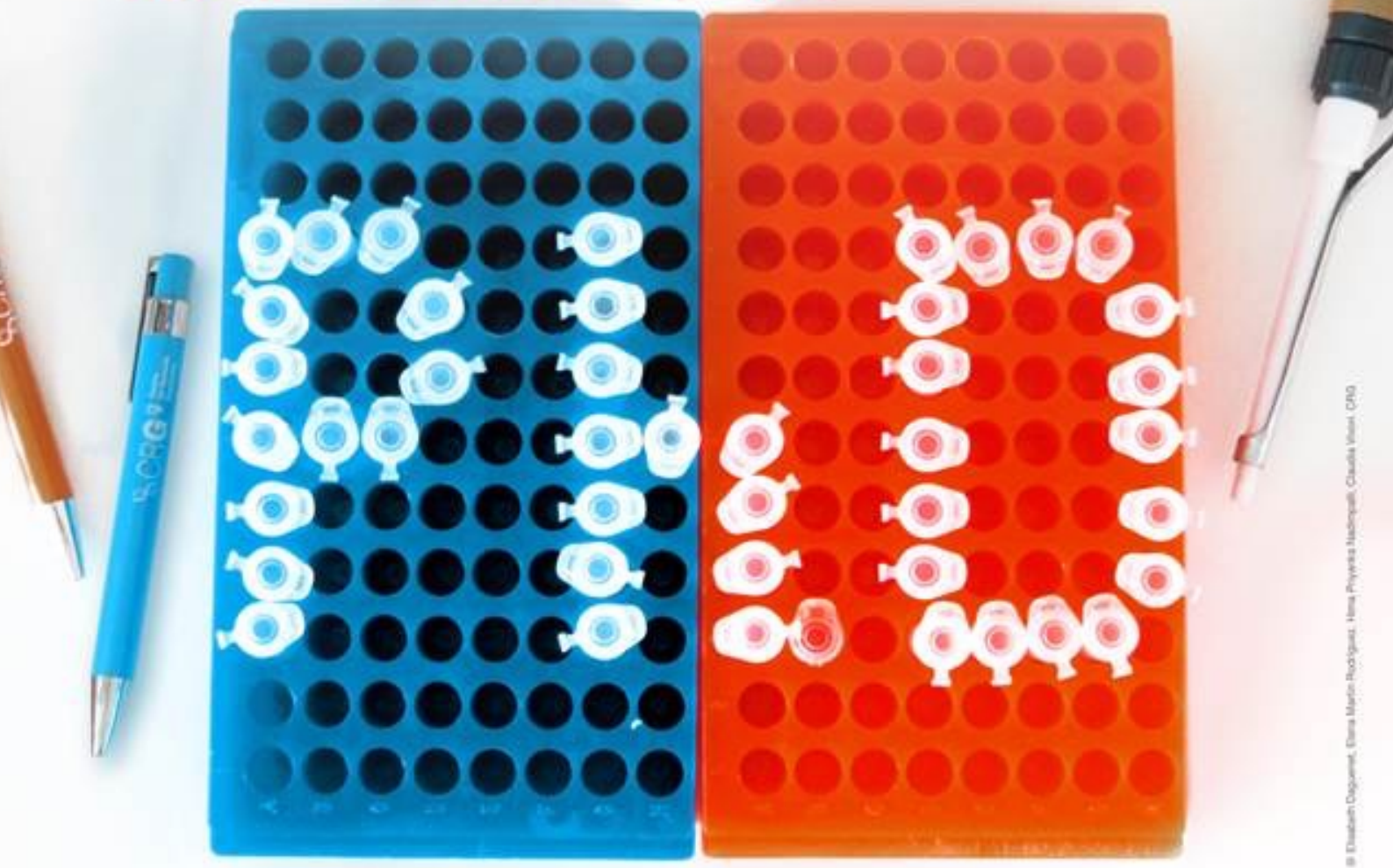
IX CRG PhD Symposium

CRG Centre for Genomic Regulation

26th & 27th November 2015



Speakers

Andrea BATTOLA
Birgit RITSCHKA
Carla BELLO
Carolina GALLO
Elie M. FINK
Ernst THUER
Hana SUSAK
Hima P. NADIMPALLI
Joan PALLARÉS
Laia CARRETÉ
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Laura DOMÈNECH
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Marc CORRALES
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EXCELENCIA SEVERO OCHOA Centro de Excelencia Severo Ochoa 2013-2017 (ref. SEV-2012-0208)

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Produced by: CRG Graduate Committee
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Contents: CRG PhD Students
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Graphic design: Claudia Vivori
Cover picture: Elisabeth Daguenet, Elena Martín Rodríguez, Hima Priyanka Nadimpalli, Claudia Vivori

IX PhD STUDENT SYMPOSIUM

Centre for Genomic Regulation (CRG)
PRBB Auditorium, Barcelona
26-27 November 2015

Programme

Thursday 26 November

14:00 **Opening words by Luis Serrano**

Session 1

14:10 Carolina Gallo Lopez
14:30 Mireia Ortega Crespo
14:50 Marc Corrales
15:10 Birgit Ritschka

15:30 **Coffee break & Poster session**

Session 2

16:30 Carla Bello Cabrera
16:50 Hana Susak
17:10 Ernst Thuer
17:30 Andrea Battola
17:50 Miquel Rosas Salvans

18:30 **End of session**

Friday 27 November

Session 3

10:00 Linus Manubens Gil
10:20 Elie Fink
10:40 Marta Inglés Ferrándiz
11:00 Laura Barba Moreno

11:30 Coffee break & Poster session

Session 4

12:00 Laura Domenech Salgado
12:20 Sebastian Ullrich
12:40 Marcos Francisco Perez
13:00 Marta Fructuoso Castellar

13:30 Lunch break

Session 5

14:30 Pol Cuscó Pons
14:50 Hima Priyanka Nadimpalli
15:10 Laia Carreté Muñoz
15:30 Joan Pallarès Albanell

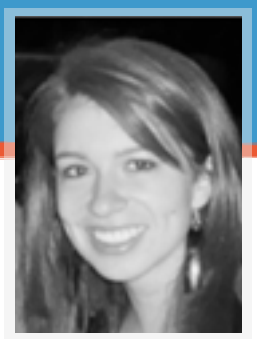
16:00 Coffee break

16:30 Closing remarks by Luciano Di Croce
Best talk / Best poster prizes

18:00 Happy hour

Talks

Increasing growth of the slow-growing, genome-reduced bacterium *Mycoplasma pneumoniae*



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The factors that govern bacterial growth rates are poorly understood. Several extrinsic factors have been described, such as nutrient availability, pH, temperature and osmolarity, but intrinsic factors are less well characterized. Nonetheless, it was shown that growth rate correlates with rRNA and tRNA gene content. Likewise, other studies have highlighted the importance of protein synthesis in the rate of bacterial growth and fitness. ***Mycoplasma pneumoniae*** is a good model organism of a minimal bacterium because it has one of the smallest genome, little genomic redundancy and it divides every 8h in laboratory conditions. Moreover, the doubling times of the ***Mollicutes*** species range from 27 min to 72 h, which makes ***Mollicutes*** class an interesting group to study growth. ***Mollicutes*** have only one or two rRNA operons and fewer tRNA genes. Furthermore, most cultivable ***Mollicutes*** lack tricarboxylic acid cycle enzymes, quinones, and cytochromes and it was shown that representative ***Mollicutes*** species from the four prominent metabolic groups were relatively deficient in adenylate energy charge and ATP concentration during their mid-exponential growth phase. We aim to study and increase the growth of ***M. pneumoniae***. Here, we doubled the wild type rRNA and tRNA gene dosages, and in the first case it resulted in shorter doubling time. Also we are planning to increase ATP production in ***M. pneumoniae*** and to compare transcriptomes from fast- and slow-growing ***Mollicutes*** species to identify candidate genes whose overexpression or deletion boost growth. Finally we will integrate all this information in a whole-cell model of ***M. pneumoniae***.

DYRK1A overexpression impact on signaling networks: a systems biology approach

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Trisomy of human chromosome 21 results in Down syndrome, the most complex and common genetic perturbation leading to intellectual disability. Dyrk1A, the dual-specificity tyrosine (Y)-phosphorylation kinase 1A, is a candidate gene to explain Down syndrome phenotypic abnormalities that regulates fundamental cellular functions. DYRK1A kinase activity in the hippocampus is normalized upon environmental enrichment in TgDyrk1A mice (only overexpressing the kinase), along with improvements in cognition. Importantly, pharmacological inhibition of DYRK1A kinase activity using EGCG, a green tea flavonol, and the combined treatment of EGCG and environmental enrichment also rescues the cognitive and the neuronal phenotype in TgDyrk1A and trisomic (Ts65Dn) mice and in Down syndrome patients. However, the molecular mechanism through which the flavonol rescues the Down syndrome phenotypes remains elusive.
As initial step to tackle this goal, we used mass spectrometry-based technologies to elucidate the changes in the proteome and phosphoproteome of TgDyrk1A mice upon different treatments that combine the use of environmental enrichment and the aforementioned flavonol. Our results show several protein abundance changes and phosphorylation events that expand multiple cellular functional networks, including functions related to neural plasticity. We are also analysing the possible transcriptional regulators that may explain the protein abundance changes, and which of them could have direct relation with Dyrk1A.

Chromosome conformation drives euchromatic position effects in Drosophila

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Genome Architecture
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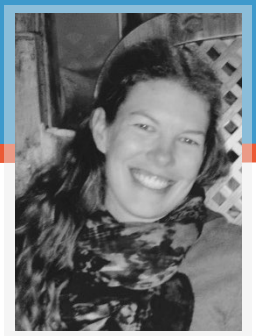
In eukaryotes, the regulation of transcription is the result of sequence-specific and context-dependent mechanisms. It has long been observed that the expression of a gene can change when it is translocated to an ectopic site in the genome, a phenomenon known as position effect. For genes transplanted near a centromere, invasion by heterochromatin was proposed as the mechanism of silencing. Position effects also take place away from the centromeres, where they were proposed to be governed by association with the nuclear lamina, chromatin composition or enhancer proximity. Here we show that position effects on chromosome arms are driven by the local conformation of the chromatin fiber. By measuring the expression of ~ 72,000 integrated reporters in the Drosophila genome, we establish that position effects on chromosome arms and at the centromeres rely on distinct mechanisms. We discovered regions where reporters have low expression, even though they are away from the lamina, actively transcribed and enhancer-rich. Those regions share with Lamina-Associated Domains (LADs) a characteristic low proportion of HiC contacts at the 5 kb scale. Our results argue that genome folding is a critical determinant of gene expression and not merely a consequence of it. They also suggest that the repressive effects of LADs can be partly attributed to a spatial conformation of the chromosome.

Senescence is a regenerative process that instructs stem cell function



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Mechanisms of Cancer and Aging
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Cellular senescence is a form of cell cycle arrest that is linked to tumor suppression and aging. It can be induced by replicative exhaustion, oncogenic signalling, DNA-damage and chemotherapeutic agents, acting to limit the proliferative capacity of damaged cells. Conversely however, it has been suggested that senescent cells also have pro-tumorigenic activity through the secretion of specific proteins, termed the senescence-associated secretory phenotype (SASP). Unexpectedly, we found that primary mouse keratinocytes undergoing oncogene-induced senescence exhibit an increase in the expression of skin stem cell-associated genes. Furthermore, we show that the SASP from oncogene-induced senescent keratinocytes induces an increase in stem cell-associated genes in a paracrine manner, suggesting that the SASP can enhance tissue stemness. Importantly, by performing hair reconstitution assays with keratinocytes exposed to senescence-conditioned media, we demonstrate that these cells have functional stem cell capacity, suggesting that senescence can enhance regeneration in vivo. However, direct transplantation of senescent cells resulted in papilloma formation, suggesting that increased exposure to the SASP might induce tumor formation through activation of aberrant tissue regeneration. Together, this work uncovers a primary function for cellular senescence in promoting tissue regeneration, but which when prolonged, can lead to pre-malignant tumor formation.

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Evolution of lncRNAs in the human lineage

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The evolution of new genes plays a key role in the creation of new phenotypes and helps to ensure the survival of the species. Among the key mechanisms responsible for the origin of novel genetic material is the duplication of genomic sequences, including whole and partial gene duplication.The increasing amount of information regarding long non-coding RNAs (lncRNAs) has raised the question of whether these RNA genes could play important roles in human evolution. Here, we evaluate the rate and impact of exon duplication on lncRNAs in the human lineage. Utilizing the data generated by the ENCODE project, we show that exon duplication is a common event among lncRNAs and that alternatively spliced lncRNA genes undergo significantly more exon duplication events than those that are not. By comparing primate genomes using whole-genome sequencing (WGS) data we have identified novel human specific lncRNA genes that were recently formed through exon duplication. Moreover, RNAseq expression analysis using publicly available data shows that some of these novel genes are expressed in the brain, suggesting a role in human brain expansion. Notably, single-nucleotide polymorphism (SNP) analysis utilizing data from the 1000 Genomes Project shows that these lncRNAs are evolutionary constrained and thus under negative selection, indicating that they are in fact functional. Overall, these results contribute to unveil the evolutionary pathway of the human lineage and other apes.

Signatures of positive selection reveal cancer driver genes across multiple tumor types

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Tumors are an evolving population of proliferative cells subjected to natural "Darwinian" selection. Positive selection of cancer traits underlies the mutational load of driver genes. Nevertheless, current approaches for cancer gene identification focus mainly on mutation frequency but neglect clonal structure and tumor evolution. Here, we describe a novel Bayesian approach, cDriver, to rank genes simultaneously using multiple signatures of positive selection. Our method models the mutation frequency in drivers (foreground) and the background mutation rate using the cancer cell fraction of observed mutations, thereby approximating positive selection on a cell instead of on a patient level. We prove that our ranking captures the best landscape of known driver genes in a non-solid tumor (CLL), in a solid tumor (BRCA) and in a PanCancer dataset under different scenarios. Moreover, we identify low-frequency mutated genes across 20 tumor types by modeling a rank-based driver gene landscape. We categorize tumor specific genes (e.g. VHL) and common driver genes (e.g. PIK3CA). Functional enrichment of top genes reveals that mitosis and cell proliferation are under positive selection across multiple tumors. Identification of new therapeutic targets can benefit from the novel genes found to be common to more tumor types than expected.

Investigating long noncoding RNA in Yeast

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An important scientific focus of the CRG lies with the studying of long noncoding RNA.
Most research in this field has been carried out in higher eukaryotes, unveiling a large variety of noncoding transcripts involved in many cellular functions.
In an effort to increase the knowledge of those elusive transcripts, we analysed them in two species of unicellular fungi. The model organism *Saccharomyces cerevisiae* and the related opportunistic human pathogen *Candida parapsilosis*. After an initial prediction and annotation, we investigate the appearance, expressional pattern, conservation, coexpression and structural formation tendencies of noncoding RNA, revealing unexpected features and possible functional classification. To validate the results of the predictions, knock outs are being performed on the most promising candidate transcripts in *C. parapsilosis*.

The role of Boi1, Boi2 and Exocyst in NoCut

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Coordination of Cytokinesis with Chromosome Segregation
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Cytokinesis is the last step of cell cycle that leads to physical separation of dividing cells that become then individuals. Importantly, chromosome segregation, which starts in anaphase, needs to be properly coordinated with cell division, in order to avoid inequalities in DNA content among the newborn cells.
Defects during DNA segregation might lead to the formation of DNA bridges that span the site of cell division. If these bridges are not resolved before cytokinesis, cell division would cut the DNA bridge, resulting in DNA damage, aneuploidies, and decreased cell viability. A recently described pathway, called NoCut, detects the aforementioned DNA bridges at the division site and delays the last step of cytokinesis, called abscission, when plasma membranes separate. This delay gives time to the cell to clear away DNA remnants from the division zone, increasing the fitness of the population.
Boi1 and Boi2 are two membrane-associated proteins involved in polarized growth and required for NoCut. Indeed, their depletion leads to failure in the NoCut-dependent abscission delay, therefore causing a "cut" phenotype. The mechanism how Boi1 and Boi2 inhibit abscission is not clear yet. My goal is to elucidate how Boi proteins inhibit abscission in response to DNA bridges. I will present evidences suggesting that Boi proteins block abscission in response to chromosome decatenation defects through the regulation of the exocyst, a multimeric complex involved in tethering post-Golgi vesicles to the plasma membrane.

RanGTP, from spindles to cilia

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Ran GTPase is the central regulator of nucleus-cytoplasm protein transport.
Ran is also involved in the regulation of the activity of a set of proteins involved in spindle assembly. Ran regulated proteins promote the formation of the chromosomal microtubules, which are absolutely necessary and sufficient for assembling a functional bipolar spindle. I have focused in DnaJB6, which seems to be involved in spindle organization. Interestingly, this protein has been related with the IFT complex, which have an essential role in cilia. Recently, RanGTP had been seen into the cilium. Ran has been associated to the transport regulation of a specific protein (kif17) into the cilium. I would like to test the hypothesis that RanGTP may control the activity of a similar set of components involved both in spindle and cilium assembly. I want to test whether Ran could be regulating DnaJB6 function in the two processes.

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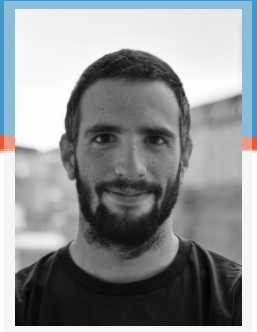
Modeling the effect of neuronal structure and connectivity alterations in Down syndrome mouse models



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Understanding behavior and cognition requires understanding the rules that determine the patterns of spatio-temporal brain activity. Those patterns derive from the emergent properties of large collections of overlapping neural circuits, with precise connectivity topography of their connections, determined by neuronal architecture. These connections are fine-tuned by short-term synaptic plasticity.
Our goal is to study how neuronal architecture and connectivity constrain the mesoscopic network activity and influence the flow and storage of information in neuronal circuits. To this aim, we propose to take advantage of pathologies, such as intellectual disabilities, in which the essential properties of the network are systematically violated. Specifically we use Down syndrome (DS) mouse models that recapitulate the neuronal morphology and connectivity disruptions (reduced synaptic connectivity, excitatory-inhibitory misbalance, reduced dendritic complexity and reduced neuronal density in specific brain regions), and the cognitive deficits in learning and memory of DS patients.
To address our question we are using a mixed experimental and computational approach, to comprehensively analyze different spatial scales, from the local connectivity in neuronal modules to the long-range connectivity in the mouse brain.
We hypothesize that the changes in connectivity caused by local morphology will affect distal projection alterations being thus sufficient for impairing the ability of neuronal circuits to perform cognitive functions. We will show an experimental and computational framework that will allow us to evaluate the Pareto optimality of neuronal networks for building cost, information storage capacity, processing efficiency, neural complexity (coexistence of functional segregation and integration) and rhythmic coupling capacity in function of the explored multi-scale neuromorphological alterations.

Olfactory Differentiation in the Drosophila Genus

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For insects such as the Drosophila fly and its larva, olfaction represents a sensory system indispensible to evaluate their environment. They use olfactory cues for foraging, habitat localization, social communication and sexual reproduction. Within the Drosophila genus certain species have evolved distinct preferences for specific host substrates while others are generalists and exploit a variety of substrates. We may therefore expect the chemical sensory systems of different species to have evolved in accordance with shifts in habitat and host specialisation. With this project we explore how peripheral encoding of odours, and the neural circuits that drive adaptive behaviour have coevolved with shifts in natural habitats and feeding niches. What are the sensory mechanisms underlying the adaptation to different ecological niches? We tackle this in a cross-species comparison of the larvae of 8 drosophilid species within the melanogaster species group. We observe differences in sensitivities of these species towards a panel of odorants and note that responses elicited by certain subsets of odours are conserved. We also observe distinct foraging and odour-search related strategies employed, which mirror their ability, or lack thereof, to proficiently localise an odour source. Seen differences and similarities also partially reflect the phylogenetic relationship between the species explored.

Role of UNR/ CSDE1 in histone mRNA metabolism

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Upstream of N-ras (UNR/ CSDE1) is an RNA-binding protein that regulates mRNA translation and stability. We have recently found that UNR promotes melanoma development by coordinating RNA regulons important for invasion and metastasis. A group of mRNAs that are regulated by UNR en mass are the histone mRNAs. iCLIP experiments revealed that UNR binds to ~80% of the histone mRNAs expressed in melanoma cells. RNA-Seq data indicate that depletion of UNR results in histone mRNA down-regulation. Intriguingly, UNR binds close to a regulatory stem-loop structure in the 3' UTR necessary for efficient translation and stability. We are currently investigating the consequences of UNR binding to histone mRNAs using reporter constructs, and the possible interplay of UNR with the histone mRNA degradation machinery.

Molecular mechanisms and functions of chromatin associated-DYRK1A

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DYRK1A is member of the DYRK subfamily of protein kinases (dual specificity tyrosine-(Y)-phosphorylation-regulated kinases), highly conserved from Drosophila to humans. Changes in DYRK1A expression levels, either by inactivation of one allele or by the presence of an extra copy when in trisomy, lead to pathological phenotypes in several organs in humans.
Recent work from our lab shows that DYRK1A is recruited to RNA-polymerase II proximal promoter regions containing a highly conserved palindromic sequence and appears to positively regulate the transcription of several of its targets. A subset of DYRK1A targets are ribosomal protein genes (RPGs), a fact that could underlie the reduction in protein translation rates induced by DYRK1A-depletion. Although the regulation of RPGs transcription has been well characterized in yeast, very little is known on the regulatory mechanisms in higher eukaryotes. Therefore, we propose to study the functional link between DYRK1A and its recruitment to the proximal promoter regions of RPGs to shed light on this issue. We have investigated how DYRK1A is recruited to its genomic loci and found that purified DYRK1A binds to single-stranded DNA containing the palindromic sequence, suggesting that DYRK1A might bind unwound DNA at the chromatin level. In addition, analysis of the ChIP-Seq data from ENCODE reveals that several transcription/chromatin-related factors, such as the co-repressor ZBTB33 and the tumour suppressor BRCA1, can be recruited to the DYRK1A-consensus motif. In fact, we have validated the presence of ZBTB33 and BRCA1 at several DYRK1A-bound RPG promoters; moreover, the interaction of DYRK1A with ZBTB33 or BRCA1 is detected in nuclear extracts by co-immunoprecipitation experiments. These findings suggest a cross-talk between DYRK1A and these proteins in the transcriptional regulation of the target RPGs, which will be further investigated.

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Deconstructing obsessive-compulsive disorder by whole exome sequencing

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Obsessive-compulsive disorder (OCD) is a neuropsychiatric condition that affects 1-3% of the population worldwide and that is listed as the tenth most disabling illnesses of any kind. Current available treatments for OCD only provide partial relief of symptoms. Therefore, improving our understanding of its biology is an essential step towards an effective and personalized therapy. Family and twin studies have demonstrated that OCD involves both environmental and polygenic risk factors. However, despite an abundance of candidate genes, linkage studies and GWAS, very little progress has been done towards elucidating the genetic causes of OCD. In this project we aim at an innovative strategy that will contribute to explain the aetiology of this disorder.
We performed an exhaustive genomic analysis of 306 unrelated OCD cases through whole exome sequencing and rare variant association study. Specifically, we implemented a unified mixed-effect model, aggregating exonic non-synonymous and splicing variants into genes and testing for association with the disorder comparing with a group of control exomes of our in-house database. We also accounted for minor allele frequencies (prioritizing rare variants) and pathogenicity score. After implementing these analyses, we identified a list of genes harbouring a higher number of mutations in cases when compared to the controls. Subsequently, we performed a Pathway Enrichment Analysis with these genes in order to find enriched pathways related with brain neurodevelopment or function.
Stronger evidence of association of the significant genes and pathways found should be obtained through replication in larger cohorts of OCD and through functional studies.

Characterization of transcriptomic variety in the human hematopoietic lineage

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The hematopoietic lineage gives rise to blood cell types involved in a variety of processes from oxygen transport to pathogen defense. As all somatic cells share approximately the same DNA content, the identity of each cell type is established by the set of genes it expresses. Which holds not only true for differentiated cells but for their precursors as well. In my thesis I am studying the transcriptomic characteristics that create cell identity and how they morph during differentiation. This does not only involve expression but also the processing of the transcripts.
Within the scope of the Blueprint project I am involved in the characterization of 15 differentiated human blood cell types. Their comparison revealed that neutrophils are transcriptionally distinct to the other cell types, both in terms of gene expression and splicing. In particular, they express fewer genes and at a lower level. Furthermore, they have lower splicing completion, which means that introns are removed in a less efficient manner. In concordance with that we found that neutrophils exhibit also lower expression levels for splicing related genes. These outstanding transcriptomic features of neutrophils might reflect their unique position in the immune system to kill bacteria but also limit their lifespan to a couple of hours.

Nutrient provisioning to embryos links parental life history to variation in progeny phenotypic outcomes

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The interaction of genetic and environmental factors is commonly thought to determine organismal development and phenotype. Nonetheless, isogenic individuals reared in the same environment display variation in many phenotypic traits.
We are using Caenorhabditis elegans as a model system to investigate how microenvironmental and ancestral cues impact on phenotypic outcomes, focussing on the role of the low-density lipoprotein orthologues, vitellogenins or yolk proteins. The six vit genes are transcribed prolifically in the adult hermaphrodite intestine. Secretion of yolk lipoprotein complexes into the body cavity and uptake by oocytes prior to fertilisation provides embryos with a bulk nutrient store.
We are using a transgenic strain carrying a translational gene fusion, vit-2::tdimer2, to probe vitellogenin uptake in embryos. Unusually for such highly expressed genes, there is much variation in maternal vitellogenin expression and embryonic vitellogenin uptake.
Reduction of vitellogenin uptake by RNAi against maternal vit leads to viable embryos which exhibit defective survival during starvation-induced larval diapause. These worms also exhibit developmental and reproductive abnormalities on recovery from extended diapause.
Vitellogenin loading into embryos is influenced by parental age or parental experience of larval diapause. These embryos in turn exhibit similar phenotypes to those observed under vit RNAi, although it remains to establish a causal link.
These results suggest that worms may modulate bulk nutrient supply to their progeny in response to their age or early life history cues, possibly influencing the phenotypic outcomes of their offspring and generating variation within isogenic populations.

Unbalanced feeding choices and metabolic mechanisms of obesity in a mouse model of Down syndrome

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Obesity is a major health threat in persons with intellectual disability. The intake of nutrients is coordinated by internal and external cues, including homeostatic requirements and the circadian clock. There is strong evidence supporting the role of major genes and minor genomic variants in causing or contributing to human obesity, which represents prevalent condition in Down syndrome (DS). This suggests that trisomy of chromosome 21 (HSA21) could confer a genetic susceptibility compromising the mechanisms controlling feeding behavior and body weight maintenance. We have characterized meal pattern and metabolic parameters in Ts65Dn mice, a validated model of DS. We show a hyperphagic meal pattern defined by longer and slower meals in Ts65Dn mice along with increased adiposity as compared to wild type littermates. The faster recovery of normal values of blood glucose could act as positive feedback signal and explain the faster re-feeding in trisomic mice. When exposed to free choice access to high-energy palatable diets, Ts65Dn show increased preference for high fat and inflexible behaviors upon restricted access, as compared to wild type mice. In such obesogenic environment (high-fat diet), Ts65Dn mice develop a dramatic pancreatic dysfunction similar to diabetes-like syndrome. We conclude that trisomy confers increased risk for metabolic complications such as diabetes that frequently co-occur with overweight and leads to compulsive and impulsive behavioral traits and unbalanced feeding choices.

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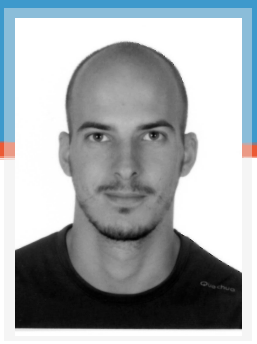
Discretizing ChIP-seq profiles



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Chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) is the standard method to investigate chromatin protein composition. As the number of community-available ChIP-seq profiles increases, it becomes more common to use data from different sources, which makes joint analysis challenging. Issues such as lack of reproducibility, heterogeneous quality and conflicts between replicates become evident when comparing data sets, especially when they are produced by different laboratories.
In this talk I will present Zerone, a ChIP-seq discretizer with built-in quality control. Zerone can merge several replicates into a single discretized profile and is able to identify low quality or irreproducible data. Zerone can detect such low quality profiles with an error rate as low as 2%. In the benchmark against other software, Zerone achieves outstanding precision, and runs on mammalian genomes in just about 5 minutes.

Novel mechanisms of cytoplasmic polyadenylation

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Cytoplasmic poly(A) tail elongation is a widespread mechanism to regulate mRNA translation known to be involved in mitosis, meiosis, cellular senescence and synaptic plasticity. The biochemistry of cytoplasmic polyadenylation has been elucidated in vertebrates, where it requires two sequence elements in the 3′ UTR of substrate mRNAs: the U-rich cytoplasmic polyadenylation element (CPE) and the AAUAAA hexanucleotide (Hex). These elements are recognized by the canonical polyadenylation factors CPEB and CPSF, respectively. In Drosophila embryogenesis, maternal mRNAs undergo cytoplasmic polyadenylation in the absence of the CPEB homolog Orb. Using Drosophila as a model system, our group has previously identified a non-canonical mechanism that operates on Toll mRNA (Coll et al., 2010). A ~180bp region in Toll 3′ UTR was found to be important for this mechanism. RNA affinity chromatography identified the siRNA processing factor Dicer-2 as a candidate for non-canonical polyadenylation. To gain insight into the non-canonical polyadenylation mechanism, we are further dissecting the cis-acting elements required for Toll polyadenylation. Furthermore, we are delineating the cytoplasmic polyadenylation map at a genome-wide level using a novel technology termed TAIL-seq. Our long-term goal is to dissect major, conserved machineries and mechanisms involved in cytoplasmic polyadenylation.

Genome variation across global isolates in the emerging fungal pathogen *Candida glabrata*

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Infections caused by pathogenic yeasts are becoming of increasing medical importance. *Candida glabrata* is one of the most common pathogenic fungi in humans, ranking as the second causative agent of candidiasis worldwide. Despite its name is distantly related to the model pathogen *Candida albicans* and belongs to the Nakaseomyces clade, more closely related to *Saccharomyces cerevisiae* (Gabaldón et al., 2013). This indicates that virulence to humans has independently and recently emerged within this clade. Considering that virulence properties can vary significantly among strains of the same species, it is important to study the detailed genetic background of pathogenic and commensal isolates.

Here, we use a genome re-sequencing approach to analyse the variability among 32 different genomes from clinical and commensal *Candida glabrata* samples sampled from different countries. We did a computational analysis for detecting single-nucleotide polymorphism, ploidy, copy number variation and genomic re-arrangements. The sequenced strains are structured in six differentiated clusters, which do not cluster by geographical origin or site of infection. Despite an overall high similarity at the sequence level, most differences between strains consist of gene losses and gains, often involving cell-wall proteins. We find evidence for active recombination between distinct subpopulations, which is remarkable for species considered as asexual. In accordance we find evidence for active mating type switching in four strains.

Gabaldón et al.: Comparative genomics of emerging pathogens in the *Candida glabrata* clade (2013). BMC Genomics 14:623.

Evaluation of non-coding RNAs role in Parkinson's disease

Joan Pallarès Albanell
Bioinformatics and Genomics Programme
Genomics and Disease
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Age-releated neurodegeneration is a highly detrimental condition in which neurons undergo a deterioration process with devastating clinical outcomes. Parkinson's Disease (PD) is the second most common among these diseases. Its primarily movement manifestations are due to dopaminergic cell loss in the Substantia Nigra Pars Compacta. Despite its clinical importance, etiology remains uncertain, with non-coding RNAs (ncRNAs) emerging as a new regulatory layer with essential roles in neuronal homeostasis and disease.

This work explores the intersection between ncRNAs deregulation and neurodegeneration in the context of PD. Our working hypothesis is that early ncRNA deregulation contributes to pathogenesis. We study different kinds of ncRNAs: long non-coding RNAs (lncRNAs) and small non-coding RNAs (sncRNAs), especially micro RNAs (miRNAs). We aim to define ncRNAs signatures at different PD stages and assess the contribution of the deregulated species to neuronal dysfunction in a PD cellular model.

Recently, our lab described the early perturbation of sncRNAs in the amygdala of presymptomatic PD patients. Our data indicate that sncRNA are also perturbed in the frontal cortex, with deregulation partially overlapping with that of the amygdala. To understand the functional consequences of these perturbations we have developed sncRNAs High-Throughput Functional Screenings (HTS) in cell cultures. We aim at identifying sncRNAs that modulate neuronal homeostasis and/or regulate oxidative stress response, a key feature in PD. We have already identified a few sncRNAs whose function is crucial to neuronal viability. Some of them are deregulated in PD, suggesting an implication in pathogenesis.

Notes

Notes

Posters

Francesco Aulicino

Discovery and characterization of Wnt-regulated LncRNAs involved in somatic cell reprogramming

01

Gene Regulation, Stem Cells and Cancer Programme
Reprogramming and Regeneration - Maria Pia Cosma

Long non-coding RNAs (lncRNAs) have been found to act in the circuitry controlling pluripotency in mouse embryonic stem cells (mESCs), but their impact on somatic cell reprogramming of mouse embryonic fibroblasts (MEFs) has not been studied so far.
We aimed at studying lncRNAs controlled by Wnt/β-catenin pathway, a well-known regulator of both mESCs pluripotency and somatic cell reprogramming. We identified 10 Wnt-regulated lncRNAs with a putative role in controlling reprogramming and pluripotency. Among them, we found that LncRNA9 is upregulated during MEFs reprogramming and its ablation dramatically reduces the number of reprogrammed clones. Furthermore preliminary data show that, in mESCs, LncRNA9 ablation compromises Wnt pathway activation. Using in silico tools such as CatRAPID, we predicted an RNA-protein interaction between LncRNA9 and β-catenin protein that we are now validating.
We are currently working on modulating LncRNA9 expression levels to assess its impact in both reprogramming and pluripotency maintenance.

Gireesh Bogu

Systematic Analysis of lincRNAs associated with human diseases

02

Bioinformatics and Genomics Programme
Computational Biology of RNA Processing - Roderic Guigó

Recent advances in high-throughput sequencing revealed thousands of novel lincRNAs (Long Intergenic noncoding RNAs) in human. However, most of their function remains unknown, especially the ones that are associated with human diseases. To address this, we analysed 8555 RNA-Seq datasets of 53 different tissues to compare disease-affected individuals with disease-unaffected (Genotype-Tissue Expression Consortium data) by accounting several confounding factors and batch effects. Here, we identified several lincRNAs associated with 19 disease-affected phenotypes across multiple tissues in human. This study provides a valuable resource of disease-associated lincRNAs along with protein-coding genes in human.

Silvina Catuara Solarz

03

Pharmacological and environmental interevention ameliorates age-associated cognitive deficits and cholinergic neurodegeneration in a mouse model of Down syndrome

Systems Biology Programme
Cellular & Systems Neurobiology - Mara Dierssen

Down syndrome (DS) individuals have a high prevalence of Alzheimer disease (AD)-like neurodegeneration and dementia. Although there is no effective therapy to improve learning and memory and prevent/delay neurodegeneration in DS, (-) epigallocatechin-3-gallate (EGCG), the most abundant polyphenol found in green tea, has gained attention as it has antioxidant and anti-inflammatory activity, and promotes the non-amyloidogenic pathway of APP through ADAM10 maturation. EGCG is also a potent inhibitor of the kinase activity of DYRK1A, a DS candidate gene whose overabundance is associated with neurocognitive symptoms and neurodegenerative phenotypes. Our laboratory has shown that EGCG ameliorates cognitive deficits both in AD and DS mouse models, and also in young adults with DS.

Here, we investigated the use EGCG as co-adjuvant to potentiate the effects of environmental enrichment (EE), a housing condition that enhances neuroplasticity and cognitive functions, in the Ts65Dn mouse model which partially mimics DS/AD pathology. We assessed age-associated hippocampal-dependent learning and memory using the Morris water maze and we generated a global learning indicator by principal component analysis. This composite measure showed that EGCG or EE alone had no significant effects when administered at the onset of cholinergic neurodegeneration. However, combined treatment of EE-EGCG significantly ameliorated Ts65Dn hippocampal-dependent learning and memory alterations. Furthermore, we assessed cholinergic-dependent recent memory by the passive avoidance test and we found that EE-EGCG induced an improvement both in WT and Ts65Dn mice. This cognitive improvement in Ts65Dn mice was accompanied by a reduction in age-dependent cholinergic neuronal loss in the medial septum, which projects to the hippocampus.

Maria Chatzou

Systematic uncertainty casts doubts on the reliability of large scale multiple sequence alignments

04

Bioinformatics and Genomics Programme
Comparative Bioinformatics - Cedric Notredame

Multiple Sequence Alignment is one of the most widely used modeling methods in biology. While it is well known that the NP-Complete nature of the problem prevents exact algorithms to be designed, it is often assumed that the available heuristic solutions based on the alignment algorithm are numerically stable enough to provide reasonably accurate models for evolutionary reconstruction. We show here this is not
the case and that all aligners able to deal with very large datasets are highly sensitive to alignment uncertainty. This issue affects on average more than 30% of the columns and it results in over 50% of tree nodes being unstable when doing phylogenetic reconstruction. We also found that local support fails at providing useful indications for systematic filtering of unstable tree branches. Our findings suggest that this effect is a direct consequence of the aligners' reliance on binary guide trees. These observations potentially challenge a large number of large-scale evolutionary studies based on such alignments.

Francesca Di Giovanni

The importance of chromosome positioning in yeast

05

Cell and Developmental Biology Programme
Coordination of Cytokinesis with Chromosome Segregation - Manuel Mendoza

Chromatin is not randomly organized inside the interphasic eukaryotic nucleus. In budding yeast nucleus, chromosomes are arranged in a so called "Rabl like structure", which results from the chromosomes arrangement in the previous mitosis. The centromeres are clustered near the spindle pole body (SPB), on the opposite side of the nucleus lies the nucleolus, and telomeres are distributed in 3-8 foci at the periphery of the nucleus via protein-protein interactions with the nuclear envelope. It has been shown that gene expression of some specific loci is affected by their positioning in the nucleus. Replication firing of early origins seems also to be influenced by the position of the respective ARS in the nucleus. These effects are limited to some specific loci, but the role of the global genome arrangement in nuclear function is still unclear.

In order to study the importance of chromosome positioning in nuclear processes and cell physiology, we use budding yeast strains carrying compound chromosomes, generated by end-to-end fusion, that are expected to dramatically change the typical "Rabl like structure" of yeast nucleus.
By combining live cell imaging and polymer modeling we show that the chromosomes positioning in the strains carrying compound chromosomes is greatly modified. We performed experiments to test the effect of such modification in gene expression, replication timing and growth rate under stress conditions. Preliminary results suggest that in yeast, chromosome positioning does not have a direct effect on DNA metabolism, but maybe could have been evolutionarily selected to create subnuclear environments in which epigenetic modifications, that are known to be important for DNA expression and replication, are favored.

Jordi Hernández Ribera

06 Mechanisms of NUMB alternative splicing regulation in lung cancer cells by RBM10 and SF1/BBP

Gene Regulation, Stem Cells and Cancer Programme
Regulation of Alternative pre-mRNA Splicing during Cell Differentiation, Development and Disease - Juan Valcárcel

Alternative splicing of NUMB exon 9 generates mRNAs encoding proteins with antagonistic functions in the NOTCH pathway and in cancer cell proliferation. Increased exon 9 inclusion is among the most frequent splicing alterations in lung cancer and mutations in the exon 9-promoting skipping factor RBM10 are among the most frequent genetic lesions in lung adenocarcinomas. We have systematically mapped regulatory sequence elements in exon 9 using modified antisense oligonucleotides and identified regulatory factors using a spliceosome-wide RNAi screen. These analyses identified two potent splicing enhancers whose effects are mediated, respectively, by SRSF6/SRSF1/hnRNP K and – surprisingly- by the Branchpoint Binding Protein (BBP/SF1). Biochemical, ex vivo and in vivo results indicate that a branchpoint-mimic sequence acts as an exonic enhancer that can mediate the effects of SF1/BBP, revealing a novel function for this protein in splicing regulation.
Our results using lung cancer cells in culture and in mouse xenografts indicate that RBM10 acts as a tumour suppressor, while mutant variants found in lung cancer promote cell proliferation and tumour growth. We have

used a battery of mutant forms of RBM10 found in lung cancer to carry out a structure/function analysis of domains and residues important for its activity in promoting NUMB exon 9 skipping. The results reveal the requirement for both of the RRMs, the second Zn-finger and OCRE domains. In addition our data indicate that while mutation of valine 354 to glutamic acid in the second RRM disrupts regulation without affecting RNA binding, a natural RBM10 variant lacking valine is equally active in promoting NUMB exon 9 skipping, suggesting that V354E is a gain of function mutant that binds to the polypyrimidine tract preceding exon 9 but fails to assemble a repressive complex. Work in progress aims to identify protein partners of RBM10 whose interaction is affected by this mutation.

Shalu Jhanwar

A generalized ML framework for *in silico* prediction of active enhancers

07

Bioinformatics and Genomics Programme
Genomic and Epigenomic Variation in Disease - Stephan Ossowski

In silico prediction of enhancers, a class of cis-regulatory elements, is computationally challenging, as they are lowly conserved and can regulate gene expression irrespective of their orientation or location within the genome. Previous studies have proposed genome-wide enhancer identification based on a combination of epigenomic marks using supervised and unsupervised machine-learning approaches. However, due to a lack of a extensive experimentally validated dataset of enhancers, interrogation of active enhancer related properties remains elusive and training of supervised classifiers has been suboptimal. In this study we use a recently published resource of experimentally validated, in-vivo transcribed enhancers (Kheradpour 2013) to gain better insights into active enhancer related properties and to train a novel supervised learning classifier for genome-wide identification of enhancers. For training purposes we included the most informative and readily available epigenomic and genomic features; such as histone marks, transcription factor binding sites, chromatin accessibility, ratio of histone marks and distance to nearest TSS. Our classifier allows for genome-wide identification of enhancers in any cell line, cell type or tissue whose epigenome has been broadly interrogated, as is the case for samples analysed by Encode, mouseEncode, Roadmap Epigenomics and the International Human Epigenome Consortium. We demonstrate that our

classifiers outperforms existing methods (e.g. RFECS) using independent validation sets based on e.g. CAGE-seq (transcribing enhancers) and ChIA-PET (enhancer-promoter chromatin interaction). Our classifier consistently achieves accuracies above 90% across cell-types and tissues as well as across mammalian species without the need to re-train the classifier for each type. In conclusion, we have demonstrated that our method trained on a single cell type is able to identify active enhancers in any mammalian cell type or tissue at high accuracy.

Lisa Johnsen

08 To cut a long story short: alternative splicing regulates a novel component of the Fld1p-Ldb16p complex in yeast

Cell and Developmental Biology Programme
Organelle Biogenesis and Homeostasis - Pedro Carvalho

Neutral lipids are stored in specialized organelles termed lipid droplets (LDs) in the cell. Lipid droplets are made at the Endoplasmic Reticulum (ER) and consist of a neutral lipid core enclosed by a phospholipid monolayer. Lipid droplet size and number are tightly regulated. In yeast, lack of FLD1, the functional homolog of human Seipin leads to supersized droplets. Fld1p forms a complex with Ldb16p at the Lipid droplet/ER contact sites and is thought coordinate the release of neutral lipids into the droplet with the enclosing phospholipid monolayer.
In our lab we have identified a third interaction partner of the Fld1p-Ldb16 complex called Ymr147-148p/Osw5p. This protein has two isoforms (Ymr147-148p and Osw5p) which are generated by alternative splicing. Overexpression of specifically one of the isoforms leads to a massive accumulation of fat in the cell. This phenotype seems to be determined by the ratio of the two isoforms. Furthermore we think that this ratio could be regulated by the metabolic state of the cell.

Verónica Lloréns-Rico

Key determinants of transcript abundance in the minimal bacterium *Mycoplasma pneumoniae*

09

Systems Biology Programme
Design of Biological Systems - Luis Serrano

The idea of creating an in silico cell, a computational representation of all the processes and their interactions occurring inside a cell, has become popular in the recent years. Although methods have been presented that allow this representation, the lack of comprehensive knowledge of even the simplest organisms hampers the development of these models. One of the least understood processes is how RNA levels are regulated to maintain cellular homeostasis and to respond to external perturbations.
Here, we focus on how transcript abundance is regulated in a reduced-genome bacterium, ***M. pneumoniae***. In prokaryotes, many efforts have been devoted to the determination of gene regulatory networks, based on trans-acting transcription factors and cis-regulatory elements. However, other mechanisms exist that may regulate transcript abundance, such as differential promoter strength or RNA degradation, or the action of small RNAs (sRNAs). We have studied the effect of each of these factors and determined that, compared to other studied bacteria, the gene regulatory network of ***M. pneumoniae*** is not the principal determinant of RNA abundances. Furthermore, we propose that the majority of small RNAs have no regulatory function in this bacterium, and that this may be a general feature in bacteria. Therefore, we estimate that other factors, such as promoter strength, RNA degradation and metabolites, are major determinants of transcript abundances in ***M. pneumoniae***. This findings will be compiled in a computational model of transcription that aims to reproduce the experimental results observed in gene expression studies.

Michael Maier

The mitotic exit network (MEN) prevents anaphase bridges

10

Cell and Developmental Biology Programme
Coordination of Cytokinesis with Chromosome Segregation - Manuel Mendoza

Anaphase chromatin bridges form when sister chromatids are not able to properly separate or condense during mitosis. They cause genomic instability as bridging chromosomes break during cytokinesis and they

are therefore thought to contribute to cancerogenesis. While these bridges are frequently found in in cancer cells, they sometimes occur spontaneously in healthy cells. What would give rise to them in seemingly normal cells is not well understood. To get an overview of which processes normally prevent the formation of anaphase bridges, I have performed a screen for mutants that display an elevated number of bridges.
This screen was performed on 1130 mutants and has, beyond the expected hits, identified components of the DNA replication machinery and the mitotic exit network (MEN) as factors that prevent bridge formation. It appears that underreplicated DNA is a general cause for anaphase bridges as mild persistent replication stress induced by hydroxyurea causes cells to enter anaphase with unresolved chromosomes. Bridges induced by the inhibition of the MEN appear to be specific to telomeres as these display a segregation defect in MEN mutants. In support of this, inactivation the shelterin complex that coordinates multiple activities required for correct telomere biogenesis and assembly of telomere/protein complexes leads to resolution of around 40% of MEN induced bridges.

Domenica Marchese

11

Post-transcriptional regulation of Parkinson's disease-associated SNCA gene

Bioinformatics and Genomics Programme
Gene Function and Evolution - Gian Gaetano Tartaglia

Alpa-synuclein is a presynaptic neuronal protein known as the major component of Lewy bodies, the pathological hallmark of Parkinson's disease [PD] (Stefanis, 2012). Recent evidence indicates that large RNA transcript isoforms of SNCA, with long 3' untranslated region (3'UTR) produced by alternative polyadenylation, are selectively linked to PD pathology (Rhinn et al., 2012, Locascio et al., 2015).
This project aims to identify RNA-binding proteins (RBPs) that specifically target two main 3'UTR isoforms of SNCA mRNA, SynS (3'UTR-570 nt) and SynL (3'UTR-2529 nt) and impact their stability and translation. The length of the 3'UTR affects alpha-synuclein expression, as observed in our gene reporter assay, with the longest 3'UTR significantly inhibiting reporter gene expression with respect to the short one. By means of protein arrays, we have identified around 20 SynS-3UTR and SynL-3UTR specific-interactors. Two of them, TIAL1 and ELAVL1/HuR, were also

confirmed by RNA affinity purification assay. TIAL1 and ELAVL1 are also capable of binding SNCA mRNA in vivo, as shown by RNA immunoprecipitation (RIP) from in vitro differentiated human neuroblastoma cell lines. Furthermore, TIAL1 knockdown in HeLa cells leads to a down-regulation of alpha-synuclein protein and mRNA, while ELAVL1 knockdown causes an increase of alpha-synuclein expression, suggesting antagonistic role in its regulation.
We are currently investigating the mechanism of post-transcriptional regulation of TIAL1 and ELAVL1/HuR measuring SNCA mRNA decay and translational rate in presence or absence of the proteins.

Elena Martín Rodríguez

Alternative splicing regulation by the SPF45-SR140-CHERP complex

Gene Regulation, Stem Cells and Cancer Programme
Regulation of Alternative pre-mRNA Splicing during Cell Differentiation, Development and Disease - Juan Valcárcel

The splicing factor SPF45 is important for alternative 3′ splice site recognition and interacts with other splicing factors to regulate alternative splicing (AS). SPF45 overexpression is present in numerous tumours and results in multidrug resistance. A functional splicing network developed in our lab links SPF45 with the splicing factors SR140 and CHERP, as their knockdowns have very similar effects on a series of AS events involved in cell proliferation and apoptosis. Interestingly, SPF45, SR140 and CHERP are all U2 snRNP-related factors. Our aim is to decipher the functional links between SPF45, SR140 and CHERP and the mechanisms of action to understand their coordinated effects on AS regulation. We have shown that the protein levels of all three proteins are decreased upon the individual knockdowns of each of them and that they can co-immunoprecipitate in HEK293 and HeLa cells. These results suggest that the three factors form a functional complex. We are now focusing on the interacting domains and their importance in AS regulation. Furthermore, SPF45, SR140 and CHERP depleted cells show a decrease in cell proliferation and a cell cycle arrest. On going experiments are studying the effects of the depletion and overexpression of the three factors in cell migration, invasion and multidrug resistance. To identify the genome-wide targets of SPF45, SR140 and CHERP we are now performing RNA-seq analysis of depleted cells. Future studies will focus on candidate AS

events involved in cell proliferation, cell cycle progression, migration or invasion as well as in multidrug resistance that can explain these phenotypes.

Pablo Prieto Barja

13

Coordinated genomic development and bet hedging strategy of the protozoan pathogen *Leishmania donovani*

Bioinformatics and Genomics Programme
Comparative Bioinformatics - Cedric Notredame

Leishmania is an important human pathogen that causes fatal diseases with increasing incidence during the last years due to global warming. Transitions during the parasite life cycle from sandflies (promastigote) to mammalian macrophages (amastigote) involve intense environmental changes leading to a process not well understood that affects the parasite genome and gene expression.
We combine in vivo parasites grown in hamster and in vitro cultured parasites in a system that triggers genome development adaptation to study how parasites evolve and adapt to environmental changes during transitions. Phenotypical outcome show culture adaptation having strong fitness cost for infectivity during genome re-arrangement.
Using DNA/RNA-seq, DNA-FISH at different stages of the development we show that chromosome copy number mosaicism develops easily in cultured populations of promastigotes, pointing to a bet-hedging strategy, while other aneuplodies occur consistently. Through SNP analysis of DNA/RNA-seq we are able to distinguish between aneuplodies that are more or less likely to occur and ancient events. Chromosome CNV observed show correlated levels of DNA/RNA except for some chromosomes, suggesting a putative unknown mechanism for silencing or transcriptional down-regulation.

Kadri Reis

DNA replication stress disrupts the inheritance of repressed chromatin in *C. elegans*

14

Systems Biology Programme
Genetic Systems - Ben Lehner

In complex eukaryotic genomes, repetitive DNA elements constitute a significant fraction of the organism's genomic complexity. These elements, made up of nucleotide sequences of various lengths and compositions can be organized in tandem, inverted and dispersed organizations. Due to their repetitive nature, these elements are often assembled into condensed and transcriptionally silent heterochromatin. Maintenance of these correct chromatin states is critical for the control of gene silencing and chromosomal stability, and prevention of mutations and translocations, often associated with various disease conditions.
In *C. elegans* transgenes introduced via transformation are usually maintained as heritable structures containing repetitive tandem arrays with hundreds of copies of the inserted DNA or its recombined fragments, which can be induced to integrate. These tandem arrays are expressed; and though physically present both in soma and germline, repetitive arrays are efficiently silenced in the germline. Interestingly, the level of expression seems to be stochastic, and the level of expression inherited to the progeny over multiple generations.
To identify genes required for the repression of repetitive DNA, we performed a genome-wide RNAi screen in *C. elegans*, using the quantitative de-repression of an integrated repetitive transgene array in somatic cells as a phenotypic assay. I will present the results of this screen and discuss the connection it revealed between transgene repression and DNA replication.

Lorenzo Rinaldi

Novel roles of Dnmt3a and Dnmt3b at enhancers regulate human epidermal stem cell function

15

Gene Regulation, Stem Cells and Cancer Programme
Epigenetic Events in Cancer - Luciano Di Croce

The dynamic acquisition of cell fates during development depends on the de novo DNA methylation catalyzed by Dnmt3a and Dnmt3b. Both proteins play important roles in adult tissue homeostasis, and their deregulation causes tumorigenesis. However, the genome-wide

localization and function of Dnmt3a/b in adult stem cells is unknown. Here, we show that Dnmt3a/b have unique patterns of genomic localization in human epidermal stem cells and their differentiated counterparts. Dnmt3a binds to the TSSs of a cohort of active genes required for the interaction of stem cells with their underlying stroma. Conversely, Dnmt3b specifically bind the gene body of genes that establish the stem cell and differentiated signatures. Intriguingly, both proteins also bind to the most active enhancers. These enhancers contain very low levels of DNA-methylation, but high amounts of DNA-hydroxymethylation. Our data support that Dnmt3b is responsible for maintaining DNA-methylation levels surrounding the enhancer center, while Dnmt3a establishes DNA-hydroxymethylation at enhancer center. Leading to a deactivation of these enhancers and spontaneous differentiation of epidermal stem cells. Altogether we show novel functions of Dnmt3a/b in promoting transcription by associating with regulatory elements.

16

Francesco Sottile

Hetero to synkaryon: how does the transition happen?

Gene Regulation, Stem Cells and Cancer Programme
Reprogramming and Regeneration - Maria Pia Cosma

Cell-cell fusion is a fundamental process that occurs during development. Indeed, homotypic cell fusion arises when two identical cells that are committed to the same fate fuse between them (e.g. syncytiotrophoblast, myotubes, osteoclast and macrophage derived giant cells).
Previous studies have demonstrated that fusion of somatic cells with embryonic stem cells (ESCs) can induce somatic nuclei reprogramming. Initially, heterokaryons characterised by the presence of two nuclei into a common cytoplasm share trans-acting factors that leads to changes in pluripotency gene expression. Finally, it has been proposed that the parental nuclei fuse to generate a synkaryon and both DNA synthesis and cell division processes are required to fully reprogram hybrids cells.
In order to address how the transition hetero to synkaryon occurs we set up an in vitro model to study heterokaryon cells. ESCs and Mesenchymal Stem Cells (MSCs) showed a prominent fusion efficiency when compared to other cell co-culture condition. However, deeper analysis of the sorted hybrids exhibited two distinct phenotypes, cell fusion and entosis. Overholtzer and

colleagues described entosis as a non-apoptotic cell death program involving the invasion of one cell into another. Furthermore, to isolate either fused or entotic cells we set up a purification protocol based on surface markers.
Finally, in our model we noticed that the heterokaryon-synkaryon transition does not involve fusion between the nuclear membrane, but cell division is necessary to mix the two different genomes into a new nucleus.

Ibrahim Tastekin

Structure-function relationships underlying Drosophila larval chemotaxis

Systems Biology Programme
Sensory Systems and Behaviour - Matthieu Louis

Animals transform time-varying sensory inputs into locomotor outputs to direct motion towards conditions favorable to their survival. Chemotaxis has become a paradigm to study how chemosensory signals are encoded and converted into patterns of behaviors actuating navigation. We are studying the neural computations underlying this process in the Drosophila larva. We aim to identify the neural circuits involved in the sensorimotor processing of olfactory stimuli. While the anatomical organizations of the peripheral olfactory circuits and the motor system are well documented, our knowledge about the neurons constituting the sensorimotor pathway remains limited. To identify these neurons, we performed two independent unbiased loss-of-function screens using GAL4-driver lines from the Kyoto Collection (1118 driver lines, Drosophila Genetic Resource Center) and the Rubin Collection (1100 driver lines screened as part of the Larval Olympiad at the Janelia Research Campus). We identified neurons in different brain regions with a function related to the control of transitions between runs and turns. Both screens highlighted overlapping populations of neurons in the subesophageal zone (SEZ). Larvae with specific subsets of SEZ neurons silenced showed a significant reduction in orientation performances without complete abolishment of chemotaxis. Using CsChrimson, we performed optogenetic gain-of-function manipulations and found that acute activation of a subset of SEZ neurons is sufficient to initiate turning maneuvers. Combining intersectional and stochastic genetic methods, we were able to isolate 3 neurons in the SEZ that are necessary and sufficient to control the rate of transition from runs to turns. This finding

corroborates the idea that the SEZ organizes transitions between motor programs in flies as well as in other invertebrates (e.g., cockroaches and locusts). Next, we demonstrated that this role of SEZ neurons could be generalized to other sensory modalities: thermosensation and vision. Our findings indicate that the SEZ operates as a general premotor center that participates in the control of action selection on the integration of sensory stimuli.

Ilda Theka

18

The role of Wnt/β-catenin signaling pathway in the regulation of the imprinted genes

Gene Regulation, Stem Cells and Cancer Programme
Reprogramming and Regeneration - Maria Pia Cosma

Wnt/β-catenin signaling is an evolutionary conserved pathway of molecular reactions that regulate many cellular and developmental aspects. Indeed Wnt/β-catenin pathway has been shown to be crucial in cell fate determination, stem cell maintenance, embryogenesis and several diseases. Moreover, our group and others have recently shown that the perturbation of this pathway can improve somatic cell reprogramming either by transcription factors (Aulicino, Theka et al., 2014) or cell-cell fusion (Lluis et al., 2009). We are now focusing our attention on the potential role of Wnt/β-catenin signaling in regulating the imprinted genes. In particular, we are investigating the connection between β-catenin and Zfp57, that has been shown to bind specifically to methylated imprinting control regions (ICRs) and protect them from demethylation. Indeed, through a large HiSeq screening, we identified Zfp57 to be upregulated in embyonic stem cells (ESCs) with stabilized β-catenin (βcat-Stab ESCs), which show higher pluripotency marker levels and higher reprogramming capacity. Successively, to understand if the upregulation of Zfp57 was translated into a change of the methylation profile, we performed Reduced Representation Bisulfite Sequencing (RRBS). βcat-Stab ESC clones show an increase of methylation at the ICRs when compared to the control clone and as a consequence, higher H3K9me3 rectruitment at the same regions. Finally, we are now addressing if Wnt/beta-catenin pathway controls the imprinted genes during the first stages of embryo development by using β-catenin knock-out models.

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