Societat Catalana de **BIOLOGIA**

II Congrés de Biologia

Organitzat per les Seccions de l'SCB:

Aqüicultura, Biofísica, Biologia del Càncer, Biologia del Desenvolupament, Biologia Molecular i Senyalització Cel·lular i Metabolisme

PROGRAMA + RESUMS DE LES COMUNICACIONS

INSTITUT D´ESTUDIS CATALANS

Carrer del Carme 47

Barcelona

3 i 4 de maig de 2018

Sis anys després del I Congrés Internacional de Biologia de Catalunya (CIBICAT), celebrat reeixidament amb motiu del centenari de la Societat Catalana de Biologia (SCB) (1912-2012), ens plau celebrar la segona edició del Congrés de Biologia de l'SCB.

Aquest Congrés té per objectiu posar en contacte investigadors de diferents àrees de recerca de casa nostra i donar una visió de la recerca biològica que s'està fent als socis de l'SCB, als estudiants universitaris i de doctorat, i a la comunitat científica en general.

A més, aquest Congrés vol commemorar el 25è aniversari de les Jornades de la Secció de Biologia Molecular i el 30è aniversari de les Jornades de la Secció de Biologia del Desenvolupament, que durant molts anys van dur a terme les seves Jornades tan recordades per molts socis de l'SCB, de manera conjunta.

Dia 3 de Maig: **Aqüicultura, Biofísica, i Biologia del Càncer**

Dia 4 de Maig: **Biologia del Desenvolupament**, **Biologia Molecular**, i **Senyalització Cel·lular i Metabolisme**

Amb la col·laboració de:

ÍNDEX

3 de maig de 2018 ⇨ **Sala Pere i Joan Coromines**

Jornada d'Aqüicultura /Avenços en recerca

Organitzada per la Secció d'Aqüicultura de l'SCB

Coordinadora: Nerea Roher

Programa

9.30-9.45	Rebuda/Welcome
9.45-10.30	*Invited speaker*: **Sonia Rey**, University of Stirling TBD
10.30-11.00	**Francois Chauvigne**, ICM, IRTA-CSIC New insights into the endocrine regulation of teleost spermiogenesis
11.00-11:30	**Noelia Carrasco**, IRTA, Sant Carles de la Rapita, Tarragona Recerca en patologia de mol·luscs: de la identificació de la problemàtica al maneig sanitari
11.30-12.00	Coffee Break
12.00-12.20	**Rosemary Thwaite,** UAB Nanopellets of recombinant viral antigens as a new approach for fish oral vaccines
12.20-12.40	**Emilio Vélez,** UB Approaches to optimize muscle growth and remodeling in gilthead sea bream
12.40-13.00	**Jie Ji,** UAB Zebrafish intubation as a model to study mucosal immunity: TNFα protein nanoparticles protect zebrafish against *M. marinum* challenge
13.00 -15.00	Lunch
15.00-15.45	Invited speaker: **Joan Oca**, UPC, Castelldefels Integració de la producció d'algues en sistemes de recirculació aqüícola
15.45-16:15	**Encarni Capilla** UB Primary culture of bone-derived mesenchymal stem cells to investigate osteogenesis in fish
16.15-16.35	**Laia Ribas,** ICM-CSIC, Barcelona TBD
16.35-16.55	**Student**
17.00-17.05	Cloenda/Closing

3 de maig de 2018 ⇨ **Sala Prat de la Riba**

Jornada de Biologia del Càncer

Tumor fibrosis and cancer associated fibroblasts

Organitzada per la Secció de Biologia del Càncer de l'SCB

Coordinador i organitzador: Oriol Casanovas / Coorganitzador: Jordi Alcaraz

Programa

9.00- 9.15	Welcome
9.15- 9.55	**Jordi Alcaraz**, UB Tumor associated fibroblasts in lung cancer: translational opportunities
9.55-10.35	**Pilar Navarro**, IMIM Regulation of tumor-stroma crosstalk in pancreatic cancer: a key role for Galectin1
10.35-11.15	**Alexandre Calon**, IMIM A TGF-beta driven program in stromal cells mediates progression to metastasis
11.15-11.45	Coffee Break
11.45-12.25	**David G. Molleví**, ICO-IDIBELL Prognostic and predictive opportunities of carcinoma-associated fibroblasts specific biomarkers
12.25-12.45	**Oscar Rodríguez**, Oncomatryx BioPharma, Bilbao DMTX invaScan™, an accurate tool for the diagnosis and prognosis of invasive tumors
13.00-14.00	Lunch
14.00-15.00	Poster Session
15.00-15.40	**Anna Labernadie**, IBEC Collective cancer cell invasion by fibroblast forces
15.40-16.20	**Josep Baulida**, IMIM Prometastatic CAF activity on the extracellular matrix is driven by Snail1 and methyltransferases
16.20-17.00	**Patricia Fernández-Nogueira**, IDIBAPS Tumor Associated Fibroblasts Contribute to HER2-Targeted Therapies Resistance in Breast Cancer through FGFR2 Activation
17.00-17.15	Closing Remarks

P1. Dysregulated collagen homeostasis by matrix stiffening and TGF-β1 in fibroblasts from idiopathic pulmonary fibrosis patients: role of FAK/Akt

Paula Duch[1], Alícia Giménez[1], Marta Puig [1], Marta Gabasa[1,2], Antoni Xaubet[2], Jordi Alcaraz[1,3]

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[2] Medical Oncology Department, Hospital Clínic of Barcelona, August Pi i Sunyer Biomedical Institute (IDIBAPS), Barcelona, Spain.

[3]CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain.

Idiopathic pulmonary fibrosis (IPF) is an aggressive disease in which normal lung parenchyma is replaced by a stiff dysfunctional scar rich in activated fibroblasts and collagen-I. We examined how the mechanochemical pro-fibrotic microenvironment provided by matrix stiffening and TGF-β1 cooperate in the transcriptional control of collagen homeostasis in normal and fibrotic conditions. For this purpose we cultured fibroblasts from IPF patients or control donors on hydrogels with tunable elasticity, including 3D collagen-I gels and 2D polyacrylamide (PAA) gels. We found that TGF-β1 consistently increased *COL1A1* while decreasing *MMP1* mRNA levels in hydrogels exhibiting pre-fibrotic or fibrotic-like rigidities concomitantly with an enhanced activation of the FAK/Akt pathway, whereas FAK depletion was sufficient to abrogate these effects. We also demonstrate a synergy between matrix stiffening and TGF-β1 that was positive for *COL1A1* and negative for *MMP1*. Remarkably, the *COL1A1* expression upregulation elicited by TGF-β1 alone or synergistically with matrix stiffening was higher in IPF-fibroblasts compared to control fibroblasts in association with larger activities of FAK and Akt. These findings provide new insights on how matrix stiffening and TGF-β1 cooperate to elicit excessive collagen-I deposition in IPF, and support a major role of the FAK/Akt pathway in this cooperation.

P2. Nintedanib selectively inhibits the activation and tumor-promoting effects of fibroblasts from lung adenocarcinoma patients

Marta Gabasa, Rafael Ikemori, Frank Hilberg, Noemi Reguart, Jordi Alcaraz

Nintedanib is a clinically-approved multikinase receptor inhibitor that, in combination with docetaxel, provides clinical benefits to advanced lung adenocarcinoma (ADC) patients but not to lung squamous cell carcinoma (SCC) patients. However, the mechanisms underlying the selective therapeutic effects of Nintedanib in ADC remain poorly understood. Of note, Nintedanib is also approved to treat patients with idiopathic pulmonary fibrosis (IPF), a rare disease characterised by an abundant desmoplastic stroma rich in pathologically activated fibroblasts. Since the tumor stroma in lung cancer is also desmoplastic, and we recently showed that tumor-associated fibroblasts (TAFs) exhibit different phenotypes in ADC and SCC, we hypothesized that TAFs may underlie the selective effects of Nintedanib in ADC. To test this hypothesis we first activated TAFs with the pro-fibrotic cytokine TGF-β1 in the presence of increasing concentrations of Nintedanib, and collected the corresponding conditioned medium. Remarkably SCC-TAFs showed very modest inhibition of a panel of fibrotic markers including α-SMA, P4HA2 and fibrillar collagens (COL1A1, COL3A) in response to Nintedanib, in striking contrast to ADC-TAFs and paired lung parenchyma fibroblasts, which were markedly affected. This was matched by a significant reduction in the abilities of the conditioned medium of ADC-TAFs but not SCC-TAFs to promote cancer cell growth and invasion in a panel of lung cancer cell lines after Nintedanib treatment. These results reveal that Nintedanib is an effective inhibitor of stromal fibrosis and its associated tumor-promoting effects in ADC, and that the poor antifibrotic response of SCC-TAFs to Nintedanib may contribute to the differential clinical benefit observed in both subtypes. Our findings also support that TGF-β signalling and aberrant TAF–carcinoma cross-talk are regulated by different mechanisms in ADC and SCC. In addition they support that preclinical models based on carcinoma-TAF interactions may help defining the mechanisms of the poor antifibrotic response of SCC-TAFs to Nintedanib and testing new combined therapies to further expand the therapeutic effects of this drug in solid tumors.

P3. Methylation analysis in Sézary syndrome (SS) and integration of exome and transcriptome data

Mar Garcia-Valero, Juan Sandoval, Georgia Escaramís, Daniel Hervás, Anna Puiggros, Blanca Espinet, Xavier Estivill, Ramon Pujol, Fernando Gallardo, Raquel Rabionet

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Sézary syndrome (SS) is an aggressive variant of cutaneous T-cell lymphoma (CTCL) characterized by the presence of neoplastic $CD4^+$ T-cells both in the peripheral blood and skin.

The aim of our study is to perform a whole-genome DNA methylation analysis in SS patients in order to identify differentially methylated regions that could be regulating the expression of pathologically relevant genes. Circulating non-neoplastic $CD4^+$ T-cells from psoriatic patients were employed as controls. These results will be integrated with available exome analysis and mRNA and miRNA expression data from the same patients.

In a cohort of 17 SS cases, with previous exome analysis of somatic mutations (12 cases), and mRNA expression analysis in $CD4^+$ T-cells (12 cases), we have performed additional miRNA expression analysis (12 cases) and DNA methylation detection with Illumina's EPIC array (10 cases and 6 psoriatic "controls"). DNA methylation analysis was performed with R Studio Bioconductor minfi package.

Our first results show >70000 CpGs with significant differential methylation between cases and controls, allowing for patient classification based on methylation patterns, where two patients with an early disease stage classify in an intermediate group. We are conducting pathway enrichment analysis of the most significant genes, and will perform convergence analysis to integrate these results with those from exome sequencing and miRNA expression data.

The epigenetic landscape of the SS is nowadays a challenge for the research community, which our results help address.

P4. Activation of Wnt signalling pathway as a therapeutic target in Rhabdomyosarcoma

Irina Giralt, Josep Roma, Isaac Vidal, Patricia Zarzosa, Natalia Navarro, Miguel F. Segura, Josep Sánchez de Toledo, Soledad Gallego

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Background: Rhabdomyosarcoma (RMS) is the most common type of soft tissue sarcoma in children and can be divided into two main subtypes: embryonal and alveolar. Wnt pathway has been related to the differentiation process of the muscle progenitor cells. This pathway was found to be inhibited in RMS, this inhibition may be caused by the expression of Wnt pathway inhibitors, such as Dickkoppf-1. These results suggest that the activation of Wnt pathway could have anti-oncogenic effects by promoting cell differentiation.

Objective: To study the antioncogenic mechanism of Wnt pathway activation in Rhabdomyosarcoma (RMS). More concretely, to elucidate the role of Dickkopf-1 (DKK-1) in the pathogenesis of RMS.

Results and conclusions: The expression levels of Wnt pathway inhibitors were evaluated in RMS cell lines and patients, showing DKK-1 as the inhibitor with highest expression. The inhibition of DKK-1 induced the activation of Wnt pathway, and decreased both cell proliferation and invasion. The activation of the pathway didn't affect the expression of its oncogenic target genes, but it increased the expression of myogenic markers such as MyoD1. These results suggested that DKK-1 inhibition may exert an anti-oncogenic effect by activating the Wnt pathway in RMS. Therefore, DKK-1 is a potential therapeutic target in RMS.

P5. Subtype specific aberrant SMAD2/3 signaling drives fibrosis in lung cancer

Ikemori R Y; Gabasa M; Reguart N; Alcaraz J.

Lung cancer is the most commonly diagnosed cancer worldwide and the leading cause of cancer-related deaths in Europe. Among them, the most prevalent subtypes are adenocarcinomas (ADC) and squamous cell carcinomas (SCC) in non-small cell lung cancer (NSCLC). In addition to cancer cells, stromal cells in the tumor microenvironment play an important role in tumor progression, being noteworthy the role of the tumor-associated fibroblasts (TAFs). Among the TAFs activation factors, the Transforming Growth Factor β1 (TGF-β1) is the major fibrosis regulator derived from cancer cells and one of the most important and well-studied cancer cell-derived factors affecting TAFs activation. Previously, our group showed that a collection of TAFs from 12 surgical patients with ADC and SCC NSCLC presented a global hypomethylation with enriched methylation in the TGF-β1 pathway compared to paired control fibroblasts (CFs). Also, we have showed that Nintedanib, a triple kinase inhibitor and anti-fibrotic drug, acts inhibiting the fibroblast-cancer crosstalk only in ADC TAFs and not in SCC TAFs. In this work, we investigated even further these findings and demonstrate a subtype specific aberrant SMAD3 pathway in SCC TAFs due to its promoter methylation. These SCC TAFs alterations lead to decreased phosphorylated SMAD3 and decreased expression of fibroblast activation markers (α-SMA, P4HA2 and collagen1a1) compared to ADC TAFs. These findings corroborate with *in vivo* analysis demonstrating that ADCs tumor samples present higher collagen deposition compared to SCCs. Also, we demonstrate that increased SMAD3 expression in ADC TAFs is correlated to the effectiveness of Nintedanib in the fibroblast activation inhibition and the fibroblast-induced tumor cell proliferation. Altogether, our results demonstrate an aberrant TGF-β1 pathway in NSCLC that translates to an altered *in vitro* fibroblast activation and treatment response and *in vivo* desmoplasia.

P6. Cancer Immunotherapy using Polypurine reverse Hoogsteen hairpins against PD-1 and PD-L1

Marlene Medina, Alex J. Félix, Carlos J. Ciudad and Véronique Noé

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Introduction: We are using immunotherapy strategies to provoke cancer cell death by inhibiting the interaction between membrane receptors in macrophages and membrane proteins expressed in cancer cells, which act as a "do not eat me" signals. Methods: To inhibit the expression of PD-1, which is an immunoinhibitory receptor mainly expressed in T cells, and PD-L1, which is overexpressed in various types of cancer cells such as PC3, HeLa and SKBR-3 cells, we designed various PPRHs targeting different regions of both PD-1 (promoter, intron 4 and exon 1) and PD-L1 (intron 1 and intron 2) genes. The ability of PPRHs to silence PD1 and PDL-1 was assessed by cell viability using MTT assays in PC3, HeLa and SKBR·3 cells and confirmed at the mRNA level by qRT-PCR and at the protein level by Western Blot in PC3 cells. Results: First, we proceeded to test different combinations of PPRHs against both targets in co-culture experiments. The most effective combination in co-culture with PC3 cells was PPRH against promoter of PD-1 and PPRH against intron 2 of PD-L1. For HeLa and SKBR3 cells the most effective combination was PPRH against intron 4 of PD-1 and PPRH against intron 2 of PD-L1. For the 3 cell lines those combinations in co-culture experiments provoked a decrease in cell viability of at least 80% compared to that of the co-culture control. This reduction in cell survival was accompanied by a decrease of 4-fold in PD-1 protein levels using PPRH versus intron 4 in THP1 cells, and 2-fold decrease in PDL-1 mRNA levels and 3-fold decrease in its protein levels using the PPRH against intron 1 of PD-L1 in PC3 cells. Conclusions: Our data support the usage of PPRHs to diminish PD1/PDL-1 interaction by decreasing the expression of both molecules thus resulting in an enhanced killing of PC3, HeLa and SBKBR-3 cells by macrophages, which might translate into beneficial effects in cancer therapy. These results corroborate the potential of PPRHs to be used in distinct immunotherapy approaches.

Acknowledgments: Work supported by grant SAF2014-51825-R

P7. Polypurine Reverse Hoogsteen Hairpins as a Gene Silencing Tool for Cancer

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Polypurine reverse Hoogsteen (PPRH) molecules are DNA hairpins formed by two polypurine strands in an antiparallel orientation, linked by a pentathymidine loop, and bound through intramolecular reverse Hoogsteen bonds. PPRHs can bind by Watson-Crick bonds to their corresponding polypyrimidine target in the dsDNA provoking a displacement of the polypurine strand of the duplex. PPRHs can be designed against the template or the coding strand of a given gene. Template-PPRHs inhibit transcription whereas a coding-PPRH against a polypyrimidine region in intron 3 of the *dhfr* gene provoked a splicing alteration by avoiding U2AF65 binding. Two PPRHs directed against the coding or template strand of the *survivin* promoter reduced the binding of transcription factors GATA-3 and Sp1, respectively.

The proof of principle of PPRHs as a therapeutic tool was established using a PPRH against survivin in a xenograft prostate cancer tumor model. The stability of PPRHs is higher than that of siRNAs. PPRHs do not induce the levels of the transcription factors nor the proinflammatory cytokines involved in the Toll-like Receptor pathway and they do not trigger the formation of the inflammasome complex.

To expand the usage of PPRHs in cancer therapy and prove their general applicability, we targeted a collection of therapeutic genes such as genes related to proliferation (DHFR, telomerase, MDM2), topoisomerases (TOP1), antiapoptotic genes (survivin, BCL2), transcription factors (MYC), protein kinases (mTOR, WEE1, CHK1), immune system (CD47, SIRPα, PD1, PDL1) and splicing factors (SF3B1). All PPRHs were effective in different cancer cells lines indicating that PPRHs can be used as therapeutic tools to target genes related to cancer progression, resistance to drugs or immunotherapy approaches.

Acknowledgments: Work supported by grant SAF2014-51825-R

P8. ABTL0812, a new therapeutic alternative for high-risk Neuroblastoma

París-Coderch, Laia[1]; Soriano, Aroa[1]; Muñoz, Pau[2]; Erazo, Tatiana[2]; Alfón, José; Pérez, Héctor[3]; Yeste, Marc[3]; Domènech, Carles[3]; Roma, Josep[1]; Lizcano, José M[2]; Sánchez de Toledo, Josep[4]; Gallego, Soledad[4]; Segura, Miguel F[1].

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The most promising therapeutic targets for the treatment of high-risk Neuroblastoma (NB) converge on or are modulated by the PI3K/AKT/mTOR pathway. This evidence provides a rational to test whether AKT/mTOR inhibitors could cover the unmet medical needs in high-risk and relapsed NB patients.

ABTL0812 is a new molecule that targets different components of the AKT/mTOR signaling axis and is currently under phase I/IIa clinical trial in adult's endometrial and non-small cell lung cancer with an excellent safety and tolerability profile.

Our preliminary results suggest that all tested NB cell lines have a similar sensitivity to ABTL0812 (IC_{50} from 30-60µM) independently of their genetic profile. By staining cells with Hoechst and Propidium Iodide, and performing Western Blots against active caspases and autophagy markers, we confirmed that cells died by autophagy and apoptosis without triggering a DNA damage response. Interestingly, ABTL0812 also induced a decrease of the major oncogenic transcription factor in NB, MYCN.

Oral administration of ABTL0812 impaired the growth of the tumors and metastatic burden of NB xenografts. Body weight follow-up and hematological analysis showed that ABTL0812 treatment was safe and well tolerated.

In conclusion, we propose that ABTL0812 could improve the efficacy and security of the therapeutic regimens used for the treatment of NB and other pediatric MYCN-driven tumors.

P9. Mechanisms of tumor malignization after anti-angiogenic therapies in Renal Cell Carcinoma

Roser Pons, Lidia Moserle, Jordi Senserrich, Mar Martinez &Oriol Casanovas
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Introduction: Targeted therapies inhibiting angiogenesis are currently used to treat several cancer types, especially those particularly refractory to standard therapies, such as renal cell carcinoma (RCC). However, these treatments fail to produce long-term durable effects in many patients, due to tumora daptation and subsequent resistance to therapy. Furthermore, preclinical data have shown that antiangiogenic therapies can switch on an invasive and metastatic phenotype as an adaptation to the inhibition of the VEGF pathway.

Materials and methods: We have developed PDX mouse models based on orthotopic implantation of human RCC tumors derived from primary biopsies. By defining different parameters of local tumor invasion, we have evaluated the effects of VEGF signalling inhibition in the invasive behaviour of RCC tumors. Furthermore, organoids embedded in Matrigel were generated from primary cell cultures derived from RCC PDX mouse models.

Results: RCC PDX mouse models recapitulate phenotypical and histological features of human tumors. Targeting the VEGF pathway in these models exacerbate the invasion in some tumors. Interestingly, the invasion after anti-angiogenic treatments was strongly linked to extracellular matrix components and interactions. Additionally, organoids embedded in Matrigel reproduce the invasive phenotype of PDX tumors under nutrient deprivation conditions.

Conclusions: The understanding of the mechanisms related to the tumor reaction to anti-angiogenic therapy represents the basis for the development of enduring anti-tumor treatments. Additionally, organoids could be used as a good model to screen molecular patterns driving tumor malignization. Then, candidates can be validated in RCC PDX mouse model so that predictive markers of response to current anti-angiogenics can be found, and also to identify new therapeutic targets aiming for improved efficacies.

P10. Methyltransferase Inhibitors Interfere with Snail1 Action on Myofibroblast Activity to Prevent Fibrosis and Metastasis

Sala, Laura

Snail1 activity is required for the myofibroblast transdifferentiation of cancer-associated fibroblasts (CAFs). The presence of CAFs that express the transcription factor Snail1 is a por prognostic marker in early breast cancers. Using TGFβ-activated fibroblasts, we describe here that Snail1 regulates methylation. Upon fibroblast activation, two related members of the protein arginine-methyl-transferase family, PRMT1 and PRMT4, interact with Snail1 to promote regulatory histone marks in the proximal fibronectin promoter. Inhibition of methyltransferases with AMI-1 or Sinefungin prevents myofibroblast activity in both cell culture and a wound-healing mouse model. Of therapeutic interest, the inhibitors are effective in blocking the exacerbated activity of fibroblasts from idiopathic pulmonary fibrosis patients; in mice, Sinefungin efficiently interfered with the fibrillary activity of breast tumor–associated fibroblasts and reduced the metastatic burden. Our data reveal a new molecular pathway induced by Snail1 and point to methyltransferase inhibitors as potential reagents for preventing fibrosis and metastasis.

P11. The atypical cyclin CNTD2 promotes colon cancer cell proliferation and migration

Abril Sanchez-Botet[1&], Laura Gasa[1&], Eva Quandt[1], Sara Hernández-Ortega[1], Nuria Masip, Javier Jiménez[1], Pau Mezquita[1], Miquel Àngel Carrasco-García[1,2], Stephen J. Kron[3], August Vidal[4,5], Alberto Villanueva[6], Mariana P.C. Ribeiro[1*], Josep Clotet[1*]

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Colorectal cancer (CRC) is one of the most common cancers worldwide, with 8–10% of these tumours presenting a BRAF (V600E) mutation. Cyclins are known oncogenes deregulated in many cancers, but the role of the new subfamily of atypical cyclins remains elusive. Here we have performed a systematical analysis of the protein expression levels of six atypical cyclins in human CRC tumours and several cell lines, and found that CNTD2 is significantly upregulated in CRC tissue compared to the adjacent normal one. CNTD2 overexpression in CRC cell lines increases their proliferation capacity and migration, as well as several stemness properties like spheroid formation capacity and anchorage-independent growth. Interestingly, CNTD2 increases tumour growth *in vivo* on xenograft models of CRC with wild-type *BRAF*, and decreases tumour growth on xenografts with mutated *BRAF*, a finding that correlated with the prognostic significance of CNTD2 in CRC patients. Accordingly, CNTD2 downregulation significantly diminished the proliferation of wild-type *BRAF* CRC cells. Our results suggest that the outcome of CNTD2 overexpression is dependent on the *BRAF* context, and that CNTD2 may represent a new prognostic factor and a promising drug target in the management of CRC.

3 de maig de 2018 ⇨ **Sala Nicolau d'Olwer**

VII Jornada de Biofísica

Organitzada per la Secció de Biofísica de l'SCB

Coordinador: Pere Garriga

Programa

14.30	Recollida de documentació
14.45	Introducció: Pere Garriga (sala Prat de la Riba)
15.00-15.45	**Anna Labernadie**. IBEC, Barcelona Collective cancer cell invasion by fibroblast forces *Compartida amb la Secció de Biologia del Càncer, Sala Prat de la Riba*

Sessió I

Moderador: **Alex Perálvarez-Marín**, UAB

15.45-16.00	**Joan-Ramon Daban**, UAB Stacked thin layers of planar chromatin explain the 3D organization of genomic DNA in condensed metaphase chromosomes
16.00-16.15	**Marc Rico-Pastó**, UB Melting enthalpy and entropy change with single molecule experiments resolution
16.15-16.30	**Marina I. Giannotti**, CIBER-IBEC Pulling lipid tubes from model membranes
16.30-16.45	**Alvaro Martínez Monge**, UB Single-molecule characterization of heterogeneous DNA ensembles
16.45-17.00	**Carlo Manzo**, Uvic Quantification of protein copy number from super-resolution images
17.00 -17.30	Pausa cafè

Sessió II

Moderador: **Carlo Manzo**, Uvic

17.30 -17.45	**Alfredo de la Escosura-Muñiz**, UPC Electrical evaluation of bacterial pathogen virulence factors using nanopores
17.45.18.00	**Xavier Viader-Godoy**, UB Length-dependence of the elastic response of single-stranded DNA
18.00-18.15	**Elena Álvarez-Marimon**, UAB Nano-X-Ray Fluorescence Studies of Alzheimer Disease Amyloid Plaques
18.15-18.30	**Marta Gironella**, UB Relaxational kinetics in red blood cell mechanics: linking physical to biological agin
18.30 -19.15	**Alexander Scholten**. University of Oldenburg (Germany) Biophysical and biochemical approaches to investigate vertebrate phototransduction in health and disease

Collective cancer cell invasion by fibroblast forces*

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Cancer-associated fibroblasts (CAFs) promote tumour invasion and metastasis. We show that CAFs exert a physical force on cancer cells that enables their collective invasion. Force transmission is mediated by a heterophilic adhesion involving N-cadherin at the CAF membrane and E-cadherin at the cancer cell membrane. This adhesion is mechanically active; when subjected to force it triggers β-catenin recruitment and adhesion reinforcement dependent on α-catenin/vinculin interaction. Impairment of E-cadherin/N-cadherin adhesion abrogates the ability of CAFs to guide collective cell migration and blocks cancer cell invasion. N-cadherin also mediates repolarization of the CAFs away from the cancer cells. In parallel, nectins and afadin are recruited to the cancer cell/CAF interface and CAF repolarization is afadin dependent. Heterotypic junctions between CAFs and cancer cells are observed in patient-derived material. Together, our findings show that a mechanically active heterophilic adhesion between CAFs and cancer cells enables cooperative tumour invasion.

**Compartida amb Biologia del Càncer*

Stacked thin layers of planar chromatin explain the 3D organization of genomic DNA in condensed metaphase chromosomes

Joan-Ramon Daban

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The 3D organization of genomic DNA in metaphase chromosomes has been one of the most challenging problems in structural biology since the discovery of the double helix. This study shows that chromosome images obtained from typical banded karyotypes and from different multicolor cytogenetic analyses can be used to obtain information about the 3D folding of chromatin within chromosomes. Chromosome bands and the connection surfaces in sister chromatid exchanges and in cancer translocations are planar and orthogonal to the chromosome axis. Chromosome stretching produces band splitting and even the thinnest bands are orthogonal and well defined, indicating that short stretches of DNA can occupy completely the chromosome cross-section. These observations impose strong physical constraints on models that attempt to explain chromatin packaging in chromosomes. The thin-plate model, which was proposed from previous experimental studies of our laboratory (1), consists of many stacked layers of planar chromatin perpendicular to the chromosome axis (2). This is the only model compatible with the observed orientation of bands, with the existence of thin bands (<1Mb), and with band splitting; it is also compatible with the orthogonal orientation and planar geometry of the connection surfaces in chromosome rearrangements. The results obtained provide for the first time a consistent interpretation of the chromosome structural properties that are used in clinical cytogenetics for the diagnosis of hereditary diseases and cancers. A complete description of this work can be found in ref. (3).

(1) JR. Daban (2011) Micron 42:733-750.
(2) JR. Daban (2014) J R Soc Interface 11:20131043
http://rsif.royalsocietypublishing.org/content/11/92/20131043
(3) JR. Daban (2015) Scientific Reports 5:14891
https://www.nature.com/articles/srep14891

Melting enthalpy and entropy change with single molecule experiments resolution

Marc Rico-Pastó[1], Marco Ribezzi-Crivellari[2,3] and Felix Ritort[1,3]

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An accurate knowledge of the thermodynamic properties of nucleic acids as a function of temperature is crucial to predict their structure and stability far away from the physiological temperature. Traditionally, molecular thermodynamic properties, such as free energy, enthalpy and entropy change have been determined by bulk experiments [1]. In the last 20 years, single molecule experiments have become powerful, accurate and bulk complementary methods to characterize thermodynamic parameters such as base pair (bp) energy contributions and folding free energies [2].

We propose a novel method to determine the enthalpy and entropy change from hopping experiments at one unique salt concentration, in contrast with the traditional DSC bulk experiments. We have carried out experiment with three different DNA hairpins, i.e. poly-GC, poly-AT and CD4 (52% GC content), in a temperature range between 5 and 50ºC to measure the enthalpy and entropy change for the folding a GC and AT bp. From our data we have observed a strong temperature dependency, what it means a non-zero heat capacity change, ΔC_p. The measured ΔC_p are, 83±2 , 40±6 and 54±3 cal/K·mol for a GC, AT and CD4 bp. Moreover, we have compared the measured folding free energy at each temperature as $\Delta G = \Delta H - T\Delta S$ with the measured one by subtracting the stretching and orienting contributions using the WLC and FJC models. Finally, an empirical formula to determine the melting temperature for two complementary DNA sequences is presented.

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Pulling lipid tubes from model membranes

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Several cellular processes, including endocytosis, membrane resealing, signaling and transcription, among others, involve conformational changes such as bending, vesiculation and tabulation. For instance, in endocytosis, the endocytic system needs to generate force enough to form an endocytic vesicle by bending the membrane bilayer. Separation of a membrane segment from the cytoskeleton as well as strong membrane bending are both involved in these mechanisms, which are also associated with the membrane chemical composition and physicochemical properties. This process is energetically comparable to pull out a membrane cylindrical tube by applying a force orthogonal to a small membrane region.[1] Both separation of a membrane segment from the cytoskeleton as well as strong membrane bending are involved in these mechanisms. In this context, these procedures can be mimicked by applying an external force with highly sensitive force transducers such as optical tweezers or atomic force microscopy (AFM).[2] The lipid tube growth is then evidenced on a constant force process in the force-distance curves (Fig. 1). This force is the growing tube force, F_{tube}.

In this work, we compare SLBs with different compositions and prove that the phospholipid state (gel or fluid) as well as the headgroup play a role on the F_{tube}, following the tendency observed on the well-established breakthrough force (F_b) characterization approach.[3,4] In addition, we evaluate the influence of the underlying substrate on F_{tube}, by comparing the tube growth from deposited vesicles and lipid bilayers supported onto silicon or mica substrates. Finally, the influence of the AFM tip chosen to perform the measurements is also studied, considering the tip radius (r_{tip}) and the retracting velocity of the tip away from the sample. We demonstrate that working with SLB models is an intermediate step between a free membrane (blebs) and a cytoskeleton supported membrane.

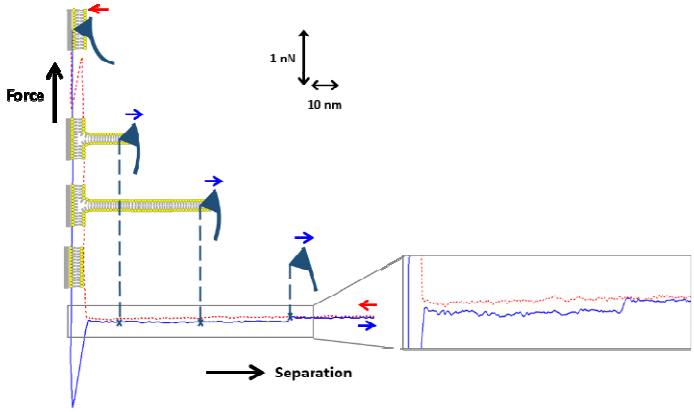

Figure 1: Example of a force-separation curve when performing an AFM-FS measurement on an SLB: approach (red dotted line) and retract (blue line). Schematic representation of the different steps.

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Single-molecule characterization of heterogeneous dna ensembles

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Heterogeneity exists across all spatial scales, from communities down to molecular level. The characterization and quantification of heterogeneous effects, although have been usually overlooked, have turned out to be fundamental in many scientific disciplines, such as cancer research. In this work, we combined single-molecule measurements using optical tweezers with fluctuation theorems to build a novel theoretical framework that allows us to quantify the folding free energy spectrum of the heterogeneous ensemble.

Quantification of protein copy number from super-resolution images

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Single-molecule-based super-resolution microscopy offers researchers a unique tool to visualize biological processes at the nanoscale. Nevertheless, providing a quantitative description of the molecular mechanisms underlying cellular function requires the precise molecular counting of protein copy numbers. Suitable calibration methods – based on the combination of biochemicals and analytical tools – represent a valuable solution to address the challenges of molecular counting using several super-resolution techniques (STORM, STED) in conjunction with immunofluorescence.

Along this line, we have recently proposed a versatile platform for calibrating fluorophore and antibody labeling efficiency based on DNA origami and GFP antibodies to quantify protein copy number in cellular contexts using localization microscopy. The combination of this calibration with image and data analysis methods, besides quantifying the average protein copy number in a cell, allows determining the abundance of various oligomeric states [1]. These quantitative approaches allow accurate studies of the stoichiometry of membrane proteins, nucleoporins and molecular motors.

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Electrical evaluation of bacterial pathogen virulence factors using nanopores

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Bacterial hyaluronidases produced by a number of pathogenic Gram-positive bacteria catalyze the degradation of hyaluronic acid (HA), initiating infections at the skin or the mucosal surfaces. It's known that streptococcus, staphylococcus, streptomyces or clostridium bacteria between others, use this enzyme as a virulence factor to destroy the polysaccharide that holds animal cells together, making easier for the pathogen to spread through the tissues of the host [1]. The interest in the detection of this enzyme is related to two different aspects: i) the evaluation of the secreted levels of enzymes for different bacterial species would allow to discriminate between Gram-positive and Gram-negative bacteria and also to classify them in terms of virulence and ii) the evaluation of the enzyme secretion inhibition would allow to propose novel antimicrobial/antivirulence agents. However, the current available tools for the detection of this enzyme are quite limited. It's a very small protein (60 kDa) which difficult its detection using traditional immunoassays, typically radioimmunoassays (RIA) and enzyme-linked immnusorbent assays (ELISA) that are expensive, time consuming and need hazardous label reagents.

Biosensors in general and the ones based on nanoporous platforms in particular, overcome most of these limitations, since they are rapid, cheap and allow label-free detection [2,3]. In this context, we propose here a novel methodology for hyaluronidase detection on anodized aluminum oxide (AAO) nanoporous membranes. The proposed analytical method based on the electrical monitoring of specific nanochannels blocking/unblocking is shown as a useful tool for the detection of hyaluronidase through immunoassays. This label-free method is rapid and cheap, avoiding sandwich assays and the use of labels. Preliminary results open the way to future applications for virulence evaluation of enzymes as well as for monitoring bacterial infection processes.

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Length-dependence of the elastic response of single-stranded DNA

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Single-stranded DNA (ssDNA) plays a major role in several biological processes, such as replication or transcription. Therefore, it is of fundamental interest to understand the elastic response of this biological polymer. Besides, force spectroscopy techniques have been widely used to study biochemical and enzymatic processes involving DNA. The interpretation of the results obtained by these experiments, such as [1], requires an accurate description of the elastic properties of ssDNA. However, elasticity of ssDNA has been less studied than that of double-stranded DNA, and a large dispersion on the elastic parameters is obtained from different methods and sequences [2].

In this work, we study the elastic properties of ssDNA using molecules with different sequences and lengths comprising 4 orders of magnitude (from 60 bases to 14kbases). Using the inextensible Worm-Like Chain model we proof that the apparent discrepancy found in the previous works arises mainly from the different range of forces used to fit long and short molecules. We have also tested sequences with different pyrimidine/purine content in order to investigate the effect of base stacking, which is known to largely change the elastic properties of homogeneous sequences [3]. Even that the stacking of bases has a minor impact in the elastic response of heterogeneous sequences, we are able to detect base stacking effect at the level of tenths of bases.

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Nano-X-Ray Fluorescence Studies of Alzheimer Disease Amyloid Plaques

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Alzheimer´s Disease (AD) is characterized by the presence of neurofibrillary tangles (NFT), senile plaques (SP) and changes in the distribution of metal ions[1].

In order to understand the relationship between characteristic secondary structures of Aβ amyloid aggregates, altered distribution of metal ions (Cu, Zn, Fe, Ca) and lipid oxidation, we used the combination of two synchrotron radiation techniques: nano-X-ray fluorescence (nano-XRF) and μFTIR at ESRF beamlines ID16B-NA and ID21, respectively, on human brain tissues of affected AD individuals and healthy controls

On the one hand, synchrotron-based infrared microscopy makes possible the *in-situ* localization (figure 1A) and structural study of amyloid aggregates in relation to other physicochemical parameters, such as tissue oxidation[2]. The infrared data was analysed using Principal Component Analysis (PCA) which allowed us to distinguish between two different types of amyloid aggregates that might correspond with dense core plaques and diffuse plaques. On the same sample areas nano-XRF measurements at 0.2 μm^2 pixel size were done at ID16B-NA (ESRF) in order to measure the metal distribution. The results indicated that Fe, Cu and Zn ion maps co-localize with the plaques with cation content within the plaques well above the level measured outside the plaques. Moreover, when dense and diffuse plaques were compared, the Fe content turned out to be higher in the dense plaques.

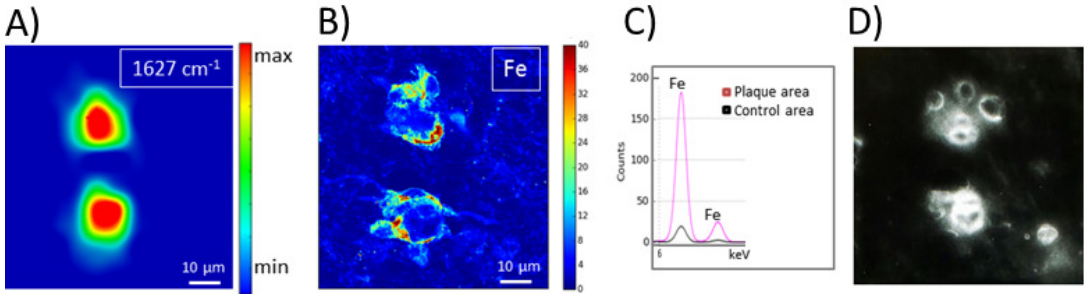

Figure 1. *ADV patient tissue analysis.* A) μFTIR map of the fibrillar β plaques (corresponding to dense plaques). B) Nano-XRF map of the same area representing Fe distribution. C) Fe content on the plaque area is significantly higher to the control area. D) After synchrotron analysis Thioflavin-S dye on the same tissue area confirmed the presence of amyloid plaques.

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Relaxational kinetics in red blood cell mechanics: linking physical to biological aging

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Red blood cells (RBC) are one of the most abundant and simplest cells in human body. Only composed of a lipid bilayer and an spectrin cytoskeleton, their shape, mechanics and aging are fundamental features to understand and treat the majority of blood diseases. In this project we study relaxational processes in the mechanics of RBC using optical tweezers. We use two different approaches in order to understand the viscoelastic response of the RBC: 1) Pulling experiments, where we pull and push the RBC at different maximum forces and different pulling velocities to extract information of the force-distance curves and; 2) Relaxation experiments, where we apply a force jump to the RBC and measure force relaxation. From these two kind of experiments we are able to characterize four different time-scales, three of them related to membrane-cortex interaction, the other one (which is the longest) shows a stiffening of the RBC that we hypothesize it is linked to aging in the RBC. The correlation between the time-scales allows us to globally understand the temporal evolution of RBC and link physical to biological aging.

Biophysical and biochemical approaches to investigate vertebrate phototransduction in health and disease

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Phototransduction means the conversion of light into a biological signal. In the vertebrate retina it takes place in specialized neuronal cells called rod and cone photoreceptor cells. The phototransduction cascade is an archetype of a G-protein coupled cascade. It requires a fine tuned balance between two second messengers: 3',5'-cyclic guanosine monophosphate (cGMP) and Ca^{2+} ions. To maintain this balance and to enable adaptation to different light conditions a system of membrane bound guanylate cyclases (GCs) and GC activating proteins (GCAPs) evolved in photorecptor cells. The main players of this system are GC-E and the GCAP isoforms 1 and 2. In the dark GCAPs sense the high Ca^{2+} concentration and inhibit the enzyme activity of GC-E. After a light stimulus [Ca^{2+}] drops, GCAPs release bound Ca^{2+} ions and switch into an activator state: GC-E produces cGMP. The activation of GC-E by GCAPs occurs in a consecutive manner: GCAP1 release it Ca^{2+} ions first, only after a strong light stimulus and more pronounced drop in [Ca^{2+}] also GCAP2 turns into its activating state. We called this mode of fine tuned adaptation the Ca^{2+}-relay model.

In order to better understand how the GC/GCAP system works on a molecular level we followed two main strategies: the first one included comparative analysis of the two isoform GCAP1 and GCAP2. The second strategy based on the characterization of GCAP1 (and GC-E) mutants that are known to cause inherited retinal degeneration in human. Knowledge about dysfunction in the impaired systems in diseases improved our knowledge on the non impaired wildtype system. Our comparative analysis included biochemical and biophysical approaches and focused on different features of the proteins: activity, structure, Ca^{2+}-affinity and GCAP/GC interaction.

Time-resolved fluorescence spectroscopy on labeled proteins showed us differences in the structural rearrangement of GCAP1 and 2 after Ca^{2+} binding. Circular dichroism was useful to find GCAP1 mutants with changed structure. To determine the Ca^{2+} affinity of the GCAPs we utilized a variety of techniques: $^{45}Ca^{2+}$ binding assay, chelator assay, isothermal titration calorimetry, and surface plasmon resonance. A newly developed technique called back-scattering interferometry gave us new insights into the binding of GCAP1 and 2 to the GC-E.

4 de maig de 2018 ⇨ **Sala Prat de la Riba**

Jornades conjuntes de Biologia Molecular i del Desenvolupament

Organitzades per

Secció de Biologia Molecular
Coordinador: Bernat Crosas

Secció de Biologia del Desenvolupament
Coordinador: Francesc Cebrià

Col·laboradors: Teresa Adell, Marta Morey i Berta Alsina

Programa

8:30-9:00	Arrival and Registration
9:00	Welcome by the Coordinators of the Sections of Molecular Biology and Developmental Biology
9.10-9:40	Origin of the sections and the joint meetings by Jaume Baguñà, Jordi Domingo and Pere Puigdomènech

Chair: TBA

9.40-10.20	**Benoît Kornmann**, ETH, Zurich Membrane contacts, what are they, what are they good for and how can they be bad?
10.20-11.00	**Anna Rubio-Cosials**, EMBL, Heidelberg Transferring antibiotic resistance: structural insights into the mechanism of a conjugative transposon
11.00-11.15	**Damià Garriga**, ALBA Synchrotron, Barcelona Molecular basis for the inhibition of poxvirus assembly by the antibiotic rifampicin
11.15-11.30	**Andrea Izquierdo-Bouldstridge**, IBMB-CSIC Histone H1 depletion triggers an interferon response in cancer cells

11.30-12.00 Coffee and Posters

12.00-12.15 **Cinthia Raquel Millan**, UPC
New drugs complexed with AT-rich DNA accumulate in kinetoplast DNA: a promising treatment against Sleeping Sickness

12.15-12.30 **Albert Torra**, VHIR-CIBERNED
Activation of Transcription Factor EB as a neuroprotective strategy for Parkinson's disease

12.30-12.45 **Sílvia Pérez-Lluch**, CRG, UPF
Natural no-coding antisense transcription along development and evolution

12.45-13.00 **Alba Ventós-Alfonso**, CSIC-UPF
Role of Zelda in the hemimetabolan insect *Blattella germánica*

13.00-13.40 **Volker Hartenstein**, UCLA, USA
Structure and development of neural circuits of the Drosophila brain: a lineage-centered approach

13:40-15:00 Lunch and Posters

Chair: TBA

15.00-15.40 **Fàtima Gebauer**, CRG
RNA binding proteins in cancer progression

15.40-16.20 **Salvador Aznar-Benitah**, IRB Barcelona
Epigenetic mechanisms in adult stem cells, and their possible impact over mutational burden of cancer stem cells

16.20-16.35 **Juan J. Fraire-Zamora**, CRG
Dorsal closure in dipterans: epithelial rupture, contraction and seaming without genetic changes in the scuttle fly Megaselia abdita

16.35-16.50 **Brenda Gavilán**, UB
Serial section Transmission Electron Microscopy (ssTEM) analysis of the acoel *Symsagittifera roscoffensis*

16.50-17.05 **Eudald Pascual-Carreras**, UB
Smed-BS is a novel peptide which inhibition produces bigger planarians or overgrowths depending on the nutritional status

17.05-18.30 Drinks and Posters

18.30 Awards and concluding remarks

Membrane contacts, what are they, what are they good for and how can they be bad?

Benoît Kornmann

ETH- Zurich, Switzerland

Intracellular organelles constitute dense and branched membrane networks that are under constant remodeling. My lab is interested in how these organelle networks are generated, distributed and regulated. We also investigate how this networked morphology is related to the organelle's activity. These highly extensive and dynamic networks cohabit in the extremely crowded cytoplasmic space. This situation leads to unwanted collisions and entanglements that needs to be resolved. We show that, in the case of mitochondria, these collisions and entanglements can be resolved by mitochondrial fission. Mechanical forces applied to mitochondrial tubules lead to the recruitment and activation of the mitochondrial fission machinery, leading to the resolution of entanglements. These results imply that a biochemical response can be triggered by a mechanical stimulus and that forces within the cells participate in the shaping of organelles. The extended morphology of several organelles might allow them to contact each other to exchange lipid molecules. Because most of the factors involved in lipid exchange are unclear or unknown, we developed a novel method that uses transposons and next-generation sequencing to interrogate the yeast genome and map in a single step all proteins and protein domains necessary for growth in a given condition. We use it to identify redundancies in lipid exchange routes, but the power of the method finds myriad of applications far beyond our usage.

Transferring antibiotic resistance: structural insights into the mechanism of a conjugative transposon

Anna Rubio-Cosials, Eike C. Schulz, Lotte Lambertsen, Georgy Smyshlyaev, Carlos Rojas-Cordova, Kristoffer Forslund, Ezgi Karaca, Aleksandra Bebel, Peer Bork, Orsolya Barabas

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Conjugative transposition drives the emergence of multidrug-resistance in diverse bacterial pathogens, yet the mechanisms are poorly characterized. The Tn1549 conjugative transposon propagates resistance to the antibiotic vancomycin used for severe drug-resistant infections. Here, we present four high-resolution structures of the conserved Y-transposase of Tn1549 complexed with circular transposon DNA intermediates. The structures reveal individual transposition steps and explain how specific DNA distortion and cleavage mechanisms enable DNA strand exchange with an absolute minimum homology requirement. This appears to uniquely allow Tn916-like conjugative transposons to bypass DNA homology and insert into diverse genomic sites, expanding gene transfer. We further uncover a structural regulatory mechanism that prevents premature cleavage of the transposon DNA before a suitable target DNA is found, and generate a peptide antagonist that interferes with the transposase-DNA structure to block transposition. Our results reveal mechanistic principles of conjugative transposition, which could help control the spread of antibiotic resistance genes.

Structure and development of neuronal circuits of the *Drosophila* brain: A lineage-centered approach

Volker Hartenstein

Department of Molecular Cell and Developmental Biology, University of California Los Angeles, Los Angeles, CA 90095

Common features of the central nervous system encountered in all bilaterian animals are the relatively high number of cells, diversity in neuronal cell types, and specificity in neuronal connections. This puts a heavy burden on the developmental process generating the central nervous system. In Drosophila, a fixed lineage mechanism plays a pivotal role in controlling neuronal diversity and connectivity. The fly brain is composed of a relatively small number of stereotyped neuronal lineages, groups of neurons descended from individual embryonic stem cells, called neuroblasts. During the course of its proliferation, each neuroblast expresses characteristic sets of regulatory genes. These genes control the differentiation of the neurons born from that particular neuroblast during a particular time interval. Through this mechanism, a lineage, or smaller subdivision of a lineage called sublineage, develops into a specific class of neurons which share common wiring properties, including the projection of their axons, branching pattern, and placement of synapses. Several discrete neuronal classes/lineages are put together into a neuronal circuit. In the first part of my talk I will delineate the lineage mechanism, concentrating on the example of a particular circuit, the anterior visual pathway (AVP). The AVP conducts input form the optic lobe to the central complex, a brain center known to process and store visual information in order to control fly locomotion. The central part of the AVP is formed by three lineages, whose neurons form several classes of highly ordered parallel and sequential elements.In the second part of my talk I will provide a progress report of our ongoing studies of neuronal wiring at the single synapse level of resolution. We are following the hypothesis that the wiring properties and choice of synaptic partners of a neuron is strictly correlated to its lineage association. In collaboration with numerous other groups we use a dataset consisting of a complete series of more than 5000 registered electron microscopic sections of an entire early larval brain (“L1 EM stack”). The reconstructed neurons are morphologically complex 3D graphs whose nodes are annotated with labels representing different types of synapses. By cross-referencing the L1 EM stack with similarly oriented stacks of confocal sections we could identify lineage identity of most neurons. The data allow one to study quantitatively the spatial relationship between synapses with different partners, and formulate hypotheses regarding neural function at the microcircuit level. We have designed software that generates 2D renderings of 3D neurons in order to help biologists analyze the wealth of data that is now available. The renderings are dendrograms that capture a neuron’s tree-like structure, and they realistically encode morphological features, such as relative length and branching depth of a side branch, and synapse locations. We hence refer to these neuron sketches as "morphological feature dendrograms" (MFDs).

RNA binding proteins in cancer progression

Fátima Gebauer

CRG Barcelona

RNA binding proteins (RBPs) are gaining attention in the oncology field for their potential to regulate essentially every hallmark of tumor development. However, to date only a few RBPs have been shown to play roles in cancer progression, in large part because RNA metabolism has been a poorly investigated aspect of cancer research. Fueled by our initial discovery that the conserved RBP UNR/CSDE1 has dedicated roles in melanoma metastasis, we have launched an unbiased genome-wide screen of RBPs for which cancer metastatic cells show specific dependencies. My talk will focus on our efforts to untangle the RBP diversity of metastatic cells.

Epigenetic mechanisms in adult stem cells, and their possible impact over mutational burden of cancer stem cells

Salvador Aznar Benitah

ICREA Professor; IRB Barcelona

Mutations are not evenly distributed throughout the genome. However, what causes the disparate genomic mutational distribution is still under debate. It has been proposed that a factor contributing to the uneven distribution of cancer mutations is the open versus closed chromatin distribution of their cell-of-origin. Importantly, mutations and expression changes of epigenetic modifiers are pervasive in human tumours, making epigenetic factors attractive as anti-tumour targets. However, if epigenetic alterations affect mutational burden, this raises the concern that targeting epigenetic factors in cancer patients might alter mutability and possibly aggravate disease progression in the long-term. Yet, a causal link between changes in chromatin accessibility in tissues and the mutational landscape of their cognate tumours has not yet been established. I will present functional data showing how altering chromatin accessibility severely affects tumorigenesis *in vivo*. I will comment the effects that changing chromatin openness has over tumor mutability. I will also discuss the implications of our results on the effect that our lifestyle (*i.e.* diet) could have on the epigenetic landscape of adult stem cells which might then influence the aggressiveness of their cognate tumors.

Molecular basis for the inhibition of poxvirus assembly by the antibiotic rifampicin

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In contrast to most enveloped viruses, poxviruses produce infectious particles that do not acquire their internal lipid membrane by budding through cellular compartments. Instead, poxvirus immature particles are generated from atypical crescent-shaped precursors derived from host ER membranes. Two key viral proteins participate to this process: A17 inserts in the membrane and is proposed to induce curvature, while D13 forms a scaffold that remodels membranes into a closed, spherical particle. The formation of these viral crescents can be inhibited by the antibiotic rifampicin, although the mechanisms underlying such inhibition remain unknown. Using a combination of X-ray crystallography, surface plasmon resonance and CPMG, a ligand-detected NMR technique, we showed that rifampicin directly binds D13 and identified its binding site. We further proved that this inhibitor directly competes with A17, evidencing an overlap of binding sites. We then used a classical fragment-based drug design approach to target the A17/rifampicin binding site in D13, screening a library of 1137 compounds by STD and CPMG NMR. This allowed the identification of 25 fragments that bind D13. Out of these, 2 molecules with unrelated structures were found to compete with both rifampicin and A17. These two lead compounds were taken for optimisation towards the design of assembly inhibitors against poxviruses.

Histone H1 depletion triggers an interferon response in cancer cells

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[#] These two authors contributed equally to this work.

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

New drugs complexed with AT-rich DNA accumulate in kinetoplast DNA: a promising treatment against Sleeping Sickness

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Approximately 70 million people distributed over a surface of 1.55 million km2 are estimated to be at different levels of risk of contracting sleeping sickness. *Trypanosoma brucei* accounts for 82.2% of the population at risk. About 6 million to 7 million people worldwide, mostly in Latin America, are estimated to be infected with *Trypansosoma cruzi*, the parasite that causes Chagas disease. We study drugs interacting with minor groove of DNA, such as the N-phenylbenzamide bis(2-aminoimidazoline) derivatives 1 The main objective was to identify their cellular target inside the parasite. We were able to demonstrate that the drugs have a clear effect on the S-phase of T. brucei cell cycle by inflicting specific damage on the mitochondrial DNA, a unique and complex structure called kinetoplast. The kinetoplast has more than 70% of AT-DNA.

C.R. Millan*, et al*. "Functional and structural analysis of AT-specific minor groove binders that disrupt DNA-protein interactions and cause disintegration of the *Trypanosoma brucei* kinetoplas" *Nucleic Acid Research* (2017) vol.45, pag.8378-8391 Doi: 10.1093/nar/gkx521

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Activation of Transcription Factor EB as a neuroprotective strategy for Parkinson's disease

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Neurotrophic factor-based therapy stands as one of the most promising disease-modifying therapeutic approach for Parkinson's disease (PD). However, axonal impairment and downregulation of their receptors may account for the lack of therapeutic success in clinical trials. An alternative to delivering neurotrophic factors to overcome these hurdles is to directly activate the intracellular signaling pathways responsible for their effect. We demonstrate that Transcription Factor EB (TFEB) overexpression in mice drives a previously unknown bona fide neurotrophic effect that involves the activation of the MAPK1/3 and AKT pro-survival pathways, giving rise to cell growth, and increased dopamine release. We also demonstrate that TFEB overexpression prevents neuronal death, increases dopaminergic function and counteracts atrophy and the associated protein synthesis decline in the MPTP mouse model of PD. It has been suggested that TFEB neuroprotective effect may be due to its capacity to boost the autophagy-lysosomal system for the clearance of protein aggregates. However, we show that knocking down the master transcriptional repressor of autophagy ZKSCAN3 is not sufficient to protect dopaminergic neurons in this model. Overall, our results suggest that TFEB activation is an alternative neuroprotective/neurorestorative strategy to neurotrophic factor-based therapies for PD and related disorders.

Natural no-coding antisense transcription along development and evolution

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The genome of *Drosophila melanogaster* is estimated to encode over two thousand lncRNAs; however, only few of them have a characterized function. Natural antisense transcripts (NATs) are fully processed lncRNAs which overlap protein coding genes on the opposite strand with or without exonic complementarity. Several roles in genomic regulation are reported for NATs in metazoa, including gene expression regulation of the overlapping protein coding gene, DNA methylation, chromatin modifications and RNA editing. Here, we have identified 855 lncRNAs overlapping 873 protein coding genes in antisense orientation, forming 953 sense-antisense (SA) pairs in the fruit fly genome. By analysing the transcriptome of different imaginal tissues at 3rd instar larvae, we have explored the relationship between NATs expression and alternative transcript usage across fly larval samples. Of the 376 SA expressed pairs involving a protein coding gene with multiple isoforms, *blistered*/*CR44811* is the one showing a highest correlation between changes in coding gene isoform usage and NAT expression. *blistered* (*bs*) gene encodes for two main isoforms: a short one, expressed mainly in the wing where the NAT *CR44811* is also expressed, and a long one, expressed in the other tissues where the NAT is silent. *CR44811* CRISPR mutant flies show a dramatic change in the *bs* isoform usage in larval and pupal wings, as well as a strong phenotype in the adult, indicating impairment of the proper wing development. Manual annotation of the *bs* locus using available RNAseq data from other species has allowed us to align both isoforms of the coding gene along development, suggesting a role of both isoforms in further species and its possible regulation through the action of the lncRNA.

Role of Zelda in the hemimetabolan insect *Blattella germanica*

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There are two modes of metamorphosis in insects: holometabolan and hemimetabolan. In the hemimetabolan (which is the ancestral mode), the embryo develops the basic adult body structure, whereas in the holometabolan, the adult body structure becomes completed in the pupal stage. Therefore, to better understand the evolution of metamorphosis it is important to study the differences in the mechanisms regulating embryo developmental processes in hemimetabolan and holometabolan species. One of those processes is the maternal-to-zygotic transition (MZT), where the maternal mRNAs are eliminated and the zygotic genome starts to be transcribed. Zelda has been described as a key protein in the MZT in holometabolan insects, such as *Drosophila melanogaster*, being involved in both maternal mRNA cleavage and activation of the zygotic genome. Conversely, information about the role of Zelda in hemimetabolan insects is very limited. In this work, we carried out a functional analysis of Zelda in the embryo of the hemimetabolan insect *Blattella germanica*, using maternal RNAi. We found that Zelda regulates the expression of early zygotic genes (involved in abdomen formation and dorso-ventral patterning), and that of miR-309, a microRNA that plays a key role in eliminating maternal mRNAs during the MZT. We have also observed that Zelda regulates the expression of epigenetic factors, like DNMT1 or Nejire. The whole results confirm that Zelda is a key protein in MZT in both hemimetabolan and in holometabolan insects. However, a key difference between both metamorphosis modes is that Zelda is expressed only in early embryogenesis in *B.germanica*, whereas in *D. melanogaster* it is expressed all along the embryogenesis. This difference could explain the different output of both types of embryo development.

Dorsal closure in dipterans: epithelial rupture, contraction and seaming without genetic changes in the scuttle fly Megaselia abdita

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The evolution of morphogenesis is generally assumed to be associated with changes in genetic patterns that lead to the spatial reorganization of tissues. I will present a case study where rearrangements of epithelial organization occur without major changes in genetic patterning. In Drosophila melanogaster, the process of dorsal closure consists of the fusion of opposing epithelial sheets where a contractile extraembryonic amnioserosa and a JNK/Dpp-dependent epidermal actomyosin cable result in a microtubule-dependent seaming of the epidermis. In the scuttle fly Megaselia abdita, dorsal closure must occur in the presence of a separate serosa, amnion and epidermis. It differs from Drosophila in morphogenetic rearrangements despite conservation of the JNK/Dpp signaling pathway. Using a quantitative approach in a non-model organism, we show that dorsal closure in Megaselia is driven by the rupture and contraction of the serosa, an epidermal actomyosin cable and the consecutive microtubule-dependent seaming of amnion and epidermis. Upon rupture, serosa cells retract and reduce their size through actomyosin apical accumulation. This process promotes the internalization of serosa cells bringing together the opposing amniotic flanks, followed by the contractile activity of an epidermal actomyosin cable that depends on JNK/Dpp signaling. The final process of dorsal closure in Megaselia depends on two sequential microtubule-dependent seaming events. First the amniotic flanks must seam at the dorsal midline, followed by the seaming of the opposing epidermal flanks. Microtubule depolymerization prevents amniotic seaming and rescue experiments resume Megaselia dorsal closure. Using high-resolution time-lapse imaging, immunostaining and molecular tools, we obtained evidence indicating that the evolutionary transition to a reduced system of dorsal closure involves the simplification of the seaming process without changing the signaling pathways of closure progression.

Fraire-Zamora JJ, Jaeger J and Solon J. (2018) Two consecutive microtubule-based epithelial seaming events mediate dorsal closure in the scuttle fly Megaselia abdita. eLife

Serial section Transmission Electron Microscopy (ssTEM) analysis of the acoel *Symsagittifera roscoffensis*

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In collaboration with S. Sprecher and V. Hartenstein laboratories from University of Fribourg (Switzerland) and University of California, Los Angeles (USA), respectively, we have undertaken a thorough study of the acoel cellular organization leading towards the 3D reconstruction of structures of major interest. Transmission Electron Microscopy serial sections (ssTEM) of the most anterior part of a *Symsagittifera roscofensis* juvenile body were taken, covering approximately the first half of the body, where the brain and beginning of the nerve cords are located. These sections were aligned in the correct order forming a stack using the ImageJ plugin TrakEM2 (developed by Albert Cardona, HHMI, Janelia Farm, USA). The software allows us the 3D reconstruction of cells and cell groups. My work in this project consists in analyzing the structure and organization of different cell types present, and how they connect with each other. This is particularly challenging in this group of animals, since the acoel body is not organized as well-delimited organs; the different cell types appear intermingled and their membranes highly folded. As acoels (together with their entire group, the phylum Xenacoelomorpha) are considered the sister group of the remaining bilaterians, this study can help us to shed some light in the evolution of organs' architectures. Our main aim is to see if there are patterns of distribution of these cellular types, how they are connected to each other and very especially to the central nervous system. We also took particular interest in the different sensory receptors and gland necks located in the epidermis, with a focus on their subtype classification, distribution and innervation. This systematic approach gives us a unique opportunity of studying the nervous system in much more detail, and aiming at a complete 3D reconstruction of the brain, nerve chords and main peripheral nerve tracks.

Smed-BS is a novel peptide which inhibition produces bigger planarians or overgrowths depending on the nutritional status

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The control of cell number is crucial to define the final body size during animal development, and also to restrict tumoral transformation in adult organisms. The cell number of any organism results from the balance between cell proliferation and cell death. Although a large number of genes are known to be involved in the control of both processes, the molecular mechanisms relating cell number and body size remain poorly understood. To further understand this relationship we study planarians, flatworms that continuously change their body size depending of food viability. In this study, we present a novel secreted peptide, Smed-Blitzschnell (Smed-BS), which inhibition produces an increase of proliferation and a decrease in cell death, thus leading to an increase in cell number. Interestingly, *Smed-bs* RNAi inhibition in starved planarians results in animals with more cells than controls but with the same body size; thus, showing a higher density of cells that are smaller. Eventually, this increase in cell density leads to overgrowths. In contrast, *Smed-bs* RNAi fed planarians growth faster than controls, since they have more cells, but those cells are of the same size than the control ones. Thus, the increase in cell number after *Smed-bs* RNAi is translated to: 1) a tumoral transformation in starved conditions, and 2) an increase in body size in animals with a rich nutritional status. Thus, the impact of an increase in cell number depends on the energetic state of cells

P1. Analysis of Nse1 function in maintenance of genomic stability in human cells

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During cell division multiple proteins are involved in replication and segregation of chromosomes to ensure an accurate transmission and distribution of genetic material to daughter cells. Failure in these key processes leads to the occurrence of aneuploidy and genomic instability, a hallmark of cancer cells. The Smc5/6 protein complex is conserved from yeast to humans and plays a crucial role in chromosome segregation and DNA repair. One of the subunits of the complex - Nse1 - contains a RING domain with ubiquitin ligase activity. Mutations in this domain impair DNA repair in budding yeast, causing hypersensitivity to genotoxic drugs. To reveal the function of Nse1 in mammalian cells we have created human cell lines carrying a deletion or a point mutation in the RING domain of Nse1 by CRISPR/Cas9 system. Both type of mutants express extremely low levels of the Nse1 protein, show slow growth and a higher death rate. Our preliminary results indicate that there is an increase in gamma-H2AX phosphorylation, a slight increase in anaphase bridges and micronuclei and a significantly increased number of BLM foci, compared to wild type cells. Besides, FACS analysis shows a higher number of cells with less than G1 DNA content in the Nse1 mutant population, suggesting a chromosome segregation defect.

Overall, we conclude that the integrity of the RING domain in the human Nse1 is important for Nse1 protein stability and for the maintenance of genomic integrity.

P2. Tissue Engineering Unit at CRG. Services for Stem Cell and Developmental Biology

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The goal for the Tissue Engineering Platform is to provide services to researchers in the latests technologies used in the fields of stem cell biology, stem cell differentiation, organoid formation and induced pluripotent stem cells (iPSCs). The Unit is constantly setting up new technologies that are emerging in the above fields. The Tissue Engineering platform works in collaboration with the Biomolecular Screening & Protein Technologies Unit at the CRG to provide CRISPR/Cas9 genome editing technology service. We provide the following services for stem cell and developmental biology researchers:

-Stem cells and iPS cells
-CRISPR/Cas9 gene editing to cell lines and directly to embryos
-Embryo micromanipulation
-2D and 3D (organoids) specific stem cell differentiation

P3. D-GADD45 as putative modulator of JNK pathway in regeneration

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Drosophila imaginal discs are a well established model system to study regeneration, both after physical damage or cell-death induction. Although little is known about the early signals driving the regenerative process, the activation of the Jun N-terminal kinase (JNK) pathway is likely to play a decisive role. Studies on the gene expression profiles of imaginal discs at different time points after cell death reveal several genes showing an expression burst right after damage and returning to normal levels early in the process. We focus on the *Drosophila* Growth arrest and DNA damage-inducible gene 45 (D-GADD45), which is a stress sensor involved in DNA repair, apoptosis and cell cycle control. Downregulation of D-GADD45 after cell death blocks the activation of the JNK pathway and severely compromises the regeneration process. We suggest that D-GADD45 activates the JNK pathway upstream of basket, promoting the signaling cascade that activates the expression of key genes involved in regeneration.

P4. How centrosome number influences collective cell migration

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Cell migration is a complex process that plays an important role both during the biological development of organisms and in disease conditions. During cell migration, cell movement is driven by the continuous reorganization and turnover of microtubules and the actin cytoskeleton. The centrosome is the major microtubule-organizing center (MTOC) in mammalian cells and centrosome abnormalities are associated with human tumours. However, the role of centrosomes in cell migration and invasiveness is still not well understood. To study cell migration during development, we use *Drosophila melanogaster* as a model organism, focusing on the embryonic development of the tracheal system, an organ whose development relies heavily in cell migration. In this study, we compare the migration patterns of the tracheal cells in wild type flies to tracheal cell migration in different mutants that have alterations in centrosome number. To approach this, we use confocal microscopy both in fixed and *in vivo* embryos.

P5. *Smed-cbp* regulates stem cells commitment and differentiation in planarians

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The plasticity and differentiation of stem cells into multiple specific lineages is an open basic question in developmental biology. CBP (CREB-binding protein) is a conserved gene that functions as transcriptional co-activator and histone acetyl transferase. In different organisms, CBP plays an important role in wide range of cellular processes, including cell proliferation and differentiation, cell death, DNA damage response and tumorigenesis. Here, we have studied the function of a CBP homologue in planarians, which have astonishing cellular plasticity thanks to their neoblasts (adult pluripotent stem cells). Because planarians have remarkable regenerative abilities they provide us an ideal scenario to understand the molecular mechanism underlying stem cell differentiation in vivo. Our data show that the silencing of *Smed-cbp* in planarians results in apparently proper blastema formation but severely impairs tissue differentiation. Analyses with specific molecular markers for neural and eye lineage-committed progenitors indicate that neoblast specialization into these cell types is largely blocked, which results in the absence of neural regeneration. In contrast, for the epidermal lineage it seems that *Smed-cbp* is not necessary for the specialization of epidermal progenitors, and opposite to what it is seen for neural lineages there is an increase in the number of differentiated epidermal cells. Overall, our results suggest that *Smed-cbp* could have a multiple function in regulating neoblast commitment and differentiation.

P6. Functional characterization of a mutation identified in an Opitz C syndrome patient

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Opitz C syndrome (OTCS, MIM #211750) is an extremely rare genetic disorder characterized by multiple malformations (e.g. trigonocephaly, congenital heart defects), contractures, variable intellectual and psychomotor delay and a high mortality rate. In a cohort of 11 patients clinically diagnosed as OTCS, mutations in 9 different genes were identified as disease-causing by whole exome sequencing (WES). Thus, OTCS could be causally heterogeneous phenotype instead of a specific entity. In this project, we aim to functionally characterize a *de novo* heterozygous missense variant found in a gene of the TRAF family in one of the patients and to assess its pathogenicity. The protein encoded by this gene has a role as E3 ubiquitin ligase in different signalling pathways mediated by Tumor Necrosis Factor (TNF) family ligands, such as the NF-κB pathway.

We have analyzed cell viability by MTT assay and while a slight increase in the patient's cell viability could be observed, there is no significant difference. *In vitro* analysis of fibroblasts from 3 patients bearing similar mutations were performed at mRNA level by qPCR and mRNASeq. Compared to control fibroblasts, patient's cells showed an altered basal expression of several genes of the NFKB pathway and gene expression patterns in response to TNFα stimulation. Results so far strongly suggest a pathogenic role of the mutation, however they do not clarify if the effect is a loss or a gain of function of the protein. Future experiments, such as co-immunoprecipitation assays with the major partner of the protein and further analysis of the mRNASeq data, may help to elucidate the impact of these mutations.

P7. EGFR and ecdysone in *Blattella germanica* oogenesis.

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Oogenesis is a crucial process to ensure continuity of the species and, therefore, it needs to be finely regulated. Even the regulation of this process is a complex network where different genes and pathways participate, it is important to know each piece of the puzzle and its function, and one of the main pieces of this puzzle is ecdysone that is already known in *Drosophila melanogaster* for stimulating the germ stem cell proliferation. Our work is focused on the epidermal growth factor receptor (EGFR) using as a model the cockroach *Blattella germanica*, a species with panoistic ovaries, the most primitive ovary type. In previous work, we found that the depletion of EGFR determines an increase in the number of germinal cells and our objective is to unveil a possible interaction between EGFR signaling and ecdysone, an interaction that must be regulating ecdysone biosynthesis or its signaling gene cascade. To measure the activity of the ecdysone biosynthesis, we pay special attention to the expression of Shadow an enzyme that triggers the final step in the biosynthesis of ecdysone. To unveil these interactions, we use the RNAi methodologies to deplete the expression levels of EGFR and Shadow, observing at microscopic levels how this depletion could affect the differentiation of the germinal cells in the germarium and by qRT-PCR quantifying the expression of those genes that can be implicated in this process.

P8. Proteasomal degradation of naturally occurring glutamine-rich peptides

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The ubiquitin proteasome system (UPS) is a central player in eukaryotic protein homeostasis. In a cellular context, ubiquitin activating, conjugating and ligating enzymes act sequentially marking the substrates for their degradation in the proteasome. In this way, ubiquitin serves as a common recognition tag among most of the proteasome substrates. As part of the UPS system, the proteasome acts as a protein degrading hub for the cell, integrating degradative signals from multiple ubiquitin ligase enzymes. This tag, however, is not imperative for protein degradation. Instead, it has been described that location to the proteasome is sufficient for protein degradation, given that the substrate features an unstructured region able to interact with the inner ATPases of the proteasome. When protein homeostasis fails, the whole cell functioning is impaired. This is often associated with cell stress, toxicity and death. Here we investigate the interaction between proteasome and disease related proteins such as huntingtin or ataxin-3. These proteins have a common trait; they all feature glutamine rich regions. In this project we propose that these characteristic domains can serve as signal sequences able to direct the proteins to the proteasome in an ubiquitin independent way. Direct targeting of toxic proteins to the proteasome serves as a model to study how aggregation prone proteins affect the proteasome system. Additionally, it is to be studied how replicating these proteasome modifications in eukaryotic cells could shed some light into new treatment for the above-mentioned neurodegenerative diseases.

P9. *In vivo* and *in vitro* models in the study of the FGF23 regulation

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Introduction: Chronic kidney disease (CKD) is a major public health problem. The fibroblastic growth factor 23 (FGF23) is a fosfaturic protein that is expressed predominantly in osteocytes and whose levels are increased in patients with CKD. The possible regulation of FGF23 by calcium and phosphorus independently remains unknown. The objective of this study is to establish the models that allow us to study it. **Materials and methods:** A. Para-thyroidectomy model (T-PTX): The animals underwent a para-thyroidectomy (T-PTX) and a tyrosine hormone replacement therapy (T4). B. Hypercalcemia model: Intraperitoneal injection of calcium gluconate monohydrate (250 mg/kg every 2 hours during 8h). C. Hypocalcemia model**:** Intramuscular injection of EDTA (150 mg/kg). The animals were sacrificed 6 hours later. *In vitro*: Primary cultures of bone marrow mesenchymal stem cells (MSCs), differentiated by a conditioned medium. **Results and conclusion:** Mice subjected to T-PTX showed a reduction in calcium levels, as well as an increase in serum phosphorus levels. In mice undergoing hypercalcemia, a significant increase in the excretion of calcium in urine was observed without changes in serum calcium levels. In mice subjected to hypocalcemia, a decrease in calcium levels was observed in both serum and urine. Finally, in *in vitro* differentiated cells (MSCs differentiated to osteocytes-like) there was an increase in gene expression of mineral matrix markers such as OSC, OSX, RUNX2, OPN and FGF23, as well as a decrease in mesenchymal markers such as COL I. We can conclude from the results obtained that the T-PTX was successful and can be used for the study of the regulation of FGF23 independently of PTH. On the other hand, models of hyper-and hypocalcemia in the doses studied exert the desired effects on the regulation of calcium levels. The individual combination of these two models with the T-PTX will allow us to study the role of calcium independently of PTH in the regulation of FGF23.

P10. The effect of stress in the retinal cells of a *Cerkl* mouse model generated by CRISPR/Cas9

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Retinal neurodegeneration, characterized by the apoptosis of photoreceptor cells, is the major cause of genetic blindness. So far, mutations in over 200 genes are associated with inherited monogenic retinal diseases (1:3000 worldwide), but we are still far from completely understanding their ethiopathology. Therefore, animal models and *in vitro* cell culture assays are essential to characterize the precise role of *CERKL*, a causative gene of retinal dystrophies. The physiological function of CERKL is yet to be determined, but it is related to stress cell resilience since its overexpression protects cells from the apoptosis triggered by oxidative stress. We generated a mouse model by causing the full deletion of the *Cerkl* gene using CRISPR/Cas9. in order to investigate the retinal effects in the oxidative/light stress response. Unexpectedly, complete ablation of *Cerkl* causes perinatal lethality in homozygosity. Therefore, to approach the CERKL function in the retina, we have generated a heterozygous knockdown/knockout mouse model ($Cerkl^{KD/KO}$) in an albino background to perform *in vivo* light stress experiments. This model is viable and fertile, and the expression of *Cerkl* has been reduced to 18%. Our results showed that CERKL localized to the stress granules formed the retina in response to stress. Moreover, the number of stress granules was notably higher in the retinas with reduced CERKL expression compared to those of wild type mice, thus supporting the CERKL role in the protection and maintenance of retinal cells.

P11. The role of ASK1 in Drosophila's wing disc regeneration

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Recent work has strengthened Drosophila imaginal discs as a model system for regeneration studies. Evidence is accumulating that oxidative stress drives the cellular responses for repair and regeneration. Apoptotic cells result in a burst of reactive oxygen species (ROS) that can propagate to neighboring cells. These ROS are known to activate JNK and p38 kinases for regenerative growth.Two key issues arise from these observations. The first is how the ROS are propagated. The second, what is the link between ROS and the stress activated protein kinases p38 and JNK. Our results reveal that Ask1 senses ROS differently in apoptotic cells and living surrounding cells. High levels of ROS are produced in apoptotic cells, which in turn generates high activity of Ask1 that turns on JNK, which is known to enhance apoptosis. Neighboring undamaged cells show low ROS levels, which are beneficial for the regenerating tissue. In these, Ask1 is activated but its activity is attenuated by Pi3K dependent Akt1 phosphorylation, as a survival signal that results in beneficial levels of p38 and JNK. Our data reveal a non-autonomously activated ROS sensing mechanism driven by Ask1 and Akt to drive regeneration in the neighboring unstressed cells.

P12. Gene-Repair of point mutations at the endogenous locus using PolyPurine Reverse Hoogsteen hairpins in mammalian cells

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Repair of point mutations in the DNA is important for the correction of monogenic diseases. Repair-PPRHs are a new powerful tool within this field. A Repair-PPRH consists of a PPRH hairpin core bearing an extension sequence at one end, which is homologous to the DNA sequence to be repaired but containing the wild type nucleotide instead of the mutated one. Previously, we designed Repair-PPRHs to correct deletions, insertions, substitutions and a double substitution present in a collection of Chinese hamster ovary (CHO) cell mutants in the endogenous *locus* of the *dhfr* gene. Surviving colonies in a DHFR selective medium (lacking glycine-hypoxanthine-thymidine) were analyzed by DNA sequencing, mRNA and protein levels and enzymatic measurements, confirming that all the *dhfr* mutants had been corrected. To explore the generality of this methodology, we attempted to repair point mutations in a different gene, that coding for adenosyl phosphoribosyl transferase (*aprt*). By using different Repair-PPRHs we were able to correct nonsense mutations caused by a single substitution. In this case, surviving colonies were obtained by applying the AAT (adenine-aminoptherine-thymidine) selection and were analyzed by DNA sequencing and by mRNA expression. No off-target effects were detected when comparing the S23 mutant and the S23 repaired with HpS23E1rep-L after sequencing with a mean coverage of 26x. These results correspond to the set of 3158 contigs longer than 100Kb totaling around 90% of the genomic sequence (2.2 Gb), indicating no major mutational differences between the two samples. We did not see either any major bias when looking at the indels (insertion and deletions together) or only at the insertions in the treated cells. Moreover, any of the insertions detected within the variation set had similarity to the sequence present in the Repair-PPRH used for the treatment. These results demonstrate that Repair-PPRHs are able to achieve a permanent correction of point mutations in the DNA sequence of mammalian cells.

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P13. Early *hippo* target genes in planarians

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Regeneration and tissue renewal are essential processes of adult homeostasis that must be tightly controlled, since its dysfunction may lead to cancer. The Hippo signaling pathway has recently emerged as a key hub in the control of cellular renewal being found systematically deregulated in tumoral processes. Several studies in animal models demonstrate the essential role of the Hippo pathway in the control of cell proliferarion, cell death and cell differentiation. However, the specific cellular function and molecular targets of the pathway remain poorly understood. Recent studies in our lab have demonstrated that *hippo* inhibition produces tumoral overgrowths in planarians, flatworms that endow a continuous tissue renewal while changing their size according to nutrients availability. In this *in vivo* context, we demonstrated that overgrowths are caused by the inability of *hippo*knockdown cells to maintain the differentiated fate, to properly cycle and to die when required. In the present study, we characterize the function of putative *hippo* target genes found deregulated in a transcriptomic analysis of *hippo* RNAi animals. The RNAi inhibition of some of these early target genes produced overgrowths similar to the ones observed after *hippo* inhibition. This connection hinters potential effectors contributing to the tumorigenic mechanisms upon *hippo* inhibition in planarians and, probably, in humans.

P14. Organ remodeling through the actomyosin cytoskeleton and programmed cell death: the trachea of Drosophila melanogaster as a case study

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Many cellular processes are in play during organ remodeling. Most of the attention has focused on the role of progenitor cells and their transformation into adult tissues and organ growth, however, little is known about the mechanisms of organ regression. An example of this process is the removal of epithelial cells during mammary gland involution at weaning. The fruit fly Drosophila melanogaster offers an advantageous system to study organ remodeling at the cellular and molecular level. The larval trachea, a functional respiratory organ, is a modular network of tubes that must remodel during metamorphosis to give rise to the adult trachea. Our current work focuses on the cellular and molecular mechanisms that orchestrate the reduction in size of the main tracheal tube, the dorsal trunk. Concomitant to the migration of progenitor cells, the modules (i.e. metameres) of the dorsal trunk undergo a sequential reduction in length that correlates with a reduction in apical cell area and the appearance of actomyosin filaments along the longitudinal axis of the tube. Upon maximum reduction in tracheal length, we observe caspase activation and a consequent loss of posterior metameres of the dorsal trunk. These events result in a considerable reduction in size of the larval trachea at the same time that progenitors migrate to form the abdominal adult trachea. We are currently exploring the interplay between cell size reduction through actomyosin contractility and the activation of programmed cell death through apoptosis. We believe that such cellular and molecular interplay leads to the regression of the tubular dorsal trunk during metamorphosis of Drosophila melanogaster, as it occurs in other flat epithelial monolayers.

P15. Generation of Sanfilippo C syndrome cellular models using CRISPR/Cas9 system

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Sanfilippo syndrome is a rare lysosomal storage disorder (LSD) caused by mutations in genes that encode enzymes involved in heparan sulfate (HS) degradation. The accumulation of HS, due to mutations in these genes, causes a progressive and severe neurodegeneration in patients, leading to an early death. There is currently no cure for this disease. The main objective of this project is to generate cellular models for Sanfilippo C syndrome using induced pluripotent stem cells (iPSC). For that purpose, we used CRISPR/Cas9 system to knock-out (KO) the *HGSNAT* gene in iPSC cells, whose mutations cause Sanfilippo C syndrome. Moreover, we optimized a protocol to differentiate iPSC into neurons, the most affected cell type in this syndrome. Combining these two novel technologies, we will be able to obtain KO and WT differentiated neurons in order to analyze differences between these isogenic lines. With this approach, possible variations due to differences in the genetic backgroundWe aim to compare levels of HS storage between lines by immunocytochemistry. Furthermore, branching and spines measurements will be performed in GFP+ isolated neurons. To refine the analysis, synapses will be quantified combining specific antibodies for pre- and post-synaptic markers. To confirm that the phenotypes observed in the KO neurons are only caused by *HGSNAT* disruption, we will carry out a rescue experiment transfecting KO cells with a plasmid bearing the *HGSNAT*-WT cDNA. After transfection, we will perform all the assays in order to test whether or not the WT phenotype has been restored. We are confident that once this cellular model is validated, it will be valuable to test potential treatments such as the use of shRNAs as a substrate reduction therapy.

P16. Role of Aquaporins in ROS diffusion following damage

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Drosophila wing imaginal discs are able to regenerate following different types of injury, which leads to the reconstruction of normal adult wings. Recent studies pointed out the importance of oxidative stress in driving the cellular response for wound repair and regeneration. Upon apoptotic stimuli or physical injuries, a burst of reactive oxygen species (ROS) is generated in the wing disc epithelium. This results in the activation of different signalling pathways (like JNK and p38) that are required for regeneration. A key issue is to unveil how reactive oxygen species (H_2O_2 in particular) spread from cells that are committed to die to the surrounding ones, however the mechanism of ROS propagation is poorly understood. In this study, we focus on two different *Drosophila* aquaporins: AQP and Drip as putative mechanisms of oxidative stress propagation. Our results indicate that, in stressing conditions, AQP is a key element of cell-to-cell communication facilitating ROS diffusion across membranes, thus allowing the onset of the regenerative stimulus. Interestingly, while AQP loss of function impairs the regenerative process, no significant defects were observed with Drip, suggesting that the function in regeneration is not shared among all aquaporins.

P17. New Cyclin D1 cytoplasmic interactors

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The cell cycle is a set of well-ordered events that allows the cell to divide into two cells genetically identical. The different stages of this cycle depends on the activity regulation of cyclins and cyclin dependent kinases (Cdk) complexes. For instance, cyclin D1 (Ccnd1)-Cdk4 complex accumulates into the cell nucleus and phosphorylates and inactivates the Retinoblastoma (RB1) transcriptional repressor. This allows the induction of a group of genes that promote progression from G1 into S phase. For many years, export of Ccnd1 to the cytoplasm was viewed as a mechanism to prevent the cell cycle entry and to reduce cellular proliferation. More recently it has been described that Ccnd1 physically interacts with proteins associated to the cell membrane, such as filamin A, PACSIN2, Ral GTPases and paxillin. Furthermore, our group has described that Ccnd1-Cdk4 complex modulates cell adhesion, migration and invasion through the regulation of some of those cytoplasmic proteins. In our lab, we have recently carried out an iTRAQ-based protein analysis that has produced a set of cytoplasmic proteins associated to the cell membrane that are new candidate interactors of Ccnd1. Among these there are a number of proteins involved in cell signalling. Here we report the validation of some of these candidates (PGRMC1, Dab2IP and Plekhh2) by co-immunoprecipitation with Ccnd1. Specifically, PGRMC1 protein (Progesterone Receptor Membrane Component 1) interacts with the N-terminal region of Ccnd1 and promotes its stabilization. In experiments in HEK 293T cells with the protein synthesis inhibitor cyclohexmide, we have observed that expressing PGRMC1 increases Ccnd1 half-life. In conclusion, we show preliminary data pointing out to a possible function of cytoplasmic Cyclin D1 in the regulation of cell signalling.

P18. Identifying wiring specificity mechanisms: what's up with the mTOR pathway?

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A fundamental requirement in the assembly of neural circuits is that neurons establish synaptic connections with their appropriate partners. In many systems, this involves extension into a particular synaptic layer, and selection of the appropriate partner among all the cells in the layer. Virtually nothing is known about the mechanisms governing the establishment of specific connections in any system. Our hypothesis is that the molecular differences that exist between neuronal subtypes, with similar developmental origin and function, contribute to their distinct connectivity. We study the differential layer selection of the closely related R7 and R8 photoreceptors. Each eye contains 750 R7 and 750 R8 cells, and the entire population of each subtype proceeds synchronously to their respective final synaptic layer during pupal development. Taking advantage of such precise coordination, we have profiled the R7 and R8 transcriptomes right before this final extension. Our bioinformatic analysis has identified differentially expressed genes between the R7 and the R8. We have focused on 229 R8 enriched genes and performed an RNAi screen. Out of 186 genes analyzed we have identified 44 candidate genes showing layer selection defects. One of them is 4E-BP, an inhibitor of translation best known for its role in the mTOR pathway. Interestingly, the mTOR pathway has been shown to have a neurogenic role uncoupled from its well-known control of cell proliferation and growth. Following this lead we present future work exploring the role of 4E-BP in wiring specificity through detailed characterization of the mutant phenotype and genetic interactions with other members of the pathway.

P19. Study of sumoylation of *TRIM28* variants associated with intellectual disability

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Intellectual disability (ID) is characterized by high clinical and genetic heterogeneity. Sporadic cases of ID are frequently related to *de novo* mutations in neurodevelopmental genes. Our group has identified a *de novo* variant (p.P654L) in the *TRIM28* gene in a sporadic case of ID as the most likely causative variant; two additional ID patients harboring *TRIM28* mutations (p.L708P and p.A180V) have been identified through *Genematcher*. *TRIM28* is a nuclear corepressor for KRAB domain-containing zinc finger proteins (KRAB-ZFPs) expressed in several tissues, including the brain. TRIM28 mediates gene silencing by recruiting CHD3, a subunit of the nucleosome remodeling and deacetylation (NuRD) complex, and SETDB1, which specifically methylates histone H3 at 'Lys-9' (H3K9me), to the promoter regions of KRAB target genes. Sumoylation/desumoylation events regulate *TRIM28*-mediated transcriptional repression, as sumoylation is required for interaction with CHD3 and SETDB1 and the corepressor activity. The mutation we detected in this patient is located in the PHD-type zinc finger domain, which directs the sumoylation of the adjacent bromodomain required for gene silencing. Two different mutations in the same domain (p.C651A and p.L709A) have previously been shown to affect *TRIM28* sumoylation. We hypothesize that the mutations detected in the ID patients will affect *TRIM28* sumoylation, and thus its repression of gene expression. In order to prove this hypothesis, we are performing directed mutagenesis and sumoylation analysis of the mutant forms of TRIM28.

P20. DNA activates the Nse2/Mms21 SUMO E3 ligase in the Smc5/6 complex

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Modification of chromosomal proteins by conjugation to SUMO is a key step to cope with DNA damage and to maintain the integrity of the genome. The recruitment of SUMO E3 ligases to chromatin may represent one layer of control on protein sumoylation. However, we currently do not understand how cells upregulate the activity of E3 ligases on chromatin. Here we show that the Nse2 SUMO E3 ligase in the Smc5/6 complex, a critical player during recombinational DNA repair, is directly stimulated by binding to DNA. We have identified a cluster of lysine residues in the coiled coil of Smc5 able to promote DNA-dependent upregulation of the Nse2 SUMO ligase activity. To test the relevance of these residues in budding yeast, we introduced different combinations of lysine to glutamic acid mutants to countercharge binding to phosphate groups of DNA. Using these *smc5-KE* mutants we show that compromising DNA binding to Smc5 sensitizes yeast cells to MMS-induced DNA damage. Accordingly, sumoylation of Smc5 itself and the Nse2-target Sgs1 protein (a homologue of the Bloom's and Werner's syndrome genes and a member of the STR complex) is impaired in *smc5-KE* cells. These defects are not due to defective binding of Nse2 to Smc5 or to impaired loading of Smc5/6 onto chromatin in *smc5-KE* alleles. Overall, we conclude that a positively charged patch in the Smc5 molecule acts as a DNA sensor in yeast, able to interact with DNA and to promote the activity of the Nse2 SUMO ligase thus ensuing repair of DNA damage. We propose that this mechanism restricts sumoylation in the vicinity of those Smc5/6-Nse2 molecules directly engaged on DNA.

P21. Role of Myoglianin in metamorphosis of *Blattella germanica*

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The onset of insect metamorphosis occurs when juvenile hormone (JH) titer decrease in the pre-adult stage. In *Blattella germanica*, this is determined in the last nymphal instar (N6). At the beginning of N6, JH production declines, the expression of Kr-h1 (a transducer of the JH anti-metamorphic action) is concomitantly reduced, and that of E93 (an adult specifier factor) starts increasing. However, it remains unclear what determines the initial decline of JH production. In the cricket *Gryllus bimaculatus*, Ishimaru and coworkers (2016) demonstrated that Myoglianin (*myo*), a homolog of *Drosophila* Myoglianin/vertebrate GDF8/11, suppresses the expression of *jhamt*. *jhamt* is a gene that encodes the enzyme JH acid *O*-methyltransferase (JHAMT), which catalyzes the last step of JH synthesis in the corpora allata (CA), which are the JH producing glands. When *myo* expression is suppressed in last nymphal instar of the cricket, expression of *jhamt* became activated and metamorphosis is inhibited. In *B. germanica*, high expression levels of *myo* were observed in the CA of the penultimate nymphal instar (N5). Moreover, using RNAi approaches, we showed that *myo* depletion in N5 up-regulated *jhamt* expression in N6, and metamorphosis became inhibited. The *myo*-depleted insects molted to a supernumerary nymph (N7) instead to molt into adults. The results suggest that high *myo* expression in N5 promotes the initiation of metamorphosis in N6 through the cessation of JH synthesis. In addition, our results also suggest a conserved role of *myo* in regulating JH production and metamorphosis, at least in hemimetabolan insects.

P22. The Spectraplakin Short-Stop is an essential microtubule regulator mediating subcellular branching

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Branching networks are a very common feature of multicellular animals and underlie the formation and function of numerous organs including the nervous system, the respiratory system, the vasculature and many internal glands. These networks vary from subcellular structures such as dendritic trees to large multicellular tissues such as the lungs. The production of branched structures by single cells, which has been better described in neurons and in cells of the respiratory and vascular systems, involves complex cytoskeletal remodelling events. In *Drosophila*, tracheal system terminal cells (TCs) and nervous system dendrites are models for these subcellular branching processes. During tracheal embryonic development, the generation of subcellular branches is characterized by extensive remodelling of the microtubule network and actin cytoskeleton, followed by vesicular transport and membrane dynamics. We have recently shown that centrosomes are key players in the initiation of subcellular lumen formation where they act as microtubule organizing centres (MTOCs) (Ricolo, et al. Cur. Biol. 2016). However, not much is known on the events that trigger the formation of these subcellular branches or what makes them choose a particular trajectory within the cytoplasm of the TC. We have identified that the spectraplakin *Shortstop* (s*hot*) is involved in the microtubule stabilisation events that lead to the formation and extension of the subcellular lumen. We observed that an excess of s*hot* induces more branching points in the embryonic tracheal TC leading to cells with extra subcellular lumina and that a *shot* loss-of-function leads to cells deficient in *de novo* subcellular lumen formation. In addition, we show that *shot* expression is intimately linked to the tip-cell fate, being modulated by the transcription factor DSRF.

P23. Differential expression of piRNAs during the ontogeny of Blattella germanica, a short germ-band, hemimetabolan insect

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Genomic stability is especially important in germ-line cells and in embryo stages for the insect development. For this reason the insect needs to control transposable elements (TEs). There is an evolutionary conserved mechanism, called piwi-interacting RNA (piRNA) system to control TEs. This system is common within species among metazoans and insects. To study piRNA system it is more suitable to use a long genome with many TEs. Thus, we use the hemimetabolan insect Blattella germanica as a reference model. In the present work we have identified and studied the expressed piRNA in B. germanica across 11 developmental stages, with a stringent process that includes non-fertilized egg, embryo, nymphs and adult females. We have been able to classify the piRNAs from B. germanica based in their biogenesis (primary and secondary pathways). We found that the majority piRNAs identified are generated from the primary pathway, although there are a small but highly expressed set of piRNAs participating in the secondary ("Ping-Pong") reamplification pathway. Furthermore, we have analysed the expression pattern of all these piRNAs identified, observing that the expression of piRNA generated in the "Ping-Pong" pathway is quite restricted to early embryo stages. In addition, an important number of piRNA clusters are exclusively expressed in late embryo and nymphal stages. This study contributes to confirm the role of the piRNA system controlling TEs in early embryogenesis, but also to show that the function of piRNAs are wider than previously thought, as we found different expression of piRNAs in different stages of development.

P24. Involvement of AGT1 in the cystinuria mouse model Slc7a9

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Cystinuria (OMIM #220100) is a rare inherited aminoaciduria characterized by urine hyperexcretion of cystine and dibasic amino acids (lysine, arginine and ornithine). Its clinical manifestation is cystine lithiasis in the urinary system due to the low solubility of cystine at physiological urine pH that induces its precipitation and, as a consequence, stone formation. Cystinuria, with a prevalence of 1:7000 births, is the most common primary inherited aminoaciduria and, to date, genetic alterations in the renal amino acid transporters, *SLC7A9* ($b^{0,+}$AT) and *SLC3A1* (rBAT) have been identified as responsible for this manifestation. However, the lack of genotype/phenotype correlation in cystinuria patients justifies the search for modulating genes. AGT1, the second renal cystine transporter encoded by *SLC7A13*, heterodimerizes with rBAT in the renal apical membrane where mediates efflux of anionic amino acids in exchange for cystine. Urine aminograms, renal expression of rBAT and AGT1 analyzed by western blot, analysis of *Slc7a13* mRNA by RT-PCR in WT and mutant (*Slc7a9*$^{-/-}$) mice of both sexes were compared to study AGT1 contribution in amino acid reabsorption. Significant differences were observed in CssC, Glu, Asp urine concentration among genders. Male mice have 30-40 times more rBAT protein than females and, as previously shown, female mice showed no AGT1 protein in kidney BBMs although *Slc7a13* mRNA was detected in kidney preparations.

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P25. Wiring specificity and cell type specific signaling downstream of widely expressed cell surface molecules: the cytoplasmic molecule Espinas

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The exquisite precision with which neurons assemble into neural circuits has fascinated neuroscientists since Ramon y Cajal described how different types of neurons established connections with specific synaptic partners. Both biochemical and genetic approaches have revealed the significant role of cell surface molecules (CSM) in wiring specificity since they are final effectors of cell-cell contact events. However, while most CSM are widely expressed, they have specific roles in some neurons and not others. Some of these situations have been explained by CSM working in distinct combinations. Alternatively, specificity could come from differential expression of other types of molecules with the potential to regulate the function and/or signaling of CSM, such as for example intracellular cytoplasmic molecules. To address wiring specificity we use as a model system the Drosophila R7 and R8 photoreceptors, closely related neurons with differential layer selection. Through RNAseq transcriptional profiling comparisons of these two cell types we have identified the cytoplasmic molecule Espinas (Esn) significantly enriched in R8 versus R7 and characterized R8 targeting defects in *esn* mutants. Esn has been shown to physically and genetically interact with the atypical cadherin CSM Flamingo (Fmi) in Da neurons were it regulates dendritic self-avoidance. Interesting, Fmi is widely expressed in the fly visual system where *fmi* mutations result in R8 targeting phenotypes while R7 targeting is not affected. Our results suggest that Fmi and Esn also work together in R8 targeting. Our working hypothesis is that Esn cytoplasmic signaling could explain the differential functional outcome of R8 versus R7 Fmi expression, highlighting the importance of cytoplasmic molecules in wiring specificity.

P26. Identification and molecular characterization of adenosine A_2A—cannabinoid CB_1 receptor heteromers in the dorsal striatum as targets for Hungtinton disease

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The dorsal striatum is a key node for many neurobiological processes such as motor activity, cognitive functions, and affective processes. The proper functioning of striatal neurons relies critically on metabotropic receptors. Specifically, the main adenosine and endocannabinoid receptors present in the striatum, ie, adenosine A_{2A} receptor ($A_{2A}R$) and cannabinoid CB_1 receptor (CB_1R), are of pivotal importance in the control of neuronal excitability. Facilitatory and inhibitory functional interactions between striatal $A_{2A}R$ and CB_1R have been reported, and evidence supports that this cross-talk may rely, at least in part, on the formation of $A_{2A}R$-CB_1R heteromeric complexes. However, the specific location and properties of these heteromers have remained largely unknown. Here, by using techniques that allowed a precise visualization of the heteromers in situ in combination with sophisticated genetically modified animal models, together with biochemical and pharmacological approaches, we provide a high-resolution expression map and a detailed functional characterization of $A_{2A}R$-CB_1R heteromers in the dorsal striatum. Specifically, our data unveil that the $A_{2A}R$-CB_1R heteromer (i) is essentially absent from corticostriatal projections and striatonigral neurons, and, instead, is largely present in striatopallidal neurons, (ii) displays a striking G protein-coupled signaling profile, where co-stimulation of both receptors leads to strongly reduced downstream signaling, and (iii) undergoes an unprecedented dysfunction in Huntington's disease, an archetypal disease that affects striatal neurons. Altogether, our findings may open a new conceptual framework to understand the role of coordinated adenosine endocannabinoid signaling in the indirect striatal pathway, which may be relevant in motor function and neurodegenerative diseases.

P27. Transcriptome analysis of Salmo trutta. Dating the whole genome and local duplication events in Salmonidae and identifying positive selected transcripts in salmo speciation

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Understanding animal adaptation and its underlying molecular basis is an important role in evolutionary biology. A prevailing hypothesis is that such adaptation has been favored by gene duplication events that provide large amounts of material for evolutionary adaptation. Evolution in vertebrates is marked by numerous duplication events, both whole-genome duplication (WGD) and local gene duplications (LGD) as well as gene losses. One example of recent whole-genome duplications in vertebrate is the salmonid-specific WGD, which occurred subsequent to the common teleost WGD event. It is assumed that WGD provided the teleost with diversification potential that can become effective much later, such as during phases of environmental change. In this study, genome-wide and local gene duplication events in the salmonids were investigated. A de novo transcriptome sequencing strategy was used to characterize the
transcriptomes of two key organs of Salmo trutta: telencephalon and muscle. We identified over 140000 sequences. Potential unique expressed transcripts were annotated
by sequence homology to databases and tissue expression was determined. An analytic workflow was designed to distinguish between orthologues and paralogues originated from LGD or from WGD events. The phylogenetic reconstruction and dating of these events was performed using the Ks ratio of neutral mutations between species. The identification of positive selected transcripts was performed using Ka/Ks ratio. Result allow dating both teleost's WGD and salmonid's WGD. In addition, we found evidence for LDG took place shortly after the speciation event. From Salmo trutta – Salmo salar speciation event a total of 158 positive selected transcripst (Ka/Ks>0.5) were characterized by anatomical structure (n=24), KEGG pathways (27) and GO orthology (101). Enrichment for Xenobiotics and Glycan metabolism pathways was detected among selected transcripts.

P28. Role of the transcription factor E93 in the oogenesis of *Blattella germanica*

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E93, also known as “adult specifier”, is the key gene for metamorphosis in both holometabolous and hemimetabolous insects. By using *Blattella germanica* as a model of hemimetablolous insect with the most ancient type of ovaries (panoistic type), our aim is to study the function this transcription factor has in both the capacitation and maturation of the ovaries, the main processes involved in the oogenesis. The expression of E93 is depleted by using interference RNA technique during the last nymphal stage. The depletion of E93 results in a change in the adult phenotype which makes us describe the adults obtained as intermediate adults (Ai), because they show both nymphal and adult structures. The ovarian changes due to the E93 depletion are also studied by immunofluorescence. We also study the changes that this depletion may cause to the other components of the Juvenile Hormone (JH) signalling pathway and the Ecdysone signalling pathway, in which E93 plays a regulation role. Ai, where JH is not produced, provides us with a unique animal model where we can study the effects E93 has in the ovary development in a JH free system.

P29. *tbx5a* in left/right asymmetry in zebrafish

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Tbx5 is a transcription factor expressed in the developing heart, eyes and anterior appendages. Mutations in human TBX5 cause Holt-Oram syndrome, a condition characterized by heart and upper limb malformations. In our lab, we identified a novel *tbx5* gene in zebrafish (*tbx5b*) that is co-expressed with its paralogue (*tbx5a*). *tbx5* paralogues downregulation in zebrafish revealed that *tbx5* genes have essential roles in the establishment of cardiac laterality, dorsoventral retina axis organization and pectoral fin development. Our data show that distinct relationships between *tbx5* paralogues are required in a tissue-specific manner to ensure the proper morphogenesis of the three organs in which they are expressed. Additionally, we uncover a novel role for *tbx5* in the establishment of heart asymmetry in zebrafish embryos. Further analysis of *tbx5a* role during left-right (LR) asymmetry establishment show that the expression of left-side markers expressed in the lateral plate mesoderm (LPM) is also randomized. We also detected a randomization of *lefty1* expression in the dorsal diencephalon in *tbx5a* morphant embryos and that the display of the endodermal structures of the liver, pancreas and gut is also affected after *tbx5a* morpholino knock-down.

To our surprise when we specifically knocked-down tbx5a in the DFCs/KV (dorsal forerunner cells / Kupffer's vesicle) lineage, responsible for LR asymmetry generation, cardiac jogging was randomized. Interestingly, we observed a stronger phenotype with this DFC-targeted injection. Furthermore, we detected by ISH and RT-PCR early *tbx5a* expression during gastrulation. Finally, a reduction on BMP signalling levels in DFC-targeted *tbx5a* morphants was observed and consistently, a putative binding site for tbx5 was found in the *bmp4* regulatory region after an *in silico* analysis, pointing towards a regulatory mechanism that would at least partially rely on BMP signalling as a downstream effector.

P30. Glial ionic homeostasis in brain development

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Glia-neuron interactions are crucial during development and the correct function of the adult nervous system. The mammalian CLCN2 chloride channel is widely expressed in the brain. While its expression in oligodendrocytes and astrocytes is related to K+ buffering in myelinated processes, its physiological role in other glial types during development is unknown. In humans, in addition to vacuolization and edema of the brain due to disruption of the myelin sheath, patients with mutations in *ClCN2* have cognitive defects and *CLCN2* has been linked to autism spectrum disorders. Together these observations suggest that *CLCN2* could play a role in the assembly if neural circuits. We turned to the Drosophila visual system as a convenient structure to address glial cell biology and glia-neuron interactions. We have detected expression of the *CLCN2* Drosophila homolog gene *ClC-a* in cortex glia and several other glial types in the developing brain. Mutations in *ClC-a* result in brain compartimentalization defects due to cortex glia impaired ionic function. We focused on the *ClC-a* expressing glial barrier, which acts a landmark for photoreceptor axon guidance. The glia-photoreceptor interaction in early development of the visual system is mediated by Slit/Robo signaling. Slit secretion from glial cells is necessary for the correct guidance of photoreceptors. Similar to optic lobe specific *slit* mutations, *ClC-a* mutant animals showed defects in photoreceptor axon guidance. Through detailed developmental analysis we have characterized the formation of the barrier, the glial types contributing to the barrier and their Slit expression in wild type and mutant animals. Our findings indicate that ClC-a is required during development for the correct assembly of the glial barrier and Slit signaling. We propose that, in addition to its relevance in adult brain physiology, glial ionic homeostasis is an important aspect of brain development.

P31. Monoamine oxidase A (MAOA) interaction with parenting practices on Callous-Unemotional Traits in Preschoolers

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Purpose: Monoamine oxidase A (MAOA) is a mitochondrial enzyme that catalyzes the degradation of several biogenic amines. A polymorphism in the *MAOA* gene (*MAOA*-uVNTR) results in differential enzyme activity: high-activity alleles (*MAOA*-H) could result in reduced dopamine, serotonin and norepinephrine availability in comparison to low-activity allele (*MAOA*-L). *MAOA*-uVNTR has been described to moderate the relationship between childhood maltreatment and aggressive and antisocial behavior. This study hypothesized that in interaction with other environmental factors such as parenting practices, *MAOA* might also be associated with increased callous-unemotional traits (CU) in preschoolers. CU traits have been associated with more severe antisocial behavior.

Methods: In a longitudinal study, data was collected from a sample of preschoolers through diagnostic interviews and questionnaires answered by parents and teachers. *MAOA*-uVNTR was genotyped in 368 Caucasian participants (51.9% male). Multiple linear regression analyses were conducted to analyze the interaction effect of *MAOA* genotypes and both positive parenting and punitive parenting practices on CU traits at two different periods (3 and 5 years old) and separately by sex.

Results: No significant interactions were found for boys. Among girls, a significant interaction effect was found for *MAOA*-LL carriers, who showed higher CU traits at age 5 when exposed to higher positive or punitive parenting.

Conclusions: This study provides the first evidence for significant MAOA × parenting effects on CU traits in preschoolers, specifically among female MAOA-LL carriers.

P32. Comparison between the operation of different kinases in the development of *Drosophila melanogaster*'s embryos

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The MAPK signalling cascades are key elements implementing cellular responses in numerous developmental and physiological processes. These cascades can act individually or in parallel and frequently display cross-regulatory interactions. Further, they implement negative and positive feedback loops that complicate the identification of their singular roles. Three main MAPK pathways, ERK, JNK and P38, have been identified characterized by the specificity of their phosphorylation targets. In order to obtain information on the functions of JNK, p38 and ERK, in the *Drosophila* embryo, we used kinase translocation reporters (KTRs). These reporters are peptides carrying a GFP fluorescent marker that when are phosphorylated by a specific kinase are exported out of the nuclei. Otherwise if this phosphorylation does not happen another a nuclear localization sequence is revealed and the fluorescent marker is translocated to the nuclei. We have generated transgenic animals carrying KTR reporters specific for JNK, ERK and P38 that can be expressed in different tissues and times using the Gal4/UAS system. Preliminary experiments targeting these sensors to the epithelia (Pannier-Gal4), the mesoderm (Mef2-Gal4) and both, the epidermis and the nervous system in restricted domains (En-Gal4) have let us to identify specific differences and dynamics in the activity of these three kinases in different tissues and processes. We are now in the way to characterize in more detail these differences. The use of these sensors will eventually allow us to infer cross-regulatory interactions between these pathways and with other signalling elements in epistatic analyses.

P33. Regulation of mitochondrial function through Hippo pathway signalling underlies tumoral transformation

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The Hippo pathway is a master regulatory network that regulates cell proliferation, cell death and cell differentiation in response to the environment. Its deregulation leads to the unbalance of this processes and to the establishment of a variety of diseases including neurodegeneration and cancer. Its targeting appears as a powerful medical tool to improve regeneration and to prevent tumoral transformation. However, its precise molecular outs and inputs remain poorly understood. Recent results from our lab demonstrate that inhibition of hippo in planarians, flatworms which continuously change their size and renew their tissues according to nutrition, leads to overgrowths. Those overgrowths are caused by a decrease in apoptosis, aberrant mitosis and the inability of cells to maintain the differentiated fate. With the aim to understand the hippo targets responsible for the tumoral transformation we performed a transcriptomic analysis of hippo RNAi animals. The results revealed an enrichment of differentially regulated genes with a mitochondrial function. RNAi inhibition of those genes produced overgrowths similar to the ones observed after hippo inhibition. We are currently analyzing whether inhibition of mitochondrial genes also deregulates apoptosis, mitosis and cell differentiation. Our findings support that mitochondrial function could be involved in the tumoral transformation that occurs after hippo inhibition.

P34. Analysis of ATPase-Dependent Binding of the Smc5/6 Complex onto Chromatin

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The three eukaryotic SMC complexes, cohesin, condensin and Smc5/6, play essential roles in genomic integrity. All SMC proteins display a rod-shaped structure with one ATPase domain at one end, connected to a hinge domain through a long coiled coil. Pairs of SMC proteins heterodimerize through their hinge domain. A kleisin subunit then connects the two ATPase heads at the other end, closing a ring-shaped structure able to entrap DNA. Previous work from our lab has shown that the Smc5/6 complex is essential to remove different type of sister chromatid junctions, including recombination and replication intermediates arising in response to replication fork damage. Their removal probably requires the engagement of Smc5/6 on chromatin. However, very little is known about what promotes Smc5/6 association with chromatin. Here we have analyzed the role of the ATPase and observed that binding of ATP is required for Smc5/6 loading onto chromatin. In fact, the ATPase activity is not only necessary to mediate the interaction with DNA but also to ensure its association with the kleisin subunit. Besides, we have observed that loading of Smc5/6 is maximal towards the end of the S phase, and can be induced by replication fork damage. Using various mutants affected in the metabolism of sister chromatid junctions, we show that binding of the complex onto chromatin is not triggered by the presence of junctions; in contrast, the complex seems to directly bind damaged forks, before they are channeled into different repair and bypass pathways. These findings suggest that the Smc5/6 complex binds early onto damaged forks in an ATPase-dependent manner, to promote the subsequent removal of junctions.

P35. Exploring the function of relevant retinal genes: animal and cellular models

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Cell fate decisions during the differentiation and maintenance of specific neuronal retinal cell types are controlled by transcriptional factors and other regulatory proteins. In order to shed light on the mechanisms regulating retinal development, we generated animal and cell models to study the role of two genes associated to human neurodegenerative disorders, namely *ATXN3*, which encodes a deubiquitinating enzyme involved in hereditary ataxia, as well as *NR2E3,* a retinal dystrophy gene that encodes a transcription factor required for cone and rod differentiation. The function of ATXN3 was analysed by observing the retinal phenotype of *Atxn3* knockout (KO) mice. A significant elongation of photoreceptor cilia and outer segments was observed in the KO retinas by immunofluorescence and transmission electron microscopy. These results were confirmed in an *in vitro* cell model, since silencing of endogenous *ATXN3* caused elongation of primary cilia. We are currently exploring the implication of ATXN3 in both cilia formation and control of phagocytosis, whose alteration is causative of retinal dystrophy. To address the function of NR2E3, we have generated several mutant alleles with small and large deletions of *Nr2e3* using the CRISPR/Cas9 system in mouse. The retinal phenotype of these mouse models is being currently analysed in wildtype, heterozygous and homozygous mouse littermates. Besides, in order to dissect the molecular function of the genome-edited alleles, *in vitro* cell models have been generated by transfection of minigenes designed to express the CRISPR-generated mutations. Our preliminary results show that both genes are relevant for retinal development and photoreceptor function.

P36. Injury, repair and regeneration of the Drosophila larval CNS

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The neuroblasts, the stem cells of Central Nervous System (CNS) of Drosophila undergo two waves of neurogenesis: the first one during embryogenesis, and the second one during the second larval stage L2. Those two waves are separated by a period of quiescence (a 24 hours window), during the first larval stage (L1) in which neuroblasts do not divide. This quiescent period gives us the opportunity of studying the reactivation of proliferative capabilities of stem-like cells during repair and regeneration. To target specific cells to necrotic death (mimicking traumatic injury), we employed the Gal4/Gal80ts system and overexpressed a mutated variant of the Glutamate Receptor (GluR1Lc), which directs the accumulation of intracellular calcium, the toxic death of cells and necrotic spreading. We alternatively employed Reaper, an apoptotic response inducer, to distinguish the behavior of the tissue to different types of insult. In first place, in search for Gal4 lines with distinct patterns of expression, we selected a set of lines (Flylight database (Janelia, HHMI)) and characterized their pattern of expression in first instar larvae. This let identifying lines active at this period with restricted patterns. We initiated our analyses employing the RN2 Gal4, which is just specifically expressed in the RP2, aCC, pCC and MP2 inter and motoneurons. Secondly, we set up genetic and dissecting protocols aimed to explore the response of the tissue. Death induction was temporally controlled and the tissue let to recover via temperature shifts. Activating expression during 12 hours at the beginning of the first instar larvae and letting them recover for another 16 hours we could monitor robust death responses. In third place, we initiated the characterization of the tissue recovery and potential regeneration by studying both cell proliferation and structural modifications at or around wounded areas. Interestingly, associated to local induced proliferation, we observed “scar-like” tubulin-rich structures. We are now set out to investigate what are those structures and how do they participate in wound recovery.

P37. The role of Wnt modulation in the derivation of mouse embryonic stem cell lines from single blastomeres isolated from 8-cell embryos

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Naïve pluripotency displayed by mouse embryonic stem cells (mESC) depends on the activation of the Wnt pathway and the inhibition of the MAPK pathway. On the other hand, epiblast stem cells, which show a primed pluripotency, depend on the inhibition of the Wnt pathway, suggesting that this pathway plays a critical role in maintaining both pluripotent states. In this study, the effect of signalling modulations on mESC derivation from isolated blastomeres from 8-cell embryos has been analysed. To do so, blastomeres were cultured with the Wnt activator CHIR99021 (CHIR), the Wnt inhibitor IWR-1-endo (IWR) and the MAPK inhibitor PD0325901 (PD), either alone or combined. Non-treated (NT) blastomeres were used as the control group. After the validation of the mESC established, Wnt transcriptional activation, assessed by AXIN2 levels, and the state of pluripotency of the mESC lines, assessed by FGF5 levels and alkaline phosphatase (AP) activity, were determined. Blastomeres from NT, IWR or CHIR groups resulted in the lowest derivation rates (1.4%-4.9%), whereas the combination of any two of the inhibitors increased the derivation rates (15%-24.7%). On the other hand, mESC lines derived from the NT, CHIR or CHIR-PD (2i) groups displayed high AXIN2 levels, corresponding to a high Wnt-transcriptional activity, whereas the lowest levels were displayed by mESC treated with IWR. Concerning FGF5 levels and AP activity, only mESC derived from CHIR- or 2i-treated blastomeres actually acquired the features of naïve pluripotency, showing basal levels of FGF5 and high AP activity. By contrast, IWR-CHIR and IWR-PD treatments induced the expression of features of the primed pluripotency state. Surprisingly, mESC derived from the NT, IWR or PD groups displayed features of both pluripotency states, suggesting that they are in an intermediate state between naïve and primed pluripotency. To conclude, the activity of Wnt pathway plays a key role in pluripotency maintenance in mESC lines established from single blastomeres, originating mESC lines displaying features of either naïve or primed pluripotent states by modulating this pathway.

P38. The regulatory genome of Drosophila regeneration

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The ability to regenerate varies greatly not only between species but also between tissues and organs or developmental stages of the same species. Differential activation of the genome, determined by a complex interplay of regulatory elements functioning at the level of chromatin, must be the initial mechanism behind these different regenerative capabilities. Resetting gene expression patterns during injury responses is, thus, shaped by the coordinated action of genomic regions that integrate the activity of multiple sequence specific DNA binding proteins. *Drosophila* imaginal discs, which show a high regenerative capacity after genetically induced cell death, are a great model to interrogate chromatin function trough the regeneration process. Using genome- wide approaches (RNA-seq and ATAC-seq) at different tissue time points after injury we have identified the regulatory elements and the expression profile dynamics governing the process. Our findings point to a global co-regulation of gene expression and provide evidence for a regeneration program driven by different types of Damage Responsive Regulatory Elements (DRRE). Among them, novel-DRRE are found acting exclusively in the damaged tissue, and cooperating with DRRE co-opted from other tissues and developmental stages. Altogether, our results decipher the regulome of regeneration and suggest the existence of a specific toolkit to drive the regenerative capacity.

4 de maig de 2018 ⇨ **Sala Pere i Joan Coromines**

4th MetNet International Annual Meeting

Organ crosstalk in the control of metabolism

Organitzadors: Joan-Marc Servitja, Rosa Gasa

Organitzada per la Secció de Senyalització de l'SCB

Coordinador: Marc Claret

Programa

8:30-9:00 Registration and documentation pickup

9:00-9:10 Welcome

Session I

Chair: Joan-Marc Servitja

9.10-9.50 **Marc Donath**,University of Basel, Switzerland
Crosstalk between resident immune cells and endocrine cells in pancreatic islets

9.50-10.30 **Maria Mittelbrunn**, CBMSO, Madrid
Immunometabolism in inflammatory diseases and aging

Short talks:

10.30-10.45 **OP1 Carlos Castaño,** CIBERDEM-IDIBAPS
Obesity-associated exosomal miRNAs modulate glucose and lipid metabolism in mice

10.45-11.00 **OP2 David Sebastian,** IRB
Regulation of mitochondrial plasticity by Mfn2 drives metabolic flexibility

11.00-11.30 Coffee Break

Session II

Chair: Laura Herrero

11.30-12.10 **Paula Mera,** Facultat de Farmàcia, UB, IBUB
Bone endocrine regulation of energy metabolism

12.10-12.50 **Manolo Tena-Sempere,** IMIBIC, Córdoba
Metabolism and reproductive function

Short talks:

12.50-13.05 **OP3 Rebeca Fernández-Ruiz,** CIBERDEM-IDIBAPS
Identification of wisp-1 as a young blood-borne factor that promotes adult pancreatic β cell proliferation

13.05-13.20 **OP4 Melisa Morcillo,** Facultat de Farmàcia, UB, IBUB
Activation of the Jun NH2-terminal (JNK) in pancreatic β cells protects against obesity-induced insulin resistance

13.30-15.00 Lunch

Session III

Chair: Rosa Gasa

15.00-15.40 **Sylvia Boj,** Foundation Hubrecht Organoid Technology, Utrecht, the Netherlands
Organoids as model systems for the study of metabolism

15.40-16.20 **Josep C. Jiménez-Chillarón,** IRSJD, Barcelona
Epigenome-wide association study in childhood obesity: Searching for new causal markers

16.20-17.00 **Mariona Graupera,** IDIBELL, Barcelona
Role of the endothelium in the control of metabolism

Short talks:

17.00-17.15 **OP5 Alicia G Gómez-Valadés,** IDIBAPS
Mitochondrial fusion protein Opa1 links mitochondrial dynamics in POMC neurons with fasting-induced adipose tissue lipolysis

17.15-17.30 **OP6 Marion Peyrou,** IBUB
Kininogen is involved in the remodeling of fat tissue

17.30-17.40 Closing act

17.40-19.30 Drinks and networking

P1. Obesity-associated exosomal miRNAs modulate glucose and lipid metabolism in mice

Carlos Castaño, Susana Kalko, Anna Novials, Marcelina Párrizas

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Maintenance of metabolic homeostasis relies on tissue cross-talk, which is disturbed by obesity. In addition to hormones and other soluble factors, cells communicate by releasing exosomes, small vesicles loaded with miRNAs that are detected circulating in blood and other biological fluids. The profile of exosomal miRNAs in blood depends on the physiological context. Hence, we focused on determining the changes induced by obesity in the profile of exosomal miRNAs in mice. In addition, we explored the role of these miRNAs in the establishment of glucose intolerance.

From a RT-PCR profiling of 378 miRNAs we observed increased levels of *miR-122*, *miR-192* and *miR-27a/b* in exosomes isolated by ultracentrifugation from the plasma of mice rendered obese by 15 weeks of high fat feeding. These data indicate that the development of glucose intolerance is associated with a modification of the population of circulating miRNAs. Surprisingly, the administration during 4 weeks of exosomes transfected with mimics of the aforementioned miRNAs induces glucose intolerance in control mice, which remain lean but show enhanced fat deposition in the epididymal depot. By bioinformatics analysis, we identified the transcription factor *ppara* as one of the main target genes of our miRNAs. Accordingly, in white adipose tissue of exosome-treated mice, the expression of *ppara* is decreased, and this is associated with a lower oxidative capacity, an increase in circulating free fatty acids and tissue inflammation. As a consequence, we observed increased expression of lipogenic genes in liver and hepatic steatosis. Liver damage is further reflected in an increase in plasma triglyceride levels.
Overall, our data suggest that obesity-associated exosomal miRNAs are novel mediators of tissue cross-talk and play an important role in the etiopathogeny of glucose intolerance and dyslipidemia.

P2. Regulation of mitochondrial plasticity by Mfn2 drives metabolic flexibility

David Sebastián, Antonio Zorzano

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Metabolic flexibility describes the ability of an organism to respond or adapt according to changes in metabolic or energy demands. Skeletal muscle plays a crucial role in energy metabolism, and therefore, has a deep impact in metabolic flexibility. In skeletal muscle, metabolic flexibility implies a good fuel selection either in the transition from fed to fasting state, switching from carbohydrate to lipid oxidation, or fasting to insulin stimulation, switching from lipid to carbohydrate oxidation. In this regard, several studies have shown that metabolic inflexibility in skeletal muscle is a key feature of insulin resistance and type 2 diabetes. Mitochondria are key organelles involved in metabolism and metabolic adaptation and therefore, they may have a prominent role in metabolic flexibility. Mitofusin 2 (Mfn2), a mitochondrial dynamics protein, is decreased in skeletal muscle of obese and type 2 diabetic subjects, and is essential for normal glucose homeostasis and healthy aging in mice. In addition, Mfn2 has been demonstrated to be crucial for a correct mitochondrial function and quality in muscle. Here we demonstrate that Mfn2 determines whole-body metabolic flexibility by controlling mitochondrial plasticity in skeletal muscle. Hence, Mfn2 protein expression is increased during fasting and it is necessary for metabolic switching from glucose to lipid oxidation in mouse skeletal muscle as well as in cultured muscle cells. These results strongly suggest that Mfn2 is an important factor in insulin resistance and type 2 diabetes by controlling metabolic flexibility in skeletal muscle.

P3. Identification of Wisp-1 as a young blood-borne factor that promotes adult pancreatic β cell proliferation

Rebeca Fernández-Ruiz, Ainhoa García, Yaiza Esteban, Berta Serra-Navarro, Joan Mir-Coll, Elena G Ruano, Ramon Gomis , Rosa Gasa

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Restoring functional β-cell mass is a current therapeutic goal in diabetes. One of the proposed strategies is to promote replication of remaining β-cells. Under normal physiological conditions, adult β-cells have a limited capacity to proliferate. This decline in replication appears to occur around weaning by mechanisms that are still poorly understood. We hypothesize that blood borne factors present in early postnatal life but absent in adulthood have an important role in this age-related decay. Identification of these factors is important to uncover novel switchers of adult β-cell replication.

In order to test whether circulating factors are important to maintain β-cell replicative activity, we performed transplants of mouse adult islets into the anterior chamber of 16-days (young) and 20-weeks old (adult) mouse recipients. Our results show that adult β-cells exhibit a significantly higher proliferation rate when transplanted in young recipients. We next compared serum from young and adult mice by using commercially available antibody arrays, revealing Wisp-1/CCN4 as one of the circulating factors that are more abundant in young than in adult serum. We confirmed our results by a specific ELISA, and surveyed *Wisp1* gene expression in several tissues in young mice, showing highest expression in bone. To test whether Wisp-1 impacts β-cell proliferation, we performed both *in vitro* experiments incubating adult islets with Wisp-1 recombinant protein, and an *in vivo* approach increasing Wisp-1 levels in the circulation of adult mice using an adenovirus-overexpressing system. In both cases we obtained a significant induction of adult β-cell proliferation.

Our results provide evidence that Wisp-1 promotes proliferation of adult β-cells, hence supporting the idea that young blood borne factors may be a useful strategy to modulate the intrinsic ability of β-cells to proliferate later in life.

P4. Activation of the Jun NH2-terminal kinase (JNK) in pancreatic β-cells protects against obesity-induced insulin resistance

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Obesity is epidemic worldwide and tightly associated to insulin resistance. Given that insulin resistance is an early trait in type 2 diabetes, obesity is a major risk factor for the development of this disease. It is well established that obesity-induced JNK activation inhibits insulin receptor signalling and, hence, promotes insulin resistance. Taking advantage of a genetically modified mouse model, MKK7D mice, we have previously shown that the activation of JNK in pancreatic β-cells interferes with the insulin paracrine action, thereby leading to a glucose intolerant phenotype due to impaired insulin release in response to hyperglycemia. Now, we show that MKK7D mice fed with a high fat diet develop obesity and glucose intolerance to a similar extend than control mice. In contrast, MKK7D mice are protected from the development of obesity-induced insulin resistance, as shown by insulin tolerant test and insulin-induced AKT activation in adipose tissue, as well as from hyperinsulinemia. Therefore, MKK7D mouse is one of the few examples in which obesity is dissociated from insulin resistance and hyperinsulinemia.

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P5. Mitochondrial fusion protein Opa1 links mitochondrial dynamics in POMC neurons with fasting-induced adipose tissue lipolysis

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Hypothalamic neurons expressing pro-opiomelanocortin (POMC) are key players in energy homeostasis. Mitochondrial dynamics in POMC neurons represent a nutrient-sensing mechanism connecting energy status with systemic metabolic adjustments. Mice lacking Opa1 (an inner mitochondrial membrane GTPase critical for mitochondrial fusion and cristae maintenance) in POMC neurons (*POMCOpa1KO*) exhibit altered glucose metabolism and impaired lipolysis that precedes the onset of obesity. Fasting-induced rise in circulating FFA was reduced, along with HSL phosphorylation, perilipin levels and *Pnpla2* (coding for ATGL) gene expression in epididymal white adipose tissue (eWAT). Furthermore, catecholamine content was selectively reduced in eWAT, suggestive of a reduced SNS tone. Interestingly, acute ICV administration of either αMSH (whose presence in PVN projections is reduced) or Ru360, a specific inhibitor of the mitochondrial calcium uniporter (MCU) described to interact with Opa1, restored lipolysis. Importantly, Ru360 treatment was able to prevent body weight gain in a chronic treatment, by restoring lipolytic inputs. Our results highlight Opa1 in POMC neurons as a critical checkpoint for the connectivity between hypothalamic POMC neurons and adipose tissue, regulating lipolysis possibly through SNS.

P6. Kininogen pathway is implicated in the remodeling of fat tissue

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Background: Brown adipose tissue (BAT) plays a beneficial role on metabolism through the induction of thermogenesis, while its inactivation is related to metabolism unbalance and favors obesity and type II diabetes. BAT might also have an endocrine function that could induce white adipose tissue (WAT) browning and affect other tissues leading to an improvement in the metabolic profile. The secreted products of the Kininogen (Kng) gene play a role in blood coagulation/pressure, pain and inflammation, but the role of these proteins in metabolism is poorly known. We observed that Kng is expressed by BAT and increased in case of BAT activation by cold exposure. Here, we aimed at unraveling the role of Kng in BAT activation and its potential role as a BAT endocrine factor.

Material and Methods: We performed *in vivo* experiments using Kng receptors knock-out (KO) mice exposed to cold or thermoneutrality, and measured various parameters using metabolic cages. We also cultured *in vitro* primary brown/white adipocytes and exposed them to Kng-derived products or β-adrenergic pathway inducers.

Results: When exposed to changes of temperature, mice deficient for Kng receptors showed an impaired brown- and beige-versus-white tissue remodeling and a lack of physiological adaptation. Indeed, KO mice under cold exposure consumed less oxygen and energy and had a reduced food intake compared to controls. They showed a dramatic impairment of induction of browning in WAT, while conversely, WAT lost the capacity of further “whitening” in response to thermoneutrality. Interestingly, Kng-derived proteins had no effect on brown and white adipocytes, indicating that the effect of Kng in mice is not cell-autonomous. However, markers of browning were increased in the KO, confirming the implication of Kng in browning.

Conclusions: All together, these data suggests that Kng is required for the plasticity of adipose tissues occurring in response to challenges in energy balance.

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