Societat Catalana de BIOLOGIA

VIII 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)

Societat Catalana de Biologia 1912-2012

BCC

VIII 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 16, 2018

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

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PROGRAM

8.20-8.50 ***Registration and documentation pickup***
8:50 ***Opening***

Session I. *Chair:* ***Albert Jordan***

9:00-9:20 **15min +5**
María José Barrero (CNIO)
Long range epigenetic remodeling in cancer

9:20-9:40 **15min +5**
Marc A. Martí-Renom (CRG-CNAG)
Structure determination of genomes and genomic domains by satisfaction of spatial restraints

9:40-10:00 **15min +5**
Esteban Ballestar (PEBC-IDIBELL)
Targeting DNA methylation changes in Immune Cells: Functional Implications

10:00-10:20 **15min +5**
Joaquim Roca (IBMB-CSIC)
Nucleosomal DNA topology measured *in vivo* cracks the "Linking Number Paradox"

10:20-10:30 **10 x 1min**
Speed poster presentations

10:30-11:10 ***Coffee break and poster session***

Session II. –BCC8. Epigenetic of retroelements and repeats
Chair: ***Sonia Forcales***

11:10-11:30 **15min +5**
Miguel Beato (CRG)
TFIIIC binding to Alu elements controls cell cycle gene expression upon serum starvation by changing genome topology

11:30-12:00 **25min +5**
Didier Trono (Ecole Polytechnique Fédérale de Lausanne)
Transposable elements, their controllers and the genesis of human-specific transcriptional networks

12:00-12:25 **20min +5**
Sara Rodriguez-Heras (GENYO, Universidad de Granada)
A new face for let-7 microRNAs as tumor suppressors: keeping L1 retrotransposition in check

12:25-12:50 **20min +5**
Tokameh Mahmoudi (Erasmus Univ. MC, Rotterdam)
HIV transcription dissected; targeting distinct steps for latency reversal

12:50 Short Talk 1 **7min +3**
Izaskun Mallona (PMPPC-IGTP)
Unveiling potentially functionalized Alu repeats in great apes

13:00 Short Talk 2 **7min +3**
Jose David Aguirre (Hebrew Univ. Jerusalem)
The dynamics of silencing: On the role of H3.3 retroviral regulation

13:10-14:30 ***Lunch and poster session***

Session III. *Chair:* ***Guillaume Filion***

14:30-14:50 **15min +5**
Guillaume Filion (CRG)
HIV and the architecture of the human genome

14:50-15:10 **15min +5**
Joan-Ramon Daban (UAB)
Planar chromatin in metaphase chromosomes: 3D structure by cryo-electron tomography and biomedical implications

15:10-15:30 **15min +5**
Laia Ribas (ICM-CSIC)
A DNA-demethylating agent (the decitabine) used for cancer treatment is able to modulate sex in the zebrafish model

15:30-15:50 **15min +5**
Jordi Bernués (IBMB-CSIC)
Histone H1 at heterochromatin prevents R-loop formation

15:50-16:00 Sponsor' talk **7min +3**
Sarantis Chlamydas (Active Motif)
Novel technologies and tools enabling epigenetics research

16:00-16:30 ***Coffee break and poster session***

Session IV. *Chair:* ***Montserrat Corominas***

16:30-16:50 **15min +5**
Lorenzo Pasquali (IJC-IGTP)
Pancreatic beta cell chromatin dynamics and non-coding genome functions

16:50-17:10 **15min +5**

Montserrat Corominas (UB)
Damage-responsive regulatory elements in regeneration

17:10-17:30 **15min +5**
Miguel A. Peinado (PMPPC-IGTP)
Untangling coregulation modules in cancer

17:30 Short Talk 3 **7min +3**
Biola Javierre (IJC)
Lineage-specific genome architecture links enhancers and non-coding disease variants to target gene promoters

17:40 Short Talk 4 **7min +3**
José Luis Sardina (CRG)
Transcription factors drive Tet2-mediated enhancer demethylation to reprogram cell fate

17:50 Short Talk 5 **7min +3**
Noelia Díaz (Max Planck Inst. for Molecular Biomedicine)
Zebrafish 3D chromatin maps reveal conserved principles of chromatin organisation throughout vertebrates

18:00 Short Talk 6 **7min +3**
Beatrice Borsari (CRG)
Exploring the relationship between expression and chromatin dynamics from a temporal perspective

18:10 Short Talk 7 **7min +3**
Irene Miguel-Escalada (IDIBAPS)
3D chromatin maps of pancreatic islets identify the targets of T2D-associated variants

18.20 Meet together for a beer

POSTERS

1. Dafni Anastasiadi (ICM-CSIC)
Genetic and environmental influences on the epigenetic component of sexual development in a teleost fish, the European sea bass

2. Raquel Casquero (IJC)
RESPONSE project: predicting biomarkers in myelodysplastic syndrome patients treated with azacitidine

3. Andrea Izquierdo (IBMB-CSIC)
Histone H1 depletion triggers an interferon response in cancer cells via activation of heterochromatic repeats

4. Eduard Casas (IGTP)
Switch of a protein-coding gene into a small RNA producing locus triggered by a transposable element insertion in the laboratory mouse

5. Javier Moraleda (ICM-CSIC)
Epigenetic changes after rearing zebrafish (Danio rerio) at high density conditions during gonadal development.

6. Maria Solà (IBMB-CSIC)
Variations of mitochondrial DNA packaging across species

7. Tian Tian (CRG)
Whsc1 links pluripotency exit with mesendoderm specification

8. Alejandro Valdivieso (ICM-CSIC)
DNA methylation levels of sex-related genes involved in the gonadal development of zebrafish (Danio rerio)

9. Stella Pappa (IBMB-CSIC)
Histone demethylase PHF2 is essential for neural progenitor proliferation and maintains genome integrity

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ABSTRACTS

Structure determination of genomes and genomic domains by satisfaction of spatial restraints

Marc A. Martí Renom (CRG-CNAG)

The genome three-dimensional (3D) organization plays important, yet poorly understood roles in gene regulation. Chromosomes assume multiple distinct conformations in relation to the expression status of resident genes and undergo dramatic alterations in higher order structure through the cell cycle. Despite advances in microscopy, a general technique to determine the 3D conformation of chromatin has been lacking. We developed a method for the determination of the 3D conformation of chromatin domains in the interphase nucleus. The general approach of our method, called TADbit [1] has been applied to study several genomes [2,3,4,5] and opens the field for comprehensive studies of the 3D conformation of chromosomal domains and contributes to a more complete characterization of genome regulation. During my presentation, I will introduce our latest research on the the study of dynamic process of transcriptional activation of the SOX2 locus during trans-differentiation [6].

[1] F. Serra, D. Baù, et al. PLOS CB (2017) 13(7):e1005665

[2] D. Baù et al. Nat Struct Mol Biol (2011) 18:107.

[3] M.A. Umbarger, et al. Molecular Cell (2011) 44:252

[4] F. Le Dily, et al. Genes & Dev (2014) 28:2151

[5] M. Trussart et al. Nature Communication (2017) 8:14665

[6] R. Stadhouders, E. Vidal, et al. Nature Genetics (2018) doi:10.1038/s41588-0

TFIIIC binding to Alu elements controls cell cycle gene expression upon serum starvation by changing genome topology

Ferrari R[1,2, 3], de Llobet LI[1,2,3], Vidal Q[1,2,3], Le Dilly F[1,2,3], Quilez J[1,2,3], Di Vona C[1,2,3], De la Luna S[1,2,3], Dieci G[4], Teichmann M[5], Beato M[1,2,3].

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Folding of the chromatin fiber in the nuclear space is crucial for eukaryotic gene regulation. Contacts between distant regulatory regions via chromatin looping are governed by architectural proteins, originally identified as transcription factors and insulators, such as CTCF and TFIIIC. While the role of CTCF in genome folding has been intensively studied in recent years, much less in known about the role of TFIIIC in this context. To explore this question, we have investigated the extensive changes in gene expression in human cells exposed to serum deprivation. We found that upon serum deprivation human TFIIIC participates in genome reprogramming by binding to elements and changing their interactions with CTCF bound to distant promoters of cell cycle genes transcribed by RNA Polymerase II (Pol2). Landing of TFIIIC on Alu-elements located close to the TSS of Pol2 genes correlates with increased histone H3 Lysine18 acetylation, without dramatic changes in transcription of the Alu-elements or the nearby genes. Depletion experiments show that the large majority of TFIIIC dependent genes is located far away from the bound Alu-elements and their Pol2-mediated expression is dependent on long-range TFIIIC mediated DNA loops that connect the Alu-elements with pre-loaded CTCF sites near cell cycle relevant genes. This interaction framework established upon serum deprivation sustains basal levels of a crucial subset of cell cycle genes enabling their rapid activation upon serum exposure. All together these results shed light on a new role of TFIIIC in the control of gene expression upon starvation stress mediated by changes in the 3D genome organization via regulation of the epigenetic state of transposable elements.

A new role for let-7 microRNAs as tumor suppressors: keeping L1 retrotransposition in check

Pablo Tristán-Ramos[1], Laura Sánchez[1], Alejandro Rubio[1], José L. García-Pérez[1,2], Sara R. Heras[1,3]

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Around 55% of the human genome is made of transposable elements whose ongoing activity continues to impact our genome. Among them, Long INterspersed Element-1 (LINE-1 or L1) are the only autonomous active retrotransposons and move by a copy and paste mechanism. Active L1s are 6kb long and encode the enzymatic machinery required for their mobilization (ORF1p and ORF2p). In most human somatic cells the expression of active L1 elements is silenced by several mechanisms. However, in a diverse group of tumors L1s are actually expressed and mobilized, generating new mutagenic insertions which can affect tumor progression. On the other hand, microRNAs (miRNAs) are endogenous ~22nt RNAs that play crucial gene-regulatory roles in eukaryotes by pairing to mRNAs of protein-coding genes to direct their post-transcriptional repression. Thus, miRNAs are involved in multiple physiological and pathological processes, including cancer. Among known miRNAs, Let-7 family members are well characterized tumor suppressors which decrease the expression of known oncogenes, and are downregulated in several types of tumors.

To characterize the potential role of microRNAs in regulating retrotransposition, we analysed high-throughput sequencing data from different human tumor samples. Interestingly, we observed an inverse correlation between the number of endogenous somatic L1 insertions and let-7 expression levels. Furthermore, using different cellular assays, we have uncovered that let-7 controls engineered L1 retrotransposition: let-7a/b depletion and overexpression increases and decreases, respectively, L1 activity in several cell lines. In order to gain insight on the mechanism underlying this regulation, we have performed genetics and biochemical assays. Our results suggest that miRNA let-7 guides the RISC complex to the mRNA of active human L1s, interfering with ORF2p translation and therefore reducing L1 retrotransposition. Overall, our results suggest that let-7 family of miRNAs could have an additional role as tumor suppressor, maintaining genome stability by regulating L1 mobilization.

Untangling coregulalion modules in cancer

Izaskun Mallona and Miguel A. Peinado

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Recent advances in molecular and computational methods allow the dissection of chromatin meta-structure in different cell models, enlightening critical processes and mechanisms governing genomic regulation. Nevertheless, most of these analyses are not feasible in clinical specimens, precluding practical applications to decipher chromatin dynamics in actual physiological and pathological conditions, i.e., cancer. We aim to develop a method to explore chromatin's functional organization in human colon cancers and their paired normal tissues obtained by standard clinicopathological procedures. DNA methylation is a principal epigenetic mark that is readily analyzable in clinical samples with feasible genome-scale techniques. By decomposition of DNA methylation profiles into comethylation networks we can infer coregulation modules that exhibit unique structural and functional signatures. Modeling DNA methylation profiles in specific modules allows the identification of potential latent drivers of chromatin dynamics and the direct stratification of new cases without further reanalysis of the whole dataset.

Supported by grant SAF2015-64521-R from MINECO, FEDER and Generalitat de Catalunya

A DNA-demethylating agent (the decitabine) used for cancer treatment is able to modulate sex in the zebrafish model

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The role of epigenetic modifications such as DNA methylation during vertebrate sexual development is far from being clear. Using the zebrafish model, we tested the effects of one of the most common DNA methyltransferase (dnmt) inhibitor, 5-aza-2'-deoxycytidine (5-aza-dC), which is approved for the treatment of acute myeloid leukaemia and is under active investigation for the treatment of solid tumours. Several dose-response experiments were carried out during two periods, including not only the very first days of development (0–6 days post fertilization, dpf), as done in previous studies, but also, and as a novelty, the period of gonadal development (10–30 dpf) in zebrafish. Early treatment with 5-aza-dC altered embryonic development, delayed hatching and increased teratology and mortality, as expected. The most striking result, however, was an increase in the number of females, suggesting that alterations induced by 5-aza-dC treatment can affect sexual development as well. Results were confirmed when treatment coincided with gonadal development. In addition, we also found that the adult gonadal transcriptome of 5-aza-dC-exposed females included significant changes in the expression of key reproduction-related genes (e.g., *cyp11a1, esr2b* and *figla*), and that several pro-female related pathways such as the Fanconi anemia or the Wnt signalling pathways were downregulated. Furthermore, an overall inhibition of genes implicated in epigenetic regulatory mechanisms (e.g., *dnmt1*, *dicer*, *cbx4*) was also observed. Taken together, our results indicate that treatment with a DNA methylation inhibitor can also alter the sexual development in zebrafish, with permanent alterations of the adult gonadal transcriptome, at least in females. Our results show the importance of DNA methylation for proper control of sexual development, open new avenues for the potential control of sex ratios in fish (aquaculture, population control), and call attention to possibly hidden long-term effects of dnmt therapy when used, for example, in the treatment of prepuberal children affected by some types of cancer.

Planar chromatin in metaphase chromosomes: 3D structure by cryo-electron tomography and biomedical implications

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1-We have used cryo-electron tomography and Synchrotron small-angle X-ray scattering to investigate the chromatin folding in metaphase chromosomes. Cryo-electron tomography has allowed us to study chromosome structure in a vitrified and close-to-native state. Our 3D reconstructions show that frozen-hydrated chromatin emanated from metaphase chromosomes is planar and form multilayered plates, as previously observed in our laboratory (1) using different techniques. Plate thickness measurements show that each single layer is ~10 nm thick, which is equivalent to the nucleosome diameter. These measurements, combined with the observation of many plates contained in the 3D reconstructions, indicate that each layer is formed by a tightly packed mononucleosome sheet. The same nucleosome organization has been observed in staked layers, but distance measurements in contacting regions between two layers show a thickness of ~16 nm. This distance is smaller than that expected for the sum of two single layers (~20 nm), and indicate that the nucleosomes from both layers are interdigitated and that in the stacked structures each layer has an apparent thickness of ~6 nm. X-ray scattering of whole chromosomes under metaphase ionic conditions shows a dominant scattering peak at ~6 nm, which can be correlated with the distance between nucleosomes interacting through their faces in interdigitated layers and to the repeated distance between adjacent layers. A complete description of this work will be available in ref. (2). All these observations reinforce previous research of our laboratory and support a compact thin-plate model (1,3) consisting of many interdigitated chromatin layers stacked along the chromosome axis.

2-Chromosome images obtained from typical banded karyotypes and from different multicolor cytogenetic analyses can also be used to gain information about the 3D folding of chromatin within chromosomes. Chromosome bands and the connection surfaces in sister chromatid exchanges and in cancer translocations are planar and orthogonal to the chromosome axis. Chromosome stretching produces band splitting and even the thinnest bands are orthogonal and well defined, indicating that short stretches of DNA can occupy completely the chromosome cross-section. These observations impose strong physical constraints on models that attempt to explain chromatin packaging in chromosomes. The thin-plate model is the only proposed structure which is compatible with the observed orientation of bands, with the existence of thin bands (<1 Mb), and with band splitting; it is also compatible with the orthogonal orientation and planar geometry of the connection surfaces in chromosome rearrangements. The results obtained provide for the first time a consistent interpretation of the chromosome structural properties that are used in clinical cytogenetics for the diagnosis of hereditary diseases and cancers. A complete description of this work can be found in ref. (4).

(1) JR. Daban (2011) Micron 42:733-750.
(2) A. Chicano, E. Crosas, BD. Engel, R. Melero, J. Otón & JR. Daban. 3D structure of chromatin plates from metaphase chromosomes by cryo-electron tomography: Layer interdigitation and face-to-face nucleosome association, manuscript in preparation.
(3) JR. Daban (2014) J R Soc Interface 11:20131043
http://rsif.royalsocietypublishing.org/content/11/92/20131043
(4) JR. Daban (2015) Scientific Reports 5:14891 https://www.nature.com/articles/srep14891

Short Talk 1

Unveiling potentially functionalized Alu repeats in great apes

Izaskun Mallona (PMPPC-IGTP)

Izaskun Mallona, Berta Martín, Mireia Jordà and Miguel A. Peinado

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About half of the human genome is composed by transposable elements. Although multiple hypotheses point out roles in genome structure and function, their contribution to genome regulation is still poorly understood. Alu repeats are restricted to the primate lineage and constitute the most abundant transposon in human. We have previously shown that unmethylated Alu repeats display epigenetic features consistent with regulatory potential in normal and cancer cells (Mallona et al, J Biomed Inform. 60:77-83, 2016; Jordà et al. Genome Res 27:118-132, 2017).

Given the high phenotypic diversity across primates, we have explored the potential regulatory roles of unmethylated Alu elements by applying Next-generation Sequencing of UnMethylated Alu (NSUMA) technique to scrutinize more than 130,000 individual Alu elements in the whole blood from four species of great apes: gorilla, chimpanzee, orangutan and human.

We estimate the rates of Alu unmethylation across species and characterize the genomic and epigenomic features of the unmethylated Alus, including sequence conservation, selective pressure, proximity to coding genes, transcription factor binding sites and chromatin modifications.

This work was supported by grants from MINECO and FEDER (SAF2015-64521-R). CERCA Programme/Generalitat de Catalunya.

Short Talk 2

The dynamics of silencing: On the role of H3.3 retroviral regulation

Jose David Aguirre (Hebrew Univ. Jerusalem)

Jose David Aguirre, Sharon Schlesinger

H3.3 is variant of the H3 histone that has been implicated in the regulation of several epigenetic processes. The H3.3 variant accumulates modifications that are associated to open chromatin and gene activation. Despite of that, studies performed in embryonic stem cells (ESC) show enrichment of the variant H3.3 also in genomic areas that should be silent, such as telomeres and endogenous retroviruses (ERVs). Histone H3.3 turnover is replication independent, but little is known about the role of its dynamic turnover in transcriptional regulation during early development. To characterize the role of H3.3 in the regulation of retroviral sequences in pluripotent cells, we used mouse ESC lines carrying a single copy of doxycycline (Dox) inducible HA-tagged version of H3.3, and monitored the rate of H3.3 incorporation by ChIP at different time points following Dox induction. To follow the changes in H3.3 dynamics following differentiation onset, we monitored the cells before and after retinoic acid (RA) induced differentiation. To study the role of H3.3 in the silencing of exogenous retroviruses, we infected the cells with two different retroviruses and examined both the retroviral expression levels and the H3.3 dynamic at the integrated provirus site. Our results suggest that specific ERVs show fast H3.3 turnover, correlating with known retroviral-silencing chromatin marks, like Trim28 and H3K9me3 binding. Moreover, in many members of this group the turnover rate slows down following differentiation, signifying an important role for H3.3 turnover rate in the retroviral silencing machinery. Additionally, our preliminary data suggest that H3.3 is involved in MLV retroviral silencing and suggest an alternative mechanism for Trim28 binding to the retroviral LTR. This study is expected to provide insights into the basic biology that underlies retroviral silencing.

Short Talk 3

Lineage-specific genome architecture links enhancers and non-coding disease variants to target gene promoters

Biola Javierre (IJC)

Human cells bears in its nucleus about 2 meters of DNA containing the genes that shape the being and the manner in which is packed regulates its function. Long-range physical interactions between regulatory elements and gene promoters play key roles in transcriptional regulation. The vast majority of interactions are uncharted, constituting a major missing link in understanding genome control. Genome-wide association studies have identified thousands of single nucleotide polymorphisms (SNPs) associated with common disorders but most of them expand non-coding regions, being difficult to be interpreted. Interestingly these non-coding SNPs cluster on DNA hypersensitivity sites, hallmark of regulatory element, pointing out a potential role of these genetic variants in the deregulation of target genes. For all these reasons, a new technique called promoter capture Hi-C (PCHi-C) has been implemented. PCHi-C allows the genome-wide systematic identification of the interacting regions that are in physical contact with 31,253 human promoters. Applying this cutting-edge technology in 17 human primary hematopoietic cell types, it has been shown that promoter interactions are highly cell type specific and enriched for links between active promoters and epigenetically marked enhancers. Promoter interactomes reflect lineage relationships of the hematopoietic tree, consistent with dynamic remodeling of nuclear architecture during differentiation. Interacting regions are enriched in genetic variants linked with altered expression of genes they contact, highlighting their functional role. With this approach, non-coding disease variants have been connected to putative target promoters, prioritizing thousands of disease-candidate genes and implicating disease pathways. These results demonstrate the power of primary cell promoter interactomes to reveal insights into genomic regulatory mechanisms underlying common diseases.

Biola M. Javierre*, Oliver S. Burren, Steven P. Wilder, Roman Kreuzhuber, Steven M. Hill, Sven Sewitz, Jonathan Cairns, Steven W. Wingett, Csilla Várnai, Michiel J. Thiecke, Frances!Burden, Samantha Farrow, Antony J. Cutler, Karola Rehnström, Kate Downes, Luigi Grassi, Myrto Kostadima, Paula Freire&Pritchett, Fan Wang, The BLUEPRINT Consortium, Hendrik G. Stunnenberg, John! A. Todd, Daniel R. Zerbino, Oliver Stegle, Willem H. Ouwehand, Mattia Frontini, Chris Wallace, Mikhail Spivakov, Peter Fraser.

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Short Talk 4

Transcription factors drive Tet2-mediated enhancer demethylation to reprogram cell fate

José Luis Sardina (CRG)

Jose Luis Sardina, Samuel Collombet, Tian V Tian, Antonio Gomez, Bruno Di Stefano, Clara Berenguer, Ralph Stadhouders, Carolina Segura-Morales, Marta Gut, Ivo G Gut, Simon Heath, Denis Thieffry and Thomas Graf.

Here we report DNA methylation and hydroxymethylation dynamics at nucleotide resolution during highly efficient transcription factor-induced somatic cell to iPS cell reprogramming. We found that gene regulatory elements of key pluripotency factors become demethylated within one day after induction of the Yamanaka factors. Throughout reprogramming we observed successive waves of hydroxymethylation at enhancers, concomitant with a decrease in methylation. This suggests active demethylation, consistent with the finding that ablating the DNA demethylase Tet2 almost completely abolished reprogramming. Three distinct transcription factors, namely C/EBP**a**, Klf4 and Tfcp2l1, were shown to interact with Tet2 and recruit the enzyme to the DNA. Some of these sites maintain high levels of 5hmC, suggesting that here hydroxymethylated cytosines act as an epigenetic mark. Surprisingly, we also discovered regions in which methylation changes preceded chromatin opening. These included sites where Klf4 was bound without leaving a detectable footprint, suggesting a novel type of pioneer factor activity.

Short Talk 5

Zebrafish 3D chromatin maps reveal conserved principles of chromatin organisation throughout vertebrates

Noelia Díaz (Max Planck Inst. for Molecular Biomedicine)

Noelia Díaz[1], Benjamín Hernández-Rodríguez[1], Kai Kruse[1], Nick Machnik[1], Alexander J Nash[2,3], Boris Lenhard[2,3], Juan M Vaquerizas[1]

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The three-dimensional organisation of chromatin within the interphase nucleus plays a fundamental role in regulating gene expression. Throughout eukaryotes, the genome folds into a hierarchical arrangement of compartments and topologically associated domains (TADs). This domain-based organisation of the genome is strongly conserved across mammals. Here, we explore the degree of conservation across vertebrates by characterising the zebrafish 3D genome. Hi-C maps of zebrafish embryos at 24 and 48 hours post fertilisation revealed that chromatin has similar organisational units to mammals, with a division of chromatin into active/inactive compartments and TADs readily apparent at the megabase scale, which can be further classified into ordinary- and loop-domains. These domains are delimited by open regions highly enriched in DNA binding motifs of known factors involved in the structural organisation of chromatin, such as CTCF and cohesin. Furthermore, structure evolution analysis using imaging recognition techniques unveiled the maintenance of chromatin conformation in regions of synteny with human and mouse, as well as blocks of conserved non-coding elements (CNEs) that coincide with TADs. Finally, comparative analysis of ohnologous genes showed a significant rewiring in 3D chromatin structure following genome duplication. These changes coincide with alterations in CNE conservation, establishing a potential association of chromatin conformation with sub- and neo-functionalisation events. Our results provide a first characterisation of the 3D genome in zebrafish and identify conserved principles of chromatin organisation across vertebrates.

Short Talk 6

Exploring the relationship between expression and chromatin dynamics from a temporal perspective

Beatrice Borsari (CRG)

Beatrice Borsari[1,2], Cecilia Klein[1,2], Ramil Nurtdinov[1,2], Emilio Palumbo[1,2], Bruna Correa[1,2], Amaya Abad[1,2], Alexandre Esteban[1,2] and Roderic Guigó[1,2]

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Combinations of various histone modifications occurring at genomic regulatory elements are key in determining the location of transcription factors, and contribute to a fine temporal and spatial modulation of transcription. To decode the temporal dynamics of histone modifications and their contribution to changes in gene expression, we rely on a seven-days model of trans-differentiation of human proB cells to macrophages. For each time point, we have analyzed the transcriptome and the profile of nine histone marks. With a dynamic programming technique we are able to reconstruct, for a given gene, pairwise alignments between its expression and chromatin time-series profiles. This methodology provides a computational framework to unravel genome-wide temporal interplays between expression and histone marks. In our model, the epigenome appears to be a more stable system when compared to the dynamic behavior of the transcriptome, which responds fast to external stimuli. Expression and signatures of active chromatin marks correlate within single time points; however, we identify groups of genes lacking temporal consistency between these two components. Genes characterized by constant expression profiles typically show increased signals of active chromatin marking, as if the presence of these marks was more related to gene transcriptional stability through time, rather than being required for the transcription initiation process. Moreover, chromatin signals show either constant or delayed profiles, which do not promptly reflect the temporal changes in expression observed for a fraction of genes. In this context, we propose a model in which active histone post-translational modifications may not be responsible for changes in gene expression, but rather cooperate for the maintenance of specific transcriptional programs throughout cell divisions.

Short Talk 7

3D chromatin maps of pancreatic islets identify the targets of T2D-associated variants

Irene Miguel-Escalada (IDIBAPS)

Irene Miguel-Escalada[1,2], Silvia Bonàs-Guarch[1,2], Goutham Atla[1,2], Joan Ponsa[3], Inês Cebola[3], Delphine Rolando[3], Claire Morgan[3], Javier García-Hurtado[1,2], Jorge Ferrer[1,2,3].

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Gene expression in each cell type requires successful communication between distal regulatory enhancers, which contain genetic variants associated to common disease such as type 2 diabetes (T2D), and their target gene promoters. 3C-based methods have experimentally demonstrated that enhancer-promoter contacts happen through chromatin loops in a highly specific fashion. Nevertheless, 3D chromatin maps in disease-relevant tissues are considerably underdeveloped.

In this project, we aimed to create improved regulatory maps and high-resolution promoter-anchored interactome maps in human pancreatic islets using promoter-capture Hi-C, allowing us to identify significant 3D regulatory interactions genome-wide. Integrative analysis of these datasets also allowed us to link non-coding T2D-associated variants to target genes and to identify enhancer domains in 3D space. These regulatory units aggregate enhancers to genes that are crucial for islet cell identity and function and concentrate T2D-associated genetic variants.

In summary, we describe the complex network of 3D enhancer-promoter interactions occurring in human pancreatic islets and demonstrate the utility of 3D maps to identify the transcriptional targets of disease-associated variants, providing new insights into the molecular mechanisms underlying T2D.

Poster 1.

Genetic and environmental influences on the epigenetic component of sexual development in a teleost fish, the European sea bass

Dafni Anastasiadi (ICM-CSIC)

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In many reptilian and piscine species, sex is a phenotypically plastic trait that can be influenced by temperature. In the European sea bass (*Dicentrarchus labrax*), the sex is determined by both genetic and environmental factors and the parental generation may produce more or less male-prone offspring. The response to temperature is mediated by the inhibition of gonadal aromatase (*cyp19a1a*) expression, the enzyme that converts androgens into estrogens, by increased DNA methylation in the gene promoter. However, the methylation levels of other genes related to sexual development and the parental effects on the epigenetically-mediated response to temperature may influence the phenotypic outcome. In this study, the offspring of four sires known to produce male- or female-prone progeny was reared at low or high temperature during the thermosensitive period. Then, in the gonads of adult fish, the methylation levels of the putative regulatory regions of 7 genes related to sexual development were measured by a custom-made multiplexed targeted bisulfite sequencing approach. We showed that the methylation of the two of the most important genes for sexual development, *cyp19a1a* and *dmrt1*, is both genetically and environmentally regulated in opposite directions according to sex. Furthermore, when we focused on the genetic component by including only the fish reared at low temperature, we observed that the CpGs of *er-β2*, *foxl2* and *nr3c1* presented unfluctuating methylation levels between sires and offspring. This result indicates a potential transmission of the methylation states across generations. On the other hand, the methylation levels of some CpGs were highly responsive to temperature. Using a combination of these CpGs we were able to achieve the first prediction of sex in a vertebrate with high accuracy based on analysis of DNA methylation pattern of a suite of selected CpGs. These results suggest a panel of CpG candidates to be used as biomarkers for sex prediction and multigenerational transmission of the methylation status. *Supported by MINECO grant AGL2016-78710-R "Epimark" to FP.*

Poster 2.

RESPONSE project: predicting biomarkers in myelodysplastic syndrome patients treated with azacitidine

Raquel Casquero (IJC)

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Myelodysplastic syndrome (MDS) is one of the most common bone marrow disorders in the elderly characterized by an ineffective hematopoiesis and strong predisposition to acute myeloid leukemia (AML). Azacitidine (AZA) is the major treatment given to high-risk MDS patients who are ineligible for bone marrow transplantation due to its severe side effects. Only 40% to 50% of AZA-treated patients show hematological improvements and complete responses are limited to 10% to 15%. The treatment is not curative and response is lost over time.

Our aim is to identify response-predicting biomarkers and novel drug targets that would be suitable for improved combinatorial therapeutic approaches. For this, we use genetic loss-of-function screening in available MDS-AML cell lines to identify AZA-sensitivity affecting genes that will be validated in primary patient samples from the first longitudinal study of AZA treatment in 100 patients. As AZA is incorporated into DNA, we will focus on chromatin and transcriptional regulators. Following a standardized protocol, we collect samples at diagnosis, after AZA treatment and, if occurring, at relapse.

Funded by ISCIII, the RESPONSE project applies the same approach to three major cancers and their current best treatment. In addition to MDS treated with AZA, this includes non-small lung cancer and colorectal cancer treated with chemotherapy.

Poster 3.

Histone H1 depletion triggers an interferon response in cancer cells via activation of heterochromatic repeats

Andrea Izquierdo (IBMB-CSIC)

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Seven linker histone H1 variants exist in human somatic cells with distinct prevalence depending on the cell type and along differentiation. H1 bind to linker DNA contributing to higher order chromatin compaction. In addition, H1 seems to be actively involved in the regulation of gene expression. It is not well known whether the different variants have specific roles. We have shown that H1 variants are not distributed uniformly along the genome and there are differences between variants, H1.2 being the one showing the most specific pattern. We have explored functions of H1 variants by inducible shRNA-mediated knock-down of each of the variants. Knock-down of each H1 variant alters expression of a different, reduced subset of genes. Combined depletion of H1.2 and H1.4 has a strong deleterious effect in the cancer cells examined, and induces a strong interferon (IFN) response with up-regulation of many IFN-stimulated genes (ISGs). Although H1 participates to repress ISG promoters, its activation upon H1 KD is mainly generated by the expression of noncoding RNA generated from heterochromatic repeats including satellites. In conclusion, redundant H1-mediated silencing of heterochromatin is important to maintain genome stability and to avoid an unspecific growth-inhibiting IFN response.

Poster 4.

Switch of a protein-coding gene into a small RNA producing locus triggered by a transposable element insertion in the laboratory mouse

Eduard Casas (IGTP)

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Transposable elements (TE) and other repeats make up nearly half of the mammalian genome and contribute to genetic variation, causing mutagenesis but also regulatory innovations. Here we report a novel impact that TEs can have on genes – they can switch a protein-coding gene into a small non-coding RNA producing locus in a tissue-specific manner.

We observed striking inter-individual variation in small RNAs mapping to Nocturnin, a protein-coding gene. This gene is host to a recent TE insertion in the mouse. We hypothesised that the TE insertion has triggered the birth of a new PIWI-interacting RNA (piRNA) producing locus in the mouse germline.

First, we confirmed that small RNA production from Nocturnin is only found in individuals with the TE insertion. We found that these small RNAs are bound by PIWI proteins in the mouse germline. We found that the TE is not acting via a transcriptional mechanism of germline-specific promoter or enhancer. Last, our results suggest that the TE signals the protein-coding transcript for piRNA processing through a non-piRNA-guided slicing mechanism.

In summary, we provide evidence consistent with the hypothesis that a TE insertion licences a protein-coding gene for processing into piRNAs through a post-transcriptional mechanism. Our findings reveal yet another way of how TEs can regulate endogenous genes and provide novel insights into the biogenesis of piRNAs in the mammalian germline.

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Poster 5.

Epigenetic changes after rearing zebrafish (Danio rerio) at high density conditions during gonadal development

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Key words: epigenetics, DNA methylation, fish, sex differentiation, stress

Stress induced by high rearing density during the gonadal development period (7 to 45 days post fertilization, dpf) in domesticated zebrafish (*Danio rerio*) caused an increase in the number of males. Moreover, the ovarian transcriptome of fish subjected to high density had more than 3,000 differentially expressed genes (DEG) when compared to non-stressed fish. Further, it is known that DNA methylation is related to the masculinization process in fish during gonadal development. Thus, the goal of this study was to determine whether there was a direct relationship between stress and DNA methylation changes in fish gonads. Zebrafish larvae were subjected to two different treatments, one group reared at low density (9 fish/liter) and another group reared at high density (74 fish/liter) during their sexual differentiation period. Once fish reached adulthood, sex ratio and biometry were recorded and gonads were dissected and kept frozen at -80ºC. In order to test how DNA methylation patterns changed due to density, a set of eleven DEG related to sex (e.g., *cyp19a1a* and *dmrt1*), stress (e.g., *β-hsd*) and DNA methylation (e.g., *dnmt1*) were selected and analyzed by Multiplex Bisulfite Sequencing (MBS) to obtain the methylation level for each CpG in the promoter regions. Sequencing data was analyzed by bioinformatics tools (e.g., Bismark software) and results discussed in relation to the ability of high density stress as an environmental factor capable to cause long-lasting effects through changes in the DNA methylation.

Poster 6.

Variations of mitochondrial DNA packaging across species

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Mitochondrial DNA (mtDNA) is packaged into nucleoprotein structures called nucleoids. Compaction of human mtDNA (h-mtDNA) relies on transcription factor A (TFAM or mtTFA) whereas in yeast (*S. cerevisiae*) mtDNA is compacted by Abf2p. In addition to mtDNA compaction, these proteins have disparate functions: TFAM is a transcription factor whereas Abf2p has been related to DNA recombination. We will present the crystal structures of these two proteins in complex with DNA, both showing a drastic bending of the nucleic acids by 180º. In addition, we will show the conformational changes of either protein when binding DNA, by small-angle X-ray scattering in solution. This will be accompanied by molecular dynamics calculations that show the molecular mechanism that maintain the stability of the complexes, which are flexible. On the other hand, human and yeast mitochondrial genomes are dissimilar. H-mtDNA is a circular molecule of 16.5 kbp with few intergenic sequences and asymmetric GC content in strands, while y-mtDNA is a 85 kbp linear molecule with extensive intergenic non-coding regions and rich in poly-adenine (poly-A) tracts. The different sequence contents endow different structural and topological properties on the DNAs and thus may impose different regulation strategies on DNA compaction. For Abf2p, the A-tracts impose a DNA structure-directed protein positioning that potentially regulates global nucleoid architecture and thus mtDNA transactions via a protein-DNA structural interplay. We will present insights into the molecular basis of mtDNA compaction based on the analysis of these distantly-related proteins and their complexes with DNA. Additionally, we explore U-turns and poly-A-tracts as components of general strategies in mtDNA compaction across species. Our multidisciplinary approach results in a comprehensive analysis of protein/DNA complex formation and suggests a universal mtDNA compaction unit.

Poster 7.

Whsc1 links pluripotency exit with mesendoderm specification

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How pluripotent cells differentiate into the various germ layers is a key question of developmental biology. Consensus holds that this is initiated by dismantling of the pluripotency gene regulatory network, allowing subsequent activation of lineage-specific networks. Here we show that the chromatin related factor Whsc1 has an unexpected dual role in pluripotency exit and germ layer specification of embryonic stem cells (ESCs). Upon induction of differentiation, a proportion of Whsc1-depleted ESCs remain entrapped in a pluripotent state and specifically fail to efficiently form mesendoderm cells, although they are still capable of generating neuroectoderm. The role of Whsc1 in these functions is independent of its histone methyltransferase activity. Mechanistically Whsc1 binds to enhancers of the mesendodermal regulators *Gata4*, *Brachyury*, *Gata6* and *Foxa2* identified using quantitative 4C technique, and activates their expression following recruitment of Brd4. Strikingly, depleting each of these lineage instructive transcription factors also delays pluripotency exit, suggesting that they at least partially mediate the effects observed with Whsc1. Hence, suppression of pluripotency and onset of differentiation are not sequential, separable processes: upregulation of mesendodermal transcription factors, coordinated by Whsc1, is required for timely egress from pluripotency. Our data instead suggest that silencing of the pluripotency regulatory network and activation of lineage-restricted networks are tightly interconnected processes.

Poster 8.

DNA methylation levels of sex-related genes involved in the gonadal development of zebrafish (Danio rerio)

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Abstract

Fish exhibit all types of reproduction systems known in vertebrates. In fish, gonadal fate is the combined result of genetic and environmental influences and some evidence indicates that epigenetic processes such as DNA methylation may have an important role. However, little is known regarding the relationship between DNA methylation and the expression of key genes involved in sexual development. In this study, we analyzed the DNA methylation of the promoter regions of canonical reproduction-related genes (*dmrt1*, *dmrt3a*, *amh*, *cyp19a1a*, *fshr*, *foxl2, ar, sf1 and fancl*) and its association with sex in 90-day-old adults of two different AB zebrafish families. DNA was extracted from their gonads and NGS libraries were prepared and sequenced using a targeted bisulfite sequencing approach. Results showed that there were significantly higher DNA methylation levels in the promoter of *amh*, *cyp19a1a,* and *foxl2* of males compared to females, while *dmrt1* and *dmrt3a* exhibited the opposite pattern. Principal Component Analysis (PCA) showed that gonadal samples could be resolved according to sex. However, a subset of males was grouped closer to females than to the rest of males, suggesting that these males were neomales (genetic females developing as phenotypic males) produced spontaneously by perturbations of the process of sexual differentiation with a yet unknown etiology. In order to further analyze these findings, we are currently analyzing the expression levels of key genes related with male and female differentiation. This study contributes to our understanding of the role of DNA methylation in gene expression regulation during critical steps of gonadal development in a vertebrate model. *Supported by MINECO grant AGL2016-787107-R "Epimark" to FP.*

Poster 9.

Histone demethylase PHF2 is essential for neural progenitor proliferation and maintains genome integrity

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Neural development is driven by transcriptional, epigenetic mechanisms and multiple signaling pathways. Many transcriptional programs are controled by histone methylation. Several histone demethylases (HDM) have been identified as important players in neural development the last years. Among others, plant homeodomain finger 2 (PHF2) is a histone demethylase that plays a role in epigenetic regulation of gene expression by demethylating H3K9me2. Although it has been shown that PHF2 can act as a tumour suppressor and can also play a role in adipocyte differentiation, very little is known about its role during early neurogenesis. It is higly expressed in neural tube and in cortex and for this reason we have characterized the phenotype of PHF2 in neural progenitor cells in vivo in the chicken embryos spinal cord as well as its function in vitro in neural progenitor cells from cortex of mouse embryos. We have seen that PHF2 is a cell cycle regulator and controls cell proliferation in vivo and in vitro. Combining ChIP seq and RNA seq experiments in NSC we found that PHF2 targets gene promoters and functions as an activator of genes implicated in G1/S transition as well as in DNA replication. PHF2 depletion leads to DNA damage as indicated by the increased γH2Ax content and to a general dysregulation of the pericentromeric heterochromatin, affecting the transcription of the satellite repeats.