Societat Catalana de BIOLOGIA

XXVI JORNADA DE BIOLOGIA MOLECULAR

Organitzada per la Secció de Biologia Molecular de la Societat Catalana de Biologia

INSTITUT D'ESTUDIS CATALANS

Carrer del Carme 47

Barcelona

11 de Juny de 2019

Organitzadors:

David Reverter (IBB-UAB)
Joan Roig (IBMB-CSIC)

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PROGRAMA

9:00-9:25 Recollida de Documentació

9:25 Benvinguda del coordinador de la Secció de Biologia Molecular

9:30-11:00 **Primera sessió (*divide*)**

Chair, Joan Roig (IBMB-CSIC)

9:30-10:15 **Martí Aldea (IBMB-CSIC)**
Holistic control by molecular networks in proliferation and aging.

10:15-10:30 Josep Tarragó-Celada (UB/IBUB)
In vitro and in vivo metabolomics analysis to target colorrectal cancer metastasis.

10:30-10:45 Patrícia Balsas Clavería (IDIBAPS)
The role of SOX11 in MCL protective microenvironment.

10:45-11:00 Ester Antón Galindo (UB/CIBERER/IBUB/IRSJD)
YWHAZ and RBFOX1: Functional studies of two autism genes to understand their role in the brain.

11.00-11.30 **Cafè i sessió de pòsters**

11.30 -13.00 **Segona sessió (*live*)**

Chair, Bernat Crosas (IBMB-CSIC)

11:30-12:15 **Vivek Malhotra (CRG)**
Collagen homeostasis in health, fibrosis and cylindromas.

12:15-12:30 Ilaria Dutto (IRB Barcelona)
ADSL is a novel regulator of ciliogenesis.

12.30-12.45 Ismael Izquierdo Villalba (IBMB-CSIC)
Gαq is a novel regulator of axonal mitochondrial transport.

12:45-13:00 Núria Martínez Gil (UB)
Functional activity of two non-coding variants in WNT16 gene.

13:00-14:30 **Dinar i sessió de pòsters**

14.30-16.15h **Tercera sessió (*study it*)**

Chair, Maria Solà (IBMB-CSIC)

14:30-15:15 **José María Valpuesta (CNB-CSIC)**
The power of cryoelectron microscopy.

15:15-15:30 Claus Schmitz (IBMB-CSIC)
Structural characterization of a bacterial response regulator involved in human pathogenesis.

15:30-16:15 **Elena Martínez (IBEC)**
Engineering tissue models to advance drug development and precise medicine.

16:15-16:30 **Break**

16:30-18:00 **Quarta sessió (*survive or die*)**

Chair, David Reverter (UAB)

16:30-17:15 **Travis Stracker (IRB Barcelona)**
The Tousled like kinases maintain genome and epigenome stability: implications for development and disease.

17:15-17:30 Jara Lascorz (IBB-UAB)
DNA activates the Nse2/Mms21 SUMO E3 ligase in the Smc5/6 complex.

17:30-17:45 Jordi Pujols Pujol (IBB-UAB)
S*ynuClean-D: discovery of a privileged molecule to tackle Parkinson's disease*.

17:45-18:00 Judit Cabana (UB)
A potential pharmacological target for cocaine addiction.

18:00-19:00 **Premis i cerveses**

PRESENTACIONS ORALS

1.

In vitro and *in vivo* metabolomics analysis to target colorrectal cancer metastasis

Josep Tarragó-Celada[1], Carles Foguet[1,2], Jordi Perarnau[1], Xavier Hernández-Alias[1], Silvia Marin[1,2], Míriam Tarrado-Castellarnau[1,2], Mariia Yuneva[3] and Marta Cascante[1,2].

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Metastasis is the process in which cancer cells from the primary tumour are able to disseminate, survive and colonize a new organ. Even though a great effort has been done to elucidate the mechanisms underlying this process, there are still a lot of unknown mechanisms, while it remains the main cause of cancer death. One of the hallmarks that raised in recent years in the study of cancer is the altered metabolism that help cells to proliferate and create a tumour. Our hypothesis is that this adaptation could be crucial also for the metastatic process and can be used as a therapeutic target. In order to evaluate how metabolism is changed during the transition from the primary tumour to an invasive and metastatic phenotype in colorectal cancer, many cancer cell lines with increasing metastatic potential have been studied. Some cell lines isolated from the primary colon tumour, others from a lymph node metastasis, and others from liver metastasis. These cell lines have been studied *in vitro* and a 13C-based metabolomics analysis using glucose and glutamine as a tracer has been performed. The results showed that the cell lines have significant differences in metabolic pathways, specifically in glycolysis and TCA. However, the metabolism of cultured cells can be different from the *in vivo* cell environment. Therefore, in order to validate the results in an *in vivo* model, three cell lines with different metastatic potential were injected subcutaneously into mice. All this metabolic characterisation is combined with other analyses such as LC/MS-based metabolomics, transcriptomics and molecular biology measurements that, with the help of a genome-scale metabolic model, will allow us to identify possible therapeutic targets for metastatic cells.

2.

The role of SOX11 in MCL protective microenvironment.

Patricia Balsas[1,2], Marta Leonor Rodríguez[1], Marta Sureda-Gómez[1], Maria C. Cid[3], Elias Campo[1,2,4], Virginia Amador[1,2]

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Mantle cell lymphoma (MCL) is considered one of the most aggressive lymphoid neoplasms with very short median survival. However, lately several studies have described a subset of patients with an indolent evolution of MCL even in absence of chemotherapy. Indolent MCL present with a predominantly leukemic nonnodal clinical disease (nnMCL) whereas conventional MCLs (cMCL) are characterized by its striking tendency to disseminate throughout the body, infiltrating the lymphoid tissues, BM, PB and extranodal sites. Although, nnMCL and cMCL correspond to the same molecular disease, they differ in a particular gene signature. The nnMCLs have very low or no expression of a subset of 13 genes that are highly expressed in cMCLs, including the transcription factor SOX11, the best characterized discriminatory gene between cMCL and nnMCL tumors. The detection of SOX11 by immunohistochemistry or RT-qPCR in larger series of patients has confirmed the relationships among its lack of expression and longer survival with stable disease in independent cohorts of patients with MCL, suggesting that in addition to its value as a diagnostic biomarker, it may be an important factor in the pathogenesis of MCL. However, the function of SOX11 and its potential target genes in lymphoid cells was poorly known.

We have demonstrated the higher engraftment and invasive capacities towards tissue niches of the SOX11-positive compared to SOX11-knokdown MCL cells in in vivo xenograft models, demonstrating the critically contribution of SOX11 to MCL progression and dissemination. Moreover, within tumor microenvironment, we have shown that SOX11 promotes tumor angiogenesis through the PDGFA-axis in MCL whose inhibition reduced tumor growth and angiogenesis in SOX11-positive MCL xenograft tumors to the same levels as the SOX11-negative ones. These results suggest that increased vascularity in SOX11-positive MCL subset may contribute to the more progressive and aggressive phenotype.

Recently, our group has shown that within a stromal microenvironment, SOX11 overexpression in MCL directly activates CXCR4 and FAK transcription. CXCR4 overexpression in SOX11-positive MCL cells and CXCL12 secreted by bone marrow stromal cells enhance FAK phosphorylation and PI3K/AKT and ERK1/2 FAK-downstream pathways activation in a SOX11-dependent manner, promoting MCL cell migration and contributing to the stromal-induced cell proliferation, survival and drug resistance. We have also demonstrated in in vitro systemic xenograft mice models that target inhibition of these pathways disturbs tumor-stromal protective interactions, facilitating the mobilization of MCL cells from their protective microenvironment to the peripheral blood and making them more accessible to conventional drugs that may help to overcome minimal residual disease and relapse commonly seen in aggressive MCL.

3.

YWHAZ and *RBFOX1*: Functional studies of two autism genes to understand their role in the brain

Ester Antón Galindo[1], Bàrbara Torrico[1], Elisa Dalla Vecchia[2], Emilio J Gualda[3], Gustavo Castro[3], Pablo Loza Alvarez[3], William HJ Norton[2], Bru Cormand Rifà[1*], Noèlia Fernàndez Castillo[1*]

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*equally contributed

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder with a multifactorial basis. Although the heritability of the disorder is high, its complex genetic architecture is still not fully understood. Our aim is to evaluate the contribution of two genes previously related to autism, *YWHAZ* and *RBFOX1*, by investigating their effect on nervous system development, neuronal activity and behaviour. For this purpose, we have used different genetic, molecular, behavioural and imaging approaches in zebrafish. We followed the expression of *ywhaz* and *rbfox1* across central nervous system development in zebrafish using *in situ hybridization* and qRT-PCR. We observed a pan-neuronal expression of *rbfox1* in the whole brain during larval stages and a restricted expression in the thalamus and hyphothalamus during adulthood. In contrast, we found a restricted expression of *ywhaz* in the cerebellum, a region that shows alterations in autistic patients. Additionally, we performed behavioural testing to evaluate social behaviour, anxiety and aggression in the *ywhaz* knockout (KO) mutant line and we found that *ywhaz* -/- animals are more anxious than *wild-type* fish and they show an altered social behaviour. We are currently phenotyping the *rbfox1* KO mutant line. Finally, we are setting up a whole-brain imaging protocol to study neuronal activity *in vivo* in larval zebrafish. The aim is to evaluate the effect of reduced *ywhaz* or *rbfox1* function on neuronal activity during development. Taken together, these approaches will provide us with in-depth information about how loss of *ywhaz* or *rbfox1* function can alter neuronal activity and behaviour with possible implications for ASD.

4.

ADSL is a novel regulator of ciliogenesis

Ilaria Dutto[1], Julian Gerhards[3], Sophia Aicher[3], Antonio Herrera[2], Cedric Boeckx[4], Melanie Philipp[3], Sebastian Pons[2], Jens Lüders[1], Travis H Stracker[1]

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Adenylosuccinate lyase (ADSL) is a homotetramer that plays a crucial role in *de novo* purine synthesis and in the purine nucleotide cycle. Mutations in *ADSL* cause Adenosuccinate lyase deficiency (ADSLD), an autosomal recessive disease characterized by a spectrum of central nervous system defects, including mental retardation, autism and microcephaly, as well as growth delay. ADSL was identified in a screen for genes that regulate brain size due to the fact that they have fixed mutations in the transition from Neanderthal to Homo sapiens. Silencing of *ADSL* in human immortilized-RPE1 by siRNA led to proliferation defects, mitotic errors and p53 activation. In addition, serum starved cells depleted for *ADSL* had less ciliated cells and shorter cilia. Defects in ciliogenesis were confirmed *in vivo* upon depletion of *Adsl* in both chicken embryos and zebrafish and were accompanied in zebrafish by defects in embryo symmetry and heart loop formation. These data suggest that ADSL is a conserved regulator of ciliogenesis and that this role may underlie some aspects of ADSLD pathology. Current progress defining the cellular functions of ADSL will be presented.

5.

Gαq is a novel regulator of axonal mitochondrial transport

Ismael Izquierdo[1], Serena Mirra[2], Antoni Parcerisas[2], Elena Rebollo[1], Cristiane Benincá[1 3], Jose Antonio Enríquez[3], Eduardo Soriano[2] and Anna M. Aragay[1]

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G proteins are the main transducers of intracellular signals from receptors at the plasma membrane[1]. Nevertheless, recent reports indicate a novel localization of G proteins at the mitochondria and other endomembranes where they regulate the physiology of these organelles[2]. In particular, the Gq subfamily is required to keep the proper balance between mitochondria fusion and fission acting at both outer and inner membrane, among other functions[3]. In order to unveil the putative effectors of Gαq that mediate those effects at the mitochondria, our group undertaken a mass-spectrometry analysis based on Gαq immunoprecipitates from cellular endomembranes using different cell lines. The "mito-interactome" study has provided further evidence that G proteins regulate the mitochondrial dynamic process through the interaction with the mitochondrial armadillo domain proteins (Alex3 and 10). Subsequent immunoprecipitation and pull-down studies demonstrated a specific interaction of Gαq with the mitochondrial Rho GTPase 1 (Miro1) and both milton adaptors TRAK1 and 2, that couple mitochondria to Kynesin and Dynein motor proteins and constitute the main regulators of mitochondrial transport in neurons.

To analyze the physiological role of those interactions, we have performed tracking analysis of mitochondria along the axons of hippocampal neurons overexpressing Gαq or its constitutive-active mutant, GαqR183C, as well as activating a Gαq-exclusive DREADD receptor with a specific agonist. The results reveal a significant arrest of mitochondrial motility upon Gαq activation, which in long term affects neuronal complexity and dendritic branching. In turn, depletion of Gαq using short-hairpin RNAs increases the number of motile mitochondria and their speed. In summary, our group postulates a new non-canonical mitochondrial function of Gαq acting as a molecular switch in neurons that would stop mitochondria with its activation at the point of the synapsis and would allow mitochondrial movement during its inactive state.

References

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2. Andreeva A V., Kutuzov MA, Voyno-Yasenetskaya TA. Gα12 is targeted to the mitochondria and affects mitochondrial morphology and motility. *FASEB J*. 2008;22(8):2821-2831.
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6.

Functional activity of two non-coding variants in *WNT16* gene

Núria Martínez Gil

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Numerous studies have highlighted the role of *WNT16* in the determination of bone mineral density (BMD). However, it is poor known its neighbor genes (CPED1 and FAM3C) with bone phenotypes. Here, we present new evidences through *in vitro* functional experiments of two variants in *WNT16*: a common CT insertion associated with BMD (rs142005327) and a rare variant (rs190011371) found only in 3 women of the complete BARCOS cohort (n = 1625), all with BMD below the mean (Martínez-Gil, N. 2018). The variant rs142005327, is located in a region of intron 2 containing typical chromatin enhancer or promoter marks, in osteoblast cell lines (ENCODE). In order to detect possible interactions of this region with other parts of the genome we have performed a 4C experiment in 3 bone cell lines (SAOS2, hFOB and MSC). We have found that the region of the CT insertion interacts with the promoter of the *CPED1* gene and with an alternate *CPED1* promoter upstream of exon 12, expressed in differentiating osteoblasts (Maynard D., R. 2018) (Figure1). Luciferases to test if this region works as enhancer or promoter are underway. Related to the rare variant rs190011371 that is located in the 3'UTR region of the *WNT16* gene. We performed luciferase reporter assays with pmirGLO Dual-Luciferase miRNA target expression vector. We observe significant differences of luciferase expression between the two alleles tested where the minor allele presents less expression (Figure 2). This agrees with the phenotype of the women carriers of the rare variant. This work provides functional evidence of the importance of expression regulatory variants.

7.

Structural characterization of a bacterial response regulator involved in human pathogenesis

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A great number of bacterial proteins involved in human pathogenesis are not yet fully described. In many cases, we know the knock-out phenotypes or related protein partners, but not the molecular basis of their activity and related enzymatic functions.
We performed structural analysis on a bacterial response regulator that belongs to a bacterial type IX secretion system and solved several structures by X-ray crystallography. These structures confirmed former bioinformatical analysis that the protein contains an N-terminal receiver domain linked to a domain of unknown function belonging to the alkaline phosphatase (ALP)-like superfamily.
Despite the active site of this ALP-like domain has a metal coordination sphere that is identical to some relatives within the ALP-like superfamily, the substrate binding pocket is unique and suggests other substrates. Therefore, we tested the binding of potential ligands to the active site of the protein and its activity by enzymatic assays, HPLC, co-crystallizations and crystal soakings.
We propose that our structures represent the first ALP-like domain involved in cyclic nucleotide signaling probably implied in bacterial biofilm formation. We will discuss the function and mechanism of the enzyme as well as further studies based on the structures we obtained.

8.

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

Jara Lascorz[1], Nathalia Varejao[1], Eva Ibars[2], Neus Colomina[2], Jordi Torres-Rosell[2], David Reverter[1]

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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 in the Smc5/6 complex a critical player during recombinational DNA repair is directly stimulated by binding to DNA. Activation of sumoylation requires the electrostatic interaction between DNA and a positively charged patch in the ARM domain of Smc5 which acts as a DNA sensor that subsequently promotes a stimulatory activation of the E3 activity in Nse2. Specific disruption of the interaction between the ARM of Smc5 and DNA sensitizes cells to DNA damage indicating that this mechanism contributes to DNA repair. These results reveal a mechanism to enhance a SUMO E3 ligase activity by direct DNA-binding and to restrict sumoylation in the vicinity of those Smc5/6-Nse2 molecules engaged on DNA.

9.

SynuClean-D: discovery of a privileged molecule to tackle Parkinson's disease

Jordi Pujols Pujol, Salvador Ventura

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Parkinson's disease (PD) is characterized by a progressive loss of dopaminergic neurons, a process that current therapeutic approaches cannot prevent. In PD, the typical pathological hallmark is the accumulation of intracellular protein inclusions, known as Lewy bodies and Lewy neurites, which are mainly composed of α-synuclein. Here, we exploited a high-throughput screening methodology to identify a small molecule (SynuClean-D) able to inhibit α-synuclein aggregation. SynuClean-D significantly reduces the in vitro aggregation of wild-type α-synuclein and the familiar A30P and H50Q variants in a substoichiometric molar ratio. This compound prevents fibril propagation in protein-misfolding cyclic amplification assays and decreases the number of α-synuclein inclusions in human neuroglioma cells. Computational analysis suggests that SynuClean-D can bind to cavities in mature α-synuclein fibrils and, indeed, it displays a strong fibril disaggregation activity. The treatment with SynuClean-D of two PD *Caenorhabditis elegans* models, expressing α-synuclein either in muscle or in dopaminergic neurons, significantly reduces the toxicity exerted by α-synuclein. SynuClean-D-treated worms show decreased α-synuclein aggregation in muscle and a concomitant motility recovery. More importantly, this compound is able to rescue dopaminergic neurons from α-synuclein-induced degeneration. Overall, SynuClean-D appears to be a promising molecule for therapeutic intervention in Parkinson's disease.

10.

A potential pharmacological target for cocaine addiction

Judit Cabana-Domínguez[1,2,3,4], Elena Martín-García[5], Laura Pineda-Cirera[1,2,3,4], Ana Gallego Román[5], Rafael Maldonado[5], Noèlia Fernàndez-Castillo*[1,2,3,4], Bru Cormand*[1,2,3,4]

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*Equally contributed

Cocaine dependence is a complex psychiatric disorder that results from the interaction of environmental and genetic risk factors. Although it is one of the most heritable psychiatric conditions (65-79%), only a few genetic risk factors for cocaine dependence have been identified and replicated so far. In a previous study, we identified an association between a genetic variant (rs1047383) in the *PLCB1* gene and drug dependence, particularly with cocaine addiction, that was replicated in an independent sample. In addition, we found that *PLCB1* is upregulated both in the nucleus accumbens of cocaine abusers and in human dopaminergic-like neurons after cocaine treatment.

In order to confirm the contribution of *PLCB1* to cocaine dependence we aimed at assessing cocaine-seeking behavior in heterozygous *Plcb1* knockout mice. First, we performed a battery of behavioral tests to study the phenotype of mutants and found that *Plcb1* +/- animals have higher levels of anxiety and altered short-term memory compared to wild-type mice. Then, mice underwent operant conditioning maintained by cocaine (0.5 mg/Kg/infusion) and no differences between genotypes were found in the acquisition of operant behavior, in the primary reinforcing effects of cocaine nor in the motivation for the drug. Interestingly, we found that *Plcb1* +/- mice showed a reduction of cue-induced reinstatement of cocaine-seeking behavior compared to wild-type mice, suggesting a reduction in the relapse levels of the heterozygous animals. Hence, we are currently assessing a pharmacological inhibitor of PLCB1 in wild-type mice as a potential therapeutic agent.

PÒSTERS

1.

Glycolytic imbalance in *LRRK2*-iPSC derived dopaminergic neurons as a potential link between Parkinson's disease and type 2 diabetes mellitus

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Background: there are evidences of Parkinson's disease (PD) and type 2 diabetes mellitus (T2DM) comorbidity, both chronic age-related diseases. These pathologic conditions present common molecular pathways such as mitochondrial dysfunction and oxidative stress, which may contribute to this concomitance and leading to shared pathogenic outcomes as inflammation and accumulation of unfolded proteins. We hypothesized that glucose and mitochondrial metabolism are altered in dopaminergic neurons (DAn) derived from induced pluripotent stem cells (iPSCs) from PD patients carrying leucine-rich repeat kinase 2 (*LRRK2*) mutations, potentially leading to defective glucose entrance or subsequent glycolytic/mitochondrial imbalance, promoting oxidative stress. **Aims:** to determine whether PD phenotype worsens in presence of pre-diabetogenic conditions, by: *i*) exploring glucose entrance into iPSC-DAn through phosphorylated-insulin-receptor-substrate-1 (pIRS-1), in order to determine if there is an alteration in glucose uptake, and *ii*) analysing lactate levels in cell supernatants as well as protein expression levels of oxidative phosphorylation system (OXPHOS), and the derived oxidative damage, in order to assess if these cells present a more glycolytic or oxidative/mitochondrial metabolism. **Methods:** the study design included iPSC-DAn from *LRRK2*-PD patients (n=2) and healthy controls (n=2) for comparison purposes. These cells were cultured during 24 hours: at basal (B: 21.25 mM) and at high glucose concentration (HG: 50 mM), the latter resembling a pre-diabetogenic environment. We quantified protein levels of pIRS-1, total IRS-1 and subunits of each enzymatic complex of OXPHOS through Western Blot (all data normalized by β-actin); lactate levels were evaluated by epoc® Blood Analysis System, oxidized proteins and total antioxidant capacity (TAC) were determined by means of OxyBlot and TAC assay kits, respectively. **Results:** *LRRK2*-PD iPSC-DAn presented trends to decreased pIRS-1 activation vs. controls, suggesting defective glucose entrance both in B (72.7% decrease of pIRS-1/β-actin; 68.5% decrease of the ratio pIRS-1/total IRS-1) and in HG conditions (75.9% decrease of pIRS-1/β-actin; 84% decrease of the ratio pIRS-1/total IRS-1). Additionally, PD cells presented trends towards biased glycolitic/oxidative metabolism vs. controls. Such metabolic switch was evidenced by increased lactate production and altered OXPHOS protein expression in both media, leading to oxidative damage, as evidenced by increased oxidised proteins and reduced TAC in PD cells. **Conclusions:** despite the limited sample of patients and the preliminarity of the data, PD cells seemed to present a defective glucose entrance, mitochondrial impairment and oxidative damage, due to metabolic disturbances. Altogether, suggest that alterations in glucose metabolism could be a potential linkage in both diseases and pre-diabetogenic environment could be a potential risk factor for PD progression.

2.

Clinical review and structural and functional analyses of DPH1 syndrome disease-causing variants

Castilla-Vallmanya, L; Urreizti, R; Mayer, K; Evrony, GD; Said, E; Cody, NAL; Plasencia, G; Gelb, BD; Grinberg, D; Brinkmann, U; Webb, BD, Balcells, S.

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DPH1 variants have been associated with an ultra-rare and severe neurodevelopmental disorder mainly characterized by variable developmental delay, short stature, dysmorphic features, and sparse hair.

The disease-causing mutation was identified in 2 undiagnosed patients through WES. A survey of all the clinical features of 17 DPH1 syndrome patients reported so far from 7 families was performed. The DPH1 enzyme activity of wild-type and of 7 disease-causing mutants was assessed through the diphtheria toxin ADP-ribosylation assay. A homology model of the human DPH1-DPH2 heterodimer was built, and molecular dynamics simulations were performed to study the effect of these variants on the catalytic sites, as well as on the interactions between subunits of the heterodimer.

Two DPH1 novel variants were identified in 2 independent families, enriching the clinical delineation of DPH1 syndrome. The enzyme assay demonstrated compromised functionality for 5 mutations (p.Leu234Pro; p.Ala411Argfs*91; p.Leu164Pro; p.Leu125Pro; p.Tyr112Cys). According to the structural model, p.Leu125Pro may affect dimerization while p.Tyr112Cys, p.Leu164Pro, p.Leu234Pro and p.Pro382Ser may interfere the binding of the iron-sulphur cluster necessary for catalysis.

The overall good correlation observed between DPH1 protein activity, structural prediction and clinical features indicate that these biochemical and structural tests may be useful tools for assessing the pathogenicity of DPH1 variants and for helping to predict the clinical severity of future DPH1 cases.

3.

Gene silencing of Thymidylate Synthase in prostate cancer cells using Polypurine Reverse Hoogsteen Hairpins

Eva Aubets[1], Alex J. Félix[1], Virginia García[2], Anna Aviñó[3], Carmen Plasencia[2], Ramon Eritja[3], Véronique Noé[1] and Carlos J. Ciudad[1]

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The Thymidylate synthase enzyme (TYMS) is used as an anti-cancer target because of its role in the synthesis the novo of dTTP and it is known to be autoregulated at the translational level. Treatment with TYMS inhibitors (e.g. 5-FU) can lead to tumor resistance through overexpression of TYMS, either by chromosome aberrations or by alteration of molecular mechanisms that regulate gene expression of this gene. It has been proposed that G-quadruplex nucleic acid sequence motifs may regulate translation as well as transcription. Therefore, we explored the incidence of G-quadruplex motifs in the 5' untranslated region (5'-UTR) of the mRNA of the thymidylate synthase gene as potential targets in cancer treatment. By using the QGRS mapper, we found a predicted G-quadruplex (G4) that was confirmed by circular dichroism and UV melting measurements for the RNA sequence. In consequence, and to develop new therapeutic agents to TYMS inhibitors, we designed a Polypurine Reverse Hoogsteen hairpin (HpTYMS-G4-T-PPRH) against the complementary strand of this G4 sequence.

First, we demonstrated the ability of binding of the PPRH to its target sequence by gel-shift assays. HpTYMS-G4-T was able to bind to the template strand of the TS ds DNA target sequence in *in vitro* assays. In addition, TYMS either purified or from nuclear extracts was able to bind to this target sequence, both as dsDNA or ssDNA sequences, whereas 2 negative control proteins (DHFR and BSA) did not produce any binding. Additionally, by using the PPRH HpTYMS-G4-T and the purified TYMS we observed that both compete with each other for the binding to the target sequence in the DNA.

Next, as TS is involved in dTTP synthesis, we compared the effect of this PPRH in PC3 cells incubated either in the presence or the absence of thymidine. The HpTYMS-G4-T PPRH was cytotoxic in both media, decreasing cell viability to less than 5% for at a concentration of 100 nM. These results raised the concept that TS could be involved in other proliferation pathways. The response of PC3 cells after treatment with HpTYMS-G4-T was dose dependent. This PPRH could be altering the transcription of TS gene at this site or the regulation of a G4 structure at this point. In fact, TYMS mRNA determination by RT-qPCR after incubation with HpTYMS-G4-T confirmed that the mRNA levels for this protein were decreased. In conclusion, our results show the ability of the designed PPRH to bind to its target sequence of TS and to decrease PC3 cells viability. Therefore, PPRHs can be considered as a new type of molecules to modulate TS expression and overcome the resistance produced by traditional treatments.

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4.

Rpn5 SUMOylation as a novel proteasome regulator

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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. 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. When protein homeostasis fails, the whole cell functioning is impaired. This is often associated with cell stress, toxicity and cell death.

In this work we study the sumoylation of Rpn5, the yeast homologue of non-ATPase regulatory subunit 12 (PSMD12). In a previous work, Alfred Vertegaal lab described a sumoylation site in the human PSMD12 (1). We thus screened the putative sumoylation sites of Rpn5 performing in vitro sumoylation assays and site directed mutagenesis we found K147-K148 as preferred modification sites.

Sumoylation is a post-translational modification that regulates a multitude of cellular processes, including cell-cycle progression, replication, protein transport and DNA damage response. In this process, similar to ubiquitylation, SUMO (small ubiquitin-like modifier) is covalently attached to target proteins in a reversible process via an enzymatic cascade.

We created a strain in which Rpn5 is substituted with the Smt3-Rpn5 chimera, that mimics Rpn5 sumoylation, to better characterize the functionality of a sumoylated proteasome and the effects on cell physiology and viability. Smt3-Rpn5 proteasomes show a normal subunit composition and regulatory particle stability. Remarkably, Smt3-Rpn5 proteasomes present a decrease in 20S particle (CP), a different distribution in RP2-CP and RP-CP subcomplexes and a decrease in LLVY-AMC degradation activity. We are particularly interested in elucidating the physiological meaning of this phenotype and how proteasome sumoylation affects this stress response. Moreover, we want to go further into the mechanism by which Rpn5 is sumoylated, deciphering which are the factors involved in the enzymatic cascade, the conditions that trigger this modification and the relationship with other post translational modification pathways.

5.

Exploring the Functional Effect of *Cis*- and *Trans*-Regulatory Elements in the Mammal Retina

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Spatio-temporal patterns of gene expression are determined by different type of regulatory elements, which vary depending on its nature and can be tissue and/or cell type specific. *Cis*-regulatory elements are non-coding regions of DNA that modulate the expression of neighbour genes, whereas *trans*-regulatory elements are transcription factors that promote or repress transcription by its direct binding to specific DNA target sites. An accurate control and integration of these transcription regulatory elements is essential for the correct function of several tissues and particularly, for the retina, due to its high sensitivity and specialization. Mutations in the genes and DNA sequences involved in the trans- and cis- regulation of retinal relevant genes are responsible for photoreceptor death and cause inherited progressive blindness.

Nr2e3 encodes an orphan nuclear receptor that function as both a transcriptional activator and repressor and is crucial in the determination of photoreceptor fate, towards cone or rod differentiation, during development. Therefore, NR2E3 is a *trans*-regulatory element of photoreceptor specific genes. As a means to understand the dysfunction of this gene in retinal human pathologies, we are currently assessing the phenotypic effect of several modified alleles generated in the last exon of *Nr2e3* in mouse by CRISPR/Cas9 and which alter or delete relevant domains of this transcription factor.

On the other hand, the complete deletion of the *Cerkl* locus, a retinitis pigmentosa causative gene, cause perinatal lethality. Our preliminary findings suggest that several *cis*-regulatory elements mapping within the *Cerkl* locus are potentially self-regulatory but can also modulate the expression of *NeuroD1*, an essential neuronal development gene located upstream *Cerkl.* To determine whether these super-enhancers are actually regulating *NeuroD1*, we are performing *in vivo* microinjection of expression reporter vectors in zebrafish embryos as well as *in vitro* assays.

To elucidate the genetic basis of retinal neurodegeneration, it is necessary to understand the *trans*-acting factors and *cis*-regulators controlling the differentiation and maintenance of specific neuroretinal cell types, such as *Nr2e3* and *Cerkl*, whose alteration trigger the onset and progression of neurodegeneration in the retina.

6.

Sustained Release of a Pharmacological Chaperone that Increases the Activity of Misfolded β-Glucocerebrosidase

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Lysosomal storage diseases (LSDs) are a group of over 70 diseases that are characterized by lysosomal dysfunction. Many LSDs originate from mutations that affect the proper folding of the enzyme in the endoplasmic reticulum (ER). The misfolded enzyme is then re-directed to the ER-associated degradation pathway instead of being directed to the lysosome. This leads to the accumulation of substrates inside the lysosome ("storage"), which ultimately results in cell dysfunction and cell death.

The use of *pharmacological chaperones (PCs)* is an emerging enzyme enhancement therapeutic approach for the treatment of protein misfolding diseases. PCs are small molecules that act by stabilizing the native conformation of the mutant enzyme, allowing more molecules to escape the ER-associated degradation pathway and ultimately resulting in increased amounts of the enzyme in the lysosome. Therefore, the activity of the mutated proteins is partially rescued, which has a favorable impact on the patient status and the rate of disease progression.

Ambroxol hydrochloride (AH) has recently been shown to act as a PC for the lysosomal enzyme β-glucocerebrosidase (GCase). Mutations in the gene encoding GCase cause Gaucher's disease, which is the most prevalent LSD. Deficiency of GCase results in the accumulation of glycosphingolipids, primarily in macrophages, and the development of hepatosplenomegaly, anemia, skeletal lesions and central nervous system dysfunctions. AH stabilizes the proper folding of GCase favoring its trafficking to the lysosome. Its effectivity has been demonstrated using both in vitro and in vivo models and is currently in clinical trials.

Since the use of PCs is a therapeutic paradigm recently launched, encapsulation into carriers to regulate their release has not yet been explored. With this aim, we have designed a multilayered system based on poly(ε-caprolactone) (PCL), which is an FDA-approved biodegradable polymer widely used for biomedical applications. We have successfully encapsulated the AH into electrospun PCL microfibers and have protected the resulting fibrous matrix with a coating that regulates its release. This protection has been achieved by sandwiching the AH-loaded matrix between two PCL nanomembranes obtained either by spin-coating or by dip-coating.

We have conducted release experiments in physiological media to evaluate the kinetics of the PC release. While we observe a fast release of AH from the original fibrous scaffolds, which occurs in less than an hour, we have been able to control this quick release with the additional external coatings. Thus, the AH release extends to weeks and months when coated fibrous scaffolds are prepared by dip-coating and spin-coating, respectively. Using a fluorogenic substrate-based assay, we have also verified that the released PC maintains its bioactivity, protecting GCase against induced thermal denaturation.

Altogether, our results demonstrate that dosage of AH to increase the activity of misfolded GCase can be regulated with these PCL-coated scaffolds.

7.

Ror2 controls Snail1 expression in tumor cells

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Wnt proteins regulate different cellular processes during development and disease, particularly in cancer. Wnt ligands interact with transmembrane Frizzled (Fz) receptors and different co-receptors, depending on which they stimulate canonical or non-canonical Wnt signaling pathways. The canonical Wnt3a binds to Fz and co-receptor LRP5/6 promoting β-catenin stabilization and transcriptional activation of β-catenin-dependent genes. In contrast, non-canonical Wnt5a ligand binds to Fz and co-receptor Ror2 and produces a decrease in ׀׀-catenin through the activation of the E3 ligase Siah2. Remarkably, although the effects on ׀׀-catenin levels are contrary, both Wnt5a and Wnt3a stimulate invasion of tumor cells.

Non-canonical Wnt ligands induce Stat3 phosphorylation and this activation is required for transcription of Epithelial-to-Mesenchymal-Transition (EMT)-associated genes (Gujral TS *et al*., 2014). We have demonstrated that canonical ligands such as Wnt3a also up-regulate Stat3 phosphorylation. For both ligands, Stat3 phosphorylation is dependent on Src and Fyn tyrosine kinases. Stat3 activation is required for stimulation by Wnt ligands of Snail1 transcription, a factor essential for EMT that increases tumor cell invasion and also chemoresistance (Lambies G *et al*., 2019); therefore, inhibition of Src or Fyn considerably retard cell invasion in response to Wnt3a and Wnt5a.

Our results indicate that in cell lines there is a clear co-relationship between Ror2 and Snail1 expression. To investigate this, we have depleted Ror2 in several cell lines. Ror2-down-regulation decreases Snail1 protein; moreover it prevents the increase in Snail1 observed in cells upon treatment with cisplatin or paclitaxel. Consequently, Ror2-depleted cells are much more sensitive to these two agents than wild-type cells. Finally, as it happens with Ror-depletion, the Src kinase specific inhibitor Dasatinib that decreases Stat3 phosphorylation also impairs the Snail1 up-regulation in response to paclitaxel.

These results suggest that activation of the non-canonical Wnt pathway through Ror2 co-receptor promotes Stat3 phosphorylation and Snail1 up-regulation, which increases resistance to antineoplastic drugs. Therefore, chemo-resistance in cells with Ror2 high expression might be partially alleviated by blocking the Ror2/Src/Fyn/Stat3 axis.

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8.

Cerkl and *Bex3*: neural genes expressed in the mammal retina that regulate mitochondrial dynamics

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The retina is the specialized region of the central nervous system that transduces light into neural signals. It is endowed with an active metabolism and displays a particular vulnerability to genetic alterations causing mitochondrial dysfunction, such as impaired energy production, mtDNA instability, disturbance of mitochondrial dynamics and mitochondrial quality control. These alterations make photoreceptors and retinal ganglion cells more susceptible to cell death.
Here we studied two genes highly expressed in central nervous system: *Cerkl* and *Bex3*, focusing on their mitochondrial distribution in mouse retinal cells and their possible role as mitochondrial dynamics regulators.
Mutations in *CERKL* cause Retinitis Pigmentosa in humans, a visual disorder characterized by photoreceptors neurodegenaration and progressive vision loss. Both in human and mouse, *CERKL* produces a wide range of mRNA isoforms that translate into proteins displaying different domains. We describe a pool of CERKL isoforms localizing at mitochondria in retinal primary culture. Moreover, we studied the impact of CERKL protein levels modulation on mitochondrial network organization by using both CERKL overexpression and knock-down models.
Bex3 belongs to the *Bex* (brain-expressed x-linked) gene family, composed of six members, *Bex1* to B*ex6*. BEX3 has been shown to interact with p75 and has been suggested to regulate NGF-mediated functions, such as survival and differentiation. Our data showed that BEX3 is expressed in embryonic retinal cultures and in adult mouse retina. Furthermore, endogenous BEX3 can localize at mitochondria, suggesting a possible role of this protein in regulating mitochondrial biology during both retinal development and in the adult.
Overall, our studies describe *Cerkl* and *Bex3* as two retinal neural genes involved in the regulation of mitochondrial function, adding knowledge to the picture of the multiple pathways controlling mitochondrial health in the mammalian retina.

9.

Targeting metabolic vulnerabilities of Cancer

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Altered cell metabolism is an established hallmark of cancer. Cancer cells rewire their metabolism to cope with boosted energy demand, enabling cancer cells rapid proliferation, survival in harsh environment, invasion, metastasis, and drug resistance. A common feature of the altered metabolism in cancer cells is the increased glucose uptake and conversion to lactate through aerobic glycolysis (`Warburg effect´) [1]. However, in the past few years this process has been shown to be more complex than simply an increase in glycolysis. Tumors require catabolites to produce ATP, maintain a reduction-oxidation (redox) balance, and generate biomass. Many biosynthetic reactions and catabolic reactions, including ATP generation, occur within mitochondria. In this regard, recent studies provide substantial evidence that mitochondrial functionality and a healthy mitochondrial metabolism are essential for tumorigenesis [2].

We aim to exploit the metabolic alterations in cancer cells following the hypothesis that aberrant mitochondrial oxidative pathways represent potential molecular targets for the development of *novel targeted cancer treatments*. By using a combination of biochemical approaches, *in vitro* studies in cultured human cancer cells and *in vivo* studies in mice, we have addressed the impact of new anticancer drugs targeting mitochondrial homeostasis alone or in combination with inhibitors of checkpoint kinases involved in DNA Damage Response. In particular, we are interested in metabolic drugs targeting key mitochondrial enzymes that are crucial for energy metabolism in cancer cells. By performing an *in vivo* genome-wide CRISPR/Cas9-based screening we aim to identify potential targets to enhance metabolism-based anticancer drugs action and efficacy, as well as biomarkers that would predict drug resistance for future therapeutic interventions.

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10.

Functional analysis of the C-terminal domain of Nse2 in protein sumoylation and DNA damage repair

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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. A prominent player during chromosome replication, repair and segregation is the Nse2 subunit in the Smc5/6 complex. Nse2 contains a C-terminal SP-RING domain, coding for an E3 SUMO ligase domain, and targets different proteins involved in various chromosomal transactions. While the NSE2 gene is essential from yeast to mice, its SP-RING domain is dispensable for growth in budding yeast. However, *nse2* mutant cells lacking its C-terminal domain are sensitive to DNA damage. Of note, 2 patients have been described bearing frameshift mutations in the C-terminal part of the NSMCE2 gene (the human orthologue of budding yeast Nse2). Their main phenotype is primordial dwarfism, extreme insulin resistance, and gonadal failure. Quite surprisingly, the truncation does not affect the RING and merely removes a short alpha helix located after the E3-ligase domain.

To characterize the role of the RING domain in cell viability, DNA damage repair and protein sumoylation, we have developed yeast strains affected in the C-terminal part of Nse2. These mutant cells bear either point mutations in conserved histidine and cysteine residues required for proper folding of the RING domain or truncations in the C-terminal alpha helix. Our results indicate that mutation of conserved residues in loop1 or loop2 of the RING domain leads to MMS-sensitivity and mild structural defects characterized by temperature sensitivity. However, these mutants are not severely affected in protein sumoylation in vivo. In striking contrast, truncation of the C-terminal alpha helix, in the presence of an intact RING domain, leads to severe growth defects, a stronger MMS and temperature sensitivity, and lower levels of sumoylation. Our results indicate a more prominent role for the C-terminal alpha helix in Nse2-dependent SUMO ligase activity and DNA damage repair than previously anticipated. In addition, our results suggest that proper folding of the SP-RING domain may play a marginal role during protein sumoylation.

11.

Expression, Solubilization and Purification of Mitochondrial Transmembrane Proteins

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I am working on a human mitochondrial transmembrane protein which has a N-terminal domain and a C-terminal domain. Since I started I produced different constructs of both domains separately because the full length protein is known to be highly insoluble. Regarding the N-terminal domain I have been able to express and solubilize it, but when it comes to purification I got degradation problems. In relation to the C-terminal domain, I haven't succeed in solubilization, so I am considering different approaches. The aim of my presentation will be to explain the different mishaps I encountered, and how I managed to solve them.

12.

Structural functional studies on human α2-macroglobulin, G-relatedα2-macroglobulin bindingprotein and human transforminggrowth factor-β2

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α2-Macroglobulins (α2Ms) regulate peptidases, hormones and cytokines [1, 2, 3]. Mediated by peptidase cleavage, they transit between a native, intact form and an activated, induced form. α2Ms have been studied over decades using authentic material from primary sources, which was limited by sample heterogeneity and contaminants [4, 5]. Here, we developed expression systems based on transient transfection in *Drosophila* Schneider 2 and human Expi293F cells, which produced pure human α2M (hα2M) at ~1.0 and ~0.4 mg per liter of cell culture, respectively. In both cases, hα2M was mainly found in the induced form. Shorter hα2M variants encompassing N-/C-terminal parts were also expressed and yielded pure material at ~1.6/~3.2 for N-hα2M and ~1.3/~4.6 for C-hα2M, mg per liter of insect or mammalian cell culture, respectively. We then analyzed the binding of recombinant and authentic hα2M to human recombinant latent transforming growth factor-β2 (pro-TGF-β2) and bacterial G-related α2M binding protein (GRAB) by surface plasmon resonance, multiple-angle laser light scattering, size-exclusion chromatography, fluorogenic labelling, gel electrophoresis and Western-blot analysis. Two GRAB molecules formed stable complexes of high affinity with native and induced authentic hα2M tetramers. The shorter recombinant hα2M variants interacted after preincubation only. In contrast, pro-TGF-β2 did not interact, probably owing to hindrance by the N-terminal latency-associated protein of the cytokine.

Transforming growth factor β is a disulfide-linked dimeric cytokine that occurs in three highly related isoforms (TGFβ1–TGFβ3) engaged in signaling functions through binding of cognate TGFβ receptors [6, 7]. To regulate this pathway, the cytokines are biosynthesized as inactive pro-TGFβs with an N-terminal latency-associated protein preceding the mature moieties [8]. Here, we obtained pure human pro- TGFβ2 at ~2.7 mg per liter of mammalian cell culture (Expi293F cells). We performed crystallization assays and obtained a different crystal form of mature TGFβ2, which adopted a conformation that deviated from previous structures. Significant differences in the wrist region lead to a distinct dimeric structure that would require significant rearrangement for binding of TGFβ receptors. This new conformation may be reversibly adopted by a certain fraction of the mature TGβ2 population and represent a hitherto undescribed additional level of activity regulation of the mature growth factor once the latency-associated protein has been separated.

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13.

Cerkl and Bex3: neural genes expressed in the mammal retina that regulate mitochondrial dynamics

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Overall, our studies describe Cerkl and Bex3 as two retinal neural genes involved in the regulation of mitochondrial function, adding knowledge to the picture of the multiple pathways controlling mitochondrial health in the mammalian retina.

14.

Neuronal differentiation and bioenergetics in adipose stem cells from patients with *LRRK2* Parkinson's disease

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Background: In vivo access of dopaminergic neurons (DaN), the definite target tissue of Parkinson's disease (PD), is not possible. Despite the great amount of cell models to study this pathology, there are important limitations from most of them. DaN derived from induced pluripotent stem cells (iPSCs) requires dedifferentiation and redifferentiation processes with high economic and temporal costs as well as low differentiation efficiency rates. On the other hand, genetic PD associated to leucine-rich repeat kinase 2 (LRRK2) mutations has been related to mitochondrial dysfunction in different cell models, but never assessed in the present model of study. **Aims:** i) to develop neuronal differentiation from neural crest stem cells (NCSC) from PD patients carrying LRRK2 mutation and ii) to characterize mitochondrial respiration in NCSC adipose stem cells. **Methods:** Explants of adipose tissue biopsies from the forearm of a control and LRRK2G2019S-PD patient were cleansed, cut in 1-2 mm2 and cultured in DMEM supplemented with pyruvate, fetal bovine serum, growth factors and antibiotics to promote growth of NCSCs. Adipose stem cells specific markers CD73, CD90 and CD105 were confirmed by flow cytometry. DaN differentiation was induced using neurobasal medium supplemented with B27, epidermal and fibroblast growth factors. The following measurements were assessed in NCSCs and DaN in parallel: Neuronal markers MAP2, BIII tubulin and tyrosine hydroxilase were determined by western blot. Proliferation rates were analyzed by hemocytometer counting. Mitochondrial function was assessed in NCSCs by high resolution respirometry. **Results:** We observed positive signals for specific markers of both adipose stem cells and neuronal cells including dopaminergic differentiation, through tyrosine hydroxilase. As expected, growth rates were lower under neuronal differentiation. Finally, preliminary results suggest decreased respiratory rates in NCSC from LRRK2-PD patients with respect to the control. **Conclusions:** Neuronal differentiation from adipose stem cells exhibit neuronal markers and lower growth rates, as expected. Lower rates of overall mitochondrial respiration in NCSC from LRRK2-PD patients may account for mitochondrial suboptimal function in the model, as observed in the disease and other cell models. Altogether, our findings suggest that DaN/NCSC may represent an adequate model of study for PD, allowing for in vivo assessment.

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15.

Molecular evidence of adenosine deaminase linking adenosine A_{2A} receptor and CD26 proteins from different cells

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Adenosine is an endogenous purine nucleoside that acts in all living systems as a homeostatic network regulator through many pathways, which are adenosine receptor (AR)-dependent and -independent. From a metabolic point of view, adenosine deaminase (ADA) is an essential protein in the regulation of the total intracelular and extracellular adenosine in a tissue. In addition to its cytosolic localization, ADA is also expressed as an ecto-enzyme on the surface of different cells. Dipeptidyl peptidase IV (CD26) and some ARs act as binding proteins for extracellular ADA in humans. Since CD26 and ARs interact with ADA at opposite sites, we have investigated if ADA can function as a cell-to-cell communication molecule by bridging the anchoring molecules CD26 and A2AR present on the surfaces of the interacting cells. By combining site-directed mutagenesis of ADA amino acids involved in binding to A2AR and a modification of the bioluminescence resonance energy transfer (BRET) technique that allows detection of interactions between two proteins expressed in different cell populations with low steric hindrance (NanoBRET), we show direct evidence of the specific formation of trimeric complexes CD26-ADA-A2AR involving two cells. By dynamic mass redistribution assays and ligand binding experiments, we also demonstrate that A2AR-NanoLuc fusion proteins are functional. The existence of this ternary complex is in good agreement with the hypothesis that ADA could bridge T-cells (expressing CD26) and dendritic cells (expressing A2AR). This is a new metabolic function for ecto-ADA that, being a single chain protein, it has been considered as an example of moonlighting protein, because it performs more than one functional role (as a catalyst, a costimulator, an allosteric modulator and a cell-to-cell connector) without partitioning these functions in different subunits.

16.

An effortless microcolony-based approach for measuring yeast replicative lifespan

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Budding yeast has been widely used in ageing research for the last 60 years[1], due to its short lifetime and the existence of multiple genetic manipulation tools. Regarding replicative ageing, the most relevant phenotype is the replicative lifespan (RLS). The existing methods for measuring RLS are manual microdissection[2] (very labor intensive and low throughput) or based on microfluidic systems[3] (requiring clean room facilities, very long timelapse microscopy and media pumping systems). Here we present an effortless technique to estimate the RLS of a large number of single yeast cells based in the Mother Enrichment Program (MEP)[4], where we infer the RLS as a function of the area of single microcolonies generated by the MEP-induced yeast when plated at low density in restrictive conditions for daughter cell's viability. We also have developed an ImageJ[5] macro to quickly measure microcolony area at a high-throughput level[6].

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17.

Role of TBC1D15 and Gq in mitophagy

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Mitochondria are the powerhouses of cells. Mitochondria produce ATP by oxidative phosphorylation, in which electron are passed through the electron transport chain. They also regulate calcium homeostasis and signaling from and to other organelles. Perturbed neuronal Ca2+ levels lead to neuronal death and are implicated in neurodegenerative diseases including AD. Although the importance of signaling pathways to control cellular homeostasis, not much was known about the role of heterotrimeric signaling pathways (GPCRs and G proteins) and mitochondria. Our research (Beninca et la., 2014) demonstrated that G proteins behave are regulators of mitochondria dynamics and physiology which is a new non-canonical function for G proteins. Utilizing a throughput analysis of proteins by immunoprecipitating enriched fractions of endomembranes our group found a collection of Gq binding partners affecting mitophagy. Here we present new data demonstrating that Gq is necessary during the process of autosomal maturation. In its absence there is an increase in mitophagy demonstrating by MitoQC probe. We also have results that demonstrate that Gq forms a ternary complex with the mitocondrial protein Fis1 and the Rab7GAP protein TBC1D15 and TBC1D17.

18.

Determinants of centrosome separation in prophase

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During prophase, centrosomes separate in a dynein and Eg5-dependant way. Dynein, a minus-end-directed motor protein complex, is in charge of pulling centrosomes along the nuclear envelope, whereas Eg5, a plus-end-directed member of the Kinesin-5 family, is capable of binding antiparallel microtubules emerging from both centrosomes and slide them apart.
To do so, Eg5 needs to accumulate around centrosomes. Taking into consideration that Eg5 is a plus-end directed protein, it is thus surprising to find it in the minus ends of microtubules. Our works aims to better understand how this localization is attained and whether the dynein complex is responsible for transporting Eg5 to the vicinity of the centrosome.

19.

Development of an in *vitro* system to test nucleotide-dependent loading onto DNA and SUMO ligase activity of an SMC complex

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Abstract

Throughout our lives, our cells have to divide several times. During each division, cells have to accurately copy their genome and equally distribute the two copies between the daughter cells. Therefore, precise chromosome disjunction is essential to maintain genomic stability. The Structural Maintenance of Chromosomes (SMC) family is a group of ring-shaped protein complexes that regulate chromosome organization and stability. Cohesin, condensin and SMC5/6 are the most relevant members of this family.

The SMC5/6 complex is mainly involved in removal of recombination and replication intermediates, thus safeguarding chromosome disjunction. To achieve this goal, SMC5/6 is equipped with various subunits, including Nse2, a SUMO E3 ligase. Current models about the function of SMC5/6 mainly derive from genetic experiments using hypomorphic mutants. However, we currently ignore how the activity of the complex is regulated. Hence, our aim is to develop an *in vitro* system to study the mechanisms used by the SMC5/6 complex to load onto DNA and to regulate its sumoylation activity.

Here we show that DNA stimulates the sumoylation activity *in vitro* using an entire SMC5/6 complex purified from budding yeast. Smc5/6 rapidly promotes polymerization of SUMO into SUMO chains of high molecular weight. Mass spectrometry analysis shows that various Smc5/6 subunits are targeted by SUMO, with some preferential sumoylation hotspots within the Smc5/6 molecule. On the other hand, we also show that the SMC5/6 loading onto DNA is highly increased in *in vitro* loading assays when the non-hydrolysable ATP analogues ADP+AlF_x and ADP+VO_4^{3-} are added to the reaction. This result suggests that the loading of SMC5/6 onto DNA requires ATP binding but not its hydrolysis. We propose that ATP-dependent binding promotes SMC5/6 loading onto DNA and subsequent activation of the Nse2 SUMO ligase.

20.

Expression, purification and crystallization of a bacterial zinc finger protein

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Brief Summary: Two bacterial Zn-finger protein homologs that activate essential genes in both *Pseudomonas aeruginosa* and in *Escherichia coli*, are expressed in *E.coli* as constructs that contain the SUMO tag to increase the protein solubility. At the end of the SUMO protein, there is the cleavage site by the SUMO protease. However, the cleavage was inefficient. We added a cleavage site for the TEV protease to these constructs. Initial trials showed that the SUMO- proteins were not cleavable neither by TEV nor SUMO, under many different conditions. One possible reason was that either SUMO cleavage site in the initial constructs, or the TEV site in the new ones, were too close to the Zn-finger protein. While addition of two aminoacids between the protein and the TEV cleavage site did not allow cleavage, high efficient cleavage was achieved for constructs that contained a linker of six aa between the protease cleavage site and the Zn-finger protein. We are currently working on purification protocols for both constructs, which include affinity chromatographies, a first step to isolate the fusion protein and a second to isolate protein of interest. Crystallization will follow with unliganded protein, or in complex with nucleotides that activate the protein, and/or with DNA sequences. *P. aeruginosa* is an opportunistic gram-negative bacteria that can grow under a variety of conditions, such as in soil, water, and in human, animal and plant host-associated environments. By virtue of its high adaptability, *P. aeruginosa* evolved to as a nocosomial infection agent that affects inmunocompromised patients or individuals with complicated conditions, such as traumatic burns or who suffer genetic illness such as cystic fibrosis {Azam, 2018 #475}. In these latter, *P. aeruginosa* invades the lungs {Davies, 2007 #474}. Indeed, its high adaptability converted this microbe in a **multidrug resistant pathogen** {Azam, 2018 #475}. Our final aim is to allow for a drug design against this target. Therefore, the crystal structure of this essential protein of interest and its homolog in *E. coli* will be pivotal to combat this pathoen with new strategies.

21.

Expression, purification and characterization of a mitochondrial nucleoid maintenance factor

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Candida albicans is a human pathogen causative of a number of infectious diseases that can lead to life-threatening disorders in immunocompromised patients. Our aim is the structural characterization of a *C.albicans* mitochondrial DNA maintenance factor. This factor is indispensable for mitochondrial DNA maintenance and for *C.albicans* survival, thus representing a potential therapeutic target. We successfully expressed and purified the protein, and identified the DNA substrates suitable for crystallization. We obtained crystals that diffract from low to medium resolution. We are currently working on structure solution by applying experimental phasing methods. Our current efforts are focused in dealing with highly anisotropic data which shows very weak anomalous signal. Strategies include addition of extra methionines by mutagenesis and heavy atom screenings. Complementary studies consist in SAXS analysis of the unbound protein and molecular microscopy imaging that shows DNA compaction by the protein.

22.

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 in the Smc5/6 complex, a critical player during recombinational DNA repair, is directly stimulated by binding to DNA. Activation of sumoylation requires the electrostatic interaction between DNA and a positively charged patch in the ARM domain of Smc5, which acts as a DNA sensor that subsequently promotes a stimulatory activation of the E3 activity in Nse2. Specific disruption of the interaction between the ARM of Smc5 and DNA sensitizes cells to DNA damage, indicating that this mechanism contributes to DNA repair. These results reveal a mechanism to enhance a SUMO E3 ligase activity by direct DNA binding and to restrict sumoylation in the vicinity of those Smc5/6-Nse2 molecules engaged on DNA.

23.

The role of Tousled like kinases in genome stability and cancer

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