 sensitizes cancer cells to the lysine H3K27me3 demethylase inhibitor (KDMi). However, EZH2i seems to reverse KDMi activity in the absence of BRG1, exposing an interesting and determining role of this histone lysine methyltransferase in the sensitization of BRG1 deficient cancer cells to the KDMi.

Conclusion:

The results will be of great value for the stratification of lung tumors according to their genetic or epigenetic background for tailored treatments, opening the possibility to use **BRG1 mutations as a potential Biomarker for personalized epigenetic target therapy in cancer**. The development of an epigenetic-based therapeutic prediction model will hopefully set the basis for future treatment of lung cancer as well as of other epithelial cancers.

ATAC-seq and RNA-seq analysis reveal new elements of planarian posterior organizer

E. Pascual-Carreras¹, M. Marín¹, S. Castillo-Lara¹, P. Coronel-Córdoba¹, M.S. Magri², J.F. Abril¹, J.L. Gomez-Skarmeta², E. Saló¹ and T. Adell¹

1. Department of Genetics, Microbiology and Statistics, Universitat de Barcelona (UB) & Institute of Biomedicine of Universitat de Barcelona (IBUB), Barcelona, Spain.

2. Centro Andaluz de Biología del Desarrollo (CABD), Universidad Pablo de Olavide, Sevilla

Organizers or signaling centers are a group of cells with the ability to specify the fate of adjacent cells, allowing a patterned growth. Although organizers are mainly studied during embryogenesis, their function is also required in adults, for instance during regeneration. Previous studies demonstrate that the amazing regenerative properties of planarians rely in their ability to regenerate specific organizers according to the polarity of the wound. Anterior and posterior facing wounds are defined by the expression of *notum* (a secreted Wnt inhibitor) and *wnt1*, respectively. The inhibition of any of those elements leads to a shift in polarity. Interestingly, during the first hours of regeneration both *notum* and *wnt1* are expressed in any wound, and it's around 36 hours that their expression becomes restricted to their respective pole. To decipher the molecular interactions that restrict the expression of *wnt1* to the posterior wound and confer the organizing activity we used genome wide approaches. ATAC-seq and RNA-seq analysis of regenerating wild-type and *wnt1* (RNAi) planarians allowed the identification of specific Cis-Regulatory Elements (CREs) and cWNT target genes related to posterior regeneration. We found that already at 12 hours of regeneration the accessible CREs in posterior and anterior wounds have essentially changed, indicating that specific posterior chromatin changes induced by amputation occur much earlier than the formation of the organizers. Furthermore, we have identified an enhancer of *wnt1* which might be conserved through evolution.

A conserved role of macroH2A1.1 histone variant in regulating nuclear NAD⁺ metabolism from amoebas to humans

Iva Guberovic*¹, Sarah Hurtado-Bagès*¹, Gunnar Knobloch², Ciro Rivera-Casas³, Marta Frigolé-Vivas⁴, Jesús García⁴, Michelle M. Leger⁵, Vanesa Valero¹, Manjinder S. Cheema⁶, Juan Ausio⁶, Iñaki Ruiz-Trillo⁵, Xavier Salvatella⁴, Jose M. Eirin-Lopez³, Andreas G. Ladurner², Marcus Buschbeck¹

Affiliations:

¹Josep Carreras Leukaemia Research Institute, Campus ICO-Germans Trias i Pujol-Universitat Autònoma de Barcelona, Badalona, Spain, ²Biomedical Center, Physiological Chemistry, Ludwig-Maximilians-Universität München, Munich, Germany, ³Institute of Water and Environment, Florida International University, North Miami, Florida, USA, ⁴Institute for Research in Biomedicine, The Barcelona Institute of Science and Technology, Barcelona, Spain, ⁵Institute of Evolutionary Biology, UPF-CSIC Barcelona, Spain, ⁶Department of Biochemistry and Microbiology, University of Victoria, Victoria, Canada

The hallmark of macroH2A histone variants is the globular macrodomain (MD) with isoform-specific metabolite-binding pockets. The binding pocket of macroH2A1.1 isoform accommodates NAD⁺ derived metabolites, such as ADP-ribose (ADPr) and poly-ADPr (PAR). NAD⁺ is one of the key metabolites which links cell energy homeostasis and transcriptional regulation. We recently discovered that the macroH2A1.1 histone variant regulates cross-organelle NAD⁺ communication without affecting gene expression. The macroH2A1.1 can bind and inhibit PARP1, the most prominent nuclear NAD⁺ consumer. This, in turn, reduces nuclear NAD⁺ usage and allows for the maintenance of the mitochondrial NAD⁺ pool, enabling optimal respiration. Although macroH2A histones were thought to originate in vertebrates, recent *in silico* analyses trace their origin back to one of the closest unicellular relatives of metazoans, *Capsaspora owczarzaki* (Co_macroH2A). It is unclear whether this ancient macroH2A has a role in cross-organelle metabolic communication or whether it evolved in higher organisms along with more complex metabolic networks. We report high homology between the MDs of Co_macroH2A and vertebrate macroH2A1.1, especially within its ADPr binding pocket. Biophysical assays reveal high affinity interaction between the Co_macroH2A MD and ADPr. A 2.0 Å resolution crystal structure substantiates the high selectivity for ADPr within the metabolite binding pocket. In addition, our assays show that Co_macroH2A binds and inhibits PARP1 *in vitro*, suggesting a role of ancient macroH2A in buffering nuclear PARP1 activity. Consistently, expression of Co_macroH2A is higher than Co_PARP1 in a dormant life stage of *C. owczarzaki*. This indicates a role of Co_macroH2A in suppressing nuclear NAD⁺ consumption in the low energy state. Taken together, our results suggest an ancient role of the histone variant macroH2A in balancing inter-organelle NAD⁺ metabolism, which may date back to the origins of multicellular life.

ABSTRACTS POSTERS

Family-dependent variation in the multigenerational effects on sex ratios in zebrafish exposed to elevated temperature: changes in the testicular epigenome of unexposed offspring

Valdivieso, A.¹, Ribas, L.¹, Monleón, A.², Orbán, L.³, Piferrer, F.^{1*}

Affiliations:

¹ Institut de Ciències del Mar, Consejo Superior de Investigaciones Científicas (ICM-CSIC), 08003 Barcelona, Spain

² Department of Genetics, Microbiology and Statistics: Section of Statistics. University of Barcelona (UB), 08003 Barcelona, Spain

³ Frontline Fish Genomics Research Group, Department of Animal Sciences, Georgikon Faculty, University of Pannonia, H-8360 Keszthely, Hungary

*** Correspondence:**

piferrer@icm.csic.es

In many organisms, sex can be determined by a combination of genetic and environmental influences. Recently accumulated evidence shows that changes in environmental conditions can affect population sex ratios through epigenetic regulation of key genes involved in gonadal development. Further, altered sex ratios can persist in the offspring even when the environmental cue is no longer present (a multigenerational effect) and that epigenetic changes tend to be paternally transmitted. However, evidence of transgenerational effects (i.e., beyond the first non-exposed generation) in subsequent generations is scarce and a matter of debate. Here, we used a laboratory strain of zebrafish, where sex determination is dependent on genetic and environmental influences, to study possible multi- and transgenerational effects of exposure to abnormally elevated temperature during the critical period of sex differentiation. To this end, we used eight different families of which we selected five to capture sufficient biological variability in sex ratio at control (28°C) and sex ratio response to elevated (35°C) temperature in the parental (P) generation. Sex ratios were determined at the non-exposed F₁ and F₂ offspring generations from parents kept at 28°C (control) and 35°C (treated). All offspring were raised at 28°C. Global DNA methylation was also assessed in males of the P, F₁ and F₂ generations derived from exposed and non-exposed fathers. Results showed a consistent increase in the number of males in the P generation in all five families but only a persisting male-skewed sex ratio in the 35°C-derived, unexposed offspring of the F₁ generation in two families, denoting family-dependent multigenerational effects. However, no transgenerational effects were observed in any of the families. Likewise, DNA methylation was significantly decreased only in the testis of the 35°C-derived males in the F₁ generation but not of the F₂ generation and, surprisingly, neither in the 35°C-exposed males of the P generation. Taken together, our results show great interfamily variation, not only in sex ratio response to elevated temperature, but also on its multigenerational effects, denoting a strong influence of genetics. Alterations in the testicular epigenome in F₁ males calls for attention to possible, previously-unnoticed effects of temperature in the unexposed offspring of heat-exposed parents in a global warning scenario.

Therapeutic potential of CREBBP/EP300 bromodomain inhibitors

Veronica Garcia-Carpizo, Sergio Ruiz-Llorente, Jacinto Sarmentero, Ana Gonzalez-Corpas and Maria J. Barrero

Centro Nacional de Investigaciones Oncologicas (CNIO)

Melchor Fernandez Almagro 3, Madrid 28029

maria.jose.barrero@gmail.com

The reported antitumor activity of the BET family bromodomain inhibitors has prompted the development of inhibitors against other bromodomains. However, the human genome encodes more than 60 different bromodomains and most of them remain unexplored. We report that the bromodomains of the histone acetyltransferases CREBBP/EP300 are critical to sustain the proliferation of human leukemia and lymphoma cell lines. EP300 is very abundant at super-enhancers in K562, a chronic myeloid leukemia cell line, and is coincident with sites of GATA1 and MYC occupancy. In accordance, CREBBP/EP300 bromodomain inhibitors interfere with GATA1- and MYC-driven transcription, causing the accumulation of cells in the G0/G1 phase of the cell cycle. The CREBBP/EP300 bromodomain inhibitors displace CREBBP and EP300 from GATA1 and MYC binding sites at enhancers, resulting in a decrease in the levels of histone acetylation at these regulatory regions and consequently reduce the expression of critical genes controlled by these transcription factors.

3D genome structure reconstruction from sparse 3C-based datasets

Julen Mendieta-Esteban¹, Irene Farabella¹, and Marc A. Marti-Renom^{1,2-3}

1- CNAG-CRG, Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Dr. Aiguader 88, 08003 Barcelona, Spain; 2- Universitat Pompeu Fabra (UPF), Barcelona, Spain; 3- Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

Julen.mendieta@cnag.crg.eu

3C technologies measure the interactions frequency between chromatin regions within the three-dimensional (3D) nuclear space in a cell or population of cells. One of such technologies is Promoter Capture HiC (pChIC) that measures interactions involving promoters, resulting in sparse interaction matrices that are rich in long-range enhancer-promoter interactions. Here, we introduce a new method to reconstruct chromatin 3D structures from pChIC data focusing at the loci level. This method combines a new normalisation protocol to handle sparse data matrices with TADdyn polymer-physics based restrain modelling. The models reconstructed by this method are highly correlated with their non-sparse counterparts, suggesting a good recapitulation of the local structure. We prove the usability of our method comparing the 3D organisation of a set of tissue-specific loci across different publicly available tissues samples.

Contribution of macrosatellite NBL2 and its associated lncRNA to neuroblastoma; promising new biomarkers and targets for therapy.

Lejuan Shi¹, Gabrijela Dumbovic^{2*}, Luca Sanna³, Sonia Forcales^{1*}

1. Department of Pathology and Experimental Therapeutics, Faculty of Medicine and Health Sciences, University of Barcelona (UB), Hospitalet de Llobregat, 08097, Catalonia, Spain.
2. BioFrontiers Institute, University of Colorado at Boulder, Boulder, CO, USA
3. Department of Biomedical Sciences, University of Sassari, Sassari 07100, Italy

*Past address: Cancer Genetics and Epigenetics, Program of Predictive and Personalized Medicine of Cancer (PMPPC), Health Science Research Institute Germans Trias i Pujol (IGTP), Badalona, Spain

Neuroblastoma is an embryonal tumor of the autonomic nervous system, probably arising from a precursor cell derived from neural-crest tissues. Therefore, neuroblastomas generally occur in very young children (diagnosis median age is 17 months). Neuroblastoma incidence is 10.2 cases per million children under 15 years of age, hence it is considered a rare disease even though it is the most common cancer diagnosed during the first year of life, and the leading cause of cancer related deaths in children under the age of 5. Despite recent advances in therapeutic approaches, 50 to 60% of patients with high-risk neuroblastoma have a relapse, and to date there are no curative regimes for those cases (long-term disease-free survival after a relapse is rarely seen). The NBL2 macrosatellite was shown to be epigenetically altered in 70% of neuroblastomas more than 20 years ago. Nevertheless, the study of the implication of this alteration in the disease pathology or response to treatment was not addressed, and as far as we know, nobody is addressing it. Our group identified NBL2 alterations in colorectal cancer (CRC), that lead to the expression of a novel lncRNA we named TNBL. We have characterized several aspects of TNBL biochemistry, and our unpublished data indicates that TNBL marks a subset of cells with chemoresistant properties and distinct gene expression profiles related to increased metastatic and angiogenesis abilities while downregulated immune response and apoptotic genes. Moreover, we have some mechanistic insights since TNBL binds to several factors involved in splicing and forms a peculiar aggregate with Sam68 bodies at perinucleolar regions. Intriguingly enough, Sam68 has been described as a marker of aggressive neuroblastoma. In neuroblastoma cell lines, we have observed that demethylated NBL2 associates with increased transcription of this locus, however we do not know whether this RNA will behave in the same manner as in CRC. Therefore, we need a better characterization of the NBL2/TNBL axis in neuroblastoma to determine whether it plays a previously unanticipated role in this disease, and whether it could represent a novel therapeutic target.

Histone H1 depletion in cancer cells promotes changes on genome architecture related to gene expression deregulation

Núria Serna¹, Mónica Salinas¹, Francesca Mugianesi², Marc Martí-Renom², Albert Jordan¹

¹ *Institut de Biologia Molecular de Barcelona (IBMB-CSIC), Barcelona, Catalonia, Spain, ajvbmc@ibmb.csic.es*; ² *Centre de Regulació Genòmica (CNAG-CRG), Barcelona, Catalonia, Spain*

Histone H1 binds to the linker DNA at the nucleosome, participating in the formation of higher-order chromatin structures. Human somatic cells may contain up to seven members of the histone H1 family contributing to the regulation of nuclear processes, apparently with certain subtype specificities.

We have previously shown that in T47D breast cancer cells, the combined knock-down of H1.2 and H1.4 subtypes (multi-H1 KD) deregulates many genes, promotes the appearance of accessibility sites genome-wide and triggers an interferon response via activation of heterochromatic repeats. Now, through the integration of chromatin immunoprecipitation followed by sequencing (ChIP-Seq), RNA sequencing (RNA-Seq) and high-throughput chromosome conformation capture (Hi-C) techniques, we aim to elucidate the biological role of different H1 subtypes in the interplay between genome architecture and gene expression. Our results support that histone H1 variants are differentially distributed in topologically associating domains (TADs) and A/B compartments. Multi-H1 KD increased TAD border definition and intra-TAD contacts, while decreased inter-TAD interactions. Moreover, TADs enriched in histone H1.2 showed major transitions from B to A compartment and changes in interactions. Multi-H1 depletion also promoted genes deregulation in 40% of TADs. Up-regulated genes accumulated within TADs presenting high H1.2/H1X ratios and low gene richness, while the opposite occurs in TADs containing down-regulated genes. Finally, TADs presenting a coordinated response to multi-H1 KD were significantly enriched compared with the expected frequencies

In conclusion, our data suggest that the equilibrium between distinct histone H1 variants is involved in maintaining the topological organization of the genome and the proper expression of particular gene programs.

Genome wide approaches reveals a putative *wnt1* enhancer with a *foxG* motif as a key element to specify posterior fate in planarians

P. Coronel-Córdoba¹, E. Pascual-Carreras¹, M. Marín¹, S. Castillo-Lara¹, M.S. Magri², J.F. Abril¹, J.L. Gomez-Skarmeta², E. Saló¹ and T. Adell¹

1. Departament de genètica, microbiologia i estadística. Universitat de Barcelona (UB) & Institute of Biomedicine of Universitat de Barcelona (IBUB), Barcelona, Spain.
2. Centro Andaluz de Biología del Desarrollo (CABD), Universidad Pablo de Olavide, Sevilla

Planarians are flatworms that can regenerate any missing part of their bodies. This huge capacity of regeneration is thanks to a large population of adult pluripotent stem cells combined with the signals that allow the proper pattern of the regenerated tissue. The signals responsible to regenerate anterior versus posterior structures according to the polarity of the regenerating fragment came from organizers (or organizing centers) that are established in any planarian wound. Previous studies show that the posterior organizer is defined by the expression of *wnt1* and the anterior by *notum* (a Wnt1 inhibitor). Importantly, at 12h of regeneration both *wnt1* and *notum* are expressed in any wound, but by 36h *notum* becomes restricted to the anterior and *wnt1* to the posterior. To understand how *wnt1* is restricted to the posterior pole we performed genome wide approaches (ATAC-Seq and RNA-Seq) and we found an enrichment of the transcription factor *foxG* motif in enhancers located near genes specifically expressed in posterior wounds, including an enhancer found in the first intron of *wnt1*. This data suggested that a context-specific regulation of this enhancer by *foxG* could be essential to restrict *wnt1* expression to the appropriate pole. To check this hypothesis we are following different approaches: 1) RNAi inhibition of *foxG*, which already demonstrated that *foxG* RNAi animals are *tailles*, since they do not express *wnt1* and lack a posterior organizer; 2) in vitro assays to demonstrate that planarian *foxG* can activate the *wnt1* enhancer; and 3) chromatin conformation capture experiments (3C) to gather evidence that this enhancer interacts with the *wnt1* promoter.

Centromeric control of nuclear envelope dynamics

Paula Escudero (IBMB-CSIC)

Cell division requires the precise coordination of crucial mitotic events that involve extensive architectural rearrangements. Metazoa generally undergo open mitosis, which implies that the nuclear envelope (NE) disassembles at prometaphase and reassembles after chromosome segregation is completed. How these processes are ultimately coordinated is unknown. This work is focus on Barrier-to-Autotintegration- Factor (BAF), a highly conserved protein that links chromatin to the nuclear envelope (NE) and plays a key role in NE reassembly after mitosis. We show that BAF associates with centromeres and is required for centromere assembly and chromosome segregation. BAF binds across centric heterochromatin in interphase cells, but, at mitosis, NHK1-phosphorylation releases BAF from heterochromatin, restricting its localization to centromeres. Centromeric BAF localization depends on the PP4-phosphatase regulatory subunit Falafel (Fifl), which has previously been shown to be recruited to centromeres by the constitutive centromere component CENP-C suggesting that PP4/Fifl maintains centromeric BAF unphosphorylated. In agreement, a phosphomutant BAF form localizes at centromeres. We also show that centromeric BAF prevents premature BAF reassociation with chromatin and regulates the onset of NE reassembly. Altogether these results show that, together with Fifl and CENP-C, centromeric BAF forms a network of interactions that is required for chromosome segregation and signals the timing of postmitotic NE reassembly, suggesting a model by which it acts as a checkpoint to coordinate these two essential mitotic events.

List of affiliations:

- Institute of Molecular Biology of Barcelona, CSIC. Barcelona. Spain
- Institute for Research in Biomedicine (IRB Barcelona). Barcelona. Spain
- Biological Research Center. Szeged. Hungary
- University of Southampton. Southampton. UK

List of coauthors:

- Mònica Torras-LLort IBMB-CSIC, IRB
- Sònia Medina-Giró IBMB-CSIC, IRB
- Paula Escudero-Ferruz IBMB-CSIC, IRB
- Zoltan Lipiszni, BRC
- Olga Moreno-Moreno, IBMB-CSIC, IRB
- Marcin R. Przewlaka, University of Southampton
- Fernando Azorin IBMB-CSIC, IRB

Transgenerational epigenetic memory imprinted by early life toxic stress in *C. elegans*

Christian Griñán-Ferré¹, Eszter Gecse², Mercè Pallàs¹ and Csaba Söti²

¹Pharmacology Section, Department of Pharmacology, Toxicology, and Therapeutic Chemistry. Faculty of Pharmacy and Food Sciences. Institute of Neuroscience, University of Barcelona (NeuroUB), Av Joan XXIII 27-31, 08028 Barcelona, Spain.

²Department of Medical Chemistry, Semmelweis University, Budapest, Hungary.

Stress exposure early in life is associated with behavioural and bodily disorders. Experiences during the critical perinatal period form permanent, imprinted memories later in life including next generations. Understanding the mechanisms underlying the enduring effects of early-life stress is an important research area. Here we report that in response to early life stresses, *Caenorhabditis elegans* nematodes form an imprinted behavioural and cellular defense memory. We show that exposing newly-born worms to toxic antimycin A exposure or repeated exposure, promotes aversive behaviour and stimulates the expression of the *hsp6* enzyme a toxin-specific cytoprotective. Learned adult defenses require memory formation during the L1 larval stage and do not appear to confer increased protection against the toxin. We found that aversive behaviour is inherited only to the F1 generation after 1 exposure to the toxic or can be passed to the F4 generation after 4 exposures to the toxic. At the molecular level we found epigenetic inheritance to the F3 generation after 1 exposure to the toxic or until the F5 generation after 4 exposures to the toxic. Thus, exposure of *C. elegans* to toxic stresses in the critical period elicits adaptive behavioural and cytoprotective responses, which form imprinted aversive behaviour and imprint a cytoprotective memory in the adulthood and the successive generations. Furthermore, transgenerational epigenetic mechanisms may explain in part this inheritance phenomenon.