SEEING AND DECODING NUCLEAR FUNCTION AND STRUCTURE

27th – 28th January 2020
PRBB Auditorium, Barcelona

BOOK OF ABSTRACTS

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Centre for Genomic Regulation, ES

Melike Lakadamyali
University of Pennsylvania, US

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Hebrew University of Jerusalem, IL

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Centre for Genomic Regulation, ES

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PROGRAMME

Monday, January 27th

13h30 Welcome by Pia Cosma

13h45 - 16h15 | Session 1
Novel super resolution approaches to visualize genome structure & dynamics
Chair: Melike Lakadamyali

13h45 | **Jan Ellenberg**
Standardised and quantitative live and super-resolution microscopy tools to analyse chromosome architecture

14h15 | **Ting Wu**
Such a lot of genome to see…

14h45 | **Eric Joyce**
Unravelling the mechanism of 3D genome folding by high-throughput FISH

15h15 | **Roman Barth**
Live-cell imaging of chromatin at nanoscale resolution

15h35 | **Jan-Michael Peters**
How Cohesin folds the genome

16h05-16h25 Coffee break

16h25 - 18h55 | Session 2
Chromatin organization & plasticity in early development & differentiation
Chair: Pia Cosma

16h25 | **Kikuë Tachibana**
Chromosome segregation and 3D chromatin structure of mammalian oocytes

16h55 | **Jiang Liu**
CTCF role in establishing 3D chromatin structure during human embryogenesis

17h25 | **María Elena Torres Padilla**
Epigenetic mechanisms of cellular plasticity and reprogramming to totipotency

17h55 | **Suzana Hadjur**
Chromatin topology regulation and stem cell state plasticity

18h25 | **Giacomo Cavalli**
3D genome organization and Polycomb proteins in development and cell differentiation

20h30 Speakers dinner

Tuesday, January 28th

09h00 - 11h50 | Session 3

Chromatin folding: from modeling to nanoscale imaging

Chair: Jérôme Solon

09h00	**Leonid Mirny** The interplay of loop extrusion and transcription
09h30	**Alba Granados Corsellas** A bag-of-visual-words model for single-molecule localization microscopy data classification
09h50	**Stephanie Portillo** Nucleosome clutches reorganization during cell differentiation and their regulation by chromatin internal parameters

10h20-10h50 Coffee break

10h50	**Pablo Gómez García** Mesoscale modelling and single molecule tracking for the study of chromatin structure and dynamics during differentiation
11h10	**Claudia Cattoglio** Genome organization dynamics at single-molecule resolution
11h30	**Yang Liu** Super-resolution imaging reveals dysregulated nanoscale chromatin folding in cancer development

12h00-14h00 Lunch break with poster session

14h00 - 16h20 | Session 4

Chromatin dynamics & transcription

Chair: Eran Meshorer

14h00	**Peter Becker** Chromosome organisation and global regulation of transcription
14h30	**Laszlo Tora** Towards the understanding the dynamics of RNA polymerase II transcription
15h00	**Marie Victoire Neguembor** Investigating the role of transcription in 3D genome folding and loop extrusion
15h20	**Karsten Rippe** Regulation of gene expression by phase separation processes
15h50	**Victor Corces** Mechanisms of transgenerational inheritance of obesity epiphenotypes

16h20-16h30 Closing remarks

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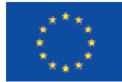
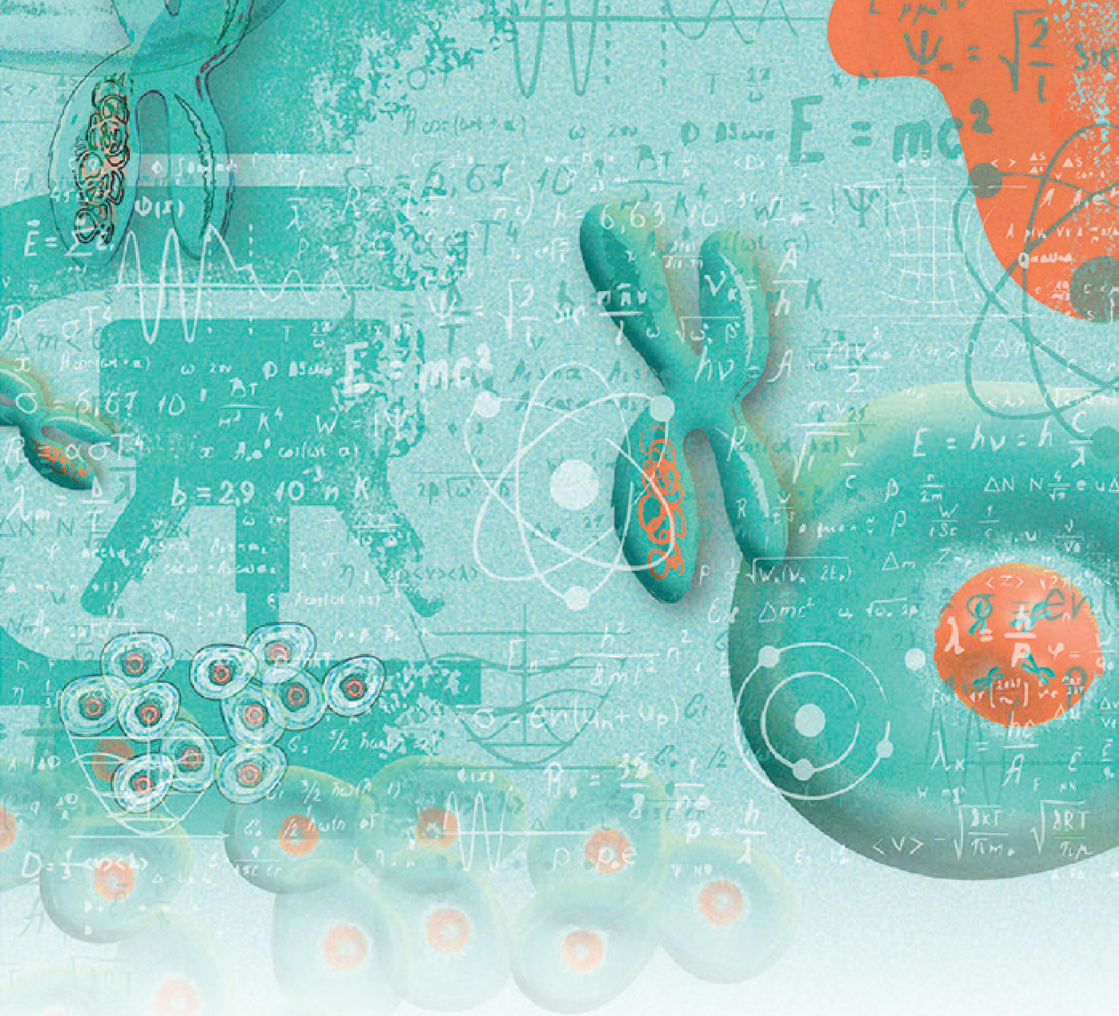




ABSTRACTS
Invited Speakers

(in order of appearance in the Programme)

Standardised and quantitative live and super-resolution microscopy tools to analyse chromosome architecture

Jan Ellenberg[1], Jonas Ries[1], Ewan Birney[2]

[1] Cell Biology & Biophysics Unit, European Molecular Biology Laboratory, Heidelberg 69117, Germany

[2] European Molecular Biology Laboratory-European BioInformatics Institute, Hinxton CB10 1SD, United Kingdom

The spatial organisation of the genome is essential for regulation of its function and mitotic chromosome compaction. However, the 3D structure and folding principles of chromosomal DNA is poorly understood in situ. New microscopy technologies are currently revolutionising our ability to unravel molecular assemblies inside cells, but often lack rigorous quantitative readouts and comparability between laboratories. We have combined absolutely quantitative live imaging with super-resolution microscopy (SRM)[1,2] to determine protein copy number and positions of single protein complexes on chromosomal DNA. We have furthermore introduced human cell lines with the nuclear pore protein Nup96 homozygously tagged with commonly used fluorescent tags, as versatile reference standards for the community[3]. Applying these novel tools allowed us to determine the abundance of the key chromosome structure regulators Condensins, Cohesins and CTCF[4,5,6] on human chromosomes throughout cell division. The finding that around 1400 individually spaced Condensin complexes organise a single human chromosomal DNA molecule, allowed us to calculate the loop sizes of mitotic chromosomes to approximately 90 kb[4]. To resolve the predicted loops, we have implemented DNA Exchange-PAINT of 10 kb spaced DNA hybridisation probes and shown that the 3D fold of 100 kb of chromosomal DNA can be resolved, while keeping overall nuclear architecture intact. We find that loop size and structure in a euchromatic region is highly variable and displays different degrees of compaction between cells[7]. These quantitative data allow us to propose new models for the folding of DNA that underlies mitotic chromosome compaction.

1 Cai et al., Nature 561:411-5
2 Walther and Ellenberg, Methods Cell Biol 145:65-90.
3 Thevathasan et al., Nature Methods 16:1045–1053.
4 Walther et al., J Cell Biol 217:2309-2328
5 Cattoglio et al., Elife 8 pii: e40164
6 Holzmann et al., Elife 8. pii: e46269
7 Oedegaard et al., in preparation.

Such a lot of genome to see...

Guy Nir[1*], Irene Farabella[2*], Huy Q. Nguyen[1*], Shyamtanu Chattoraj[1*], David Castillo Andreo[2*], Jelena Erceg[1*], Jumana AlHaj Abed[1*], Anton Goloborodko[3,a*], Cynthia Pérez Estrada[4*], Carl G. Ebeling[5*], Nuno M. C. Martins[1*], S. Dean Lee[1], Son C. Nguyen[1,b], Evan R. Daugharthy[1,6], Paul Reginato[1,3], Taehyun Ryu[1], Mohammed A. Hannan[1], Job Dekker[7], Jeff A. Stuckey[5†], Leonid A. Mirny[3†], George M. Church[1,8†], Erez Lieberman Aiden[4,9,10†], Marc A. Marti-Renom[2,11,12†], C.-ting Wu[1,8†]

[1] Harvard Medical School, Boston, MA, USA
[2] CNAG-CRG, Barcelona, Catalonia, Spain
[3] Massachusetts Institute of Technology, Cambridge, MA, USA
[4] Baylor College of Medicine, Houston, TX, USA
[5] Bruker Nano, Inc., USA
[6] Currently, ReadCoor, Cambridge, MA, USA
[7] University of Massachusetts Medical School, Worcester, MA, USA
[8] Wyss Institute for Biologically Inspired Engineering, Boston, MA, USA
[9] Rice University, Houston, TX, USA
[10] Broad Institute, Cambridge, MA, USA
[11] Universitat Pompeu Fabra, Barcelona, Catalonia, Spain
[12] ICREA, Barcelona, Catalonia, Spain
[a] Currently, Institute of Molecular Biotechnology, Vienna, Austria
[b] Currently, University of Pennsylvania, Philadephila, PA, USA

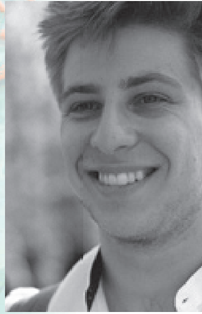
This presentation will provide a summary of our work using Oligopaints, HOPs, and OligoSTORM (GN, IF, CPE, CGE, SDL, JAS, ELA, MAMR) and the development of a new technology called OligoFISSEQ (HQN, SC, DCA, SCN, ERD, PR, TR, GMC, MAMR) to view chromosomes, in situ, using wide-field and super-resolution microscopy. Time permitting, it may also include an update of our studies of somatic homolog pairing using haplotype-resolved Hi-C as well as OligoSTORM (JE, JAA, AG, JD, LM) and of centromere structure using OligoSTORM (NMCM). Work carried out in my own laboratory was supported by the dedicated contributions of MAH and awards from EMBO to JE (ALTF 186-2014) and NIH to GMC (RM1HG008525) and CtW (R01HD091797, R01GM123289, DP1GM106412).

Unraveling the mechanism of 3D genome folding by high-throughput FISH

Eric Joyce

Department of Genetics, Penn Epigenetics Institute, Perelman School of Medicine, University of Pennsylvania

The emerging model is that metazoan genomes are arranged into a nested hierarchy of unique structural features, driven by a division of labor amongst the few architectural proteins that we know of (e.g. CTCF, cohesin, condensin). During interphase, cohesin is essential for chromatin looping while recent work from our lab and others suggests that condensin II is important instead for large-scale chromosome folding. However, the mechanism by which condensin mediates chromosome folding during interphase remains unknown. This highlights our need to identity additional factors to determine how these molecular machines, including condensin II, cooperatively guide the genome through the cell cycle and development. To this end, we have combined two technologies that use fluorescent in situ hybridization (FISH). The first is a technology for high-throughput FISH (Hi-FISH), and the other, called PENNie probes, is a new Oligopaint-based probe that reduces the cost and increases the resolution of FISH. Using a combination of these tools, we are conducting imaging-based screens for novel architectural proteins in Drosophila and human cells. This work is providing new insights into the mechanisms underlying condensin regulation and chromatin folding during interphase.



Live-cell imaging of chromatin at nanoscale resolution

Roman Barth

Delft University of Technology. The Netherlands

Chromatin conformation regulates gene expression and thus constant remodeling of chromatin structure is essential to guarantee proper cell function. To gain insight into the spatio-temporal organization of the genome, we employ high-density photo-activated localization microscopy and deep learning to obtain temporally resolved super-resolution images of chromatin in living cells. In combination with high-resolution dense motion reconstruction, we confirm the existence of elongated ~ 45 to 90 nm wide chromatin 'blobs'. A computational chromatin model suggests that these blobs are dynamically associating chromatin fragments in close physical and genomic proximity and adopt TAD-like interactions in the time-average limit. Experimentally, we found that the chromatin structure exhibits a spatio-temporal correlation extending ~ 4 μm in space and tens of seconds in time, while chromatin dynamics are correlated over ~ 6 μm and outlast 40 s. Notably, chromatin structure and dynamics are closely interrelated, which may constitute a mechanism to grant access to regions with high local chromatin concentration.

How cohesin folds the genome

Jan-Michael Peters

Research Institute of Molecular Pathology (IMP), Vienna, Austria

Eukaryotic genomes are folded into loops and topologically-associating domains (TADs), which contribute to chromatin structure, gene regulation and recombination. These structures depend on cohesin, a ring-shaped DNA-entrapping ATPase complex which has been proposed to form loops by extrusion. Such an activity has been observed for condensin, which forms loops in mitosis, but not for cohesin. I will provide evidence that human cohesin rapidly extrudes DNA into loops in a process that depends on cohesin's ATPase activity but not on topological entrapment of DNA by cohesin.

Chromosome segregation and 3D chromatin structure of mammalian oocytes

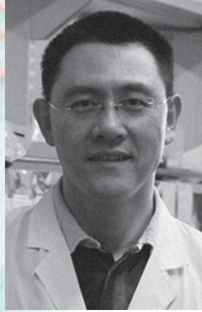
Emmanouella E. Chatzidaki[1], Johanna Gassler1, Sean Powell[1], Kikuë Tachibana[1,2]

[1] Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna BioCenter, Vienna, Austria

[2] Max Planck Institute of Biochemistry (MPIB), Martinsried, Germany

Maternal age is the most important risk factor for trisomic pregnancies. Most trisomies are caused by chromosome missegregation during the meiotic divisions of oocytes. Multiple mechanisms contribute to the increase in aneuploid eggs and fetuses at advanced maternal age. It is therefore considered unlikely that a single approach could prevent the maternal age effect. Using three experimental lines of evidence, we show that ovulation reduction is sufficient to prevent egg aneuploidy in aged mice. To test our hypothesis that ovulations contribute to oocyte ageing and promote chromosome missegregation, we generated mice that undergo few or no ovulations due to lack of the G-protein coupled receptor 54 (Gpr54), which is essential for puberty onset. Remarkably, egg aneuploidy is reduced 3-fold in pre-pubertal Gpr54-/- compared to Gpr54+/+ aged females. A reduction in egg aneuploidy was also observed when reducing ovulations by hormonal contraception or successive pregnancies. We show that ovulation suppression correlates with retention of chromosomal Rec8-cohesin, which is essential for sister chromatid cohesion in oocytes. This implies that ovulations are linked to cohesin deterioration. This work provides the first experimental evidence that Ovulation suppression reduces egg aneuploidy and suggests that hormonal contraception can reduce the risk of trisomic pregnancies like Down's syndrome at advanced maternal age.

Using our single-nucleus Hi-C (snHi-C) method, we discovered that ageing alters 3D chromatin organization in long-lived single cells. We show that chromatin loops depend on Scc1-cohesin, rather than Rec8-cohesin, in oocytes. Given that Rec8 deteriorates with age, we tested if and how higher-order chromatin structure changes. Unexpectedly, the size of average extruded loops increases with age and this is reduced by ovulation suppression. We propose that cohesion mediated by Rec8-cohesin limits loop extrusion by Scc1-cohesin. We conclude that reducing ovulations leads to retention of chromosomal Rec8, which maintains interphase chromatin structure and promotes chromosome segregation and production of euploid eggs.



CTCF role in establishing 3D chromatin structure during human embryogenesis

Jiang Liu,

Beijing Institute of Genomics, CAS

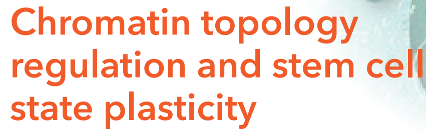
In a cell's interphase cycle, chromatin is arranged in a hierarchical structure within the nucleus, which plays an important role in regulating gene expression. However, the dynamics of 3D chromatin structure during human embryogenesis remains unknown. Here, we report that human sperm do not have CTCF and TAD structure, which differs from mouse sperm. After human fertilization, TAD structure in embryos is obscure, and then is then gradually established through embryonic development. We also find that A/B compartmentalization is lost in human 2-cell embryos, and is reestablished during embryogenesis. Notably, blocking zygotic genome activation (ZGA) can inhibit TAD establishment in human embryos, which differs from that in mouse and Drosophila. Interestingly, CTCF is very low before ZGA, and then is highly expressed at the ZGA stage when TADs are observed in human embryos. Consistently, TAD structure is significantly reduced in CTCF knock-down embryos, suggesting TAD establishment requires CTCF expression during ZGA in human embryos. Taken together, CTCF plays a key role in the establishment of 3D chromatin structure during human embryogenesis.

Epigenetic mechanisms of cellular plasticity and reprogramming to totipotency

Maria Elena Torres-Padilla[1]

[1]Institute of Epigenetics & Stem Cells, Helmholtz Centre Munich, Munich, Germany

In mammals, the terminally differentiated sperm and oocyte fuse to create a totipotent zygote upon fertilisation. The mechanisms underlying the epigenetic reprogramming towards totipotency that follows fertilisation are not fully understood, and the molecular features of totipotent cells remain scarce. Embryonic cells remain totipotent only for a restricted time window. During this time, embryonic cells are characterised by an atypical chromatin structure and reactivation of specific families of retrotransposons. Recently, it was reported that totipotent-like cells arise in ES cell cultures in vitro. Like in the embryo, these cells are characterised by the expression of MERVL LTR retrotransposons. To address how the expression of these elements is regulated during the transition between totipotent and pluripotent states, we first examined histone modifications and chromatin structure in early mouse embryos. Remarkably, we have found that specific features of embryonic chromatin are also present in totipotent-like cells in vitro. Based on this analysis, we have begun to decipher key molecular regulators of repetitive elements in the embryo, and how they contribute to shaping the regulatory programme of the newly formed embryo. Our results have identified candidate proteins that regulate chromatin function and expression of these elements and show that they can induce totipotency. We are currently examining the role of these molecules in sustaining totipotency in the embryo. We will present our latest results that reveal a new role for chromatin integrity in promoting epigenetic reprogramming and sustaining molecular features of totipotent cells.



Chromatin topology regulation and stem cell state plasticity

Dubravka Pezic, Wazeer Varsally, Samuel L Weeks, Suzana Hadjur

University College London Cancer Institute, Paul O'Gorman Building, 72 Huntley Street, WC1E 6BT, London UK

Cell populations consist of mixtures of cells in distinct cellular states which is critical for responsiveness of the system to changing environments. Interconversion between states underlies biological processes such as stem cell control and cancer development. A full understanding of cellular identity requires knowledge of both the transcriptional states and the mechanisms that control state stability. Using heterogenous embryonic stem cell (ESC) populations, we reveal a role for 3D chromatin topology regulation in cell state plasticity. Cohesin is a ubiquitously expressed, multi-subunit protein complex that has fundamental roles in cell biology including dynamic 3D chromosome topology in interphase. The complex is composed of Smc3, Smc1, Rad21 and one of either Stag1 or Stag2 which together form a ring-like structure. Cohesin directly interacts with CTCF through the Stag subunit of the complex and together they physically tether chromatin loops to create insulated chromatin domains. Here, we show that the proteins which regulate cohesin's association with chromatin, but not the core cohesin ring itself, play important roles in cell state switching. The levels of the Stag protein paralogs are regulated during ESC differentiation. Stag1 is the dominant version in naive ESCs and has a critical role in defining the balance between the pluripotent state and differentiation. Further, cell-state specific promoters and alternative splicing drive expression of Stag1 protein variants. Modulating the relative levels of Stag1 variants in ESCs dictates state interconversion probabilities. Mechanistically we show that variants have specialised roles in 3D chromatin topology that are functionally non-redundant. Altogether, our results highlight the importance of diversifying cohesin regulator protein pools (paralogs and variants) and maintaining a homeostatic balance of these to ensure chromatin landscape dynamics and thus cell state plasticity.

3D genome organization and Polycomb proteins in development and cell differentiation

Giacomo Cavalli[1,2]

[1] Institute of Human Genetics, CNRS, 34396 Montpellier Cedex 5, France

[2] University of Montpellier, Montpellier, France

The eukaryotic genome folds in 3D in a hierarchy of structures, including nucleosomes, chromatin fibers, loops, chromosomal domains (also called TADs), compartments and chromosome territories that are highly organized in order to allow for stable memory as well as for regulatory plasticity, depending on intrinsic and environmental cues. Polycomb Group (PcG) and trithorax group (trxG) proteins form multimeric protein complexes that regulate chromatin via histone modifications, modulation of nucleosome remodeling activities and regulation of 3D chromosome architecture. These proteins can mediate epigenetic inheritance of chromatin states but also dynamically bind to some of their target genes, thereby affecting cell proliferation and differentiation in a wide variety of biological processes. Polycomb group proteins form two main complexes, PRC2 and PRC1, which coregulate a subset of their target genes, whereas we have shown that other processes, such as cell proliferation, are regulated specifically by one of the complexes. Our progress in these fields will be discussed.

Acknowledgements

This work was supported by the European Research Council Advanced Investigator grants (3DEpi), by the Horizon 2020 program (MuG), by the Association pour la Recherche sur le Cancer, by the Fondation pour la recherche médicale (FRM), by INSERM and the ITMO Cancer (MMTT project), by the INCa, and by the CNRS.

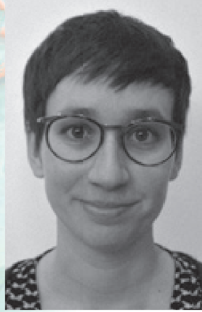
The interplay of loop extrusion and transcription

Leonid Mirny,

Massachusetts Institute of Technology (MIT)

I will review our recent progress in understanding the loop extrusion and its interference with other biological processes. Recently, we found that, in both bacteria and higher eukaryotes, the process of transcription can serve as a directional boundary to extrusion, slowing down and pushing back extruding SMCs; the process that we described as the "moving barrier mechanism". An important feature of the mechanism is that an SMC tracks with a polymerase, allowing to maintain a continuous contact between a distal regulatory element looped by the SMC and the elongating polymerase.

We also examine potential role of loop extrusion in mediating enhancer-promoter interactions and show that extrusion can control direct contacts (i.e. ~10nm proximity) because it brings loci along the chromosomal fiber into immediate proximity within the cohesin complex. We further find that extrusion can mediate and alter frequency of direct contacts by more than ten-fold while generating only two-fold relative enrichment on Hi-C maps of more coarse contact. Furthermore, we show that by exploiting its ability to scan across long genomic regions, loop extrusion can both facilitate and target direct contact through selective placement of extrusion barriers. We find further support of extrusion-mediated contacts in the new Micro-C data.



A bag-of-visual-words model for single-molecule localization microscopy data classification

Alba Granados[1], Jérôme Solon[2]

[1] Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, 08003 Barcelona, Spain

[2] IKERBASQUE, Basque Foundation for Science. E-48011, Bilbao, Spain.

I will review our recent progress in understanding the loop extrusion and its interference with other biological processes. Recently, we found that, in both bacteria and higher eukaryotes, the process of transcription can serve as a directional boundary to extrusion, slowing down and pushing back extruding SMCs; the process that we described as the "moving barrier mechanism". An important feature of the mechanism is that an SMC tracks with a polymerase, allowing to maintain a continuous contact between a distal regulatory element looped by the SMC and the elongating polymerase.

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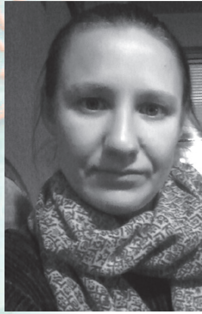
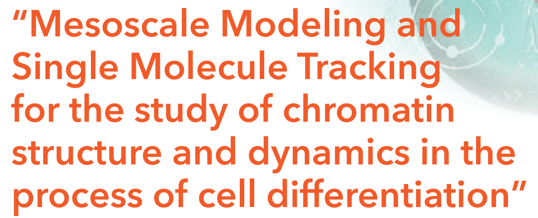


Nucleosome clutches reorganization during cell differentiation and their regulation by chromatin internal parameters

Stephanie Portillo-Ledesma and **Tamar Schlick**

Department of Chemistry, New York University, New York, NY 10003, USA.

Nucleosomes, the building block of chromatin, cluster together when chromatin folds in the cell to form heterogeneous groups termed "clutches". These structural units add another level of chromatin regulation to the cell, for example during cell differentiation. To gain further insights into chromatin reorganization during differentiation, we simulate chromatin fibers typical of mouse embryonic stem cells (mESC) and neural progenitor cells (mNPC) using our nucleosome-resolution chromatin mesoscale model. In agreement with previous super-resolution microscopy results, we find that nucleosome clutches are larger and more compact in differentiated cells. Moreover, the mNPC chromatin fiber is overall more compact than mESC and exhibits higher-order folding motifs such as hierarchical looping. These results indicate a direct correlation between the nucleosome clutches organization and the open and closed state of the chromatin fiber. To understand the specific mechanisms regulating nucleosome clutches and interpret the clutch patterns observed, we explore the effects of chromatin fiber internal parameters on clutch patterns by modeling fibers that differ in nucleosome positions, linker histone density, or histone tail acetylation levels. We find that the main factor regulating clutch size is nucleosome placement, with linker histone density and acetylation levels important modulators as well. These results shed light on the chromatin reorganization at the nucleosome level during cell differentiation and its regulation by internal parameters. We suggest a new epigenetic mechanism by which chromatin internal parameters can regulate transcriptional activity via the three-dimensional folded state of the genome



“Mesoscale Modeling and Single Molecule Tracking for the study of chromatin structure and dynamics in the process of cell differentiation”

Pablo Gómez García

Centre for Genomic Regulation (CRG) Barcelona, Spain

"STORM imaging revealed that nucleosomes are arranged in groups with heterogeneous sized termed "clutches" and that those clutches are smaller and less densely compacted in mouse embryonic stem cells (mESCs) compared to mouse neuronal progenitor cells (mNPCs), in correlation with the more open chromatin state of mESCs. In this work we applied modelling and Single Molecule Tracking (SMT) to compare the structure of synthetic fibers and local nucleosome dynamics with the super resolution images of chromatin fiber in mESCs and mNPCs. First, using mesoscale chromatin modeling, we simulated the spatial arrangement of chromatin fibers corresponding to the pluripotency gene Oct4 in mouse ESCs (mESCs) and mouse NPCs (mNPCs), taking into account nucleosome positions from MNASE-Seq data, the ratio of linker histone H1 per nucleosome, and the amount of histone tail acetylation. The resulting folded fiber configurations showed higher compaction of the overall fiber and of the nucleosome clutches in mNPCs compared to mESCs, recapitulating the super resolution imaging data. Then we further used SMT both at short (15ms) and long (500ms) exposure times to show that nucleosome turn over and local dynamics within the chromatin fiber correlate with the structural features observed in super-resolution data and the polymer models. Nucleosomes are less stable, turn over faster, explore larger volumes and are more mobile in mESCs than in mNPCs."

Genome organization dynamics at single-molecule resolution

Claudia Cattoglio[1,2]

[1] Department of Molecular and Cell Biology, Li Ka Shing Center for Biomedical and Health Sciences, CIRM Center of Excellence, UC Berkeley, Berkeley CA 94720, USA

[2] Howard Hughes Medical Institute, University of California, Berkeley CA 94720, USA

Mammalian genomes are folded into Topologically Associating Domains (TADs), DNA regions spanning hundreds of kilobases defined by a high 3D-interaction frequency. CTCF and cohesin have emerged as key causal regulators of mammalian TADs and of the chromatin loops that hold them together. We are interrogating the dynamics of CTCF and cohesin using a combination of super-resolution and live-cell single-molecule imaging, genome editing, biochemistry and genomics. We find that CTCF and cohesin form a rapidly exchanging complex, suggesting that chromatin loops frequently break and reform during each cell cycle. Both proteins exhibit unusual anisotropic diffusion in the nucleus, as they are transiently trapped in zones of ~200 nm in size. These zones correspond to CTCF clusters, and trapping inside them increases the efficiency of CTCF's DNA-target search mechanism. We further demonstrate that CTCF anisotropic diffusion at the 200-nm scale is largely due to an internal RNA-binding region downstream of its zinc finger domain (RBRi). Deletion of the RBRi thus results in impaired target search efficiency, but also reduces CTCF clustering and alters chromatin architecture and gene expression. Taken together, our work underlines the highly dynamic nature of genome organization and reveals critical roles for RNA interactions in regulating CTCF nuclear topology, target search and chromatin looping.

Super-resolution imaging reveals dysregulated nanoscale chromatin folding in cancer development

Yang Liu, Jianquan Xu, Hongqiang Ma, Douglas J. Hartman, Sarah J. Hainer

Biomedical and Optical Imaging Laboratory, Departments of Medicine and Bioengineering, University of Pittsburgh, Pittsburgh PA 15213, USA

Mammalian genomes are folded into Topologically Associating Domains (TADs), DNA regions spanning hundreds of kilobases defined by a high 3D-interaction frequency. CTCF and cohesin have emerged as key causal regulators of mammalian TADs and of the chromatin loops that hold them together. We are interrogating the dynamics of CTCF and cohesin using a combination of super-resolution and live-cell single-molecule imaging, genome editing, biochemistry and genomics. We find that CTCF and cohesin form a rapidly exchanging complex, suggesting that chromatin loops frequently break and reform during each cell cycle. Both proteins exhibit unusual anisotropic diffusion in the nucleus, as they are transiently trapped in zones of ~200 nm in size. These zones correspond to CTCF clusters, and trapping inside them increases the efficiency of CTCF's DNA-target search mechanism. We further demonstrate that CTCF anisotropic diffusion at the 200-nm scale is largely due to an internal RNA-binding region downstream of its zinc finger domain (RBRi). Deletion of the RBRi thus results in impaired target search efficiency, but also reduces CTCF clustering and alters chromatin architecture and gene expression. Taken together, our work underlines the highly dynamic nature of genome organization and reveals critical roles for RNA interactions in regulating CTCF nuclear topology, target search and chromatin looping.

Chromosome organisation and global regulation of transcription

Peter B. Becker

Biomedical Center, LMU Munich, Germany

Mammalian genomes are folded into Topologically Associating Domains (TADs), DNA regions spanning hundreds of kilobases defined by a high 3D-interaction frequency. CTCF and cohesin have emerged as key causal regulators of mammalian TADs and of the chromatin loops that hold them together. We are interrogating the dynamics of CTCF and cohesin using a combination of super-resolution and live-cell single-molecule imaging, genome editing, biochemistry and genomics. We find that CTCF and cohesin form a rapidly exchanging complex, suggesting that chromatin loops frequently break and reform during each cell cycle. Both proteins exhibit unusual anisotropic diffusion in the nucleus, as they are transiently trapped in zones of ~200 nm in size. These zones correspond to CTCF clusters, and trapping inside them increases the efficiency of CTCF's DNA-target search mechanism. We further demonstrate that CTCF anisotropic diffusion at the 200-nm scale is largely due to an internal RNA-binding region downstream of its zinc finger domain (RBRi). Deletion of the RBRi thus results in impaired target search efficiency, but also reduces CTCF clustering and alters chromatin architecture and gene expression. Taken together, our work underlines the highly dynamic nature of genome organization and reveals critical roles for RNA interactions in regulating CTCF nuclear topology, target search and chromatin looping.

Towards the understanding the dynamics of RNA polymerase II transcription

Laszlo Tora[1], Sascha Conic[1], Dominique Desplancq[2], Alexia Ferrand[3], Veronique Fischer[1], Kishore Babu N.[5], Graham D. Wright[4], Nacho Molina[1], and **Etienne Weiss[2]**

[1]Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Illkirch, France
[2]Institut de Recherche de l'ESBS, UMR 7242, Illkirch, France.
[3]Imaging Core Facility, Biozentrum, University of Basel, Basel, Switzerland.
[4]Institute of Medical Biology, A*STAR, Singapore, Singapore.
[5]School of Biological Sciences, Nanyang Technological University, Singapore.

Initiation of transcription by RNA polymerase II (Pol II) is the outcome of a number of sequential events beginning with the binding of activators to their binding sites, which will trigger the recruitment of coactivator complexes and general transcription factors (GTFs) at promoters to allow the loading of Pol II into the preinitiation complex. TFIID is the first GTF that is involved in promoter recognition. During transcription initiation coactivators play multiple crucial roles. To better characterize the dynamic behavior of GTFs and Pol II, we used our recently developed versatile antibody-based imaging approach (VANIMA) that allows non-harmful delivery of organic dye-conjugated antibodies, or antibody fragments, into different metazoan cell types. VANIMA permits live imaging of the labelled probes bound to their endogenous targets. By using conventional and super-resolution imaging we show dynamic changes in the distribution of several nuclear transcription factors (i.e., Pol II, TFIID subunits such as TAF10). Quantifications of Pol II foci shows that they range from 10−3 μm3 to ~1.6 × 10−2 μm3, with only 34% of the foci having the smallest volume. TAF10 foci are in general smaller than those of Pol II, with 55% of the spots showing the smallest volume. Interestingly, ~3% of the Pol II foci are larger than 10−2 μm3. Live-cell measurements show that the larger Pol II clusters are dynamic and are constantly dissociating to smaller foci and re-associating again over time. However, after inhibition of transcription the percentage of larger Pol II clusters is decreasing by a factor of 4. Thus, our results show that when transcription elongation is inhibited the larger Pol II containing clusters dissociate, and suggest that Pol II molecules become mobile.

Using VANIMA and super resolution imaging we were also able to visualize and quantify the number of γH2AX labelled foci before and after DNA damage. Our quantifications show that DNA damage increased the number of γH2AX foci by ~80-100-fold in treated cells, suggesting that labelling with transduced Fab fragments allows precise analysis of chromatin modifications upon replication stress. Thus, VANIMA allowed us to uncover novel biological information based on the dynamic behaviour of transcription factors or posttranslational modifications in the nucleus of single live cells.

Investigating the role of transcription in 3D genome folding and loop extrusion

Maria Victoria Neguembor[1], **Álvaro Castells-García**[1, &], **Laura Martin**[1, &], **Chiara Vicario**[1, &], **Pablo Aurelio Gómez-García**[1], **Jumana AlHaj Abed**[2], **Alba Granados**[1], **Ruben Sebastian-Perez**[1], **Francesco Sottile**[1], **Jérôme Solon**[1, 3], **Chao-ting Wu**[2, 4], **Melike Lakadamyali**[5, *, $] and **Maria Pia Cosma**[1, 3, 6, *, $]

[1] Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, 08003 Barcelona, Spain

[2] Department of Genetics, Harvard Medical School, Boston (Massachusetts), United States of America.

[3] Universitat Pompeu Fabra (UPF), Dr Aiguader 88, 08003 Barcelona, Spain

[4] Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston (Massachusetts), United States of America.

[5] Perelman School of Medicine, Department of Physiology, University of Pennsylvania, 19104 Philadelphia (Pennsylvania), United States of America.

[6] Institució Catalana de Recerca i Estudis Avançats (ICREA), Pg. Lluís Companys 23, 08010 Barcelona, Spain

[&] These authors contributed equally to the work, names are listed in alphabetical order

[$] Co-senior authors

Understanding how the genome is folded and how the 3D genome organization regulates gene activity is a key area of investigation to understand life. Yet, our knowledge of the mechanisms controlling genome folding is still at its infancy. In particular, while it is thought that genome organization can regulate gene transcription, whether gene transcription can in turn shape genome organization is largely underexplored. Likewise, the mechanisms and driving forces controlling cohesin mediated loop extrusion are still largely unknown. Using super-resolution microscopy and single molecule tracking, we investigate the impact of transcriptional activity on genome organization as well as the crosstalk between transcription and cohesin. We show that transcription has an essential role in shaping 3D genome organization and loop extrusion by influencing cohesin function. Thanks to improved spatial resolution provided by STORM microscopy, we shed light into the organisation of loops thus providing single-cell information of nuclear organization at the nanoscale level.

Regulation of gene expression by phase separation processes

Karsten Rippe

German Cancer Research Center (DKFZ) and Bioquant, Division of Chromatin Networks, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

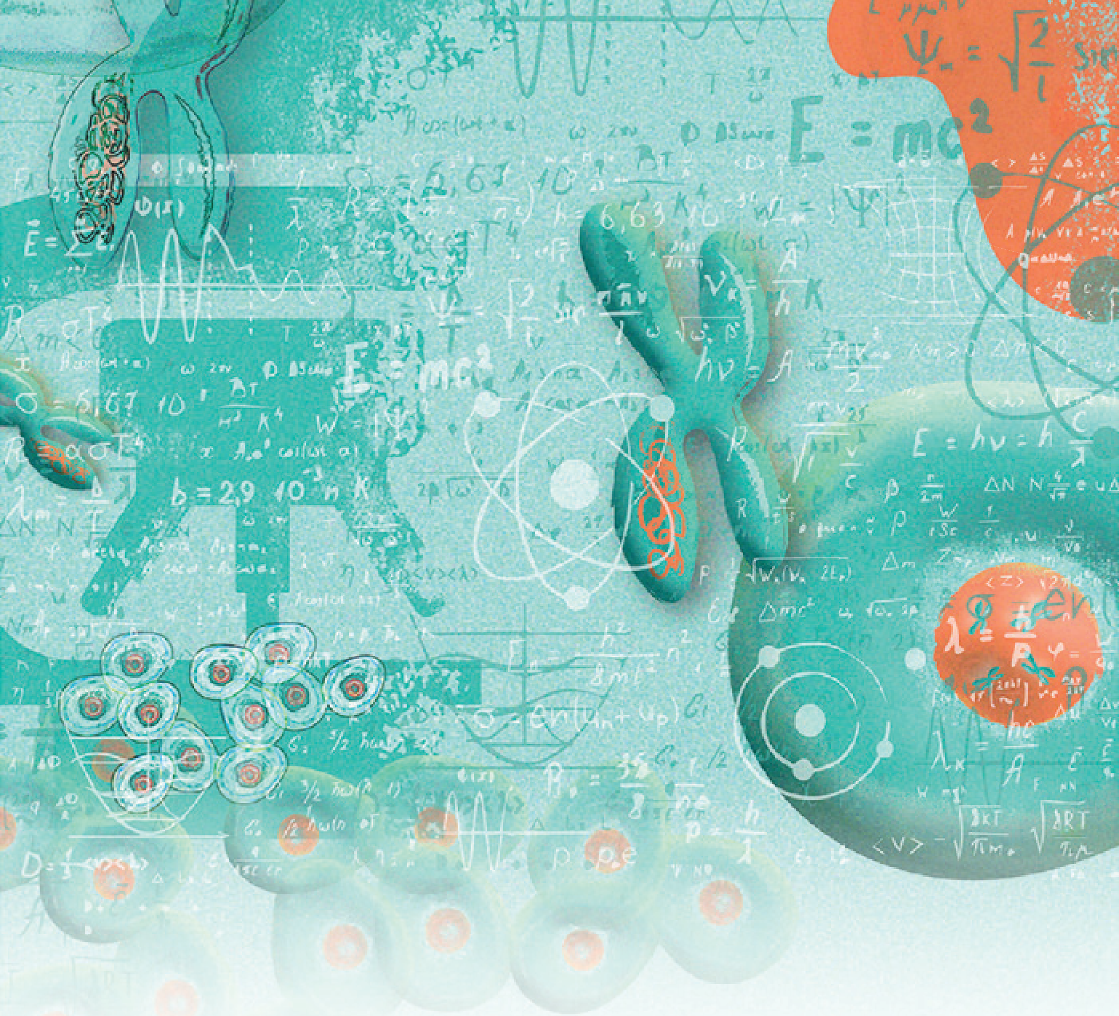
The eukaryotic nucleus lacks internal membranes and within seconds proteins can translocate through it by diffusion. Nevertheless, the cell establishes specific patterns of active and silenced chromatin domains that are characterized by a distinct distribution of chromosomal proteins and certain patterns of histone modifications and DNA methylation marks. However, the mechanisms that drive the formation of these chromatin subcompartments are only partly understood. We have dissected the dynamic properties of pericentric heterochromatin domains as well as an inducible array of ~200 reporter genes as model systems for silenced and active nuclear subcompartments. By investigating their physio-chemical properties like protein binding dynamics, internal mixing and response to the recruitment of transcriptional activators, the contribution of phase separation processes was assessed. Our findings on the mechanisms that underlie chromatin compartmentalization have a number of functional implications for chromatin state transitions, heterochromatin spreading, epigenetic memory and regulation of transcription.

Mechanisms of transgenerational inheritance of obesity epiphenotypes

Victor G. Corces

Emory University, Atlanta, GA 30322, USA

Mechanisms by which epiphenotypes are transmitted between generations through the paternal germline remain poorly understood. Most promoters in mouse sperm contain RNAPII and are flanked by positioned nucleosomes marked by a variety of active histone modifications. The sperm genome is bound by transcription factors, including Mediator, FoxA1, ER and AR. These proteins are found at promoters, enhancers, and super-enhancers. CTCF and cohesin are also present in sperm DNA, where they mediate interactions that organize the sperm genome into domains overlapping extensively with those found in mESCs. This information suggests that epigenetic information present in mammalian sperm could be altered by environmental factors to cause novel phenotypic outcomes in the next generation. When pregnant females are exposed to endocrine disruptor chemicals, their progeny show a variety of phenotypes, including obesity. This phenotype is transmitted between generations in the absence of further exposure. Approximately 68 new protein binding sites are present in the sperm and fat tissue of obese mice from the F1 through the F4 generations. These new binding sites correspond to CTCF, RA, and ER, suggesting that effects of these proteins on 3D chromatin organization and transcription of specific genes are responsible for the establishment and transmission of epiphenotypes.



ABSTRACTS

Posters

Lysosomal degradation ensures accurate chromosomal segregation to prevent genomic instability

Eugènia Almacellas[1,2], Charles Day[3,4], Santiago Ambrosio[5], Albert Tauler[1,2*] and Caroline Mauvezin[2*]

[1] Department of Biochemistry and Physiology, Faculty of Pharmacy, University of Barcelona, Barcelona 08028, Spain.

[2] Metabolism and Cancer Laboratory, Molecular Mechanisms and Experimental Therapy in Oncology Program (Oncobell), Bellvitge Biomedical Research Institute IDIBELL, L'Hospitalet del Llobregat 08908, Spain.

[3] Hormel Institute, University of Minnesota, Austin, Minnesota, 55912, USA.

[4] Neuro-Oncology Program, Mayo Clinic, Rochester, Minnesota, 55905, USA.

[5] Department of Physiological Sciences, Faculty of Medicine and Health Sciences, University of Barcelona, L'Hospitalet del Llobregat 08907, Spain.

Lysosomes, as primary degradative organelles, are the end-point of different converging pathways including macroautophagy. To date, lysosome function has mainly focused on interphase cells, while their role during mitosis remains controversial. Mitosis dictates the faithful transmission of genetic material among generations, and perturbations of mitotic division lead to chromosomal instability, a hallmark of cancer. Heretofore, correct mitotic progression relies on the orchestrated degradation of mitotic factors, which was mainly attributed to ubiquitin-triggered proteasome-dependent degradation. Here, we show that mitotic transition does not only rely on proteasome-dependent degradation, as impairment of lysosomes increases mitotic timing and leads to mitotic errors, thus promoting chromosomal instability. Furthermore, we characterized an atypical nuclear phenotype, the toroidal nucleus, as a novel biomarker for genotoxic screenings. Our results establish lysosome-dependent degradation as an essential event to prevent genomic instability.

CTCF and the emergence of 3D chromatin structure

MJ. Andreu[1], M. Portela[1], A. Álvarez[1], D. Giménez[2], C. Badia-Careaga[1], M. Tiana[1], A. Cuadrado[2], A. Losada[2] and M. Manzanares[1]

[1] Centro Nacional de Investigaciones Cardiovasculares (CNIC), Calle de Melchor Fernández Almagro 3, 28029 Madrid, Spain.

[2] Centro Nacional de Investigaciones Oncológicas (CNIO), Calle deMelchor Fernández Almagro 3, 28029 Madrid, Spain.

The generation of high-resolution chromatin interaction maps has revealed that the genome folds into distinct megabase-scale modules termed topologically associated domains (TADs) that show a direct relationship to gene expression and regulation within them. CTCF is a ubiquitously expressed and highly conserved chromatin architectural protein enriched at the boundaries of these domains. However, depletion of CTCF protein in different systems does not cause a dramatic disassembling of the organization of the genome. Given the mostly stable nature of TAD organization, we are studying the requirement of CTCF when 3D chromatin structure is first assembled: during mouse preimplantation development. By genetically eliminating the maternal and/or zygotic contribution of CTCF, we will examine the structure of the genome that has not been exposed to CTCF at all. To do so, we are setting up single-embryo HiC in wild-type mouse embryos to then perform this technique in maternal and maternal-zygotic mutant embryos. Maternal-zygotic mutants die at the late blastocyst stage, when they suffer a block in proliferation. Unsuspectedly, maternal mutants are viable up to adulthood. Transcriptional analysis of maternal-zygotic mutant blastocysts shows a clear downregulation of metabolism, DNA replication and protein processing together with an overexpression of ribosomal and mRNA processing proteins and proteins involved in ubiquitination. On the other hand, maternal mutants do not differ from control embryos. Our results show that CTCF is not necessary for the first phases of mouse preimplantation development, but is later absolutely required for proper developmental progression. Ongoing work will inform us how these changes relate to the early assembly of chromatin structure.

ISR8 enhancer region affects IFNα- and NF-κB- mediated responses

Marina Barriocanal[1], Nerea Razquin[1], Celia Prior[1], Puri Fortes[1,2,3]

[1] CIMA/UNAV. [2] IDISNA. [3] CIBEREhd Pamplona. Spain.

The study of the interferon (IFN)α-induced cell transcriptome has showed altered expression of several long non-coding RNAs (lncRNAs). One example is *ISR8* (IFN Stimulated RNA 8). This gene is located close to IFN regulatory factor 1 (*IRF1*) coding gene and transcribes for a lncRNA induced after IFNα treatment, as well as, by IRF1 or NF-κB transcription factors. Depletion or overexpression of ISR8 RNA does not lead to deregulation of IRF1 or other IFN-stimulated genes (ISGs). Surprisingly, disruption of *ISR8* locus with CRISPR-Cas9 genome editing, results in cells that fail to stablish a successful IFNα response. Moreover, in these cells ISGs and pro-inflammatory cytokines are not induced by IRF1 or the NF-κB subunit RELA. Then, *ISR8* locus may play a relevant role in IFNα and NF-κB pathways. Interestingly, ISR8-disrupted cells have functional IFNα/NF-κB pathways, as IFNα treatment and IRF1 or RELA overexpression induce luciferase expression from exogenous episomic promoters. The defects observed in ISR8-disrupted clones are not due to general gene silencing, as the aberrant IFNα and NF-κB responses are also observed after treating these cells with methyltransferase or histone deacetylase silencing inhibitors. Moreover, ChIP experiments performed on ISR8-disrupted and control cells suggest that the promoter region of several ISGs is active and accessible to the transcription machinery. We believe that we have identified a loci required to build proper long-range interactions required to stablish complete antiviral and inflammatory responses. This is relevant for human health, as several SNPs located in the ISR8 region are associated with human diseases caused by a dis-regulated immune response. In summary, our results show that genomic disruption in the ISR8 region leads to silencing of ISGs and pro-inflammatory genes expressed elsewhere in the genome.

Rapid reactivation of the inactive X in iPSC involves a unique intermediate chromosome structure

Moritz Bauer[1, 2*], **Enrique Vidal**[1, 2*], **Stefan Pinter**[3], **Guillaume Filion**[1, 2] and **Bernhard Payer**[1,2]

[1] Gene Regulation, Stem Cells and Cancer Program, Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain

[2] Universitat Pompeu Fabra (UPF), Barcelona, Spain

[3] Department of Genetics and Genome Sciences, Institute for Systems Genomics, University of Connecticut Health Center, USA

The functional interplay of 3D genome organisation and transcription remains a highly debated topic and can be exemplified by the process of X chromosome inactivation. The inactive X chromosome in mammals is characterized by a unique transcriptionally silenced state and a distinct chromosome conformation, defined by attenuation of topologically associating domains and a partitioning into two mega-domains. However, whereas the dynamics of X chromosome inactivation have been studied intensively, much less is known about its reversal, X chromosome reactivation (XCR). We here present work on XCR, using a novel neural precursor cell-based iPSC reprogramming system, that allowed us to isolate large numbers of cells that undergo successful reprogramming, addressing common shortcomings of conventional MEF reprogramming systems. This enabled us to investigate the combined dynamics of transcriptional reactivation, chromatin opening and chromosome restructuring *in vitro*. Similar to XCR in the epiblast, we are able to show that XCR *in vitro* happens in a short window of time. Moreover, using Hi-C, we observed that during reprogramming, the X chromosome forms a distinct structural intermediate, with the reappearance of topologically associating domains, while features of the two mega-domains are still present. To summarize, our novel iPSC system allows us to define the kinetics of XCR *in vitro* and sheds light on the relationship between transcription and chromosome structure.

Genome organization in and around the nucleolus

Cristiana Bersaglieri, Jelena Kresoja-Rakic, Dominik Bär, Shivani Gupta, Rostyslav Kuzyakiv, Raffaella Santoro

Department of Molecular Mechanisms of Disease, University of Zurich, Switzerland

In eukaryotic cells, the higher-order organization of the genome is functionally important to ensure correct execution of gene expression programs. Increasing evidence indicated that large-scale folding of chromatin affects gene expression by locating genes to specific subnuclear compartments that are either stimulatory or inhibitory to transcription. The nuclear periphery and the nucleolus are two important nuclear landmarks where repressive chromatin domains are often located. However, while the role of the nuclear periphery in genome organization has been well documented, the function of the nucleolus remains yet elusive. Indeed, while domains associating with the nuclear periphery (LADs) have been identified and characterized, nucleolar-associated domains (NADs) remained under-investigated.

Here we present a new methodology that allows the identification of NADs. We used this method to map NADs during embryonic stem cell differentiation. Genome contacts with the nucleolus revealed broad continuous regions, indicating compartmentalization around the nucleolus that changes according to cell state. Comparative HiC data confirmed that these a large fraction of these NADs interact with the nucleolar ribosomal RNA (rRNA) genes and these interactions occur at inter and intra-chromosomal level. Data will be presented describing the method in details and the features of NADs that in combination with the analysis of expression and chromatin states of NADs and LADs will help to move toward the obtainment of a comprehensive functional map of genome compartmentalization, clarifying its role in the regulation of gene expression and cell function.

Crosstalk between chromatin organization and transcription using Hi-M

Espinola S.[1], Goetz M.1, Fiche J.B.[1], Schaeffer M.[1], C. Houbron.[1], Lagha M.[2], Nollmann M.[1]

[1] Centre de Biochimie Structurale, CNRS UMR 5048, INSERM U1054, Université de Montpellier, Montpellier, France.

[2] Institut de Génétique Moléculaire de Montpellier, CNRS UMR 5535, Université de Montpellier, Montpellier, France 4Neuro-Oncology Program, Mayo Clinic, Rochester, Minnesota, 55905, USA. 5Department of Physiological Sciences, Faculty of Medicine and Health Sciences, University of Barcelona, L'Hospitalet del Llobregat 08907, Spain

Simultaneous observation of 3D chromatin organization and transcription at the single cell level and with high spatial resolution may hold the key to unveil the mechanisms regulating embryonic development, cell differentiation and even disease. We have recently developed Hi-M, a technology that allows for the sequential labelling, 3D imaging and localization of multiple genomic DNA loci together with RNA expression in single cells within whole, intact Drosophila embryos. Using Hi-M, we now aim to investigate how chromatin folds into topologically associating domains (TADs) during Drosophila early embryogenesis, focusing in the relationship between enhancer-promoter (E-P) contacts and transcriptional activity. Our results demonstrate that emergence of TADs arises at nc14, just before the MBT transition. In addition, we were able to identify specific interactions between regulatory elements (E and P). We show that an enhancer is able to contact two promoters, and that promoter-promoter interactions are widespread.

Cohesin and condensin extrude DNA loops in a cell-cycle dependent manner

Stefan Golfier[1,2,3], Thomas Quail[1,2,3], Hiroshi Kimura[4], Jan Brugués*[1,2,3]

[1] Max Planck Institute of Molecular Cell Biology and Genetics, 01307 Dresden, Germany

[2] Max Planck Institute for the Physics of Complex Systems, 01187 Dresden, Germany

[3] Centre for Systems Biology Dresden, 01307 Dresden, Germany

[4] Cell Biology Center, Institute of Innovative Research, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8503, Japan

DNA loop extrusion by structural maintenance of chromosomes complexes (SMCs) has been proposed as a mechanism to organize chromatin in interphase and metaphase. However, the requirements for chromatin organization in these cell cycle phases are very different, and it is unknown whether loop extrusion dynamics and the complexes that extrude DNA also differ. Here, we used *Xenopus* egg extracts to reconstitute and image loop extrusion of single DNA molecules during the cell cycle. We show that loops form in both metaphase and interphase, but with distinct dynamic properties. Condensin extrudes asymmetric loops in metaphase, whereas cohesin extrudes symmetric loops in interphase. Our data show that loop extrusion is a general mechanism for the organization of DNA, with dynamic and structural properties that are molecularly regulated during the cell cycle.

Exploring nanoscale chromatin structure in living cells and multicellular organism by quantitative FLIM-FRET Microscopy

David Llères[1], Claire Dupont[1], Aurélien Perrin[1,2], Aymeric Bailly[2], Robert Feil[1]

[1] IGMM, CNRS, University of Montpellier, Montpellier, France

[2] CRBM, CNRS, University of Montpellier, Montpellier, France.

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Mesoscale modeling and single molecule tracking for the study of chromatin structure and dynamics in the process of cell differentiation

Julen Mendieta-Esteban[1], Irene Farabella[1], 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.

STORM imaging revealed that nucleosomes are arranged in groups with heterogeneous sized termed “clutches” and that those clutches are smaller and less densely compacted in mouse embryonic stem cells (mESCs) compared to mouse neuronal progenitor cells (mNPCs), in correlation with the more open chromatin state of mESCs. In this work we applied modelling and Single Molecule Tracking (SMT) to compare the structure of synthetic fibers and local nucleosome dynamics with the super resolution images of chromatin fiber in mESCs and mNPCs. First, using mesoscale chromatin modeling, we simulated the spatial arrangement of chromatin fibers corresponding to the pluripotency gene Oct4 in mouse ESCs (mESCs) and mouse NPCs (mNPCs), taking into account nucleosome positions from MNASE-Seq data, the ratio of linker histone H1 per nucleosome, and the amount of histone tail acetylation. The resulting folded fiber configurations showed higher compaction of the overall fiber and of the nucleosome clutches in mNPCs compared to mESCs, recapitulating the super resolution imaging data. Then we further used SMT both at short (15ms) and long (500ms) exposure times to show that nucleosome turn over and local dynamics within the chromatin fiber correlate with the structural features observed in super-resolution data and the polymer models. Nucleosomes are less stable, turn over faster, explore larger volumes and are more mobile in mESCs than in mNPCs

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

Julen Mendieta-Esteban[1], Irene Farabella[1], 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.

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.

Investigating the role of transcription in 3D genome folding and loop extrusion

Maria Victoria Neguembor[1], Álvaro Castells-García[1,&], Laura Martin[1,&], Chiara Vicario[1,&], Pablo Aurelio Gómez-García[1], Jumana AlHaj Abed[2], Alba Granados[1], Ruben Sebastian-Perez[1], Francesco Sottile[1], Jérôme Solon[1,3], Chao-ting Wu[2,4], Melike Lakadamyali[5,*,$] and **Maria Pia Cosma[1,3,6,*,$]**

[1] Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, 08003 Barcelona, Spain

[2] Department of Genetics, Harvard Medical School, Boston (Massachusetts), United States of America.

[3] Universitat Pompeu Fabra (UPF), Dr Aiguader 88, 08003 Barcelona, Spain

[4] Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston (Massachusetts), United States of America.

[5] Perelman School of Medicine, Department of Physiology, University of Pennsylvania, 19104 Philadelphia (Pennsylvania), United States of America.

[6] Institució Catalana de Recerca i Estudis Avançats (ICREA), Pg. Lluís Companys 23, 08010 Barcelona, Spain

[&] *These authors contributed equally to the work, names are listed in alphabetical order*

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Connecting broken DNA molecules

Davide Normanno

Cancer Research Centre of Marseilles (CRCM) (Marseilles, France)
davide.normanno@crg.eu
www.davidenormanno.com

Cancer incidence is inevitably rising in our aging society as a consequence of prolonged accumulation of genetic mutations due to environmental and lifestyle factors. To increase favourable prognosis and therapies efficacity, a promising strategy is the development of potent and specific inhibitors of vital cellular processes, such as DNA repair. To this end, in-depth knowledge, at the molecular level, of the mechanisms ensuring genome integrity is fundamental as well as the understanding of proteins diffusion and drugs transport inside cells.

Here, I will first present recent results elucidating the molecular mechanisms, and the regulation, of DNA repair processes obtained using single-molecule micromanipulation and imaging tools [1,2]. I will then discuss how nuclear factors diffuse and locate their target sites in the nucleus of human cells [3]. Finally, I will present recent work on the transport of nanosized objects in living cells that has permitted to formulate a general model of cytoplasmic diffusivity, which might hinder efficient drugs delivery [4].

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[4] Etoc *et al.*, *Nature Materials* **17**, 740 (2018)

Single-cell chromatin accessibility profiling reveals the regulatory programs underlying early mouse organogenesis

Blanca Pijuan-Sala[1,2], Nicola K. Wilson[1,2], Jun Xia[3], Xiaomeng Hou[4], Rebecca L. Hannah[1,2], Sarah Kinston[1,2], Fernando J. Calero-Nieto[1,2], Olivier Poirion[4], Sebastian Preissl[4], Feng Liu[3], Berthold Göttgens[1,2]

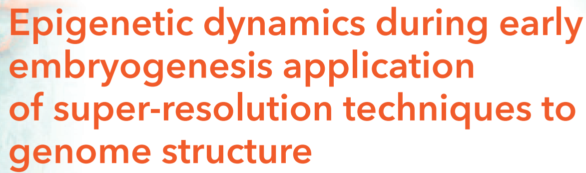
[1] Department of Haematology, University of Cambridge, Cambridge, UK.

[2] Wellcome-Medical Research Council Cambridge Stem Cell Institute, University of Cambridge, Cambridge, UK.

[3] State Key Laboratory of Membrane Biology. Institute of Zoology, Chinese Academy of Sciences. Beijing, China 100101.

[4] Center for Epigenomics, Department of Cellular and Molecular Medicine, University of California, San Diego, School of Medicine, La Jolla, CA, USA.

During early mouse embryonic development, pluripotent cells rapidly diversify into a wide range of cell types that will form the major organs of the organism, yet what regulatory programs underlie the establishment of cellular diversity remains ill-defined. To comprehensively delineate the chromatin accessibility landscapes underlying early organogenesis, we have profiled 19,453 single nuclei collected from mouse embryos at 8.25 days post-fertilisation using single-nucleus ATAC-seq. Our dataset has allowed determining which open chromatin regions contribute to the identity of each lineage. Motif enrichment analysis together with an examination of gene expression using our previously published single-cell transcriptomic atlas of this developmental stage have helped identify known and novel transcription factors that may play a role in the establishment of particular cell types. By combining our dataset with available ChIP-seq experiments, we have also discovered two new TAL1-bound haemato-endothelial enhancers, which we have validated using transgenic mouse assays. Furthermore, pseudotime inferences have helped define the chromatin dynamics underlying endothelial establishment and the transcriptional behavior of members of the ETS family, which have provided new insights that have been subsequently validated *in vivo* in zebrafish. In conclusion, single-cell chromatin accessibility maps of this developmental stage provide a baseline to decipher the regulatory programs underlying mammalian organogenesis.



Epigenetic dynamics during early embryogenesis application of super-resolution techniques to genome structure

Marta Portela [1, 2], Maria Jose Andreu [1], Miguel Manzanares [1,3]

[1] Centro Nacional de Investigaciones Cardiovasculares Carlos III, Madrid, Spain

[2] Universidad Autonoma de Madrid, Madrid, Spain

[3] Centro Biología Molecular Severo Ochoa

Totipotency is the capability of a cell to differentiate towards any possible cell fate. In mammals, this property is unique to the zygote, which results from the fusion of the male and female gametes. As the zygote divides, this cellular plasticity is gradually lost and serial differentiation events occur. This transition is accompanied not only by changes in gene expression but also by changes in chromatin organization such as DNA methylation, histone modifications, histone variants and nucleosomes positioning. Nowadays, our understanding of chromatin organization is mainly indirectly inferred from in vitro biochemical assays that give little information regarding the spatial distribution of these modifications inside the nuclei. In this context it is necessary to develop of new strategies to study chromatin spatial organization. Therefore, to address these questions in the context of the early development and the first lineage decisions we have pursued an optical study based on super-resolution, imaging of post-translational modifications of H3K4me3 and H3K9me3 related to active and repressed chromatin states respectively. We obtained information about the distribution and characteristics of these histone marks, showing differences between more and less differentiated cell types. Finally, this study offers a new and complementary tool to study this issue, providing the opportunity to reach a comprehensive understanding of how chromatin is structured at early developmental stages.

MALL is a nuclear protein that regulates LEM-protein localization

Armando Rubio-Ramos, Leticia Labat-de Hoz, Javier Casares-Arias, Miguel Bernabé-Rubio, Leonor Kremer, Isabel Correas and Miguel A. Alonso

Department of Cell Biology and Immunology, Centro de Biología Molecular Severo Ochoa, 24049, Madrid, Spain

Purpose of the study

To characterize the integral protein MALL and investigate its function in the nucleus

Summary of results

The MAL-like protein (MALL), also referred to as BENE, is a tetraspanning membrane protein belonging to the MAL family of specialized protein traffic machinery. We have recently generated a specific mAb to human MALL and found that MALL is selectively expressed in a restricted range of cell lines. Confocal microscopic and cell fractionation analyses indicate that endogenous MALL localizes to the nucleus. Time-lapse experiments and confocal microscopy reveal that MALL overexpression causes mislocalization of barrier-to-autointegration factor (BAF) from the nucleus. It is known that BAF interacts with LEM domain-containing proteins such as emerin and LAP2β MALL overexpression also causes emerin and LAP2β-GFP dispersal from the nuclear envelope, whereas the distributions of lamin B membrane receptor, the HP1β factor or lamins A and B, remain unaffected. Despite these alterations, the sealing of the nucleus after mitosis was not affected, since the reporter GFP-NLS accumulates normally in the nucleus. The analysis of cells overexpressing MALL reveals a significant increase in the number of binucleated cells. MALL expression knockdown or MALL knockout increases the number of nuclear invaginations containing lamin A. Our results suggest that MALL levels affect the targeting of LEM proteins to the nuclear envelope, which in turn is necessary for proper cytokinesis.

Conclusions

-MALL is an integral protein of the nuclear envelope of mammalian cells

-MALL overexpression perturbs the nuclear envelope localization of LEM-family proteins and increases the formation of binucleated cells

De novo heterochromatin formation during the transition from totipotent to pluripotent state

Ruben Sebastian-Perez[1], Sergi Aranda[1], Martina Pesaresi[1], Shoma Nakagawa[1], Luciano di Croce[1,2,3], Maria Pia Cosma[1,2,3]

[1] Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology, Dr. Aiguader 88, 08003 Barcelona, Spain.

[2] Universitat Pompeu Fabra (UPF), Barcelona, Spain.

[3] Institució Catalana de Recerca i Estudis Avançats (ICREA), Pg Lluis Companys 23, 08010 Barcelona, Spain

Heterochromatin formation is key to ensuring proper genome function during early development. However, the molecular mechanisms underlying de novo heterochromatin establishment remain largely unknown, mostly due to the minuscule amounts of material available during embryogenesis. Recently, exogenous overexpression of the transcription factor Dux was reported to be sufficient to induce early embryonic-like (2C-like) cells from pluripotent stem cells (PSCs). While PSCs have defined heterochromatic foci, 2C-like cells lack chromocenters. Therefore, the study of the exit from the 2C-like state back to pluripotency could help decipher the mechanisms of heterochromatin formation at the molecular level. Here, we performed DNA-mediated chromatin pull-down to identify a cluster of proteins that were selectively displayed from the chromatin-bound proteome of 2C-like cells. Notably, this protein cluster was enriched in cell cycle regulators and DNA replication-related factors. Interestingly, compared to PSCs, 2C-like cells displayed a cell cycle profile characterized by a shorter S and a prolonged G2/M phases; this further supported the hypothesis of the absence of a chromatin-bound replication machinery. Additionally, cell cycle arrest induced by epigenetic inhibitors and cell cycle checkpoint modulators increased the fraction of 2C-like cells, suggesting a strong relationship between cell cycle regulation and the establishment of the 2C state. We are functionally validating the most promising candidates from the chromatin pull-down with a CRISPR-Cas9 loss-of-function screen to identify key contributing factors acting in the exit from the 2C-like state back to pluripotency. To summarize, our data suggests a fundamental role of cell cycle regulators and DNA replication in both 2C-like state emergence and heterochromatin formation. Importantly, our results could explain both in vitro and in vivo observations about mouse embryogenesis.

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

Núria Serna[1], Mónica Salinas[1], Francesca Mugianesi[2], Marc Martí-Renom[2], Albert Jordan[1]

[1] Institut de Biologia Molecular de Barcelona (IBMB-CSIC), Barcelona, Catalonia, Spain

[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.

Turnover of activator binding and phase separation regulate gene expression induction and co-activator recruitment

Jorge Trojanowski[1,§], Lukas Frank[1,§], Anne Rademacher[1] and **Karsten Rippe[1,*]**

[1] Division of Chromatin Networks, German Cancer Research Center (DKFZ) and Bioquant, Heidelberg, Germany

§ Co-first authors

Induction of gene transcription is regulated by transcription factors (TFs) in a multistep process. The DNA-binding domain (DBD) and the (trans-)activation domain (AD) of TFs have been thought to independently determine the binding and activation properties. However, the recently discovered ability of ADs of some TFs to form phase-separated liquid droplets, raises the question whether chromatin binding and activation properties are truly independent. Here, we employed a toolbox of synthetic transcription activation complexes with different propensities to form liquid droplets based on CRISPR/dCas9, the activation domains VP16 and VPR and optogenetic modules. We measured the activator residence times at a reporter gene promoter by fluorescence recovery after photobleaching (FRAP) and followed the production of RNA and the recruitment of the co-activator BRD4. The activator complex residence time was dependent on both its direct target binding domain and the phase-separation propensity of the AD. BRD4 recruitment showed differences for activator compositions with different residence times and was enhanced by liquid droplets. Moreover, ADs with a higher phase-separation propensity induced transcription faster. However, for some constructs artificial light-induced liquid droplets had a repressive effect on transcription. We conclude that phase-separation properties of the AD affect both the binding and activation properties of a TF in a complex manner.

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