IV 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)

Societat Catalana de BIOLOGIA

BCC

INSTITUT D´ESTUDIS CATALANS

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18 de març de 2014

IV 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 18, 2014

IEC. carrer del Carme, 47, Barcelona

Prat de La Riba hall

Sponsored by

Covalab

Active Motif

IMPPC

PROGRAM

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

8.55 ***Opening***

Session I. *Chair:* ***Alex Vaquero***

9.00-9.20
Alfred Cortés (CRESIB)
Epigenetic variation in malaria parasites. sex, drugs, and ... survival

9.20-9.40
Dave Monk (PEBC)
Genome-wide characterization of imprints. implication for patients with multi-locus methylation defects

9.40-10.00
Eulàlia de Nadal (UPF)
Control of stress-induced long non-coding RNAs by the Hog1 SAPK

10.00-10.20
Rafael Oliva (IDIBAPS, UB)
Chromatin structure and epigenetics of the sperm cell

10.20-10.40
Josep Jiménez (Htal. St Joan de Deu)
Transgenerational inheritance of diabetes risk. Epigenetics on board?

10.40-11.10 ***Coffee break and poster session sponsored by Covalab***

Session II. *Chair:* ***Albert Jordan***

11.10-11.30
Manel Esteller (PEBC)
Epigenetics in Health and Disease

11.30-11.50
Marian Martínez Balbas (IBMB-CSIC)
Histone demethylases. new regulators of nervous system development

11.50-12.10
Maribel Parra (PEBC)
HDAC7. A novel master regulator in B cell development

12.10-12.30
Alicia Roque (UAB)
Linker histone ex vivo partial phosphorylation impairs chromatin aggregation

12.30-12.50
Ferran Azorín (IBMB-CSIC, IRB)
Analyzing histone H1 functions in Drosophila

13.00-14.45 ***Lunch at restaurant and poster session***

Session III-BCC4. Polycomb – new insight into an epigenetic paradigm
Chair: ***Montserrat Corominas***

14.45-15.05
Diego Pasini (IEO, Italy)
Novel activities of Polycomb Group Proteins in proliferation and identity control.

15.05-15.25
Holger Richly (IMB, Germany)
Epigenetic regulation of Nucleotide Excision Repair

15.25-15.40 Short Talk 1
Nicola Iovino (IHG, Montpelier, France)
Epigenetic Inheritance Through The Germline

15.40-16.00
Malte Beringer (CRG)
A novel interactor of Polycomb group proteins in embryonic stem cells.

16.00-16.15 Short Talk 2
Emilio Lecona (CNIO, Madrid)
USP7 regulates the stability of PRC1 complexes

16.15-16.35
Marcus Buschbeck (IMPPC)
A Polycomb complex facilitates gene activation during ES cell differentiation

16.35-17.00 ***Coffee break and poster session sponsored by Active Motif***

Session IV. *Chair:* ***Ferran Azorín***

17.00-17.20
Tanya Vavouri (IMPPC)
The small RNA content of human sperm

17.20-17.40
Sandra Peiró (IMIM)
Chromatin dynamics in EMT

17.40-18.00
Sergio Alonso (IMPPC)
Exploring DNA methylation alterations in colorectal cancer.

18.00-18.20
Roderic Guigó (CRG)
Transcriptional activation without chromatin marking in developmentally regulated genes.

18.30 Farewell

Secretaries of SCB

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Epigenetic variation in malaria parasites: sex, drugs, and ... survival

Alfred Cortés

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In malaria parasites, stochastic epigenetic variation within isogenic populations lies at the basis of phenotypic plasticity. Stochastic transitions between transcriptionally permissive and repressive chromatin states are an intrinsic property of a subset of genes (clonally variant genes) that play an important role in the adaptation of malaria parasites to changes in their environment. We have characterized the role of specific clonally variant genes in parasite survival: the transcriptional state of the clonally variant *clag3* genes determines the ability of a parasite to survive in the presence of some toxic compounds, whereas expression of another clonally variant gene determines survival of the parasite to heat-shock mimicking malaria fever episodes. We also found that the clonally variant gene *pfap2-g* acts as a master regulator that determines the asexual or sexual fate of a parasite. Altogether, our results indicate that malaria parasites rely on stochastic transcriptional switches controlled at the epigenetic level for fundamental processes in which individual parasites can make a choice between alternative options.

Genome-wide characterization of imprints: implication for patients with multi-locus methylation defects

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Differential methylation between the two alleles of a gene has been observed at imprinted regions, where the methylation of one allele occurs on a parent-of-origin basis, the inactive X-chromosome in females, and at those loci whose methylation is driven by genetic variants. We have extensively characterized imprinted methylation in a substantial range of normal human tissues, reciprocal genome-wide uniparental disomies and hydatidiform moles, using a combination of whole genome bisulphite sequencing and high-density methylation microarrays. This approach allowed us to define methylation profiles at known imprinted domains at base-pair resolution, as well as identifying 24 novel loci harbouring parent-of-origin methylation, 18 of which are restricted to the placenta. We observe that the extent of imprinted differentially methylated regions (DMRs) is extremely similar between tissues, with the exception of the placenta. Further we profiled all imprinted DMRs in sperm and embryonic stem cells derived from parthenogenetically-activated oocytes, individual blastomeres and blastocysts to identifying primary DMRs and reveal the extent of reprograming during pre-implantation development. These results have important implications for individuals with imprinting disorders with underlying multi-locus methylation defects, including Transient Neonatal Diabetes Mellitus patients with ZFP57 mutations.

Control of gene expression by the Hog1 SAPK

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Exposure of yeast cells to high osmolarity results in the activation of the Hog1 Stress-Activated Protein Kinase (SAPK), which is required to generate a set of osmoadaptive responses. The p38-related Hog1 SAPK is a key element for reprogramming gene expression in response to osmostress by acting on hundreds of stress-responsive genes. Hog1 is recruited to the osmoresponsive genes by specific transcription factors and serves as a platform to recruit RNA polymerase II and associated factors. Hog1 is also present at the ORFs of stress-responsive genes where it stimulates strong chromatin remodeling. In addition, Hog1 associates and controls the induction of a novel set of lncRNAs in response to osmostress. One of the genes expressing a stress-induced lncRNA in antisense orientation is *CDC28,* the CDK1 kinase that controls the cell cycle in yeast. Induction of the *CDC28* lncRNA permits the increase on the levels of Cdc28 allowing cells to re-entry more efficiently cell cycle after stress. Elucidating the control of gene expression by the Hog1 SAPK should help to understand how eukaryotic cells implement a massive and rapid change on their transcriptional capacity in response to adverse conditions.

Chromatin structure and epigenetics of the sperm cell

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Most of the mammalian sperm cell DNA is packaged by protamines while a small fraction (~1-8%) remains associated with nucleosomes enriched at loci of developmental importance (Hammoud et al. Nature 2009, 460:473–8; Arpanahi et al., Genome Res 2009, 19:1338–49; Oliva et al. Proteomics 2009, 9, 1004–17;). But the distribution of other proteins, in addition to protamines and histones, in the different sperm chromatin fractions had not yet been explored (Castillo et al 2014. Andrology, advanced dec 10 2013; Amaral et al. Hum Reprod Update 2014, 20:40-62). Therefore we have performed a detailed proteomic and genomic characterization of the sperm chromatin in order to increase the epigenetic knowledge of the male germ cell and determined whether quantitative alterations in chromatin proteins were present in infertile patients. Two different sperm chromatin fractions were obtained from sperm nuclei through the dissociation of histones using a saline treatment and the proteins were identified by mass spectrometry. Sperm DNA was additionally subjected to fractionation by endonucleases and deep genome sequenced confirming appropriate dissection of the sperm chromatin. Our results indicate that the sperm cell chromatin delivers to the offspring a rich combination of histone variants, transcription factors, chromatin-associated and chromatin-modifying proteins differing in chromatin affinity, which may be involved in the regulation of histone-bound paternal genes after fertilization. The differential proteomic results suggest that alterations in the proteins involved in chromatin assembly and metabolism may originate epigenetic errors during spermatogenesis, resulting in inaccurate sperm epigenetic signatures, which could ultimately prevent embryonic development (Azpiazu et al., 2014, Human Reproduction, in press). Funded by Ministerio de Economía y Competitividad (BFU2009-07718 and PI13/00699), European Union (ITN-GA-2011-289880) and Fundación Salud 2000 (SERONO13-015).

Transgenerational inheritance of diabetes risk: Epigenetics onboard?

Josep Jiménez

Obesity and type 2 diabetes have a heritable component that is not attributable to genetic factors. Instead, epigenetic mechanisms may play a role. We have developed a mouse model of intrauterine growth restriction (IUGR) by in utero malnutrition. IUGR mice developed obesity and glucose intolerance with ageing. Strikingly, offspring of IUGR male mice also developed glucose intolerance. Here we show that in utero malnutrition of F1 males influenced the expression of lipogenic genes in livers of F2 mice, partly due to altered expression of Lxra. In turn, Lxra expression is attributed to altered DNA methylation of its 5' UTR region. We found the same epigenetic signature in the sperm of their progenitors, F1 males. Our data provides evidence of the transmission of an epigenetic modification, via the sperm, that is stably maintained in somatic tissues (liver) of the offspring and that contributes, in part, to the development of metabolic dysfunction in the second-generation offspring.

Histone demethylases: new regulators of nervous system development

Asensio-Juan E[1], Fueyo R[1], Balada M[1], Gallego C,[1] Lois S[2], de la Cruz X[2,3] and Martinez-Balbás MA[1]
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Histone methylation is a regulatory mark that serves to control the transcriptional programs. In the last years several histone demethylases (HDM) have been identified as important players in neural development and function and their molecular mechanisms of action are starting to be underscored. PHF8 is a recently identified HDM that removes H4K20me and H3K9me2 marks. Interestingly, mutations on the PHF8 catalytic domain lead to mental retardation and autism. Although the activity of PHF8 is well characterized in vitro, the molecular mechanisms responsible for its role in nervous system development and function are not clearly established. In this talk we will discuss a new function of PHF8 fine tuning the transcriptional activity of genes to properly respond to external signals.

HDAC7: A novel master regulator in B lymphocyte development

Maribel Parra
Cancer Epigenetics and Biology Program (PEBC)
Bellvitge Biomedical Research Institute (IDIBELL)

Within the hematopoietic system, mature B lymphocytes are generated as the result of complex cell lineage commitment and differentiation processes. Each cellular transition is tightly regulated at the transcriptional level. The central role of lineage-specific transcription factors in positively regulating the distinct differentiation processes underlying B cell development is well established. Increasing evidence highlight the relevance of transcription factors in silencing lineage-inappropriate genes. However, the mechanisms by which B cell transcription factors mediate the process of gene silencing to acquire and maintain the cellular identity is poorly understood. Using and *in vitro* reprogramming system we have recently reported that HDAC7 is critical in maintaining the genetic identity of B lymphocytes. Definitive proof of the crucial role of HDAC7 in B cell development requires the evaluation of its function in vivo, in whole organisms. To address this, we have generated a conditional HDAC7 knockout mouse model for specific deletion of HDAC7 in B cell progenitors. We have found that HDAC7-deficient mice show a significant block in B cell development at the pro-B cell stage, indicating that HDAC7 is an essential transcriptional repressor in B lymphopoiesis. Mechanistically, HDAC7 is recruited to MEF2 binding sites located at the promoters of lineage inappropriate genes in pro-B cells. Importantly, we show that the absence of HDAC7 results in the up-regulation of relevant genes characteristic of macrophages and T lymphocytes. In conclusion, we have identified HDAC7 as a novel master regulator in B lymphocyte development.

Linker histone ex vivo partial phosphorylation impairs chromatin aggregation

Alicia Roque, Rita Lopez, Inma Ponte and Pere Suau

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Linker histones are involved in chromatin higher-order structure and in gene regulation. The histone H1 complement in chicken erythrocytes is composed of six different subtypes, named H1.01, H1.02, H1.03, H1.10, H1.1L and H1.1R plus the differentiation-specific isoform H5, which amounts about 70% of the total linker histones. Histone H1 is phosphorylated in a cell cycle-dependent manner by CDKs, which recognize the consensus sequence (S/T)-P-X-(K/R). During interphase linker histones are present as a mixture of unphosphorylated or partially phosphorylated species. Partial phosphorylation is thought to be involved in chromatin relaxation. We have succesfully phosphorylated erythrocyte chromatin ex vivo with CDK2. HPCE and MALDI-TOF analyses showed that the number of phosphate groups increased with the time of phosphorylation, reaching, in the case of H5, 54% of phosphorylated species (mono and diphosphorylated) after overnight phosphorylation. Tandem MS after proteolytic digestion showed that in soluble chromatin the (S/T)-P-X-(K/R) motifs were unphosphorylated in all linker histones while several of these motifs were found phosphorylated after different times of phosphorylation. The effect of linker histones partial phosphorylation on chromatin aggregation induced by $MgCl_2$ was studied by Dynamic Light Scattering (DLS) and qPCR coupled to micrococcal nuclease digestion. DLS allowed the quantification of the aggregated population of molecules as well as the determination of the hydrodynamic diameter of the aggregates. The results showed differences between the phosphorylated and unphosphorylated chromatin. Phosphorylated chromatin was characterized by lower percentages in volume of aggregated molecules and aggregates with lower hydrodynamic diameter. Nuclease accesibility of ß-globin and rhodopsin genes in phosphorylated and unphosphorylated chromatin samples was quantified by qPCR after a micrococcal nuclease digestion. qPCR results showed that phosphorylated chromatin samples displayed higher nuclease accessibility than the unphosphorylated samples, confirming DLS findings. Taken together, these results indicate that linker histone phosphorylation impaired chromatin aggregation.

Epigenetic Inheritance Through The Germline

Nicola Iovino

IGH, Montpelier, France

In sexually reproducing organisms, propagation of the species relies on specialized haploid cells (spermatocyte and oocyte) produced by germ cells. During their development, in the adult germline, the female and male gametes undergo a complex differentiation process that requires transcriptional regulation and chromatin reorganization. After fertilization, the gametes then go through extensive epigenetic reprogramming, which resets the cells to a totipotent state essential for the development of the embryo. Several histone modifications characterize distinct developmental stages of gamete formation and early embryonic development, but it is unknown whether these modifications have any physiological role.

In Drosophila, during normal oogenesis, one of the 16 diploid cells of the developing germline cysts enters meiosis and becomes the oocyte. The others 15 turn into polyploid nurse cells that nurture and prepare the oocyte for fertilization. Using a combination of genetic, gene expression studies, and ChIP analysis we have identified the Polycomb PRC2 complex as a critical determinant of the oocyte fate in Drosophila independently of PCR1 complex. In the absence of PRC2 components, the oocyte undergoes a transdetermination process that transforms it into a polyploid cell of nurse-like identity. Cyclin E and the cyclin dependent kinase inhibitor, dacapo, are the two cell cycle genes that must be directly repressed by PRC2 in order to prevent the oocyte from undergoing transdetermination (Iovino et al., Dev Cell, 2013).

After fertilization, embryos develop from the fusion of two haploid gametes. The gamete genomes, after fusion, remain physically separate and undergo distinct chromatin changes. The paternal genome exchanges protamines with histones, while the maternal genome maintains some of the histone modifications acquired during oocyte growth, such as methylation on H3K27me3 and H3K9me3 (N. I. and G. C., unpublished). The acquisition of developmental potency is thought to arise from reprogramming of the parental gametes' epigenetic state, although a thorough understanding of this mechanism is still lacking. Furthermore, the extent to which chromatin is reprogrammed or maternally inherited in early embryogenesis remains unknown, leaving open the question of how much epigenetic information from the mature gametes is retained in the embryo and is required for totipotency.

We have recently shown by using a sensitive ChIP-seq strategy that works well with a small number of nuclei and by immune fluorescence experiments, that PRC2-associated repressive states are maternally transmitted to the zygote; they are present on the totipotent nuclei in the embryo and are required for successful embryonic development. (N. I. and G. C., unpublished).

USP7 regulates the stability of PRC1 complexes

Emilio Lecona, CNIO, Spain

USP7 is a protein deubiquitinase with an essential role in development. It targets many proteins and cellular processes, of which the p53-MDM2 has been the best studied. Our results show that USP7 is essential for maintaining the levels of key factors in PRC1. RING1B, the common catalytic subunit of all PRC1 complexes, and BMI1, a specific component of canonical PRC1, are stabilized by USP7. Inhibition of USP7 affects both canonical and non-canonical PRC1 complexes, resulting in a reduction of ubiquitinated histone H2A, one of the mediators of repression by PRC1. Additionally, ChIP data show that USP7 co-occupies PRC1 target genes. We have further detected a direct interaction of USP7 with SCML2, a Polycomb group protein that associates with canonical PRC1 complexes. SCML2 is essential for the correct subnuclear localization of USP7 and bridges this deubiquitinase to canonical PRC1. In the absence of SCML2, USP7 cannot interact and stabilize BMI1, while RING1B remains unaffected. Thus, USP7 is a general regulator of the stability of PRC1, with SCML2 having an essential role in bringing this deubiquitinase to canonical complexes. These data suggest that the stabilization of PRC1 by USP7 mediates its essential functions in development through the maintenance of the levels of ubiquitinated histone H2A.

A Polycomb complex facilitates gene activation during ES cell differentiation

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IMPPC, Institute for Predictive and Personalized Medicine of Cancer, Spain

The Polycomb Group Proteins (PcGs) form two major protein complexes known as polycomb repressive complexes 1 and 2 (PRC1 and PRC2). Their function is essential for the maintenance of transcriptional repression during embryogenesis. The chromobox homolog 8, Cbx8, which is part of the PRC1 complex, has long been characterized as a repressor of gene transcription. The genome wide profiling of Cbx8 during the early steps of mouse embryonic stem (mES) cells differentiation provided us with surprising results involving Cbx8 in gene activation. During mES cells differentiation the protein level of Cbx8 increases. Loss of Cbx8 resulted in a reduction and/or delay in the activation of differentiation genes most of which are direct Cbx8 target genes. While Cbx8 protein level increases, the protein level of Cbx7, another chromobox homolog part of PRC1 complex, quickly drops when cells differentiate. On target genes Cbx8 replaces Cbx7 by binding to the H3K27me3 mark during differentiation. The replacement of Cbx7 by Cbx8 is essential but not sufficient to drive the differentiation of mES cells. Upon sustained gene activation Cbx8 is occupancy is lost.
Taken together our results point out a novel function of the Polycomb protein Cbx8 in gene activation. Based on our observations we would like to suggest a model in which Cbx8 is part of a transitional complex replacing fully repressive Cbx7-containing PRC1 at the onset of differentiation and the concomitant activation of key regulatory genes.

Exploring DNA methylation alterations in colorectal cancer

Sergio Alonso, IMPPC

Cancer cells exhibit both hypermethylation of normally unmethylated CpG sites, mostly at CpG islands, and demethylation of normally methylated CpG sites, generally associated to repetitive elements and gene bodies. In each cell division, cells must replicate the methylation pattern of over 28 million CpG sites, a process catalyzed by constitutive DNA methyltransferases. This mechanism, however, is not foolproof and mistakes unavoidably accumulate after the thousands of mitosis that stem cells undergo during the lifespan of the individuals. It is now clear that DNA methylation alterations decisively contribute to carcinogenesis and cancer progression.

Our studies during recent years have focused on the DNA methylation alterations in colorectal cancers (CRC). We found that DNA methylation alterations accumulate during aging, and precede genomic damage (copy number gains and losses). Moreover, the level of DNA hypomethylation is a predictor of survival in CRC patients. We also found frequent DNA methylation alterations in the colonic mucosa of ulcerative colitis patients, which are at higher risk for CRC. Many of these alterations resemble those typically found in CRC, suggesting a possible contributing mechanism for carcinogenesis in these individuals. More recently, we discovered that global demethylation of the non-tumor colonic mucosa of CRC patients is an independent predictor of the development of metachronous tumors, supporting the concept of an epigenetic field for cancerization in which preexistent epigenetic damage favors tumor initiation in certain regions of the colonic mucosa.

All these findings together provide support to an epigenetic-initiated model for carcinogenesis for some CRCs, in which DNA methylation alterations, and in particular global DNA demethylation, relentlessly and unavoidably accumulate in the stem cells during aging, well before malignant transformation and clonal expansion. When some particular genomic regions reach certain levels of DNA demethylation, the normal mitotic process may be hindered and thus increase the probability of mitotic error. This in turn may lead to genomic damage (aneuploidy) and predisposing the cells to further genetic alterations that ultimately lead to the deregulation of the control of cell division. Under the right genomic and environmental background, the cells will transform and initiate an uncontrolled clonal expansion. The epigenetic alterations that took place before transformation, together with novel alterations taking place during the tumor progression, will be then exposed by the clonal expansion of the cell of origin.

The small RNA content of human sperm

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At the end of mammalian sperm development, sperm cells expel most of their cytoplasm and dispose of the majority of their RNA. Yet, hundreds of RNA molecules remain in mature sperm. The biological significance of the vast majority of these molecules is unclear. To better understand the processes that generate sperm small RNAs and what roles they may have, we sequenced and characterized the small RNA content of sperm samples from two human fertile individuals. We detected nearly two hundred microRNAs, some of which are highly abundant. The most abundant class of small non-coding RNAs in sperm are PIWI-interacting RNAs (piRNAs). The most abundant sperm piRNAs target LINE1 retrotransposons. This is in agreement with previous experiments showing that PIWI is required for LINE1 repression in adult testes. Our results show that LINE1 targeting piRNAs remain in mature sperm. Surprisingly, we found that human sperm cells contain piRNAs processed from pseudogenes. Clusters of piRNAs from human testes contain pseudogenes transcribed in the antisense strand and processed into small RNAs. Several human protein-coding genes contain antisense targets of pseudogene-derived piRNAs in the male germline and these piRNAs are still found in mature sperm. Our study provides the most extensive dataset and annotation of human sperm small RNAs to date and is a resource for further functional studies on the roles of sperm small RNAs. In addition, we propose that pseudogene-derived human piRNAs may regulate expression of their parent gene in the male germline.

Temperature effects on global DNA methylation patterns during larval development in the European sea bass

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Fish represent good animal models for the purposes of studying environmental effects like temperature on epigenetic marks, i.e. DNA methylation. Recently, we showed that in one-year-old European sea bass, the promoter of gonadal aromatase had higher levels of DNA methylation if the fish had been raised at high temperature during larval development when compared to fish raised at natural temperature (Navarro-Martín et al. 2011. *PLoS Genetics* 7(12): e1002447). In the present study, we expanded our knowledge by investigating the effects of temperature on global methylation during larval development in three different experiments. Two periods of temperature treatment were considered: (1) from 0 to 15 days post fertilization (dpf) and (2) from 20 to 60 dpf. The first period included: (a) a temperature (dose)-response experiment (experiment 1) with three groups of fish treated with low (LT, 13.5^{o}C), intermediate (IT, 17.5^{o}C) or high (HT, 19.5^{o}C) temperature, and (b) eight groups of fish which either had experienced a temperature switch at 15, 120 or 240 hours-post-fertilization from low to high or vice versa, or had been raised at constant, low or high temperature (experiment 2). We used the Methylation Sensitive Amplified Polymorphism (MSAP) technique in order to detect changes in global DNA methylation between treatments. Statistically significant differences were found in experiment 1 between the three temperature-response groups and between all 8 groups of experiment 2. In contrast, the second treatment period (20-60 dpf; experiment 3) did not produce any significant differences between groups. Real-time PCRs were performed for several genes related to epigenetic regulatory mechanisms or that are important for several developmental processes. Our results show that the temperature treatment during the first 15 dpf affected significantly the methylome of the European sea bass while the treatment from 20 to 60 dpf did not, and that the expression of specific genes is altered. Together, these experiments illustrate the importance of the early environment in eliciting epigenetic changes in gene expression that will probably have lifelong phenotypic consequences. *Supported by MINECO grant "Epigen-Aqua" (AGL2010-15939) to F.P.*

Methylation plotter: a web tool for dynamic visualization of DNA methylation data

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Cytosine methylation in CpG dinucleotides is an important mechanism involved in the regulation of multiple biological processes including pathological conditions. While there is a wide range of methodologies to evaluate DNA methylation, bisulfite-treated DNA sequencing is the gold standard to determine DNA methylation at the single CpG level. The functional implications of DNA methylation states are often determined not by a single CpG site at the regional level. Therefore, the interpretation and application of this sort of data require further analysis that is highly benefited by the implementation of visualization tools.

Methylation plotter is a Web tool that allows the visualization of methylation data in a user-friendly manner and with publication-ready quality. The user is asked to introduce a file containing the methylation status of a genomic region. This file can contain up to 100 samples and 100 CpGs. Optionally, the user can assign a group for each sample (i.e. whether a sample is tumoral or normal). After the data upload, the tool produces different graphical representations of the results following the most commonly used styles to display this type of data. They include an interactive plot that summarizes the status of every CpG site and for every sample in lollipop or grid styles. Methylation values ranging from 0 (unmethylated) to 1 (fully methylated) are represented using a gray color gradient. The user can select different types of arrangement of the samples in the display: in example sorting by overall methylation level, by group, by unsupervised clustering or just as provided.

Apart of the detailed plot, Methylation plotter produces a methylation profile plot that summarizes the status of the scrutinized region, a boxplot that sums up the differences between groups (if any) and a dendrogram that classifies the data by unsupervised clustering. Coupled with this analysis, descriptive statistics and testing for differences at both CpG and group levels are provided.

The implementation is based in R/shiny, providing a highly dynamic user interface that generates high quality graphics without the need of writing R code. Methylation plotter is freely available at http://gattaca.imppc.org:3838/methylation_plotter/ .

DNA methylation of transcription factor binding sites

Yulia A. Medvedeva[*,1], Abdullah Khamis[1], Ivan V. Kulakovskiy[2,3], Wail Ba-Alawi[1], Md. Shariful I. Bhuyan[1], Hideya Kawaji[4,5,6], Timo Lassmann[4,5], Alister R.R. Forrest[4,5], Matthias Herbers[4], FANTOM consortium, Vladimir B. Bajic[2,#]

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DNA methylation in promoters is tightly linked to gene repression. Yet, the question remains whether DNA methylation is a cause or consequence of gene repression. In the former case, DNA methylation may affect the affinity of transcription factors (TFs) towards their binding sites (TFBSs). In the latter case, gene repression caused by chromatin modification, is stabilized by DNA methylation. Both above-mentioned scenarios have been supported by non-systematic evidences and have not been tested for a wide spectrum of TFs.

In this study we found that for 16.6% of cytosines methylation profile and the expression profile of neighboring transcriptional start site show significant negative correlation. We name CpG that correspond to such cytosines “traffic lights”. CpG “traffic lights” are mostly located within CpG islands in gene promoters. We hypothesize that if CpG “traffic lights” are not induced by average methylation of a silent promoter, they may affect binding of TFs to their binding sites and therefore regulate transcription. We observed a strong selection against CpG “traffic lights” within TFBSs, more pronounced for “core” position of the TFBS, supporting the damaging role of CpG “traffic light” for a TFBS. Surprisingly, we found selection to be stronger for repressors then for activators or multifunctional TFs. We conclude that single cytosine methylation may play a role in transcriptional regulation. At the same time, blocking of TFBS by selective methylation is likely to be restricted to special cases and cannot be considered as a general regulatory mechanism of methylation-dependant transcription. This puts into a different perspective the current common perception of the link of methylation and gene expression.

This work is part of the FANTOM5 project, providing genome-wide expression data across various cell types using cap analysis of gene expression (CAGE).

Crystallographic Studies of Complexes of Human HMGB1 and AT-rich DNA

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High Mobility Group proteins act as architectural proteins that affect cellular functions by modulating chromatin structure and thereby gene expression in eukaryotic cells (Hock *et al.*, 2007; Catez and Hock, 2010). HMGB proteins are one of the three members of this family and are defined by their DNA binding motifs: the HMG-boxes. The HMG-box domain, found also in other proteins, has an L-shaped fold consisting of three α-helices (Thomas and Travers, 2001). HMGB proteins contain one or more HMG-boxes and have little or none sequence specificity for DNA binding (Stros, 2010).

We have performed electrophoretic mobility shift assays (EMSA) using HMGB1 and different oligonucleotides, mostly AT-rich oligonucleotides. We have worked with fragments corresponding to the two HMG-box domains (Box-A and Box-B) and also with a fragment containing both domains (Box-A+B).

Furthermore, we have performed crystallographic assays with these three fragments of the HMGB1 protein with these oligonucleotides. We have obtained crystals of diverse morphology and we show the diffraction data obtained.

Catez F. and Hock R. (2010). Binding and interplay of HMG proteins on chromatin: Lessons from live cell imaging. Biochim Biophys Acta 1799, 15–27.

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Thomas J.O. and Travers A. A. (2001). HMG1 and 2, and related 'architectural' DNA-binding proteins. Trends Biochem Sci. 26, 167–174.

The trithorax protein

Ash2 in transcriptional regulation

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Trimethylation of histone H3 at lysine 4 (H3K4me) is associated with transcriptional activation and requires several cofactors, including the trithorax protein Ash2. Mutations in *ash2* cause a variety of pattern formation defects in the Drosophila wing and we have recently found that *ash2* mutants also have 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 mark set by the histone methyltransferase Trr in these genes. We propose here that, in addition to its role in facilitating H3K4 trimethylation, *ash2* plays complex-specific functions depending of its association to different histone methyltransferases. Prominent among putative roles are the regulation of transcription initiation, chromatin remodeling, or modulation of splicing efficiency. In fact, the reduction of RNA Polymerase II phosphorylated at serine 5 (PolIIS5P) observed in mutant flies points to a fast escape from stalling in the absence of *ash2*. Quantitative RT-PCR and RNASeq approaches in *ash2* mutants are currently underway to clarify the function of ASH2/H3K4me3 during transcript elongation and/or maturation.

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Societat Catalana de BIOLOGIA

Història

Fundada l'any 1912 amb el nom de Societat de Biologia de Barcelona, i essent la primera filial de l'Institut d'Estudis Catalans, tingué com a primer president el doctor August Pi i Sunyer. Al principi, la Societat es dedicà fonamentalment al terreny de la medicina, i en no gaires anys assolí un paper destacat com a institució científica.

L'any 1938 hi hagué les darreres activitats de la vella Societat de Biologia de Barcelona; el final de la Guerra Civil espanyola comportà l'acabament del primer període de les seves activitats públiques.

L'any 1954 tingué lloc una primera sessió, clandestina, amb una conferència del professor Josep Trueta sobre poliomielitis.

No és fins a l'any 1962, quan es complia el cinquantè aniversari de la seva creació, que la Societat no reprengué a poc a poc la seva activitat pública, amb el nom actual de Societat Catalana de Biologia.

Actualment, la Societat Catalana de Biologia està afiliada a la Secció de Ciències Biològiques de l'Institut d'Estudis Catalans. Es regeix per una assemblea de socis que voten les candidatures dels membres del consell directiu al qual tots hi poden participar.

Objectius

- Reunir els professionals i els estudiosos de les ciències de la vida que se senten units per una llengua i una cultura comunes i difondre el desenvolupament de la ciència que es fa las Països Catalans arreu del món mitjançant les relacions entre científics i institucions científiques.

- Difondre el desenvolupament de la ciència catalana arreu del món, per promoure els contactes entre científics, institucions científiques i estudiants de les ciències de la vida i afavorir l'intercanvi d'idees i la col·laboració.

- Contribuir a divulgar les idees i les noves aplicacions de la ciència per tal que arribin a tothom.

Beneficis de ser soci

- Formar part d'una societat científica de renom; la societat científica més antiga, amb més socis i més activa del nostre país.
- Rebre les publicacions i revistes en paper gratuïtament a domicili.
- Gaudir de descomptes substancials en la inscripció a les jornades organitzades per diverses seccions de la SCB.
- Tenir places reservades en actes que tenen aforament limitat.
- Gaudir de descomptes en les publicacions que edita tant l'IEC com totes les seves societats filials.
- Rebre les novetats i la programació d'activitats de la Societat per correu electrònic.
- Poder participar en l'organització d'activitats de les seccions especialitzades.
- Tenir veu i vot a les assemblees generals de la Societat.

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NOTES