III Jornada de Cromatina i Epigenètica

Organitzada per la Secció de Biologia Molecular de la Societat Catalana de Biologia (SCB)

amb el Barcelona Chromatin Club (BCC)

INSTITUT D´ESTUDIS CATALANS

Carrer del Carme, 47

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4 de març de 2013

III Annual Chromatin and Epigenetics symposium

Organized by the Molecular Biology section of the

Catalan Society of Biology (SCB)

and the Barcelona Chromatin Club (BCC)

March 4, 2013

IEC: carrer del Carme, 47, Barcelona

Prat de La Riba hall

Sponsored by:

PROGRAM

8.30-9.00 ***Registration and documentation pickup***

8:55 ***Opening***

Session I. *Chair: Maribel Parra (PEBC-IDIBELL)*

9.00-9.25 **20min+5**
Jorge Ferrer (IDIBAPS-UB)
Epigenomic regulation in beta cells and diabetes

9.25-9.50 **20min+5**
Joan A. Subirana (Eng.Quim.-UPC, Evol.Genom.-IMIM-PRBB)
Junk DNA in nematodes: satellites and centromeres

9.50-10.15 **20min+5**
Francesc Piferrer (ICM-CSIC)
Epigenetics and sex determination

10.15-10:40 **20min+5**
Joan Ausió (Univ. Victoria, Canada)
H2A.Z: the story of histone variant with an epigenetic role and involvement in prostate cancer

10.40-11:05 **20min+5**
Ma José Barrero (CMRB-PRBB)
Histone variant deposition reveals reprogramming-initiation events

11:05-11.30 ***Coffee break and poster session sponsored by Covalab***

Session II. *Chair: Ferran Azorin (IBMB-IRB-PCB)*

11.30-11.55 **20min+5**
Albert Jordan (IBMB-CSIC-PCB)
Determinants of the specificity of human histone H1 subtypes

11.55-12.20 **20min+5**
Joan-Ramon Daban (UAB)
Planar metaphase chromatin: Nanomechanical properties and self-assembly

12.20-12.45 **20min+5**
Alejandro Vaquero (PEBC-IDIBELL)
The role of Sirtuins in signaling stress conditions to chromatin

12.45-13.10 **20min+5**
Joaquim Roca (IBMB-CSIC-PCB)
The topology of nucleosomal DNA

13.10-13.35 **20min+5**
Montserrat Corominas (IBUB-UB)
The trithorax protein ASH2 acts as an Ecdysone Receptor coactivator

13.35-15:00 Lunch at restaurant and poster session

Session III. *Chair: Dave Monk (PEBC-IDIBELL)*

15:00-15.25 **20min+5**
Esteban Ballestar (PEBC-IDIBELL)
Epigenetic control in a B cell proliferation model

15.25-15.50 **20min+5**
Miguel A. Peinado (IMPPC)
Zooming in and out of cancer epigenome

Session IV-BCC. Dimensions of Nuclear Organization

Chair: Marcus Buschbeck & Julien Douet (IMPPC)

15.50-15.55
BCC presentation

15.55-16.30 **30min+5**
Cristina Cardoso (Technische Universität Darmstadt, Germany)
Gene positioning and expression in stem cells and reprogramming: does location matter?

16.30-16.55 **20min+5**
Melike Lakadamyali (ICFO)
High-resolution microscopy of chromatin

16:55-17.30 Coffee break and poster session sponsored by Active motif

17.30-17.55 **20min+5**
Heinrich Leonhard (Ludwig Maximilians Univ. Munchen, Germany)
What a difference the envelope makes

17.55 -18.10 **12min+3**
Tom Sexton (IGH, Montpellier, France)
The three-dimensional landscape of the Drosophila genome

18.10-18.35 **20min+5**
Miguel Beato (CRG-PRBB)
Chromatin structural dynamics in hormone action

18.35-19:00 **20min+5**
Guillaume Filion (CRG-PRBB)
Chromatin and topologically associated domains (TADs)

19.00-19.25 **20min+5**
Marc A. Marti-Renom (CRG-PRBB, CNAG-PCB)
Determination of hormone induced structural changes in TADs

19.25 Farewell

ABSTRACTS ORAL COMMUNICATIONS

JUNK DNA IN NEMATODES: SATELLITES AND CENTROMERES

Juan A. Subirana[a] and Xavier Messeguer[b]

Centromere sequences in the genome are associated with the formation of kinetochores, where spindle microtubules grow in mitosis. Centromere sequences usually have long tandem repeats (satellites). In holocentric nematodes it is not clear how kinetochores are formed during mitosis, they are distributed throughout the chromosomes, For this reason it appeared of interest to study the satellites in nematodes, in order to determine if they offer any clue on how kinetochores are assembled in these species. We have studied the satellites in the genome of six nematode species. We have found that the presence of satellites depends on whether the nematode chromosomes are holocentric or monocentric. It turns out that holocentric nematodes are unique because they have a large number of satellites scattered throughout their genome. Their number, length and composition is different in each species: they apparently have very little evolutionary conservation. In contrast no scattered satellites are found in the monocentric nematode *Trichinella spiralis*. It appears that the absence/presence of scattered satellites in the genome distinguishes monocentric from holocentric nematodes. We conclude that the presence of satellites is related to the holocentric nature of the chromosomes of most nematodes. We have found that some satellites do have clear centromeric features.

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EPIGENETICS AND SEX DETERMINATION

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Epigenetic mechanisms provide organisms with the ability to integrate genomic and environmental information to modify the activity of their genes for generating a particular phenotype. During development, cells differentiate, acquire and maintain identity through changes in gene expression. This is crucial for sex determination and differentiation, which are among the most important developmental processes for the proper functioning and perpetuation of species. Here, I summarize studies showing how epigenetic regulatory mechanisms, including DNA methylation, histone modifications and non-coding RNAs, contribute to sex determination and gonadogenesis in plants, invertebrates and vertebrates. Special emphasis will be made on temperature as an environmental epigenator. It will be illustrated how the temperature experienced during early development influences the developmental course, leading to a particular sexual phenotype in lower vertebrates. Finally, several areas where epigenetic research may bring new discoveries and applications related to sex determination will be briefly discussed. These include research in the etiology of several disorders of sexual development in humans, the control of reproduction in animal farm production, and in our understanding of the environmental vs. genetic influences on sex determination of sensitive species in a global change scenario.

H2A.Z: THE STORY OF A HISTONE VARIANT WITH AN EPIGENETIC ROLE AND INVOLVEMENT IN PROSTATE CANCER

Juan Ausió
Department of Biochemistry and Microbiology, University of Victoria, Victira , bitish Columbia, Canada.

Within chromatin, the histone variant H2A.Z plays a role in many diverse nuclear processes including transcription, preventing the spread of heterochromatin and epigenetic transcriptional memory. The molecular mechanisms of how H2A.Z mediates its effects are not entirely understood. However, it is now known that H2A.Z has two protein isoforms in vertebrates, H2A.Z-1 and H2A.Z-2, which are encoded by separate genes and differ by 3 amino acid residues. They are expressed across a wide range of human tissues, they are both acetylated at lysine residues within the N-terminal region and they exhibit similar, but non-identical, distributions within chromatin. The phylogenetic analysis of the promoter regions of H2A.Z-1 and H2A.Z-2 as well as our biochemical and gene expression data indicate that they have evolved separately during vertebrate evolution and may have acquired a degree of functional independence. We have also shown that total H2A.Z and an acetylated form of H2A.Z is mainly present at the prostate specific antigen (PSA) enhancer and promoter in prostate cancer cell lines where the gene is expressed, but the levels decrease during rapid cycles of transcription. Treatment of prostate cancer cells with androgen results in increased H2A.Z levels due to up-regulation of the H2A.Z-1, but not the H2A.Z-2 gene. This up-regulation is likely the result of increased MYC transcription factor binding that occurs in response to androgen at the H2A.Z-1 promoter. Furthermore, we show that in a LNCaP xenograft model of prostate cancer progression, there is a significant increase of H2A.Z protein in castration resistant LNCaP tumors resulting from increased expression of the H2A.Z-1 gene. This suggests that expression of H2A.Z-1 is indicative of prostate cancer progression to androgen independence.

HISTONE VARIANT DEPOSITION REVEALS REPROGRAMMING-INITIATION EVENTS

Maria J. Barrero
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Chromatin structure plays fundamental roles in the regulation of gene expression during development and contributes to define and preserve cell identity. However, adult somatic cells can be reprogrammed to pluripotency by the overexpression of critical transcription factors. This process entails overcoming the epigenetic barriers that prevent the reactivation of the endogenous pluripotency genes. In order to identity critical players that participate in the maintenance of these barriers, we compiled genome wide expression data from several independent reprogramming experiments and extracted a list of chromatin related candidates that showed differential expression between human induced pluripotent (iPS) cells and somatic cells. Among them, we identified histone variants that are differentially expressed in somatic and pluripotent cells. These variants play essential roles in preserving somatic cell identity by preventing early chromatin changes that take place during reprogramming initiation. Overall, our results provide basic knowledge about the epigenetic mechanisms that govern cell identity and plasticity, which lay at the basis of important diseases such as cancer.

DETERMINANTS OF THE SPECIFICITY OF HUMAN HISTONE H1 SUBTYPES

Albert Jordan

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Seven linker histone H1 variants exist in human somatic cells (H1.0, H1.1 to H1.5, and H1X), with distinct prevalence depending on the cell type analyzed and along differentiation, that 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 or regulate specific promoters. We have explored this by inducible shRNA-mediated knock-down of each of the H1 variants in a human breast cancer cell line. Rapid inhibition of each H1 variant was not compensated by changes of expression of other variants. Thus, specific phenotypes are observed in breast cancer cells depleted of individual histone H1 variants. Moreover, knock-down of each H1 variant alters expression of a different, reduced subset of genes, with more genes being repressed than activated suggesting a local positive role of H1 on gene expression control. By taking advantage of specific antibodies for H1 variants and HA-tagged recombinant H1 variants-expressing cell lines, we have investigated the distribution in particular promoters and genome-wide of the different H1 variants, as well as the role of some H1 post-translational modifications. Finally, we have explored changes on the histone H1 variants content during differentiation of human embryonic stem cells and reprogramming of keratinocytes.

PLANAR METAPHASE CHROMATIN: NANOMECHANICAL PROPERTIES AND SELF-ASSEMBLY

Joan-Ramon Daban[1], Maria Milla[1], Isaac Gállego[1], Gerard Onzins[2], Xavier Sisquella[3] and Xavier Fernandez-Busquets[4]

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We had previously investigated the structure of chromatin plates observed in partially denatured chromosomes and proposed that metaphase chromosomes are formed by stacked thin chromatin layers orthogonal to the chromatid axis (1). More recently, we have applied atomic force microscopy (AFM) and friction force measurements at the nanoscale (nanotribology) to analyze the properties of these planar structures in aqueous solutions at room temperature (2). Our results show that high concentrations of NaCl, EDTA, and extensive digestion with protease and nuclease enzymes cause plate denaturation. Native plates under structuring conditions (5 mM Mg^{2+}) have a relatively high friction coefficient ($\mu \approx 0.3$), which is markedly reduced when high concentrations of NaCl or EDTA are added ($\mu \approx 0.1$). Protease digestion increases the friction coefficient ($\mu \approx 0.5$), but the highest friction is observed when DNA is cleaved by micrococcal nuclease ($\mu \approx 0.9$), indicating that DNA is the main structural element of plates. The conclusion is that planar chromatin is a flexible and mechanically resistant two-dimensional network, which presumably allows the safe storage of DNA during mitosis. Furthermore, we have found using electron microscopy that fragments of chromatin fibers (obtained from metaphase chromosomes digested with micrococcal nuclease) associate spontaneously forming multilaminar structures identical to the thin plates found previously in partially denatured chromosomes (3). Under metaphase ionic conditions, the fragments that are initially folded forming the typical 30-nm chromatin fibers are untwisted and incorporated into growing plates. These results demonstrate that metaphase chromatin has the intrinsic capacity to self-organize as a multilayered planar structure. A chromosome structure consistent of many stacked layers of planar chromatin avoids random entanglement of DNA, and gives compactness and a high physical consistency to chromatids.

(1) Daban, Micron 2011, 42:733-750.

(2) Gállego et al., Biophys J 2010, 99:3951-3958.

(3) Milla and Daban, Biophys J 2012, 103:567-575.

THE ROLE OF SIRTUINS IN SIGNALING STRESS CONDITIONS TO CHROMATIN

Alejandro Vaquero
Chromatin Biology Group, Cancer epigenetics and Biology Program (PEBC), Bellvitge Biomedical Research Institute (IDIBELL)

Detecting and efficiently responding to potentially fatal environmental changes is a major challenge for all organisms. Said changes, such as calorie restriction, are intimately associated to metabolic fluctuations and certain forms of stress (e.g. oxidative or genotoxic). Chromatin is a crucial response mediator of survival response signaling and is one of the two primary targets of most triggered protective measures; the other target is mitochondria.

Over the past decade, evidence has suggested that the proteins from the Sir2 family, or *Sirtuins*, are major players in sensing and coordinating the responses geared to chromatin and to mitochondria. The importance of the role of Sirtuins in these processes is reflected by their involvement in a wide range of human pathologies including cancer, endocrine-associated such as diabetes, and neurodegenerative diseases, among others. Mammals have seven Sirtuins, denoted SirT 1 to 7. These exhibit great functional diversification, which has led to two different enzymatic activities (NAD^+-dependent deacetylation and ADP-ribosylation of proteins), a myriad of substrates (histone and non-histone proteins) and a highly diverse pattern of cellular localization (nucleus, nucleolus, cytoplasm and mitochondria). Sirtuins SirT1 to 7 have adapted evolutionarily by acquiring new roles, including deacetylation of histone and non-histone proteins in gene expression, cell survival and metabolic regulation. Our work aims to understand the way Sirtuins signal oxidative and metabolic stress to chromatin and how this function modulates chromatin function and integrity. Here we present evidences regarding the functional implications of SIRT1 and SIRT2 in the control of chromatin structure. Our studies support a role for both Sirtuins as protectors of genomic integrity in conditions of stress.

THE TOPOLOGY OF NUCLEOSOMAL DNA

Ofelia Díaz-Ingelmo, Belén Martínez, Xavier Fernández, Joana Segura, Antonio Valdés, Joaquim Roca

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The nucleosome, the main structural fold of eukaryotic chromatin, is far from being the well-defined and inert histone-DNA complex depicted in most textbooks. On one side, nucleosome particles are very heterogeneous due to differences in DNA base-pair sequence, histone variants and their multiple biochemical modifications. Moreover, histones may combine with other DNA binding proteins to form "nucleosome-like" complexes. On the other side, we showed in recent years that nucleosomes have huge conformational plasticity to absorb DNA twisting waves and to accommodate positive or negative DNA supercoils. Thus, deciphering the complex interplay between chromatin structure and function will not be possible without understanding the mechanical properties and DNA topology constrains associated to specific nucleosome particles.

To initiate the above, we revisited here the "linking number paradox" of nucleosomal DNA, which states: While biochemical and structural data show that DNA coils ~1.7 negative turns around the histone octamer, topological analyses indicate that each nucleosome constrains in average only ~1 negative DNA supercoil. To approach the paradox, we have examined the DNA topology (DNA linking number) of yeast circular mini-chromosomes containing different nucleosome number and types. We found that native yeast chromatin constrains ~1.3 negative supercoils per nucleosome (more than commonly assumed). Our results show also that a yeast centromere, a paradigm of non-canonical nucleosome-like particle, does constrain ~ 0.5 positive supercoils, thus in partial agreement with previous reports.

THE TRITHORAX PROTEIN ASH2 ACTS AS AN ECDYSONE RECEPTOR COACTIVATOR

Albert Carbonell*, Enrique Blanco, Florenci Serras and Montserrat Corominas

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The molting hormone ecdysone triggers chromatin changes via histone modifications that are important for gene regulation. On hormone activation, the ecdysone receptor (EcR) binds to the SET domain–containing histone H3 methyltransferase trithorax-related protein (Trr). Methylation of histone H3 at lysine 4 (H3K4me), which is associated with transcriptional activation, requires several cofactors, including Ash2. It was already known that mutations in *ash2* cause a variety of pattern formation defects in the *Drosophila* wing and we have recently found that *ash2* mutants have also severe defects in pupariation and metamorphosis due to a lack of activation of ecdysone-responsive genes. This transcriptional defect is caused by the absence of the H3K4me3 marks set by Trr in these genes. We will present evidence that the Ash2 protein interacts with Trr and is required for its stabilization. Thus we propose that Ash2 functions together with Trr as an ecdysone receptor coactivator.

EPIGENETIC CONTROL IN A B CELL PROLIFERATION MODEL

Henar Hernando[1], Claire Shannon-Lowe[2], Abul B. M. M. K. Islam[3], Fatima Al-Shahrour[4], Javier Rodríguez-Ubreva[1], Virginia C. Rodríguez-Cortez[1], Biola M. Javierre[1], Cristina Mangas[5], Agustín F. Fernández[5], Maribel Parra[6], Henri-Jacques Delecluse[7], Manel Esteller[8], Eduardo López-Granados[9], Mario F. Fraga[5,10], Nuria López-Bigas[3] and Esteban Ballestar[1]

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Infection of B cells with Epstein-Barr virus (EBV), which is highly prevalent in humans, is an excellent model to investigate the molecular mechanisms associated with the acquisition of unlimited growth, associated with disease. EBV-associated changes in B cells are relevant to the development and progression of lymphomas and lymphoproliferative disorders in immune-suppressed individuals, and various autoimmune disorders. We investigated the effects of experimental EBV infection of B cells on DNA methylation and histone modifications. Integrated analysis of DNA methylation, histone modification and expression datasets during this transition has helped us to understand the biology of this process and the relevance of epigenetic events during B cell transformation. For instance, we have observed hypomethylation of around 250 genes, but no hypermethylation. Gene ontology analysis, expression profiling, and high-throughput analysis of the presence of transcription factor binding motifs and occupancy revealed that most genes undergoing hypomethylation are active and display presence of NFkB p65 and other B cell-specific transcription factors. Promoter hypomethylation was associated with upregulation of genes relevant for the phenotype of proliferating lymphoblasts. In parallel, analysis of histone modifications revealed a remarkable decrease and redistribution of heterochromatin marks. Loss of H4K20me3 occurred at constitutive heterochromatin repeats. For H3K27me3 and H3K9me3, high-throughput comparison revealed a decrease in these marks in thousands of genes. Moreover, DNAse-seq data comparison between resting and proliferating cells revealed increased accessibility in thousands of genomic sites. Both loss of H3K27me3 and increased accessibility are associated with gene activation. Surprisingly, no connection between changes in heterochromatic histone modifications and accessibility is observed, since genomic sites that lose H3K27me3, H3K9me3 and H4K20me3 do not display different accessibility to nucleases in resting and proliferating B cells. Our findings provide a novel perspective on the mechanisms and relationship between different epigenetic marks during conversion of resting to proliferative B cells.

ZOOMING IN AND OUT OF CANCER EPIGENOME

Miquel A. Peinado (IMPPC)

Cancer cells exhibit multiple epigenetic changes with global DNA hypomethylation accompanied by local hypermethylation as the most prominent and characteristic change. We have analyzed DNA methylation at the genome scale in human colorectal cancers and have investigated the distribution of changes considering separately different genomic elements. Beyond the global DNA hypomethylation affecting most of the genome, we have identified chromosomal regions with specific patterns of differential methylation and exhibiting distinctive structural and functional features. Our results suggest that colon cancer epigenome is reorganized in a consistent manner and may provide new insights into the mechanisms deregulated during the cancer process.

THE THREE-DIMENSIONAL LANDSCAPE OF THE DROSOPHILA GENOME

Tom Sexton[1], Eitan Yaffe[2], Ephraim Kenigsberg[2], Frederic Bantignies[1], Benjamin Leblanc[1], Caroline Jacquier[1], Michael Hoichman[2], Aubin Thomas[1], Hugues Parrinello[3], Amos Tanay[2] and Giacomo Cavalli[1]

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The first part of the talk will give an overview of our recently published findings (Cell 148, 458-72; 2012), where we used Hi-C to assess the chromosome folding patterns within Drosophila embryonic nuclei. We describe the organisation of the genome into distinctly folded “topological domains”, which are mirrored by the histone modification states and transcriptional activity of the underlying chromatin, suggesting an intimate link between genome structure and functional outputs. The second part of the talk will discuss preliminary Hi-C results from different null mutants of genes of the Polycomb group of transcriptional repressors. These mutants have very specific effects on chromosome organisation; whereas topological domains are largely unperturbed in these mutants, there is a drastic loss of long-range inter-domain contacts between Polycomb-regulated genes. This finding suggests that certain groups of transcriptional modulators may use spatial networks to co-ordinately regulate cohorts of developmental genes.

CHROMATIN DYNAMICS AND GENE REGULATION

Miguel Beato, Francois LeDily, Roni Wright, Silvina Nacht, Guillermo Vicent, Cecilia Ballaré, Roser Zaurin, Andy Pohl and Jofre Font

CRG & UPF, Barcelona

We are exploring how different levels of chromatin structure contribute to orchestrate the transcriptional response of breast cancer cells to the steroid hormones estrogens and progestins. We found that core histones and linker histones contribute to stabilize hormone receptor docking by interacting with the receptor-associated chromatin modifying enzymes. These include protein kinases activated by crosstalk with membrane-anchored hormone receptors, histone methylases and demethylases, histone acetyltransferases and deacetylases, poly(ADP)-ribose polymerases and glycohydrolases, and ATP-dependent chromatin remodeling complexes.

Within one minute of hormone addition the receptor-associated kinase MSK1 catalyzes the phosphorylation of H3 at S10 and the removal of a repressive complex containing HP1β, CORES T, LSD1, HDACs and the non-coding RNA SRA. The repressive complex is targeted to a subset of hormone inducible genes by non-liganded hormone receptor and is stabilized by an interaction of HP1β with H3K9me3. Upon removal of the repressive complex, a combination of at least 6 receptor-associated enzymes (CDK2, PARP1, PARG, NURF, MLL2/3, PLU1) catalyze the trimethylation oh H3 at K4 - which anchors the NURF complex - and the phosphorylation and parylation of linker histone H1, leading to its displacement. In a subsequent cycle receptor-recruited PCAF and BAF catalyze acetylation of H3 at K14 and the displacement of histone H2A/H2B dimers. Steroid hormones also repress numerous target genes using the same repressive complex that silences inducible genes prior to hormone addition.

We have used Hi-C analysis and high-resolution microscopy to study how folding of the chromatin fiber in topological associating domains (TADs) and chromosomal compartments contributes to regulation of gene expression in breast cancer cells. We found that TADs of around 1 Mb size demarcate regions with distinct epigenetic signatures and that hormone induced and repressed genes cluster and segregate into a subset of TADs that behave as units of transcriptional response. Our final goals are to understand how the 3D organization of chromatin is established and whether it contributes to cell identity and to the hormonal response.

DETERMINATION OF HORMONE INDUCED STRUCTURAL CHANGES IN TADS

Marc A. Marti-Renom
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Structural Genomics Group, Centre de Regulació Genòmica (CRG, http://crg.eu), Barcelona, Spain.

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 new method for the determination of the 3D conformation of chromosomes in the interphase nucleus, which combines 5C experiments with the computational Integrative Modeling Platform (IMP) [1]. During our presentation, we will present the application of our methods to the study of the hormone induced changes in the so-called Topologically Associated Domains in the human genome.

[1] Baù, D. & Marti-Renom, M. A. *Genome structure determination via 3C-based data integration by the Integrative Modeling Platform*. Methods 58, 300–306 (2012).

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Rubén Azpiazu[1], Judit Castillo[1], Alexandra Amaral[1], Josep Maria Estanyol[2], Marta Guimerà[3], Josep Lluís Ballescà[3], Juan Balasch[3] and Rafael Oliva[1].

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Assisted reproduction through in vitro fertilization (IVF) is a widely used technique for treating male sterility or infertility. However it is also well known that, even in infertile patients having apparently normal sperm cells, IVF may fail to result in good quality embryos and pregnancy, pointing to a potential involvement of the genetic or epigenetic information. We initiated this project with the goal to identify the differential proteins present in an altered amount in normozoospermic sperm samples not resulting in pregnancy after IVF as compared to those present in normozoospermic sperm samples which resulted in pregnancy. Towards this objective Swim-up recovered sperm cells were used for IVF and the remaining unused fraction was collected and frozen. After the assisted reproduction results we selected 10 normozoospermic sperm samples which had resulted in pregnancy and 10 which did not result in pregnancy. Both sets of 10 samples were pooled; the proteins extracted, and labeled the “Pregnancy” group with the isobaric taq TMT-127 and the “No pregnancy” group with the isobaric taq TMT-126. Both pools were then pooled, separated the proteins and identified them using LC-1D coupled to MS/MS. A total of 1957 proteins were identified, many of which were novel proteins (proteins not previously identified as part of the human sperm cell). Of these 22 proteins were increased and 38 decreased in the “No pregnancy” group as compared to the “pregnancy” group. Of relevance a substantial proportion of the proteins were related to the chromatin, thus supporting a potential epigenetic involvement in normozoospermic male sterile patients undergoing IVF treatment. These results now open up the possibility to gain further insight into their function and pathogenic mechanisms leading to male sterility. Supported by a grant from the Ministerio de Economía y Competitividad to RO (BFU2009-07718) and a IDIBAPS fellowship to RA.

GENOMIC AND PROTEOMIC DISTRIBUTION IN THE NUCLEOHISTONE AND NUCLEOPROTAMINE DOMAINS IN HUMAN SPERMATOZOA

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It is known that about 90% of human sperm DNA is packaged by protamines, and that the remaining 10% is associated with nucleosomes, which are enriched at loci of developmental importance. We initiated the present project with the goal to perform a detailed proteomic characterization of the nucleohistone and nucleoprotamine domains within the human sperm chromatin in order to identify additional layers of epigenetic information. Normozoospermic human sperm samples were purified and subjected to chromatin fractionation either using micrococcal nuclease (MN) digestion (Hammoud et al., 2009, Nature 460:473-8) or 0.65M NaCl extraction followed by EcoRI/BamHI digestion (Arpanahi et al., 2009, Genome Res 19:1338–49). MN ~150 bp nucleosomal band, MN ~70 bp band (corresponding to the nucleosome dyad axis) and EcoRI/BamHI digested regions were then deep genome sequenced and the corresponding protein fractions were subjected to detailed proteomic characterization through protein fractionation, peptide separation, and mass spectrometry (LC-MS/MS). Our sequencing results indicate a good correspondence between the MN digested fraction as compared to the EcoRI/BamHI fraction, suggesting that both methods are valid to dissect the nucleohistone-nucleoprotamine domains, with implications for interpreting the proteomic results. A total of 475 proteins were identified in the NaCl soluble fraction, while 108 proteins were identified in the NaCl insoluble fraction. Many histone variants and chromatin associated proteins identified had not been previously described. In addition, the results of the MN ~150 bp nucleosomal band as compared to the MN ~70 bp band are not equivalent, indicating the presence of sperm nucleosomal chromatin regions more "open" than others. Our proteomic and deep genome sequencing results add further consistence to the epigenetic constitution of the sperm cell and open the possibility to further characterize the specific protein-DNA interactions and to identify their function. Supported by the Ministerio de Economía y Competitividad to RO (BFU2009-07718).

DEVELOPMENT OF A SKIN CANCER MODEL FROM ADULT STEM CELLS

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Cancer cells are characterised by epigenetic and metabolic alterations. To provide us with novel insight into their role and interplay in the context of cancer, we have established a step-wise skin cancer model.
For this, we modified the transformation protocol from the Weinberg lab in two ways. First, we separated immortalization from transformation to be able to obtain cells with an intermediate transformation phenotype. Then, we selected the most malignant subpopulation by collecting the ones that grew in attachment free *in vitro* conditions. However, these were discarted since they no longer supported xenograft growth. On the other hand, the ones that were put through a round of xenograft growth gave bigger and faster growing tumours than their transformed unselected counterparts. The histopathologic analysis of these tumours revealed a variety of phenotypes and some of them resemble human SCC. Others were undifferentiated carcinomas that reexpressed the differentiation SCC marker p63 when subjected to a second round of xenograft growth.
We will apply metabolomic, epigenomic and transcriptomic approaches to this model to identify chromatin and metabolic changes occuring during oncogenic transformation.

DNA METHYLATION LANDSCAPE DURING MUSCLE-LINEAGE COMMITMENT AND TERMINAL DIFFERENTIATION

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Cellular differentiation involves widespread epigenetic reprogramming, including modulation of DNA methylation patterns. DNA methylation is a covalent biochemical modification restricted to CpG dinucleotides in mammals that controls chromatin structure and gene expression. We have investigated the role of DNA methylation during muscle-lineage commitment and muscle terminal differentiation. To elucidate genome-scale methylation changes we have developed AIMS-Seq technique (Amplification of Intermethylated Sites followed by ultrasequencing), a method that assays DNA methylation at more than 77.000 CpGs throughout the genome. Performing AIMS-Seq on mouse embryonic stem cells, muscle stem cells, terminal differentiated myotubes and adult muscle tissue allowed us to identify several trends during muscle-lineage commitment and muscle terminal differentiation.

The global DNA methylation pattern is preserved between ESCs, muscle stem cells, terminal differentiated myotubes and adult muscle tissue. However, at the same time, we observed a gain of DNA methylation during muscle lineage commitment and an interesting loss of DNA methylation in unique non-enriched CpG regions. Ongoing studies are being addressed to confirm such changes and to determine its possible functional implications.

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A DIFFERENTIAL PROTEOMIC DISTRIBUTION IS PRESENT IN THE HUMAN SPERM CHROMATIN

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About 85-95% of the DNA in the mature sperm cell is packaged by protamines (NP domain), while 5-15% remains associated with histones (NH domain). It is known that the NH domain is significantly enriched in important developmental genes, suggesting a potential epigenetic role during the first steps of embryo development. The aim of the present study was to contribute to a more detailed characterization of the human sperm nucleus establishing the putative differential distribution of the proteins in the sperm chromatin.
Sperm nuclei were isolated from a normozoospermic semen sample, which was previously purified from contaminating cells. The chromatin was fractionated with a saline treatment, using a standard approach, and the proteins extracted from soluble and insoluble fractions were then analysed by liquid chromatography followed by tandem mass spectrometry (LC-MS/MS).
Proteomics analysis resulted in the identification of 502 proteins, 475 found in the soluble fraction (SF) and 27 in the insoluble fraction (IF) of the sperm chromatin. The IF was mainly formed by structural proteins while in the SF the most abundant protein group was involved in protein synthesis, modification, folding and degradation. Among the proteins identified in the SF, “novel” histone variants and zinc finger proteins were detected. These results open up the possibility of further characterizing these proteins and their associated genes to complete the epigenetic knowledge of the human sperm chromatin and its potential contribution to embryo development.
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MACROH2A REGULATES THE COMMITMENT OF ADULT AND EMBRYONIC STEM CELLS TO DIFFERENTIATION

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The exchange of a canonical histone for a variant protein can be considered as the most extensive chromatin alteration occurring at the level of the nucleosome. The variants of histone H2A form the largest group of identified histone variants, which might be reflective of the more accessible position of the H2A-H2B dimer within the nucleosome. Among all histone variants macroH2A proteins differs most from their canonical counterpart since it possesses an additional extranucleosomal macrodomain [1]. But what is the function of this unique and facultative nucleosome component?
Here, we have analyzed macroH2A in adult and in embryonic stem cells. In mouse embryonic stem cells macroH2A1 and particularly its splice variant macroH2A1.2 is the predominantly expressed form of macroH2A. We found that knockdown of macroH2A1 does not affect self-renewal of embryonic stem cells but limits their capacity to differentiate [2]. MacroH2A1-deficient mES cells form defective embryoid bodies that are reduced in size and lack stage-specific cavitation. More strikingly, loss of macroH2A provokes a massive expansion of immature tissue in ES-derived teratomas [2]. Although a repressive function for macroH2A has been well documented [1], we found to our surprise that in ES cells macroH2A is required for the proper activation of bivalent target genes supporting a poorly recognized function in transcriptional activation [3]. Similar to mES cells, macroH2A also promoted the differentiation of human keratinocytes [2] and was required for proper zebrafish development [4]. Together these results establish macroH2A as an important chromatin regulator of cell fate transitions.
Taken our results together with the recent observation that macroH2A constitutes part of the cellular resistance to reprogramming [5], a picture emerges in which macroH2A plays a general function in the establishement and maintenance of differentiated epigenomes [3].

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IBF-1 AND IBF-2 ARE NOVEL SEQUENCE SPECIFIC DNA BINDING PROTEINS REQUIRED FOR INSULATOR FUNCTION IN *DROSOPHILA MELANOGASTER*

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Insulators or boundaries are genome elements experimentally defined by their ability to block enhancer-promoter interactions and/or to serve as barriers against the spreading of the silencing effects of heterochromatin. Recent data suggest that these elements have a broader role in the nuclei by mediating intra- and inter-chromosomal interactions to regulate transcription. Several proteins have been described in Drosophila to mediate insulator activity: CTCF, BEAF, Su(Hw) and CP190. Each one of those proteins binds to a different subset of insulators, all of them having in common CP190 binding.

In order to identify new proteins involved in insulator activity we purified the complex formed by CP190. Using Mass Spectrometry we identified the components of the complex. We found other known insulator proteins like CTCF, Su(Hw) or Mod(mdg4). We also identified Nurf-38 (a component of NURF complex) and Z4 (an euchromatin binding protein). And we identified two previously uncharacterized proteins that we named Ibf-1 and Ibf-2 after Insulator Binding Factor 1 and 2. We have studied these two proteins and showed that not only they colocalize in chromatin with CP190 (and partially with the other insulator proteins) but also they have enhancer-blocking activity and their lack causes a misregulation in gene expression of several genes. They contain a DNA-binding domain (BED zinc finger) and are capable to bind directly to DNA through the recognition of a specific DNA consensus motif. Overall our results show that we have identified two new *Drosophila* insulator proteins that form complex with CP190.

ZRF1 CONTROLS RETINOIC ACID PATHWAY AND REGULATES LEUKEMOGENIC POTENTIAL IN ACUTE MYELOID LEUKEMIA CELLS

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Cellular differentiation is controlled by the activation of diverse transcriptional programs, which are regulated mainly by transcription factors and epigenetic modifications. We recently identified the protein ZRF1, which specifically binds one of these modifications, the histone H2A-ubiquitin, and facilitates the transcriptional activation of Polycomb target genes. Using acute myeloid leukemia cells, we show here that ZRF1 regulates retinoic acid (RA) induced differentiation, proliferation and cell death. Depletion of ZRF1 gives rise to an increase in the differentiation status in basal conditions and, on the other hand, reduces the potential of the cells to fully differentiate upon RA administration. Consistently, its overexpression enhances the differentiation potential upon RA treatment. Moreover, depletion of ZRF1 notably reduces proliferation and enhances apoptosis in these cells. At the molecular level, we show that ZRF1 interacts with the retinoic acid receptor alpha (RARα), both *in vitro* and *in vivo*, and binds to RA-target gens. By using expression microarray, we show that ZRF1 regulates the expression of more than 40% of RA-targets. In basal conditions, depletion of ZRF1 produces an upregulation of around 30% of RA-targets while, upon RA-induced differentiation, 30% of RA-targets are downregulated in ZRF1 depleted cells. We also show that ZRF interact with HDAC proteins, and thus regulates histone acetylation levels. Finally, we show that ZRF1 depletion strongly reduces leukemogenicity in a murine model of acute myeloid leukemia. Our results suggest that ZRF1 controls RA-regulated transcription and it plays an important role in the regulation of leukemic cell biology and leukemogenic potential.

EPIGENETIC REPROGRAMMING AND INCREASED TRANSCRIPTION OF A TANDEMLY REPEATED SATELLITE ELEMENT IN COLORECTAL CANCER

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Hypomethylation at repetitive elements has been described in several types of cancer. We have identified a frequently hypomethylated region in colon tumors that belongs to the family of a moderately repetitive pericentromeric element called SST1. This SST1 element shows severe demethylation in 7% of colorectal cancer patients while moderate demethylation of SST1 is present in 22% of the tumors. In colon cancer cell lines with different SST1 methylation profiles, we observed that hypomethylated SST1 co-occurs with high levels of H3K27me3 and lower levels of H3K9me3, while methylated SST1 correlates with low H3K27me3 and high H3K9me3 levels. Furthermore, induced demethylation of SST1 by 5-Aza-dC treatment leads to an H3K27me3 increase and reduced H3K9me3 levels at the SST1 element. These data suggests that upon DNA demethylation, the SST1 element looses heterochromatin features being reprogrammed to a more relaxed and plastic architecture. In accordance with this observation, we have detected an increased transcription from this element upon 5-Aza-dC treatment. The ncRNA originating from the SST1 element is predominantly non-polyA.

This chromatin reprogramming at the SST1 pericentromeric region could be used as a prognostic marker for colorectal cancer. Further studies are needed to clarify the mechanism causing SST1 severe demethylation, the specific crosstalk between DNA methylation and H3K27me3 and the role that SST1 ncRNA could play in the initiation and development of colorectal cancer.

THE EFFECT OF GLOBAL DNA DEMETHYLATION ON GENE REGULATION AT THE GENOME SCALE

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Understanding the epigenetic mechanisms that rule regulation of gene transcription is fundamental to develop novel and innovative therapies for diseases as cancer. DNA methylation is one of the most important determinants of gene regulation and the usage of demethylating compounds as 5-Aza-dC in the clinic makes the study of the consequences and side effects of such treatments mandatory. Demethylating agents are being used in haematological malignancies treatments and its effects are being studied in solid tumours treatments, renal insufficiencies, platinium-based drug resistance in ovarian cancer patients and in activation of HIV-1 latently infected cells. The molecular mechanisms mediating the response to epigenetic therapies are poorly known and the genome-wide effect of removing methyl groups has not been determined yet. Thus, our main goal in this study is to understand fully the consequences of genome demethylation as a tool to design effective and relatively non-toxic therapeutic strategies as well as to gain knowledge about gene regulation and the role and interaction of the different players involved. To determine gene deregulation due to 5-Aza-dC we analyzed the colon cancer cell line HCT116 as a model. Our results indicate that genes with certain promoter properties are more sensitive to changes in DNA methylation than others. Our aim is to gain better understanding of the sequence and chromatin context of genes that are prone to activation upon loss of DNA methylation.

REPETITIVE DNA ASSOCIATED TRANSCRIPTIONAL DEREGULATION IN HYPOMETHYLATED HCT116 CELLS BY ANALYZING RNASEQ DATA

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Repetitive DNA elements are abundant in Eukaryotic genomes and they make up more that 50% of the human genome. The expression of these sequences is generally suppressed by DNA methylation and other epigenetic mechanisms. Nevertheless, they undergo hypomethylation in certain cell developmental stages and disease states such as cancer. In order to characterize the repetitive DNA sequences that get expressed as a result of hypomethylation and their effect on the expression of nearby exons, we analyzed RNASeq data from 5-aza-2'-deoxycytidine-treated (AZA) and untreated human colorectal carcinoma HCT116 cells. We identified a number of repeats that become activated upon AZA treatment. In particular, we found several satellite repeats that become highly active in cells treated with the demethylating agent. In addition, we found more than a thousand exons that are near repeats and are upregulated in AZA treated cells. Our results suggest that DNA methylation directly represses specific human repeat families more than others, and that genes that neighbour these repeats are prone to deregulation in hypomethylating conditions.

IDENTIFICATION OF PROTEINS ASSOCIATED TO CENH3 ^{CID}- NUCLEOSOMES IN *DROSOPHILA*

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Centromere identity and function is regulated epigenetically by the deposition of a centromere-specific histone H3 variant (CenH3). CenH3-nucleosomes act as platform for recruitment of other centromere associated proteins, such as the multi- protein complexes NAC and CAD. These complexes form a platform for the assembly of kinetochore proteins during mitosis. In *Drosophila,* little is known about proteins associated to CenH3-nucleosomes. Here, we have performed purification of CenH3 ^{CID}-TAP nucleosomes and subsequent analysis of their associated proteins by mass spectrometry. We will present our preliminary characterization of some of these novel proteins.

THE EPIGENOMIC AND CIS-REGULATORY CODE OF HUMAN PANCREATIC ISLET-CELLS

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Understanding the regulatory interactions that determine genome activity in pancreatic β-cells can shed light on the genetic underpinnings of type 2 diabetes (T2D) susceptibility, and provide insights to program β-cells for replacement therapies. We have now created integrated genome-wide maps of key epigenomic features, transcription factor occupancy sites, and gene transcription in human pancreatic islet cells. We show that although islet-selective transcription factors bind to thousands of ubiquitously expressed promoters and to transcriptionally silent regions, cell-specific transcription is linked to interactions of the islet transcription factor network with arrays of genomic regions enriched in H3K4me1 and H3K27ac. We use these integrated maps to uncover highly cell-specific regulatory sequence codes, and show that sequence variation at islet-cell cis-regulatory elements is associated with T2D risk and fasting glycemia. These findings illuminate how islet-cell transcription factor networks interact functionally with the epigenome, and indicate that sequence variation throughout the islet regulome contributes to T2D susceptibility.

CHROMATIN MARKING IS ASSOCIATED TO TRANSCRIPTIONAL STABILITY DURING FLY DEVELOPMENT

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We have monitored the transcriptome by RNASeq and several components of the epigenome by ChIPSeq in *Drosophila melanogaster* Wing and Eye-antenna imaginal discs. We have found that transcription is generally associated to a highly structured state of the chromatin, and that constitutively expressed genes are strongly marked with H3K36me3 and H3K4me3. However, in contrast to the canonical role of these marks, tissue specific genes are mostly void of them, even when exhibiting very high expression levels. We have similarly found that stably expressed genes during fly development are strongly marked, while stage specific genes lack activating chromatin modifications. Strong chromatin marking is also associated to a tighter regulation of alternative splicing in stably expressed genes. Consistently, we have also found a direct positive association between nucleosome occupancy and exon inclusion. Our results are supportive of a model in which a highly structured and strongly marked chromatin state is associated to stable, tightly controlled production of RNA during development, while a more relaxed, unmarked state would permit rapid gene activation—albeit resulting in less regulated RNA production. This model seems to have been conserved through metazoan evolution, since it is also consistent with data obtained from *Caenorhabditis elegans* development.

THE EARLY EMBRYO AND GERM LINE SPECIFIC LINKER HISTONE H1 VARIANT OF *DROSOPHILA*, DBIGH1, REGULATES ZYGOTIC GENOME ACTIVATION

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Linker histone H1 is a main component of eukaryotic chromatin that promotes folding of the nucleosomes into a higher-order chromatin structure, namely the "30nm chromatin fibre". An important characteristic of histone H1 is its high heterogeneity, as most species contain multiple H1 variants that play redundant as well as specific functions during development and differentiation. In particular, several germ line and early embryonic H1 variants have been reported in metazoans. In *Drosophila melanogaster*, histone H1 complexity is much reduced since, previous to this work, a single somatic dH1 variant was identified. Here, we identify the early embryo and germ line specific histone H1 variant of *D. melanogaster*, dBigH1. During early embryogenesis, dBigH1 is very abundant prior to cellularisation, which marks the time of the maternal-to-zygotic (MTZ) transition, when the maternal contribution declines and transcription of the zygote's genome is progressively switched on. At cellularisation, dBigH1 is replaced by somatic dH1 in the soma, but not in the primordial germ cells (PGC) that have a delayed MTZ and, at this stage, remain transcriptional quiescent. Consistent with a contribution to MTZ regulation, dBigH1 is detected in the PGC as long as they remain transcriptionally inert and, furthermore, a loss-of-function *bigH1*^{100} mutation shows premature MTZ both in the soma and the PGC. Mutant embryos, which dye at cellularisation, show increased levels of active RNApol II forms and zygotic transcripts, along with strong DNA damage and a high incidence of mitotic deffects. In the adult, dBigH1 is present both in ovaries and testis, showing a distinct pattern of expression, which indicates a contribution to gametogenesis.

HP1 MANTAINS HETEROCHROMATIN STRUCTURE THROUGH REGULATION OF SUV39H1 STABILITY

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Eukaryotic DNA is packed into chromatin in order to allow a proper management of all DNA associated functions. Chromatin is organized in the nucleus as a heterogeneous structure that can be divided in active open euchromatin and "closed" compacted heterochromatin. Among the different types of heterochromatin, constitutive heterochromatin (CH) is basically located in telomeres and pericentromeric regions, performing basically a structural role and their integrity for genomic stability.

Sirtuin 1, a NAD-depenent deacetylase (SirT1), promotes the formation of facultative heterochromatin by deacetylating H4K16Ac, H3K9Ac, and H1K26Ac and by establishing heterochromatin markers via interaction with the histone methyltransferase Suv39h1, a keystone of chromatin organization. Suv39h1-depenent trimethylation of H3K9 is essencial for maintenance of both pericentromeric and telomeric constitutive heterochromatin. Our previous studies have shown that the interplay between SirT1 and Suv39h1 is also crucial for pericentromeric heterochromatin formation. SirT1 is involved in the control of global levels of Suv39h1 by increasing its half-life through inhibition of Suv39h1 lysine 87 poliubiquitination. This turn increases Suv39h1 turnover in constitutive heterochromatin and ensures genome integrity. Stress conditions that lead to SirT1 upregulation, also induce higher levels of Suv39h1 in SirT1-dependent manner in vivo.

Here we show that there are other proteins involved in regulation of Suv39h1 stability,and therefore of the heterochromatin structure, such as the heterochromatin protein 1 (HP1). HP1 is a component of heterochromatin primarily responsable for gene silencing and the formation and maintenance of heterochromatin. The binding of HP1 to constitutive heterochromatin depends on the enzymatic activity of methyltransferase Suv39h1, which trimethylates H3 at lysine 9. In mammals there are three isoforms: HP1α, HP1β and HP1γ. HP1α and HP1β localize to heterochromatin, while HP1γ is in both heterochromatin and euchromatin. We determined that HP1 also has a similar effect that SirT1 on Suv39h1 stability. Interestingly, we have observed a differential role between the three isoforms of HP1 in the binding to, and regulation of the stability of Suv39h1 in the context of constitutive heterochromatin. Our results show that this mechanism may be very important for the adaptation of CH to conditions of stress and thus, to the maintenance of genome stability.

ROLE OF SIRT6 IN CHROMATIN FUNCTION

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The members of the Sir2 family, or sirtuins, constitute the class III of HDACs. They participate in the response to many forms of stress through their requirement of NAD+ as a cofactor in their enzymatic activity. Mammals have seven sirtuins (SirT1-SirT7). Among them, SirT6 is a nuclear protein involved in both genomic stability and metabolic homeostasis. Interestingly, SirT6 regulates the majority of these processes through an effect on chromatin, based on deacetylation of a histone mark, H3K9ac. However, the mechanism involved has not been characterized. In this work we aim to study the consequences of SirT6 function in chromatin organization. We show that SirT6 overexpression produces gene silencing, which fits with the role of SirT6 as a repressor, shown by previous reports. Our studies show that SirT6 interacts with a H3K9-specific methyltransferase that we have identified as Suv39h1. Suv39h1 trimethylates H3K9 and is essential in the establishment and maintenance of pericentromeric and telomeric constitutive heterochromatin. Interestingly, Suv39h1 was previously found to interact with SirT1 in the context of constitutive and facultative heterochromatin formation. Our work shows that the functional relationship between Suv39h1 and SirT6 is quite different from the already described between SirT1 and Suv39h1. SirT6 does not regulate Suv39h1-dependent constitutive heterochromatin foci as Sirt1, and the interacting domains of Suv39h1 with SirT1 and SirT6 are different. Finally, SirT6 mediates a posttranslational modification dependent on its enzymatic activity. Our studies have identified and characterized this modification. This work expands significantly our understanding about the mechanisms of action of sirtuins in chromatin regulation in response to these compromising situations.

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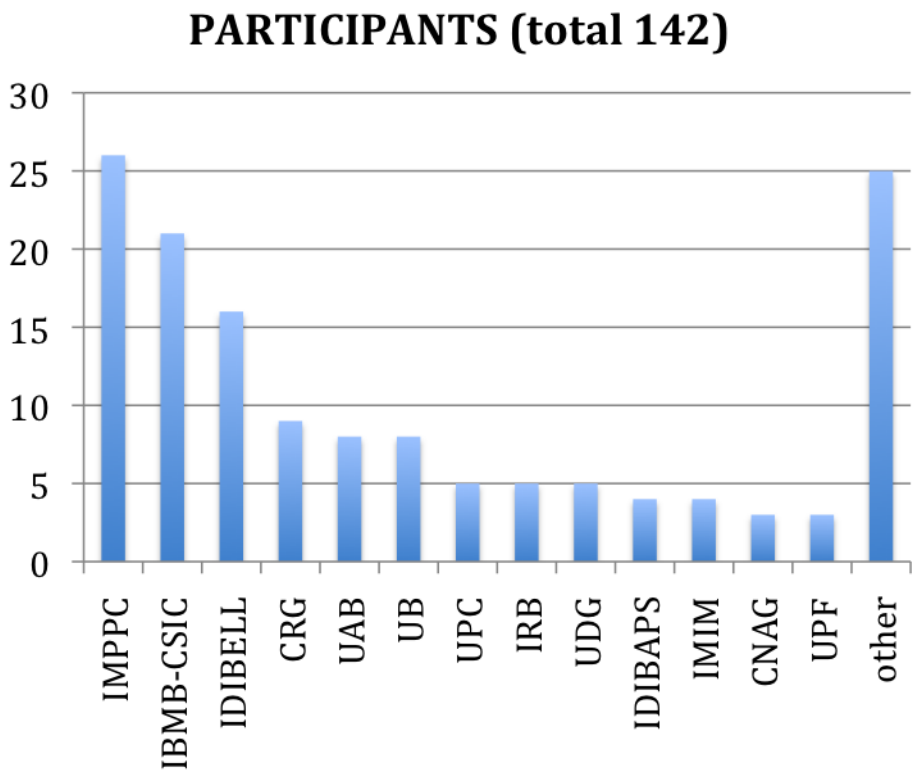
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